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By

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

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EXPRESSION PROFILING AND FUNCTIONAL CHARACTERIZATION OF SELECTED OIL PALM GENES IN HOST-MICROBIAL INTERACTION

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Chairman : Ho Chai Ling, PhD

Institute : Institute of Tropical Agriculture

Basal stem rot is a major disease of oil palm caused by a pathogenic fungus, *Ganoderma boninense*. It reduces the oil palm yield and causes severe economic loss to the oil palm industry. To better understand oil palm defence system during the host-pathogenic interactions, the gene expression profiles of eleven defence-related cDNAs in oil palm treated with *G. boninense*, *Trichoderma harzianum*, or mycorrhizas were studied. These cDNAs encode putative bowman-birk serine protease inhibitors (EgBBI1 and 2), defensin (EgDFS), dehydrin (EgDHN), early methionine labelled polypeptides (EgEMLP1 and 2), glycine rich RNA binding protein (EgGRRBP), isoflavone reductase (EgIFR), metallothionein-like protein (EgMT), pathogenesis-related protein-1 (EgPRP), and type 2 ribosome inactivating protein (EgT2RIP). These cDNAs were chosen because they are related to plant defence and were differentially expressed in oil palm upon inoculation by mycorrhizas or *G. boninensen* in a previous study. In this study, the transcript abundance of EgIFR and EgBBI2 increased in *G. boninense*-treated roots at 3 and/or

6 weeks post inoculation (wpi). While the gene expression of EgT2RIP, EgBBI1, and EgDFS increased in *G. boninense*-treated roots at 6 and/or 12 wpi. Meanwhile, EgDHN was up-regulated at all three time points. These reveal that these genes could have different roles at different stages during the infection. Transcript profiles in leaves showed two candidate genes encoding EgEMLP1 and EgMT with different profiles in *G. boninense*-treated leaves compared to that of *T. harzianum*. They may have the potential to be developed as biomarkers for early detection of *G. boninense* infection. Comparison of the transcripts expression profiles in the roots inoculated by *G. boninense*, *T. harzianum*, and mycorrhizas showed that some of the transcripts were increased by specific fungi (EgBBI1 and EgMT were up-regulated by *G. boninense* while EgPRP was up-regulated by mycorrhizas). However, EgDFS and EgT2RIP were up-regulated by all three fungi probably as a result of plant general defence mechanism. The putative functions of these cDNAs were identified by sequence analyses with other homologous proteins. SignalP predicted that EgBBI1 and 2 , EgDFS, EgPRP, and EgT2RIP are secretory proteins. The complete open reading frames (ORFs) of EgBBI1, EgDFS, EgIFR, EgPRP, and EgT2RIP were cloned for recombinant protein production. EgDFSm, EgT2RIPm-CA, and EgT2RIPm-CB were sub-cloned after removal of signal peptide and linker peptide sequences. Soluble recombinant proteins were obtained for EgDFSm, EgIFR, EgT2RIPm-CA while partially soluble protein was obtained for EgT2RIPm-CB. The recombinant EgDFSm managed to inhibit the growth of *G. boninense* mycelium by inhibiting the assimilation of starch, possibly by acting on α-amylase or calcium channel of the fungus. The butanol fraction of oil palm root extract treated with EgIFR showed some differences in its chemical profiles when analysed using a

reverse-phase-high performance liquid chromatography (RP-HPLC). Lastly, the recombinant EgT2RIPm-CA and CB that formed EgT2RIPm-AB was found to be toxic to both mammalian cell lines, MCF-7 and MCF-10. In conclusion, the findings of this study have provided insights on the molecular events that happened during the plant-microbe interaction as well as functional roles of some proteins encoded by these cDNAs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PROFIL EKSPRESI DAN PENCIRIAN FUNGSI GEN-GEN OIL PALM TERPILIH DALAM INTERAKSI HOST DENGAN MIKROB

