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DETEKSI DINI *GANODERMA* PADA KELAPA SAWIT DENGAN TEKNIK *ELISA*

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ABSTRAK

Poliklonal-poliklonal antibodi dibuat dengan jalan mengimunisasi kelinci dengan ekstrak protein miselia dari isolat tunggal Ganoderma *dan campuran 9 isolat* Ganoderma. *Kespesifikan kedua antibodi tersebut diuji dengan menggunakan* enzyme-linked immunosorbent assay *tidak langsung terhadap contoh akar kelapa sawit dan jamur-jamur saprofitik yang berasosiasi dengan akar kelapa sawit sakit. Akar kelapa sawit sehat tidak memperlihatkan reaksi antibodi. Namun, antibodi bereaksi silang lemah dengan jamur-jamur saprofitik. Kedua antibodi dalam sistem* enzyme-linked immunosorbent assay *tidak langsung dapat mendeteksi keberadaan jamur* Ganoderma *pada kelapa sawit sehingga berguna untuk deteksi dini jamur tersebut.*

Kata kunci: *ELISA*, deteksi dini, kelapa sawit, poliklonal antibodi, *Ganoderma*

PENDAHULUAN

Jarak tanam kelapa sawit yang biasa digunakan adalah 9m x 9m x 9 m dengan umur ekonomis 25 tahun. Untuk tanam ulang, bibit kelapa sawit ditanam di antara bekas tunggul kelapa sawit. Mula-mula penyakit busuk pangkal batang (BPB) hanya ditemukan pada tanaman kelapa sawit tua pada generasi pertama, tetapi akhir-akhir ini penyakit BPB juga ditemukan di tanaman muda pada generasi kedua (10). Dengan demikian cara penularan penyakit BPB dapat diasumsikan sebagai berikut: pada tanaman muda terjadi lewat kontak akar sehat dengan jaringan kelapa sawit yang terinfeksi, sedangkan pada tanaman tua lewat kontak akar sehat dengan akar yang terinfeksi. Masa inkubasi penyakit memerlukan waktu beberapa tahun dan gejala penyakit hanya terlihat pada stadia infeksi lanjut. Bila gejala penyakit tampak, lebih dari separuh jaringan *bole* telah membusuk dan tanaman tidak dapat diselamatkan.

Salah satu faktor penghambat utama dalam pengendalian penyakit BPB adalah terbatasnya metode diagnostik untuk mendeteksi kehadiran penyakit BPB sedini mungkin. Pada saat ini hanya ada dua metode yang digunakan untuk deteksi dini penyakit BPB, antara lain a) metode kolorimetrik dengan menggunakan *ethylene diamine tetra acetic acid* (*EDTA*) untuk mendeteksi *G. lucidum* pada tanaman kelapa. Metode ini berdasarkan peningkatan nilai kepadatan optikal cairan ekstraksi tanaman terinfeksi sebanding dengan intensitas penyakit (6), b) Teknik pengeboran batang kelapa sawit sakit pada ketinggian 5-10 cm dari permukaan tanah kemudian contoh ditumbuhkan pada media

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khusus untuk *Ganoderma* (2). Pengoperasian teknik konvensional ini membutuhkan banyak waktu dan akurasinya rendah. Untuk itu, adanya suatu teknik deteksi yang cepat, murah, tinggi akurasinya, spesifik dan dapat digunakan secara massal untuk mendeteksi jamur *Ganoderma* pada tanaman kelapa sawit akan sangat membantu dalam pengambilan keputusan untuk pengendalian penyakit BPB secara tepat. Suatu pendekatan baru untuk deteksi dini penyakit BPB tampaknya mengarah pada metode serologi seperti *enzyme-linked immunosorbent assay* (*ELISA*) karena teknik ini memenuhi persyaratan yang diinginkan.

Salah satu faktor penting dalam pengembangan metode serologi untuk mendeteksi *Ganoderma* pada tanaman kelapa sawit adalah kespesifikan antibodi yang dihasilkan. Antibodi harus dapat mengenali target yang dituju dan tidak bereaksi silang dengan jamur-jamur lain bukan target. Dengan menggunakan teknik *ELISA* beberapa jamur akar yang telah berhasil dideteksi, antara lain adalah jamur *Heterobasidion annosum* (Fr.) Bref., jamur penyebab penyakit busuk akar pada tanaman konifer baik menggunakan polikonal antibodi (1) maupun monoklonal antibodi (4) dan jamur *Armillaria*, jamur penyebab penyakit busuk akar pada tanaman berkayu dengan menggunakan monoklonal antibodi (3, 8).

Tujuan penelitian ini adalah untuk menghasilkan poliklonal antibodi dengan menggunakan ekstrak protein miselia sebagai antigen untuk mendeteksi keberadaan jamur *Ganoderma* pada kelapa sawit di lapangan.

BAHAN DAN METODE

ELISA

Persiapan antigen, imunisasi dan prosedur *ELISA* dilakukan berdasarkan Utomo dkk. (11). Dua macam poliklonal antibodi digunakan untuk mendeteksi *Ganoderma* pada akar kelapa sawit. PAb-*mix* dihasilkan dari campuran 9 isolat *Ganoderma* dan PAb-3 dihasilkan dari isolat *Ganoderma* SP1 (SP: Sei Pancur, Sumatra Utara, Indonesia). Kedua macam poliklonal antibodi tersebut diuji terlebih dahulu reaksi silangnya terhadap 5 jamur saprofitik selain *Ganoderma*. Prosedur *ELISA* diterapkan pada percobaan lapangan 1,2, dan 3.

