



UNIVERSITI PUTRA MALAYSIA

**EXPRESSION OF CHITINASES AND GLUCANASES IN OIL PALM IN
RESPONSE TO *GANODERMA* AND *TRICHODERMA* TREATMENTS**

YE OH KEAT AI

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**MASTER OF SCIENCE
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By

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July 2011

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Cell wall degrading enzymes (CWDEs) such as chitinases and glucanases are produced by plants during the infection of pathogens as part of their defense mechanism. In this study, the full length complementary DNA (cDNA) sequences encoding two chitinases (EgChit3-1 and EgChit5-1) and one (EgGlc5-2) glucanase from the oil palm root have been isolated by Rapid Amplification of cDNA Ends – Polymerase Chain Reaction (RACE-PCR). Meanwhile the 5'- terminal cDNA sequence encoding a chitinase (EgChit1-1) was successfully isolated. Sequence analysis was performed on the three full-length cDNA sequences obtained from this study and two full-length cDNA sequences encoding glucanases (EgGlc1-1 and EgGlc5-1) obtained from the expressed sequence tags (ESTs) of oil palm root cDNA library. Analyses of the cDNA sequences revealed that EgGlc1-1 is related to the glycosyl hydrolase (GH) family 3 while EgGlc5-1 and EgGlc5-2 are related to the GH family 17. EgChit1-1 was categorized in the GH family 19 whereas EgChit3-1 and EgChit5-1 are related to the GH family 18. Real time reverse transcription polymerase chain reaction (RT-PCR) was performed to detect the transcript levels of the isolated glucanases and chitinases using oil palm seedlings treated with

Ganoderma boninense PER71, *Trichoderma harzianum* T32 singly and in a combination of *Ganoderma* and *Trichoderma* at 3, 6 and 12 weeks post infection (wpi). Lesions caused by *Ganoderma* infection were only observed at 6 wpi and 12 wpi in *Ganoderma*-treated seedlings. The *Trichoderma* colony forming unit (CFU) estimation in *Ganoderma-Trichoderma*- and *Trichoderma*-treated seedlings increased gradually from 3-12 wpi. The expression of EgGlc1-1 was induced by *Trichoderma* in *Trichoderma*-treated seedlings at 3, 6 and 12 wpi. The expression of EgGlc5-1 and EgGlc5-2 was elevated in the roots of control seedlings while suppressed treated seedlings at 3, 6 and 12 wpi. The transcript encoding EgChit1-1 increased in response to *Ganoderma* infection at early stage (3 wpi). The transcript of EgChit3-1 and EgChit5-1 were increased at later stages of infection in roots of *Ganoderma*- and *Trichoderma*-treated seedlings. The findings of this study provide an insight to the gene expression of a few glucanases and chitinases during colonization of *Ganoderma* and *Trichoderma* on oil palm seedlings. Not all glucanases and chitinases in this study played a role in oil palm defense. Different glucanases and chitinases were regulated differently in oil palm seedlings when they were exposed to *Ganoderma*, *Trichoderma* singly and in a combination of *Ganoderma-Trichoderma*.

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

PENGEKSPRESAN KITINASE DAN GLUKANASE DALAM KELAPA SAWIT TINDAK BALAS RAWATAN *GANODERMA* DAN *TRICHODERMA*

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Enzim penguraian dinding sel seperti glukunase dan kitinase dirembes oleh tumbuhan semasa jangkitan patogen sebagai sebahagian daripada mekanisme pertahanan. Dalam penyelidikan ini, jujukan penuh DNA pelengkap (cDNA) yang mengekod dua kitinase (EgChit3-1 and EgChit5-1) dan satu glukunase (EgGlc5-2) dari akar kelapa sawit telah dipencil dengan menggunakan RACE-PCR. Sementara itu, jujukan penghujung- 5' DNA pelengkap yang mengekod kitinase (EgChit1-1) telah berjaya dipencilkan. Analisis jujukan telah dilakukan ke atas tiga jujukan penuh DNA pelengkap yang diperolehi dari penyelidikan ini dan dua jujukan penuh DNA pelengkap yang mengekod glukunase (EgGlc1-1 dan EgGlc5-1) yang diperolehi daripada tag jujukan terekspres (ESTs) perpustakaan DNA pelengkap akar kelapa sawit. Analisis jujukan DNA pelengkap mendapati EgGlc1-1 mempunyai kaitan dengan GH famili 3 manakala EgGlc5-1 dan EgGlc5-2 mempunyai kaitan dengan hidrolase glikosil (GH) famili 17. EgChit1-1 dikategorikan dalam GH famili 19 sementara EgChit3-1 dan EgChit5-1 dikaitkan dengan GH famili 18. PCR masa nyata transkripsi terbalik dijalankan untuk mengesan tahap transkrip glukunase dan kitinase menggunakan anak-anak pokok kelapa sawit yang telah dirawat dengan

Ganoderma boninense PER71, *Trichoderma harzianum* T32 dan satu kombinasi *Ganoderma-Trichoderma* selama 3, 6 dan 12 minggu selepas rawatan (wpi). Lesi akibat jangkitan *Ganoderma* hanya kelihatan pada anak pokok yang dirawat dengan *Ganoderma* pada 6 wpi dan 12 wpi. Anggaran pembentukan unit koloni *Trichoderma* (CFU) dalam anak pokok yang dirawat dengan *Ganoderma-Trichoderma* dan *Trichoderma* telah meningkat secara berperingkat dari 3 hingga 12 wpi. Pengekspresan EgGlc1-1 diaruh oleh *Trichoderma* dalam anak pokok yang dirawat dengan *Trichoderma* pada 3, 6 dan 12 wpi. Pengekspresan EgGlc5-1 dan EgGlc5-2 meningkat dalam akar anak pokok kawalan manakala terhalang dalam anak pokok yang dirawat pada 3, 6 dan 12 wpi. Transkrip yang mengekod EgChit1-1 meningkat kesan tindak balas terhadap jangkitan *Ganoderma* pada 3 wpi. Transkrip EgChit3-1 dan EgChit5-1 meningkat pada peringkat akhir jangkitan dalam akar anak pokok yang dirawat dengan *Ganoderma* dan *Trichoderma*. Keputusan yang diperoleh dari penyelidikan ini memberikan pengetahuan tentang pengekspressan beberapa gen glukunase dan kitinase dalam anak pokok kelapa sawit semasa jangkitan *Ganoderma* dan *Trichoderma*. Bukan semua glukunase dan kitinase dalam penyelidikan ini mempunyai peranan dalam pertahanan kelapa sawit. Glukanase dan kitinase yang berlainan dikawal-atur secara berbeza dalam anak pokok kelapa sawit apabila anak-anak pokok didedahkan kepada *Ganoderma*, *Trichoderma* dan kombinasi *Ganoderma-Trichoderma*.

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I certify that a Thesis Examination Committee has met on 18 July 2011 to conduct the final examination of Yeoh Keat Ai on her thesis entitled “Expression of Chitinases and Glucanases in Oil Palm in Response to *Ganoderma* and *Trichoderma* Treatments” in accordance with the Universities and University College 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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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 institutions.

YEOH KEAT AI

Date: 18 July 2011



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