UNIVERSITI PUTRA MALAYSIA

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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By

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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

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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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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

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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

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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.

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

ISOLASI KITINASE cDNAs DAN PENGEKSPRESANNYA DALAM ANAK POKOK KELAPA SAWIT *Elaeis guineensis* Jacq. DALAM INTERAKSI DENGAN *Ganoderma boninense* Pat. DAN *Trichoderma harzianum* Rifai

Oleh

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Reput pangkal batang (RPB) yang disebabkan oleh kulat *Ganoderma boninense* adalah penyakit serius yang memberi kesan kepada kelapa sawit (*Elaeis guineensis*). Ia merupakan ancaman utama terhadap industri kelapa sawit. Penyakit ini tidak dapat dikesan pada peringkat awal. Apabila jasad buah telah dikesan, penyakit ini sudah tidak menunjukkankesan kepada sebarang rawatan kimia. Kulat lain, *Trichoderma harzianum* telah dibuktikan mempunyai efikasi yang tinggi dalam mengawal jangkitan *Ganoderma* dalam kelapa sawit hanya pada peringkat awal jangkitan. Oleh itu, sehingga kini masih belum ada langkah kawalan yang mencukupi untuk mengawal penyakit ini. Oleh itu, sebagai langkah kawalan jangka masa panjang, meningkatkan sistem pertahanankelapa sawit terhadap *G. boninense* adalah satu pilihan alternatif. Protein 'pathogenesis-related' (PR) bagi kitinase tumbuhan milik repetoir mekanisme pertahanan tumbuhan yang dipercayai membentuk respons pertahanan awal dalam tumbuhan. Oleh yang demikian, protein PR bagi kitinase tumbuhan mungkin terlibat dalam reaksi kelapa sawit terhadap jangkitan oleh *G. boninense*. Matlamat kajian ini adalah untuk mengkaji potensi peranan pengekspresan kitinase mRNA dan aktiviti enzim dalam kelapa sawit yang dijangkiti

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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

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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, ekpresi kitinase berkurangan sebaliknya aktiviti enzim kitinase sedikit menaik. Data ekspresi cDNA juga telah menunjukkan kitinase berterusan diekspresi dalam tisu akar, bukannya 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 bergantng 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.

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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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TABLE OF CONTENTS

		Page
ABSTRACT		**iii**
ABSTRAK		**vi**
ACKNOLEDGEMENTS		**ix**
APPROVAL		**xi**
DECLARATION		**xiii**
LIST OF TABES		**xvii**
LIST OF FIGURES		**xviii**
LIST OF ABBREVIATIONS		**xxi**
CHAPTER 1		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	5
	2.1 The Oil palm (*Elaeis guineensis* Jacquin)	5
	2.1.1 Taxonomy of the oil palm	5
	2.1.2 Botany of the oil palm	6
	2.1.3 Uses of the oil palm	7
	2.1.4 Diseases of oil palms	7
	2.2 BSR in oil palms	8
	2.2.1 *Ganoderma boninense*	8
	2.2.2 BSR symptoms and disease development	9
	2.2.3 Control of BSR	10
	2.2.3.1 Trenches	10
	2.2.3.2 Replanting	11
	2.2.3.3 Chemical control	11
	2.2.3.4 Biological control	12
	2.3 Biocontrol by *Trichoderma* spp.	12
	2.3.1 Biocontrol mechanism of *Trichoderma* spp.	14
	2.3.1.1 Competition for nutrition and living space	14
	2.3.1.2 Biocontrol by the mycoparasitism	15
	2.3.2 Plant growth enhancement by *Trichoderma* spp.	16
	2.3.3 Induction of plant defence by *Trichoderma* spp.	16
	2.3.4 Plant root colonization by *Trichoderma* spp.	17
	2.3.5 Production of antibiotics and secondary compounds by *Trichoderma* spp.	18
	2.3.6 Other uses of *Trichoderma* spp.	19
	2.4 Interaction between Plants and Microbes	19
	2.5 Plant defence mechanism	20
	2.6 Pathogenesis-related proteins	23
	2.7 Plant chitinases	25
	2.7.1 Classification of plant chitinases	25
	2.7.1.1 Chitinase Classes I, II, and III	26
	2.7.1.2 Chitinase Classes IV, V, VI and VII	26
	2.8 Functions of plant chitinases	27