Persiapan contoh akar

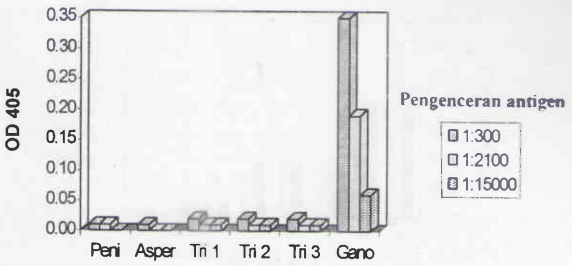
Contoh akar kelapa sawit dengan tahun tanam 1988 diambil dari kebun Sei Pancur. Daerah-daerah kosong karena serangan *Ganoderma* dipilih sebagai contoh percobaan. Tanaman yang tampak sehat (tidak ada gejala penyakit, pembusukan pada pangkal batang dan tubuh buah *Ganoderma*) di sekitar daerah kosong dipilih untuk diambil akarnya. Contoh akar diambil pada kedalaman 15-20 cm di dekat pangkal batang dengan menggunakan cangkul atau kapak. Akar-akar sehat dan sakit dipisahkan, dicuci, ditimbang dan ditumbuk dalam lumpang besi pada suhu kamar. Suspensi contoh akar diencerkan dengan ekstrak bufer (1:3), disentrifugasi 13.000 x g selama 10 menit pada 4 °C. Supernatan dipipet ke dalam tabung plastik dan disimpan pada suhu -20 °C sampai digunakan. Untuk konfirmasi, diambil sebanyak 4 contoh akar sakit untuk diisolasi. Akar sakit dipotong kecil-kecil, dicelupkan ke dalam 5 % NaOCl selama 2 menit, dikering-

anginkan dan diinkubasikan pada *Potato Dextrose Agar* (PDA) pada suhu kamar. Sebanyak 5 jenis jamur saprofitik yang diisolasi dari akar sakit diuji reaksi silangnya dengan PAb-*mix* dan PAb-3.

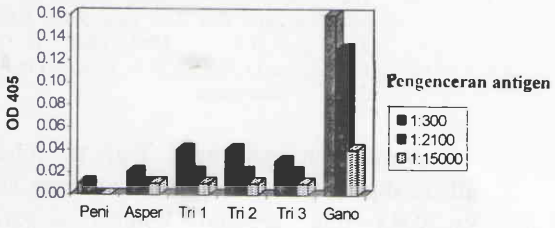
HASIL DAN PEMBAHASAN

Uji reaksi silang

Kendala utama dalam penggunaan metode serologi dalam pendeteksian suatu patogen pada jaringan tanaman adalah hal yang menyangkut tingkat kespesifikan antibodi mengenali patogen target. Satu atau beberapa situs tambat dari suatu jamur kemungkinan bisa dimiliki oleh jamur yang berlainan jenis. Untuk menghindari nilai positif palsu dalam pendeteksian patogen target, antibodi yang digunakan perlu diuji reaksi silangnya dengan jamur-jamur lain yang berasosiasi dengan jaringan tanaman sakit. Tingkat kespesifikan PAb-*mix* dan PAb-3 telah diuji terhadap 5 jamur saprofitik dominan yang berasosiasi dengan akar kelapa sawit sakit. Kelima jamur saprofitik tersebut adalah *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp 1, *Trichoderma* sp 2 dan *Trichoderma* sp 3. Tingkat reaksi silang PAb-*mix* dengan kelima jamur saprofitik relatif rendah, hanya 3 - 6 % (Gambar 1), sedangkan PAb-3 tingkat reaksi silangnya lebih tinggi yaitu 6 - 25 % (Gambar 2). Reaksi silang dengan jamur lain juga dilaporkan pada waktu pendeteksian *Verticillium dahliae* (7), *Sclerotinia sclerotiorum* (5), *Spongospora subterranea* (13) dan *Phytophthora cryptogea* (9). Tingkat reaksi silang yang rendah dari PAb-*mix* dengan jamur saprofitik yang berasosiasi dengan jaringan sakit kelapa sawit akan memudahkan evaluasi hasil dari contoh yang diuji.



Gambar 1. Reaksi silang PAb-mix dengan jamur saprofitik (berdasarkan nilai OD)

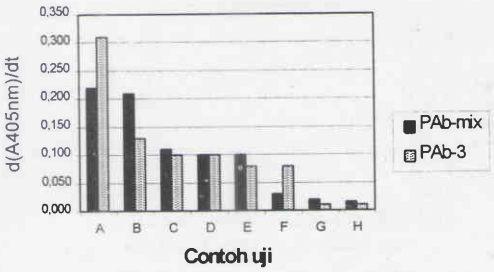


Gambar 2. Reaksi silang PAb-3 dengan jamur saprofitik (berdasarkan nilai OD)

Contoh akar kelapa sawit

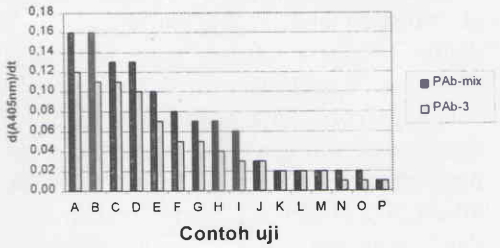
Antigen *Ganoderma* diekstraksi dari akar kelapa sawit dengan menggunakan lumpang dan alu besi. Dengan metode ekstraksi tersebut kepekatan antigen dapat diatur pengencerannya pada saat akhir ekstraksi. Van de Koppel dan Schots (12) dalam percobaannya menunjukkan bahwa konsentrasi antigen yang diekstraksi dengan menggunakan palu lebih tinggi jika dibandingkan dengan menggunakan *blender* karena penggunaan *blender* memerlukan banyak cairan untuk ekstraksi.

