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ISOLATION OF CHITINASE cDNAs AND THEIR EXPRESSIONS IN OIL PALM *Elaeis guineensis* Jacq. SEEDLINGS IN INTERACTION WITH *Ganoderma boninense* Pat. AND *Trichoderma harzianum* Rifai

MST. LAILA NAHER

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By

MST. LAILA NAHER

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

September 2011
DEDICATIONS

This Thesis is Special Dedicated to

My Parents, Nasim Ali and Hosne Ara Begum

Late Professor Fridah Abdullah and

My beloved husband Saynul Islam
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

ISOLATION OF CHITINASE cDNAs AND THEIR EXPRESSIONS IN OIL PALM Elaeis guineensis Jacq. SEEDLINGS IN INTERACTION WITH Ganoderma boninense Pat. AND Trichoderma harzianum Rifai

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MST. LAILA NAHER

September 2011

Chairman : Professor Tan Soon Guan, PhD

Faculty : Science

Basal stem rot (BSR), which is caused by the fungus Ganoderma boninense, is a serious disease that affects the oil palm (Elaeis guineensis), and it is a major threat to the oil palm industry. The disease escapes early detection. When fruiting bodies are detected the disease is too advanced to respond to any chemical treatments. Another fungus, Trichoderma harzianum has been proven to have high efficacy in controlling Ganoderma infection in oil palms only at early stage of infection of slightly infected palms. Therefore, to date there is no adequate control measure to control this disease. So, as a long term control measure, improvement of the oil palm defence system against G. boninense is the alternative option. The pathogenesis-related (PR) protein of plant chitinases belongs to the repertoire of plant defense mechanisms that are believed to constitute the early defense response in plants. Thus, the plant chitinases may be involved in the oil palm’s reaction to infection by G. boninense. The goal of this study was to investigate the potential role of chitinase mRNA’s expression and enzyme activity in oil palms infected by G. boninense strain PER71 as well as in
samples treated with *T. harzianum* strain FA1132, which is a biocontrol agent used to combat BSR disease. The effectiveness of *T. harzianum* as a biocontrol agent for BSR disease and as a potential enhancer of plant growth as well as defence response in plants was also investigated. The disease severity index value (DSI) showed that *G. boninense* infected the oil palm root as early as at week 5 with DSI = 8.3% whereas physical symptoms appeared in leaves at week 8 with DSI = 11.11%. In contrast, no disease symptoms were observed in plants treated with *T. harzianum*, either alone or in combination with *G. boninense*, and root and leaf weights markedly increased in these treatments. Although the chlorophyll concentration was not increased in *T. harzianum* treated plants but after 2 weeks post inoculation it was higher than in control plants and plants treated with only *Ganoderma*.

Three chitinase classes were selected for cDNA isolation and characterization: EgCHI1, EgCHI2, and EgCHI3 were cloned and sequenced, and their expression patterns were analyzed. Nucleotide data indicated that the EgCHI1 and EgCHI2 sequences belong to glycoside hydrolase family 19, which contains chitinase classes I, II, and IV, and the EgCHI3 sequence belongs to glycoside hydrolase family 18, which consists of chitinase classes III and V. BLAST analysis revealed that EgCHI1 was highly similar (78%) to a class I chitinase from *Arabidopsis thaliana* (AAF29391.1); EgCHI2 was highly similar (78%) to a class II chitinase from *Fragaria X Ananassa* (AAF00131.1); and EgCHI3 was highly similar (72%) to a class III chitinase from *Bambusa oldham* (ABW75909.1). Expressions of these three cDNAs were observed in oil palm seedlings treated with *G. boninense* or *T. harzianum* either alone or together at time points of 2, 5, and 8 weeks post inoculation (wpi), respectively. For seedlings treated with *G. boninense* alone, the
transcripts levels of all three chitinases in roots tissues were highest at 5 weeks post inoculation when the infection was visible, whereas chitinase enzyme activity was highest at 2 weeks post inoculation. At 8 weeks post inoculation when G. boninense infection was established, the chitinase expression was decreased but the chitinase enzyme activity slightly increased. The cDNAs expression data also revealed that the chitinases were constitutively expressed in root tissues but not in leaf tissues. Moreover, cDNA expression and the activity of enzyme were higher in root tissues compared to leaf tissues. EgCHI1 expression was up-regulated until 8 weeks post inoculation in roots and leaves of oil palms treated with a combination of G. boninense and T. harzianum. In conclusion, this study found that all three chitinases were expressed in the presence of G. boninense or T. harzianum but that the timing of chitinase expression varied depending on the type of treatment.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

ISOLASI KITINASE cDNAs DAN PENGEKSPRESAN NYA DA LM ANAK POKOK KELAPA SAWIT Elaeis guineensis Jacq. DALAM INTERAKSI DENGAN Ganoderma boninense Pat. DAN Trichoderma harzianum Rifai