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2.8.1 The role of chitinases in defense 27
2.8.2 Chitinases in symbiosis 28
2.8.3 Chitinases in nodule formation 28
2.8.4 Other functions of chitinase 29
2.9 Chitinase expression at the site of infection in plants 30
2.10 Chitinases-enhanced disease resistance in transgenic plants 31
2.11 Antifungal activity of chitinases 32
2.12 Measurement of gene expression using Real-Time PCR 33

3 ISOLATION OF THE COMPLEMENTARY DNA (cDNA) OF CHITINASE CLASSES I, II, AND III FROM THE OIL PALM
3.1 Introduction 36
3.2 Materials and methods 38
3.2.1 RNA extraction 38
3.2.2 Formaldehyde denaturing gel electrophoresis 39
3.2.3 DNase treatment 40
3.2.4 Design of gene-specific primers 41
3.2.5 Synthesis of first strand 5' and 3' RACE cDNA 42
3.2.6 Isolation of EgCHI1, EgCHI2, and EgCHI3 cDNAs 43
3.2.7 Purification of DNA from agarose gel 44
3.2.8 Preparation of *Escherichia coli* DH5alpha 45
3.2.9 Cloning and Transformation 45
3.2.9.1 Plasmid DNA extraction 46
3.2.9.2 Restriction enzyme digestion of plasmid DNA 47
3.2.10 DNA Sequencing and analysis 47
3.3 Results 49
3.3.1 Total RNA extraction 49
3.3.2 Molecular cloning of oil palm chitinases 50
3.3.2.1 Sequence analysis of EgCHI1 50
3.3.2.2 Sequence analysis of EgCHI2 55
3.3.2.3 Sequence analysis of EgCHI3 59
3.4 Discussion 64

4 ANALYSIS OF EgCHI1, EgCHI2, AND EgCHI3 cDNA EXPRESSIONS AND CHITINASE ENZYME ACTIVITY IN OIL PALM SEEDLINGS TREATED WITH GANODERMA AND TRICHODERMA EITHER ALONE OR IN COMBINATION
4.1 Introduction 66
4.2 Material and methods 68
4.2.1 Plant materials 68
4.2.1.1 *Ganoderma boninense* culture 68
4.2.1.2 Preparation of *Ganoderma* wood block inocula 68
4.2.1.3 *Trichoderma harzianum* culture 69
4.2.1.4 Preparation of carrier for Tricho-mulch 69
4.2.1.5 Preparation of *Trichoderma* conidial suspension 69
4.2.1.6 Experimental treatments and layout 70
4.2.1.7 Plant treatments in the greenhouse 71
4.2.2 *Trichoderma* colony forming unit (CFU) measurements 72

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4.2.3 Sample collection 72
4.2.4 Chlorophyll determination 73
4.2.5 Assessment of disease signs and symptoms 73
4.2.6 Statistical analysis 74
4.3 RNA extraction 75
4.3.1 First-strand cDNA synthesis 75
4.3.2 Primer design and optimization of reverse transcript RT PCR for Real-Time RT PCR 76
4.3.3 Real-Time RT-PCR 77
4.3.4 Validation of real-time amplification efficiencies 78
4.3.5 Expression analysis of oil palm cDNAs 79
4.3.6 Protein extraction 80
4.3.7 Chitinase enzyme activity analysis 80
4.4 Results 82
4.4.1 Inocula used for plant treatments 82
4.4.2 Estimation of *T. harzianum* colony forming units in soil 86
4.4.3 Determination of chlorophyll concentration 89
4.4.4 Estimation of root and leaf weights 90
4.4.5 Disease severity 92
4.4.6 RNA extraction 98
4.4.7 Optimization of RT-PCR and validation of amplification efficiencies for the comparative $\Delta\Delta C_T$ method 99
4.4.8 Expression profiles of EgCHI1, EgCHI2, and EgCHI3 101
4.4.9 Chitinase Enzyme activity in root and leaf tissues 106
4.5 Discussion 108
4.5.1 The use of biocontrol agent *Tharzianum* strain FA1132 as an enhancer for oil palm growth 108
4.5.2 Expression profiles of chitinases cDNAs and enzyme activity in oil palms in response to pathogenic and non pathogenic fungi 111

5 SUMMARY, CONCLUSIONS, AND RECOMMENDATION 116

REFERENCES 121
APPENDICES 145
BIODATA OF STUDENT 178

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