Percobaan lapangan 1. Cairan perasan dari akar sakit dan sehat dievaluasi dengan PAb-*mix* dan PAb-3. *Slope* dari nilai optikal densitas per jam dihitung dan dinyatakan sebagai $d(A_{405nm})/dt$. Perbandingan $d(A_{405nm})/dt$ dari akar sakit dan akar sehat dapat dilihat pada Gambar 3.



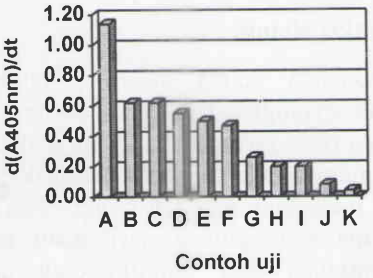
Gambar 3. Contoh akar sakit dan sehat dari percobaan lapangan 1 yang diuji dengan PAb-*mix* dan PAb-3 berdasarkan $d(A_{405\ nm})/dt$. PAb-*mix*: A = *Ganoderma* (1:3,900), B,D,E,F = akar sakit, C = batang sakit, G = esktraksi bufer dan H = akar sehat. PAb-3: A,B,C,D,F = akar sakit, E = *Ganoderma* (1:3,900), G = esktraksi bufer dan H = akar sehat

Percobaan lapangan 2. Pada percobaan ini dipilih pohon dengan akar yang sakit saja dan sebagai kontrol negatif digunakan akar sehat. Selain akar sakit dan sehat, juga diuji kelima jamur saprofitik. Perbedaan $d(A_{405\ nm})/dt$ dari akar sehat dibandingkan dengan akar sakit, positif kontrol, ekstraksi bufer dan jamur saprofitik dapat dilihat pada Gambar 4.



Gambar 4. Akar sakit dan akar sehat dari percobaan lapangan 2 serta jamur saprofitik dievaluasi dengan PAb-*mix* dan PAb-3 berdasarkan d(A405 nm)/dt. PAb-*mix*: A = *Ganoderma* (1:15,000), B-I = akar sakit, J-N = jamur saprofitik, O = ekstraksi bufer dan P = akar sehat. PAb-3: A - G, J = akar sakit, H = *Ganoderma* (1: 15,000), I,K - N = jamur saprofitik, O = esktraksi bufer dan P = akar sehat

Percobaan lapangan 3. Cairan perasan dari contoh akar kelapa sawit dievaluasi dengan PAb-*mix* dan PAb-3 dengan waktu inkubasi substrat yang berbeda (Tabel 1). Perbandingan $d(A_{405\ nm})/dt$ dari akar sakit dan sehat dapat dilihat pada Gambar 5.



Gambar 5. Akar sakit dan sehat dari percobaan lapangan 3 dievaluasi dengan PAb-*mix* (pengenceran 1: 500) berdasarkan $d(A_{405\ nm})/dt$. A - C, C - I = akar sakit, D = *Ganoderma* (1 : 15,000), J = ekstraksi bufer dan K = akar sehat

Ambang batas positif atau negatif dari suatu contoh akar ditentukan berdasarkan nilai $d(A_{405\ nm})/dt$ dari akar sehat. Bila nilai $d(A_{405\ nm})/dt$ dari contoh lebih besar tiga kali daripada akar sehat, contoh tersebut dipertimbangkan sebagai contoh positif. Hasil analisis menunjukkan bahwa nilai $d(A_{405\ nm})/dt$ dari akar sehat selalu rendah dan nilai $d(A_{405\ nm})/dt$ dari akar sakit cenderung selalu tinggi. Perbandingan nilai $d(A_{405\ nm})/dt$ dari akar-akar sakit terhadap nilai $d(A_{405\ nm})/dt$ dari akar sehat bervariasi, berkisar antara 3 sampai 29 kali untuk PAb-*mix* dan 3 sampai 31 kali untuk PAb-3. Dari 19 contoh akar sakit yang dianalisis dengan PAb-*mix*, hanya satu contoh akar sakit yang dipertimbangkan sebagai contoh negatif. Hasil analisis yang hampir serupa juga ditunjukkan oleh PAb-3, kecuali

Tabel 1. Nilai optikal densitas dari contoh akar dari percobaan lapangan 3 yang diuji dengan PAb-*mix* dengan waktu inkubasi substrat yang berbeda

No.	Contoh akar	OD_{405}, PAb-*mix*				$d(A_{405})/dt$ dari PAb-*mix*
		1 jam	2 jam	3 jam	4 jam	
1.	Pohon 2, sakit	0,47	1,52	3,09	3,89	1,14
2.	Pohon 5, sakit	0,26	0,67	1,32	2,12	0,62
3.	Pohon 6, sakit	0,26	0,66	1,31	2,12	0,62
4.	*Ganoderma* (1:15,000)	0,26	0,60	1,21	1,90	0,55
5.	Pohon 3, sakit	0,23	0,56	1,07	1,72	0,50
6.	Pohon 1, sakit	0,23	0,55	1,03	1,64	0,47
7.	Pohon 4, sakit	0,17	0,34	0,61	0,96	0,26
8.	Pohon 7, sakit	0,15	0,28	0,49	0,76	0,20
9.	Pohon 8, sakit	0,15	0,27	0,47	0,72	0,20
10.	Ekstraksi *buffer*	0,13	0,18	0,26	0,37	0,08
11.	Sehat	0,11	0,13	0,18	0,23	0,04

Catatan : - Pohon 5. pohon terinfeksi dengan gejala serangan dan tubuh buah jamur *Ganoderma*
- Pengenceran antibodi 1:500
- Akar sehat : nilai optikal densitas dari rata-rata 8 pohon

pada pohon 8 dari percobaan lapangan 2, hasil analisis dengan PAb-*mix* menunjukkan contoh positif, sedangkan dengan PAb-3 menunjukkan contoh negatif. Beberapa akar kelapa sawit sakit yang dianalisis dengan *ELISA* dan menunjukkan contoh positif, setelah dilakukan isolasi secara konvensional ditemukan jamur *Ganoderma* (Tabel 2). Contoh akar sakit tetapi hasil analisis *ELISA* menunjukkan contoh negatif kemungkinannya karena serangan hama pada perakaran yang kemudian dilanjutkan oleh kolonisasi jamur-jamur saprofitik.

