UNIVERSITI PUTRA MALAYSIA

METABOLITE PROFILING AND DEFENSE GENE EXPRESSION OF SUSCEPTIBLE AND TOLERANT OIL PALM SEEDLING PROGENIES AT EARLY STAGE OF GANODERMA BONINENSE INFECTION

NUSAIBAH BINTI SYD ALI

ITA 2012 9

METABOLITE PROFILING AND DEFENSE GENE EXPRESSION OF SUSCEPTIBLE AND TOLERANT OIL PALM SEEDLING PROGENIES AT EARLY STAGE OF *GANODERMA BONINENSE* INFECTION

By

NUSAIBAH BINTI SYD ALI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2012

Dedicated to my parents, my husband and Boney

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for Doctor of Philosophy

METABOLITE PROFILING AND DEFENSE GENE EXPRESSION OF SUSCEPTIBLE AND TOLERANT OIL PALM SEEDLING PROGENIES AT EARLY STAGE OF *GANODERMA BONINENSE* INFECTION

By

NUSAIBAH BINTI SYD ALI

December 2012

Chairman: Associate Professor Datin Siti Nor Akmar Abdullah, PhD

Institute: Institute of Tropical Agriculture

Knowledge on the localized and systemic defense mechanism during *Ganoderma boninense*-oil palm interaction is of primary importance for detecting basal stem rot disease. Thus the objectives of this study include i) to establish and confirm *G. boninense* infection on oil palm root seedlings using artificial inoculation method, ii) to identify secondary metabolites accumulated in oil palm roots at early stages of *G. boninense* infection and assess their antifungal activities and iii) to profile expression of key genes for the biosynthesis of the identified compounds involved in defense mechanism. In this study, oil palm seedlings were artificially infected with *G. boninense* infested rubber wood blocks. Establishment of *G. boninense* infection at early stages in tolerant and susceptible oil palm seedling progenies were examined through scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Metabolites present in oil palm roots during oil palm-*G. boninense* pathogenic interactions were investigated via gas chromatography-mass spectrometric (GC-MS) analysis. Antifungal assays were carried out to associate the involvements

of total metabolites and single metabolite from inoculated oil palm roots extracts in defense mechanism against *G. boninense* infection. Gene expression analysis of *anthranilate synthase α-* subunit1 (*EGASα*-1) *and stearoyl-ACP-desaturase*3 (*SAD*3) genes from tryptophan and fatty acid pathways respectively were conducted with quantitative reverse transcriptase-polymerase chain reaction. A rapid outer layer colonization of hyphae was observed in the susceptible progeny compared to tolerant progeny through SEM. Cell wall degradation was observed through TEM as early as 24 hpi before penetration of *G. boninense* hyphae. Total metabolite *in vitro* study evaluating inhibitory activity of oil palm root methanolic against *G. boninense* showed that tolerant progeny extracts gave a higher inhibition rate with 100 % at 72 and 96 hpi, where else in susceptible extracts, 100% inhibition were only achieved at 96 hpi. An alkaloid metabolite, quinoline was found to have a much more rapid and elevated accumulation in the roots of tolerant progeny (56.4% at 72 hpi) compared to susceptible progeny (43.9% at 144 hpi) at early stages of *G. boninense* infection. Quinoline gave an EC_{50} of 0.211µg/ml and showed an increasing antifungal activity with increasing quinoline concentration against *G. boninense*. The level of *EGASα*-1 gene expression reached a maximum at 120 hpi in tolerant and susceptible progeny, whereby it was 3.0-fold and 1.5-fold higher respectively. Expression of *SAD*3 gene in infected roots was 5.3-fold and 1.7-fold in susceptible and tolerant progeny respectively at 120 hpi. This indicates *SAD*3 gene is constitutively expressed in oil palm roots and their expression levels were influenced by biotic stress. These findings showed that oil palm-*G. boninense* interaction induces biochemical defense and activates pathogenesis related genes as a form of early defense mechanism.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMPROFILAN METABOLIT DAN PENGEKSPRESAN GEN BAGI PROGENI KELAPA SAWIT YANG TIDAK RENTAN DAN RENTAN PADA PERINGKAT AWAL JANGKITAN *GANODERMA BONINENSE*

Oleh

NUSAIBAH BINTI SYD ALI

Disember 2012

Pengerusi: Professor Madya Datin Siti Nor Akmar Abdullah, PhD

Institut: Institut Pertanian Tropika

Pengetahuan dalam mekanisme pertahanan penyakit yang setempat dan sistemik semasa interaksi *G. boninense*-anak benih kelapa sawit adalah sangat penting untuk mengesan penyakit BSR. Oleh itu, objektif-objektif bagi kajian ini termasuklah; i) untuk menghasilkan jangkitan *Ganoderma boninense* dan pengesahkannya pada akar anak benih kelapa sawit dengan menggunakan teknik inokulasi artifisial, ii) untuk mengidentifikasi metabolit-metabolit sekundari yang terkumpul di dalam akar anak benih kelapa sawit pada peringkat awal jangkitan *G. boninense* serta mengukur aktiviti antikulat metabolit-metabolit tersebut dan iii) untuk memprofilkan pengekspresan gen-gen utama yang terlibat dalam sintesis metabolit-metabolit pertahanan yang telah diidentifikasi. Dalam kajian ini, blok kayu getah yang telah dikolonisasi oleh *G. boninense* telah digunakan untuk menjangkiti anak benih kelapa sawit. Peringkat awal jangkitan *G boninense* pada anak benih kelapa sawit telah diperiksa melalui mikroskopi pengimbasan electron (SEM) dan mikroskopi transmisi