Oleh

MST. LAILA NAHER

September 2011

Pengerusi : Profesor Tan Soon Guan, PhD
Fakulty : Sains

oleh *G. boninense* stren PER71 serta sampel yang dirawat dengan *Trichoderma harzianum* stren FA1132, yang merupakan agen kawalan biologi yang digunakan untuk melawan penyakit RPB. Keberkesanan *T. harzianum* sebagai agen kawalan biologi bagi penyakit RPB dan potensinya sebagai peningkat pertumbuhan pokok juga dikaji. Nilai indeks keparahan penyakit (DSI) menunjukkan *G. boninense* menjangkiti akar kelapa sawit seawal minggu kelima (DSI=8.3%), manakala simptom fizikalnya muncul dalam daun pada minggu kelapan sebanyak (11.11%). Sebaliknya, tiada simptom penyakit diperhatikan dalam pokok yang dirawat dengan *T. harzianum* sama ada sendirian atau digabungkan dengan *G. Boninense*. Malahan berat akar dan daunnya didapati meningkat dalam rawatan tersebut. Walaupun kepekatan klorofil tidak meningkat dalam pokok yang dirawat dengan *T. harzianum*, tetapi kepekatannya selepas dua minggu inokulasi adalah tinggi berbanding pokok kawalan dan pokok yang dirawat dengan *Ganoderma* sahaja.

Tiga kelas kitinase telah dipilih untuk cDNA isolasi dan pencirian: EgCHI1, EgCHI2, dan EgCHI3 telah diklon serta disekuens dan paten ekspresinya telah dianalisis. Data nukleotida menunjukkan sekuens EgCHI1 dan EgCHI2 tergolong dalam famili 19 hidrolas glikosida, yang mempunyai kitinase kelas I, II, dan IV, sekuens EgCHI3 tergolong dalam famili 18 hidrolas glikosida yang mempunyai kitinase kelas III dan V. Analisis BLAST menunjukkan EgCHI1 mempunyai yang kesamaan tinggi (78%) dengan kitinase kelas I daripada *Arabidopsis thaliana* (AAF29391.1); EgCHI2 mempunyai kesamaan yang tinggi (78%) dengan kitinase kelas II daripada *Fragaria X Ananassa* (AAF00131.1); and EgCHI3 mempunyai yang kesamaan tinggi (72%) dengan kitinase kelas III daripada *Bambusa oldham* (ABW75909.1). Pengekspresan tiga cDNA ini telah diperhatikan dalam anak pokok
kelapa sawit yang dirawat dengan *G. boninense* atau *T. harzianum* sama ada sendirian atau bersama selepas inokulasi pada minggu ke-2, 5 dan 8. Bagi anak pokok yang dirawat dengan *G. boninense* sendirian, tahap transkrip bagi tiga kitinase dalam tisu akar adalah tinggi pada minggu ke-5 selepas inokulasi ketika jangkitan telah dapat diperhatikan, sedangkan aktiviti enzim kitinase adalah terbesar selepas inokulasi pada minggu ke-2. Pada minggu ke-8 selepas inokulasi dan ketika jangkitan *G. boninense* telah ketara, ekspresi kitinase berkurangan sebaliknya aktiviti enzim kitinase sedikit menaik. Data ekspresi cDNA juga telah menunjukkan kitinase berterusan diekspresi dalam tisu akar, bukan hanya dalam tisu daun. Ekspresi EgCHI1 telah diselaras sehingga minggu ke-8 selepas inokulasi dalam akar dan daun kelapa sawit yang dirawat dengan kombinasi *G. boninense* dan *T. harzianum*. Sebagai kesimpulan, kajian ini mendapati ketiga-tiga kitinase telah diekspresi dalam kehadiran *G. boninense* atau *T. harzianum* tetapi masa pengekspresan kitinase adalah berbeza bergantung kepada jenis rawatan.
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I certify that an Examination Committee met on ___ to conduct the final examination of Laila Naher on her Doctor of Philosophy thesis entitled “Isolation of chitinase cDNAs and their expressions in oil palm seedlings *Elaeis guineensis* Jacq. during interaction with *Ganoderma boninense* Pat. and *Trichoderma harzianum* Rifai ” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree.

Members of the Examination Committee are as follows:

**Norhani bt Abdullah, PhD**  
Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Rosimah bt Nulit, PhD**  
Faculty of Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Mohd. Puad bin Abdullah, PhD**  
Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Anthony James Whalley, PhD**  
Professor  
School of Pharmacy and Biomolecular Science  
Liverpool John Moores University  
United Kingdom  
(External Examiner)

---

**BUJANG KIM HUAT, 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 fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Tan Soon Guan, PhD**
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

**Ho Chai Ling, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

**Umi Kalsom Yusuf, PhD**
Professor
Faculty of Science
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 equations 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 other institutions.

MST. LAILA NAHER

Date: 6 September 2011
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