Hasil percobaan pencampuran antara *Ganoderma* dengan jamur saprofitik menunjukkan bahwa keberadaan jamur saprofitik bersama-sama dengan *Ganoderma* akan mengurangi kepekaan PAb-*mix* dan PAb-3 dalam mengenal antigen *Ganoderma*, kecuali keberadaan *Trichoderma* spl bersama-sama dengan *Ganoderma* justru akan meningkatkan kepekaan PAb-3 dalam mengenal antigen *Ganoderma* (Tabel 3). Gejala menurunnya kepekaan kedua antibodi tersebut kemungkinan akibat persaingan ruang antara antigen *Ganoderma* dengan jamur saprofitik pada fase padat atau dengan tambahan protein dari jamur saprofitik akan menutupi atau menghalangi situs tambat *Ganoderma* untuk dikenali oleh antibodi.

Tabel 2. Konfirmasi isolasi jamur *Ganoderma* dan hubungannya dengan nilai *ELISA*

No	Contoh akar	Isolasi *Ganoderma*	$d(A_{405nm})/dt$
1	Pohon 2, lapangan 2	+	0,10[a]
2	Pohon 4, lapangan 2	+	0,07[a]
3	Akar sehat		0,01[a]
4	Pohon 1, lapangan 3	+	0,47[b]
5	Pohon 6, lapangan 3	+	0,62[b]
6	Akar sehat		0,04[b]

Catatan : - Akar sehat : nilai rata-rata dari 8 pohon
- [a] Pengenceran PAb-*mix* = 1:5.000
- [b] Pengenceran PAb-*mix* =1:500

Tabel 3. Nilai OD dari PAb-*mix* dan PAb-3 terhadap *Ganoderma* dalam campuran dengan jamur saprofitik

No	Antigen	OD_{405} PAb-*mix* 4 jam	OD_{405} PAb-3 4 jam
1.	*Ganoderma* (1:3,900)	0,79	0,26
2.	*Gano : Tricho* 1 (1:3.900)	0,68	0,31
3.	*Gano : Peni* (1:3,900)	0,48	0,23
4.	*Gano : Asper* (1:3,900)	0,44	0,23
5.	*Gano : Tricho 2* (1:3,900)	0,35	0,24
6.	*Gano : Tricho 3* (1:3,900)	0,31	0,20
7.	Extraction buffer	0,14	0,13

Catatan : - Pengenceran PAb-*mix* dan PAb-3 = 1:5.000
- *Ganoderma* : jamur saprofitik = 1: 3.900 (pengenceran jamur saprofitik = 1:300)

Pendeteksian dengan metode *ELISA* memerlukan banyak waktu terutama pada pembentukan konfigurasinya. Dengan memodifikasi dan penyederhanaan *ELISA* yang menyangkut pengurangan waktu untuk diagnosis contoh, metode tersebut akan dapat digunakan untuk menganalisis contoh dalam jumlah yang besar. Pengembangan uji dengan *ELISA* selanjutnya diarahkan pada pembuatan *kit* yang spesifik terhadap *Ganoderma*.

KESIMPULAN

Meskipun hanya sedikit informasi kuantitatif tentang jumlah *Ganoderma* pada akar kelapa sawit yang dapat diperoleh, kedua poliklonal antibodi PAb-*mix* dan PAb-3 mampu mendeteksi keberadaan *Ganoderma* pada jaringan akar kelapa sawit sakit secara kualitatif dan tidak bereaksi silang dengan jaringan akar sehat. Selain itu kedua antibodi tersebut hanya bereaksi silang lemah dengan jamur saprofitik yang diuji sehingga akan mempertinggi akurasi dalam pendeteksian jamur *Ganoderma* pada contoh akar.

Dalam penelitian ini ekstraksi contoh akar dilakukan secara sederhana dan antibodi hanya dimurnikan secara parsial tetapi hasil yang diperoleh cukup memuaskan, karena itu teknik *ELISA* dapat bermanfaat untuk deteksi dini gangguan *Ganoderma* secara kuantitatif jika penetapan secara pasti tidak diperlukan.

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DAFTAR PUSTAKA

1. AVRAMENKO, R.S. 1989. A serological study of strains of *Heterobasidion annosum* (Fr.) Bref. 1. *Heterobasidion annosum* from the common pine. Mikologiya I Fitopatologiya 23 : 225 - 233.

2. DARUS, A,, A.S. IDRIS and H. KHAIRUDIN. 1993. Confirmation of *Ganoderma* infected

palm by drilling technique. Proceeding of the 1993 PORIM International Palm Oil Congress, 20 - 25 September 1993, Kuala Lumpur, Malaysia.

3. FOX, R.T.V. and K. HAHNE. 1989. Prospects for the rapid diagnosis of *Armillaria* by monoclonal antibody ELISA. *In* D.J. MORRISON (ed.) Proceedings of the Seventh International Conference on Root and Bud Rots, August 1988, British Columbia.

4. GALBRAITH, D.N. and J.W. PALFREYMAN. 1994. Detection of *Heterobasidion annosum* using monoclonal antibodies. *In* A. SCHOTS, F.M. DEWEY AND R. OLIVER (eds.). Modern Assays for Plant Pathogenic Fungi : Identification, Detection and Quantification. CAB International, Oxford, UK.