Oleh

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Reput pangkal batang adalah penyakit utama kelapa sawit yang disebabkan oleh kulat patogenik, *Ganoderma boninense*. Ia mengurangkan hasil minyak sawit dan menyebabkan kerugian yang besar kepada industri minyak sawit. Untuk memahami sistem pertahanan kelapa sawit semasa interaksi host-patogen, profil ekspresi sebelas gen yang berkaitan dengan sistem pertahanan kelapa sawit telah dikaji ke atas kelapa sawit yang telah dirawat dengan *G. boninense*, *Trichoderma harzianum*, atau mikoriza. DNA komplemen (cDNA) yang dikaji mengekod putatif perencat protease Bowman-Birk (EgBBI1 dan 2), defensin (EgDFS), dehydrin (EgDHN), polipeptida berlabel methionine (EgEMLP1 dan 2), protein pengikat RNA yang kaya dengan glisin (EgGRRBP), isoflavon reduktase (EgIFR), metallothionein (EgMT), protein berkaitan dengan patogenesis-1 (EgPRP), dan protein perencat ribosom jenis ke-2 (EgT2RIP). cDNA tersebut dipilih kerana mereka adalah berkaitan dengan sistem pertahanan kelapa sawit dan menunjukkan perubahan tahap ekspresi di kelapa sawit yang diinolukasi dengan mikoriza atau *G. boninense* dalam satu kajian yang lepas.

Dalam kajian ini, transkrip EgIFR dan EgBBI2 didapati meningkat dalam akar pada 3 dan/atau 6 minggu selepas inokulasi (wpi) *G. boninense*. Selain itu, transkrip EgT2RIP, EgBBI1, dan EgDFS didapati meningkat dalam akar pada 6 dan/atau 12 wpi selepas inokulasi *G. boninense*. Sementara itu, transkrip EgDHN meningkat pada ketiga-tiga masa dikaji. Ini menunjukkan bahawa gen-gen tersebut mempunyai peranan yang berbeza di peringkat jangkitan yang berbeza. Profil transkrip dalam daun menunjukkan dua calon gen yang mengekod EgEMLP1 dan EgMT mempunyai profil yang berbeza selepas inokulasi *G. boninense* berbanding dengan *T. harzianum*. Transkrip-transkrip tersebut mempunyai potensi untuk diperkembangkan sebagai petanda biologi untuk mengesan jangkitan awal *G. boninense*. Perbandingan profil transkrip-transkrip akar yang diinokulasi *G. boninense*, *T. harzianum*, dan mikoriza menunjukkan bahawa sesetengah ekspresi transkrip ditingkatkan oleh fungi yang spesifik (ekspresi transkrip EgBBI1 dan EgMT dipertingkatkan oleh *G. boninense* sahaja manakala ekspresi transkrip EgPRP dipertingkatkan oleh mikoriza sahaja). Walaupun begitu, ekspresi transkrip EgDFS dan EgT2RIP didapati dipertingkatkan oleh ketiga-tiga fungi mungkin hasil daripada mekanisme pertahanan yang umum. Putatif fungsi cDNA yang dikaji ditentukan dengan membanding bebenang asid amino dengan protein-protein homologi yang lain. SignalP meramalkan bahawa EgBBI1 dan 2, EgDFS, EgPRP, dan EgT2RIP adalah protein-protein sekretori. Bingkai bacaan terbuka (ORFs) daripada EgBBI1, EgDFS, EgIFR, EgPRP, dan EgT2RIP telah diklonkan untuk menghasil protein-protein rekombinan. EgDFSm, EgT2RIPm-CA, dan EgT2RIPm-CB telah disubklon selepas penyingkiran peptida isyarat dan peptida pencantum. Protein-protein rekombinan yang larut telah diperolehi bagi EgDFSm, EgIFR, EgT2RIPm-CA, manakala protein rekombinan

separa larut telah diperolehi bagi EgT2RIPm-CB. Rekombinan EgDFSm berjaya merencat pertumbuhan *G. boninense* miselium melalui penghalangan asimilasi kanji yang mungkin bertindak pada α-amilase atau saluran kalsium fungi. Pecahan butanol ekstrak daripada akar kelapa sawit yang telah dirawat EgIFR menunjukkan beberapa perubahan profil kimia setelah dianalisasi dengan menggunakan fasa terbalik kromatografi cecair berprestasi tinggi (RP-HPLC). EgT2RIPm-AB yang terbentuk daripada gabungan EgT2RIPm-CA dan CB adalah toksik kepada kedua-dua titisan sel mamalia, MCF-7 dan MCF-10. Sebagai kesimpulannya, hasil kajian ini telah memberi gambaran di tahap molekul semasa interaksi tumbuhan dengan mikrob serta fungi-fungsi beberapa protein yang dikodkan oleh cDNA tersebut.

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I certify that a Thesis Examination Committee has met on 23rd July 2013 to conduct the final examination of Tan Yung Chie on his thesis entitled "Expression Profiling and Functional Characterization of Selected Oil Palm Genes in Host-Microbial Interaction" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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

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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.

TAN YUNG CHIE

Date: 23 July 2013

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