elektron (TEM). Pengesanan dan pengecaman metabolit yang hadir dalam akar anak benih kelapa sawit semasa interaksi anak benih kelapa sawit dengan *G. boninense* telah dilakukan menggunakan alat gas kromatografi-spektrometrik jisim (GC-MS). Analisis anti-kulat telah dijalankan untuk mengaitkan penglibatan metabolit total dan metabolit tunggal dalam ekstrak akar anak benih kelapa sawit yang dijangkiti dalam mekanisme pertahanan terhadap jangkitan *G. boninense*. Analisis pengekspresan gen *anthranilate α-subunit* 1 (*EGASα*-1) dan *stearoyl-ACP-desaturase*3 (*SAD*3) daripada laluan triptofan (Trp) dan asid lemak masing-masing telah dijalankan dengan kaedah kuantitatif transcriptase berbalik-tindakbalas rantaian polimerase (qRT-PCR). Pengkolonian hifa yang lebih banyak pada lapisan luar akar anak benih kelapa sawit yang tidak rentan berbanding yang rentan telah dibuktikan melalui analisis SEM. Selain itu, TEM telah mengungkai bahawa sebelum penetrasi hifa *G. boninense* ke dalam dinding sel akar anak benih kelapa sawit, degradasi dinding sel telah berlaku seawal 24 hpi. Oleh itu, kajian anti-kulat ekstrak metanol metabolit total yang berbeza melalui faktor progeni dan pelbagai rawatan menunjukkan bahawa kadar perencatan kulat *G. boninense* yang dipamerkan oleh ekstrak daripada progeni yang rentan lebih tinggi berbanding ekstrak yang tidak rentan. Keputusan kajian *in vitro* ekstrak metabolit total menunjukkan kadar perencatan pertumbuhan *G. boninense* adalah 100% oleh ekstrak progeni rentan (72 dan 96 hpi) dan ekstrak progeni tindak rentan (96 hpi). Salah satu daripada metabolit alkaloid, quinoline telah didapati mempunyai pengumpulan lebih pesat dan maksimum dalam akar anak benih yang rentan (56.4% pada 72 hpi) berbanding progeni yang tidak rentan (43.9% pada 144 hpi). Quinoline memenuhi semua kriteria sebagai alkaloid *phytoalexin* dan memberikan EC_{50} 0.211 µg/ml serta mempamerkan aktiviti perencatan yang semakin meningkat dengan peningkatan kepekatan quinoline terhadap *G. boninense*. Tahap pengekspresan gen

EGASα-1 mencapai kadar maksimum pada 120 hpi dalam progeni rentan dan tidak rentan, di mana ia adalah 3.0-kali ganda dan 1.5-kali ganda masing-masing. Pengekspresan gen *SAD*3 dalam akar yang dijangkiti adalah 5.3-kali ganda dan 1.7-kali ganda dalam progeni yang rentan dan tidak rentan masing-masing pada 120 hpi. Penemuan-penemuan dalam kajian ini menunjukkan interaksi anak benih kelapa sawit dengan *G. boninense* mendorong tindak balas pertahanan biokimia di dalam akar anak benih kelapa sawit yang telah dijangkiti sebagai salah satu bentuk mekanisme pertahanan.

I certify that an Examination Committee has met on date of viva voce to conduct the final examination of Nusaibah Binti Syd Ali on Doctor of Philosophy degree thesis entitled "Metabolites profiling and defense gene expression of susceptible and tolerant oil palm seedling progenies at early stage of *Ganoderma boninense* infection" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy. Members of the Examination Committee were as follows:

Mohamed Hanafi bin Musa, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Norhani bt Abdullah, PhD
Professor
Faculty of Biotechnology and Bimolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Wong Mui Yun, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Monica L. Elliott, PhD
Professor
University of Florida Ifas
United States
(External Examiner)

Seow Heng Fong , PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Datin Siti Nor Akmar Abdullah, PhD
Associate Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Chairman)

Idris Abu Seman, PhD
Principle Research Officer
Biology Division
Malaysian Palm Oil Board
(Member)

Sariah Meon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Mohammad Pauzi Zakaria, PhD
Professor
Faculty of Environmental Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NUSAIBAH BINTI SYD ALI

Date: 4 December 2012

TABLE OF CONTENTS