5. JAMAUX, I. and D. SPIRE. 1994. Development of a polyclonal antibody-based immunoassay for the early detection of *Sclerotinia sclerotiorum* in rapeseed petals. Plant Pathology 43 : 847 - 862.

6. NATARAJAN, S., R. BHASKARAN and N. SHANMUGAM. 1986. Preliminary studies to develop techniques for early detection of Thanjavur wilt in coconut. Indian Coconut Journal 17 : 3 - 6.

7. PLASENCIA, J., R. JEMMERSON and E.B. BANTTARI. 1996. Production and characterization of monoclonal antibodies to *Verticillium dahliae* and development of a quantitative immunoassay for fungal biomass. Phytopathology 86 : 170 - 176.

8. PRIESTLEY, R., C. MOHAMMED and F.M. DEWEY. 1994. The development of monoclonal antibody-based ELISA and dipstick assays for the detection and identification of *Armillaria* species in infected wood. *In* A. SCHOTS, F.M. DEWEY AND R. OLIVER (eds.). Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification.CAB International, Wallingford, UK.

9. SADOWSKA-RYBAK, M., H. ECKS and P. SCHICKEDANZ. 1996. Detection of *Phytophthora cryptogea* Pethybr. Et Laff. In gerbera by serological methods. Journal of Plant Diseases and Protection 103: 8 - 14.

10. SINGH, G. 1991. *Ganoderma* - the scourge of oil palm in the coastal areas. Planter 67: 421 - 444.

11. UTOMO, C., F. NIEPOLD and C. MOLLERS. 1997. Kajian keragaman *Ganoderma* pada tanaman kelapa sawit dengan teknik ELISA. Jurnal Penelitian Kelapa Sawit, 5(1): 27-40.

12. VAN DE KOPPEL, M.M. and A. SCHOTS. 1995. Monoclonal antibody-based double antibody sandwich-ELISA for detection of *Verticillium* spp. in ornamental. Phytopathology 85: 608 - 612.

13. WALLACE, A., N.A. WILLIAMS, R. LOWE and J.G: HARRISON. 1995. Detection of *Spongospora subterranea* using monoclonal antibodies in ELISA. Plant Pathology 44: 355 - 365.

Early detection of *Ganoderma* in oil palm by ELISA technique

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Abstract

Polyclonal antibodies were produced in rabbits with mycelial protein from single Ganoderma *isolate and with a mixture of 9* Ganoderma. *The specificity of two different polyclonal antibody batches was tested by using indirect enzyme-linked immunosorbent assay against oil palm root samples and saprophytic fungi commonly isolated from diseased oil*

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palm roots. Sap of healthy root did not give an antibody reaction. However, a low cross-reaction with five saprophytic fungi was observed. Both polyclonal antibodies in the indirect enzyme-linked immunosorbent assay accurately detected Ganoderma *in oil palm tissue so it will be useful for the early detection of the fungi.*

Keywords : ELISA, early detection, oil palm, polyclonal antibody, *Ganoderma*

Introduction

The density of oil palm planting is commonly 9m x 9m x 9m with the economic lifespan of 25 years. For replanting, new palm seedlings are planted between the former old stands. Previously, basal stem rot (BSR) caused by *Ganoderma* was reported to occur only on old palms in the first planting cycle, however, recently BSR was found to attack also young palms in the second planting cycle (10). These disease incidences lead to the assumption of the mode of infection of BSR that infections in young palm is as a result of the contact of the healthy root with the infected tissue of previous planted palms. In older palms, the infection occurs by root contact with diseased neighboring palms. The incubation period of this disease is several years and unfortunately, the disease symptoms only appear at a late stage of infection. When this happens, more than half of the bole tissue has been decayed and usually this infected palm can not be cured.

One of the limiting factors in controlling the disease is the lack of reliable diagnostic method to detect early symptoms of BSR disease. Only a few methods have been developed for early diagnosis of BSR: a) A colorimetric method using ethylene diamine tetra acetic acid (EDTA) to detect *G. lucidum* in coconut, the causal agent of Thanjavur wilt disease. This method is based on the increase of optical density of the sap from infected palm parallel to the increase in the disease intensity (6), b) A drilling technique where the diseased stem of oil palm is collected by drilling into the diseased area at 5-10 cm height from the soil surface. Samples are then grown on media selective for *Ganoderma* (2). These conventional methods are time-consuming and the accuracy is not very high. Therefore, the availability of a rapid, inexpensive, accurate diagnosis, which is specific and readily adapted to large scale testing for demonstrating the presence or absence of *Ganoderma* in oil palm at an early stage of infection would benefit the detection and also decisions for taking appropriate control measures. A new approach for the early detection of BSR could be the use of serological methods such as the enzyme-linked immunosorbent assay (ELISA). This method would fulfill most of above-mentioned requirements.

To date, immunological methods have not been widely applied for the detection of root-infecting fungi. The development of simple assay systems such as ELISA for *Ganoderma* detection would not only be important to scientists, but would also be of practical benefit to the oil palm growers. One important section in developing ELISA for *Ganoderma* is to generate antibodies that can detect

Ganoderma in the palm tissue and also do not react with non pathogenic fungi from the soil or plant materials. Successful researches of root-infecting fungi detection in infected plant by ELISA have been reported previously, e.g. detection of *Heterobasidion annosum* (Fr.) Bref., one of the most common basidiomycete organisms responsible for the decay of conifer by polyclonal antibodies (1) and by monoclonal antibodies (4) and the serological detection of *Armillaria*, a root rot disease in woody plant by monoclonal antibodies (3, 8).

The aim of this research was to raise polyclonal antibodies against soluble mycelial protein in order to detect the presence or absence of *Ganoderma* in oil palm trees in the field.

Materials and Methods

ELISA

Antigen preparation, immunization and ELISA protocol were done as reported earlier (11). Two different polyclonal antibodies (PAbs) were used to detect *Ganoderma* in oil palm roots. A PAb-mix was raised against the mixture of 9 *Ganoderma* and PAb-3 raised against *Ganoderma* SP1. The two polyclonal antibody were firstly tested for their cross reaction against 5 saprophytic fungy other than *Ganoderma*. The ELISA procedure was applied on field experiment 1,2, and 3.

Root samples preparation

Root samples of oil palm were taken from Sei Pancur estate. The planting year of oil palm was 1988. The vacant areas due to *Ganoderma* attack of the oil palms were selected as trial samples. Healthy looking oil palm (no visual disease symptom of *Ganoderma*, no decayed tissue in the base and no fruitingbody of *Ganoderma*), which next to the vacant areas were chosen for sampling. Root samples were collected from the field by cutting oil palm roots in the soil at a depth of 15 - 20 cm near the basal trunk with a hoe or an axe. Healthy and diseased roots were separated, washed with tap water, weighed and ground in a metal mortar and with a pestle at room temperature. A sample of the suspension was diluted with extraction buffer (1:3), centrifuged at 13,000 x g for 10 min at 4 °C. The supernatant was pipetted into a plastic tube and stored at -20 °C until use. Diseased roots from 4 oil palm samples were selected and isolated for confirmation. Diseased roots were cut in pieces, soaked in 5 % of NaOCl for 2 min, dried and incubated on Potato Dextrose Agar (PDA) at room temperature. For control, five saprophytic fungi that isolated from diseased oil palm roots were also tested by PAb-mix and PAb-3 for the evaluation of the cross reactivity.

Results and Discussions

Cross-reactivity test

A major problem in using immunoassay was the lack of specificity forwards plant pathogenic fungi. Fungi are very complex organism that contain numerous antigens, many of which are also shared by unrelated fungi. Thus, thorough cross-reactivity tests against unrelated fungi that could be present in the plant tissue were performed. This kind of

test is necessary in order to avoid false positive values due to serological cross-reaction of unrelated fungi before the ELISA is applied routinely to detect a certain pathogenic fungus in plant tissue. The specificity of PAb-3 and PAb-mix was tested against 5 saprophytic fungi commonly isolated from diseased oil palm roots and or which are associated with the fruitingbodied of *Ganoderma*. The saprophytic fungi were determined to be *Penicillium* sp, *Aspergillus* sp, *Trichoderma* sp1, *Trichoderma* sp2 and *Trichoderma* sp3. Cross-reaction of PAb-mix against 5 saprophytic fungi tested was low (only 3-6 %) as shown in figure 2 whereas PAb-3 gave higher cross-reaction (6-25 %) against 5 saprophytic fungi tested (figure1). Cross-reaction with other fungi was also reported with *Verticillium dahliae* (7), *Sclerotinia sclerotiorum* (5), *Spongospora subterranea* (13) and *Phytophthora cryptogea* (9). The low cross-reaction of the PAb-mix with saprophytic fungi which are associated with infected oil palm will facilitate the evaluation of the results of oil palm samples in comparison with PAb-3.

Oil palm root samples

In this study, a metal mortar and pestle was used to obtain *Ganoderma* antigens from oil palm roots. The root

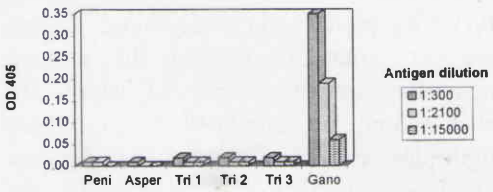

Figure 1. Cross-reaction of Pab-3 with saprophytic fungi was reflected as OD values.

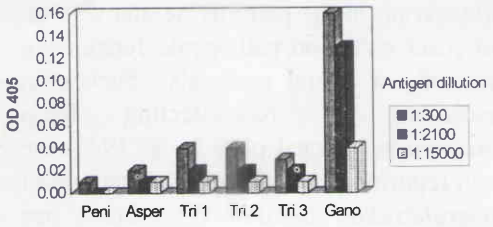

Figure 2. Cross-reaction of Pab-mix with saprophytic fungi was reflected as OD values.

crushing method gave concentrated antigens because the dilution of root samples was performed at the end of the grinding. Van de Koppel and Schots (12) showed that antigen extractions from plant tissues through hammer crushing resulted in a higher optical density readings compared with the blender. However, in the latter method, the antigen was higher diluted.

Field trial 1. The sap of healthy and diseased roots was evaluated with PAb-mix and PAb-3, and the results are shown in figure 3. The slope of the absorbance values per hour was calculated and presented as $d(A_{405\ nm})/dt$. The ratio of $d(A_{405\ nm})/dt$ of diseased root to $d(A_{405\ nm})/dt$ of healthy root is shown in figure 3.

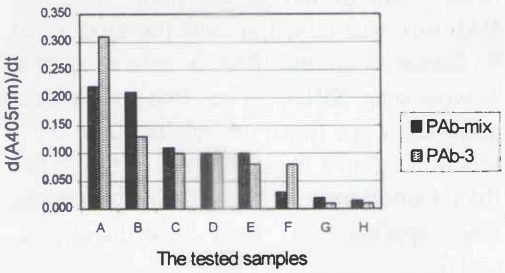

Figure 3. Diseased and healthy root from field trial 1 was tested against PAb-and PAb-3 based on $d(A_{405nm})/dt$. PAb-mix : A = *Ganoderma* (1:3,900), B,D,E,F = diseased root, C = decayed stem, G = extr. buffer and H = healthy root. PAb-3 : A,B,C,D,F = diseased root, E = *Ganoderma* (1:3,900), G = extr.buffer and H = healthy root

Field trial 2. For the samples, palms with diseased roots were selected for the tests. Healthy roots were taken as a negative control. The Sap of diseased and healthy roots as well as five saprophytic fungi was assessed with PAb-mix and PAb-3. The difference of d (A_{405nm})/dt of healthy root was compared with diseased roots, negative control, positive control, extraction buffer and saprophytic fungi (figure 4).

Field trial 3. Sap of oil palm root samples was evaluated in the ELISA by using PAb-mix and PAb-3 with different incubation time of substrate (table 1). The ratio of d (A_{405nm})/dt of diseased roots to d (A_{405nm})/dt of healthy roots was compared (figure 5).

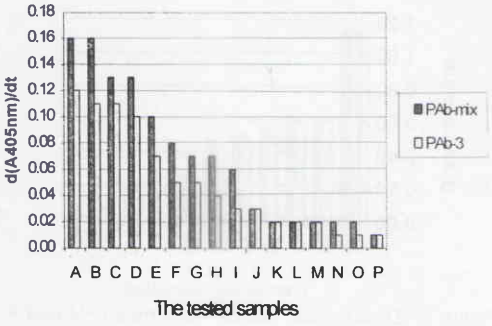

Figure 4. Diseased, healthy root from field trial 2 and saprophytic fungi were evaluated with PAb-mix and PAb-3 based on d(A405nm)/dt. PAb-mix, A = *Ganoderma* (1:15,000), B - I = diseased roots, J - N = saprophytic fungi, O = extr. buffer and P = healthy root. PAb-3, A - G, J = diseased roots, H = *Ganoderma* (1:15,000), I, K - N = saprophytic fungi, O = extr. buffer and P = healthy root

Table 1. OD values of PAb-mix obtained against various root samples from field trial 3 with different incubation time of substrate

No.	Root samples	OD_{405}, PAb-mix				$d(A_{405})/dt$ of Pab-mix
		1h	2h	3h	4h	
1.	Palm 2, diseased	0.47	1.52	3,09	3.89	1.14
2.	Palm 5, diseased	0.26	0.67	1.32	2.12	0.62
3.	Palm 6, diseased	0.26	0.66	1.31	2.12	0.62
4.	*Ganoderma* (1:15,000)	0.26	0.60	1.21	1.90	0.55
5.	Palm 3, diseased	0.23	0.56	1.07	1.72	0.50
6.	Palm 1, diseased	0.23	0.55	1.03	1.64	0.47
7.	Palm 4, diseased	0.17	0.34	0.61	0.96	0.26
8.	Palm 7, diseased	0.15	0.28	0.49	0.76	0.20
9.	Palm 8, diseased	0.15	0.27	0.47	0.72	0.20
10.	Extraction buffer	0.13	0.18	0.26	0.37	0.08
11.	Healthy	0.11	0.13	0.18	0.23	0.04

Notes : - **Palm 5, the diseased palm with disease symptoms and fruitingbody of *Ganoderma***
- **Antibody dilution 1:500**
- **Healthy: OD values of the average of 8 palms**

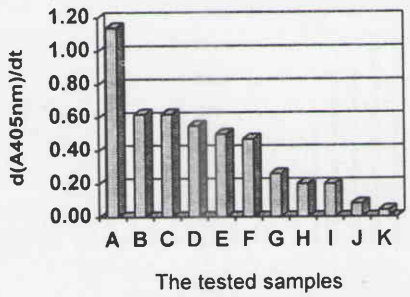

Figure 5. Diseased and healthy roots from field trial 3 were evaluated with PAb-mix (dilution 1:500) based on d (A_{405nm})/dt. A – C, E – I = diseased roots, D = *Ganoderma* (1:15,000), J = extr. buffer and K = healthy root

A positive and a negative threshold was set in the ELISA tests by calculating the d(A_{405nm})/dt of the healthy roots. If the d(A_{405nm})/dt values of the samples were three times higher than that of the healthy root, the sample was considered as positive. Routinely low d(A_{405nm})/dt values were obtained when extracts from healthy root tissue were used and consistently high d(A_{405nm})/dt values were obtained from diseased oil palm root. The ratio of d(A_{405nm})/dt of diseased roots to d(A_{405nm})/dt of healthy root varied from 3 to 29 times for PAb-mix and 3 to 31 times for PAb-3. Of the 19 diseased root samples analyzed, only one sample tested by PAb-mix was considered to be negative. The diseased roots tested by PAb-3 showed similar results, except for palm 8 sampled from field No.2. This sample was showed a positive reaction when using the PAb-mix while it was negative when using PAb-3. These data were confirmed by applying conventional methods. The isolation of *Ganoderma* mycelia from four diseased root samples confirmed the applicability of the ELISA tests (Table 2). The root which appeared to be diseased but gave a negative result in the ELISA tests were probably attacked by insects and subsequently colonized by saprophytic fungi.

In experiments where *Ganoderma* and saprophytic fungi were mixed, the presence of saprophytic fungi reduced the sensitivity of PAb-mix and PAb-3 in recognizing *Ganoderma* antigens. However, in the presence of *Trichoderma* 1 the OD_{405} values of PAb-3 in detecting *Ganoderma* increased compared to *Ganoderma* alone (Table 3). This interference phenomenon was likely a result from a competition between *Ganoderma* and saprophytic fungi antigens for a finite number of binding sites in the solid phase or it might also be the case that the additional protein from saprophytic fungi would promote a binding (blockage of antigenic sites) or a protection of the antigenic sites of *Ganoderma*. The implications of the existence of saprophytic fungi within the diseased root samples other than a *Ganoderma* infection would tend to produce false positive values whereas in the diseased root samples caused by *Ganoderma* there would be lower OD_{405} values than the actual OD_{405} values.

A major constraint of the use of ELISA is the time required to perform the configuration. With several modifications and simplifications of ELISA especially in reducing the time required for sample diagnosis, this test would have the merit of easy implementation and application to numerous root samples. This test has a high potential in the future for the development of testing kit specific to *Ganoderma* in oil palm.

Table 2. Confirmation of *Ganoderma* isolation from unhealthy root and it relationship to ELISA values

No.	Root samples	*Ganoderma* isolation	$d(A_{405nm})/dt$
1.	Palm 2, field 2	+	0.10[a]
2.	Palm 4, field 2	+	0.07[a]
3.	Healthy		0.01[a]
4.	Palm 1, field 3	+	0.47[b]
5.	Palm 6, field 3	+	0.62[b]
6.	Healthy		0.04[b]

Notes : - Healthy roots : the average of 8 palms
- [a] PAb-mix dilution 1:5,000
- [b] PAb-mix dilution 1:500

Table 3. OD values of PAb-mix and PAb-3 raised against *Ganoderma* in the mixture with saprophytic fungi

No.	Antigen	OD_{405}	
		PAb-mix 4h	PAb-3 4h
1.	*Ganoderma* (1:3,900)	0.79	0.26
2.	*Gano : Tricho* 1 (1:3,900)	0.68	0.31
3.	*Gano : Peni* (1:3,900)	0.48	0.23
4.	*Gano : Asper* (1:3,900)	0.44	0.23
5.	*Gano : Tricho 2* (1:3,900)	0.35	0.24
6.	*Gano : Tricho 3* (1:3,900)	0.31	0.20
7.	Extraction buffer	0.14	0.13

Notes : - PAb-mix and PAb-3 dilution 1:5,000
- *Ganoderma* : saprophytic fungi = 1:3,900 (in saprophytic fungi dilution 1:300)

Conclusions

Although only little information was obtained to quantify the amount of *Ganoderma* in the infected roots, both PAb-mix and PAb-3 were able to detect *Ganoderma* in the root tissue qualitatively and did not cross react with healthy root tissue. Only low cross-reaction of the antibodies obtained with saprophytic fungi represented an advantage in the detection of *Ganoderma* in root samples.

This study reports on a simple processing of root samples by macerating with extraction buffer. The antisera were prepared in a relatively crude antisera form, however, this procedure produced expedient results in root sample testing. Therefore, the applied indirect ELISA procedure can be useful as qualitative routine detection tool for early detection survey of *Ganoderma,* where accurate quantitation is not necessary.

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References

1. AVRAMENKO, R.S. 1989. A serological study of strains of *Heterobasidion annosum* (Fr.) Bref. 1. *Heterobasidion annosum* from the common pine. Mikologiya I Fitopatologiya 23 : 225 - 233.

2. DARUS, A., A.S. IDRIS and H. KHAIRUDIN. 1993. Confirmation of *Ganoderma* infected palm by drilling technique. Proceeding of the 1993 PORIM International Palm Oil Congress, 20 - 25 September 1993, Kuala Lumpur, Malaysia.

3. FOX, R.T.V. and K. HAHNE. 1989. Prospects for the rapid diagnosis of *Armillaria* by monoclonal antibody ELISA. *In* D.J. MORRISON (ed.) Proceedings of the Seventh International Conference on Root and Bud Rots, August 1988, British Columbia.

4. GALBRAITH, D.N. and J.W. PALFREYMAN. 1994. Detection of *Heterobasidion annosum* using monoclonal antibodies. *In* A. SCHOTS, F.M. DEWEY AND R. OLIVER

(eds.) Modern Assays for Plant Pathogenic Fungi : Identification, Detection and Quantification. CAB International, Oxford, UK.

5. JAMAUX, I. and D. SPIRE. 1994. Development of a polyclonal antibody-based immunoassay for the early detection of *Sclerotinia sclerotiorum* in rapeseed petals. Plant Pathology 43 : 847 - 862.

6. NATARAJAN, S., R. BHASKARAN and N. SHANMUGAM. 1986. Preliminary studies to develop techniques for early detection of Thanjavur wilt in coconut. Indian Coconut Journal 17 : 3 – 6.

7. PLASENCIA, J., R. JEMMERSON and E.B. BANTTARI. 1996. Production and characterization of monoclonal antibodies to *Verticillium dahliae* and development of a quantitative immunoassay for fungal biomass. Phytopathology 86 : 170 – 176.

8. PRIESTLEY, R., C. MOHAMMED and F.M. DEWEY. 1994. The development of monoclonal antibody-based ELISA and dipstick assays for the detection and identification of *Armillaria* species in infected wood. *In* A. SCHOTS, F.M. DEWEY AND R. OLIVER (eds.). Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification. CAB International, Wallingford, UK.

9. SADOWSKA-RYBAK, M., H. ECKS and P. SCHICKEDANZ. 1996. Detection of *Phytophthora cryptogea* Pethybr. Et Laff. In gerbera by serological methods. Journal of Plant Diseases and Protection 103: 8 – 14.

10. SINGH, G. 1991. *Ganoderma* - the scourge of oil palm in the coastal areas. Planter 67: 421 – 444.

11. UTOMO, C., F. NIEPOLD and C. MÖLLERS. 1997. Study on the variability of *Ganoderma* in oil palm by ELISA technique. Indonesian Journal of Oil Palm Research, 5(1): 27-40.

12. VAN DE KOPPEL, M.M. and A. SCHOTS. 1995. Monoclonal antibody-based double antibody sandwich-ELISA for detection of *Verticillium* spp. in ornamental. Phyto-pathology 85: 608 – 612.

13. WALLACE, A., N.A. WILLIAMS, R. LOWE and J.G: HARRISON. 1995. Detection of *Spongospora subterranea* using monoclonal antibodies in ELISA. Plant Pathology 44: 355 – 365.

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