UNIVERSITI PUTRA MALAYSIA

CLONING AND CHARACTERIZATION OF A NOVEL TRANSCRIPT ENCODING A RNA-BINDING PROTEIN FROM OIL PALM (Elaeis guineensis Jacq.)

YEAP WAN CHIN

FBSB 2015 7

CLONING AND CHARACTERIZATION OF A NOVEL TRANSCRIPT ENCODING A RNA-BINDING PROTEIN FROM OIL PALM (*Elaeis guineensis* Jacq.)

By

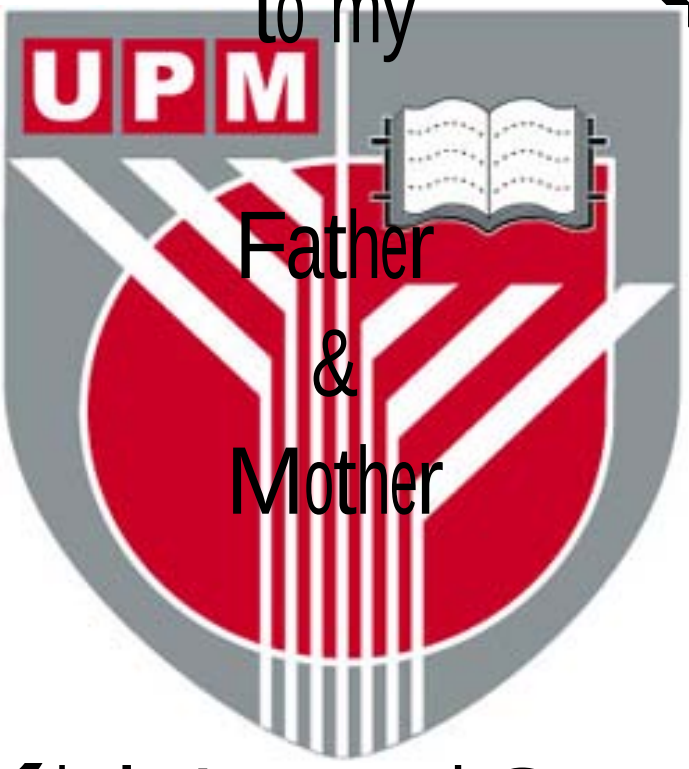
YEAP WAN CHIN

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

May 2015

Specially Dedicated

to my



Father

&

Mother

For their Love and Supports

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CLONING AND CHARACTERIZATION OF A NOVEL TRANSCRIPT ENCODING A RNA-BINDING PROTEIN FROM OIL PALM (*Elaeis guineensis* Jacq.)

By

YEAP WAN CHIN

May 2015

Chairman: Associate Professor Ho Chai Ling, PhD
Faculty: Biotechnology and Biomolecular Sciences

RNA-binding proteins (RBPs) have been implicated as regulatory proteins involved in the post-transcriptional processes of gene expression in plants that influence floral development, circadian rhythms, hormone signaling, plant growth, abiotic stress response and tolerance. RBPs have received much attention in *Arabidopsis*, tobacco and rice. Oil palm (*Elaeis guineensis* Jacq.) is the most efficient oil-yielding crop in the world. Environmental stresses have a major impact on oil palm production mainly plant growth, physiology and oil yield. However, the biological functions of RBPs in post-transcription regulation of gene expression in response to stresses are still poorly understood in oil palm. This study aimed to understand the regulation of target transcripts by a RBP from oil palm at post-transcriptional level, the regulatory factors associated with the target RBP in the ribonucleoprotein complex and the involvement of RBP in post-transcriptional RNA mechanism in response to environmental stimuli in oil palm. In this study, a gene designated as *EgRBP42*, encoding a plant heterogeneous nuclear ribonucleoprotein-like RBP was isolated from oil palm. *EgRBP42* was identified from an expressed sequence tag (EL684239) from the oil palm female inflorescence. EgRBP42 protein consists of two N-terminal RNA recognition motifs and a glycine-rich domain at the C-terminus. The upstream region of *EgRBP42* has multiple light-responsive, stress-responsive and flower development related regulatory elements. Real-time RT-PCR analysis showed that *EgRBP42* was expressed in all oil palm tissues tested, including leaf, shoot apical meristem, root, female inflorescence, male inflorescence and mesocarp with the lowest transcript level in the roots. EgRBP42 protein interacted with transcripts associated with stress responses, transcription and translation. Validation of consensus sequence of interactive transcripts binding to EgRBP42 indicated that EgRBP42 binds to the AG-rich region on its interactive transcripts. Three variants of *EgRBP42* D W W Untranslated regions were detected from

oil palm leaf tissue. The accumulation of *EgRBP42*, its interacting transcripts DQG LWV DOWHUQDWLYH WUDQVFULSWV were up-regulated (> 2 fold change) by abiotic stresses, including salinity, drought, submergence, cold and heat stresses in leaf discs (short-term stress treatment for 30 min to 28 hr) and leaves from oil palm seedlings (long-term stress treatment for 7 days). Co-immunoprecipitation and yeast II hybrid interaction studies showed that EgRBP42 protein interacted with various regulatory factors involved in transcription, nucleocytoplasmic transport, mRNA degradation and translation. The protein accumulation of EgRBP42 was up-regulated (> 2-fold change) by heat, cold, drought and salinity in oil palm seedlings exposed to long-term stress treatments for 7 days. Collectively, the data suggested that EgRBP42 is responsive to various abiotic stresses. It is potentially a nucleocytoplasmic transporter of stress-responsive mRNAs from nucleus to cytoplasm for their rapid translation in response to heat, cold and drought stresses. This study provided information on post-transcriptional regulatory mechanisms of EgRBP42 in oil palm. Hence, EgRBP42 may be useful for the engineering of stress tolerant oil palm.

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

PENGKLONAN DAN PENCIRIAN TRANSKRIP NOVEL YANG MENGEKODKAN PROTEIN PENGIKATAN-RNA DARIPADA KELAPA SAWIT (*Elaeis guineensis* Jacq.)

Oleh

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Protein pengikatan-RNA (RBPs) adalah protein pengawalatur yang terlibat dalam proses pasca transkripsi pengekspresan gen dalam tumbuh-tumbuhan dari segi perkembangan bunga, irama sirkadian, isyarat hormon, perkembangan tumbuhan, tindakbalas dan ketahanan terhadap tekanan abiotik. RBPs mendapat banyak perhatian dalam bidang penyelidikan pertumbuhan seperti *Arabidopsis*, tembakau dan beras. Kelapa sawit (*Elaeis guineensis* Jacq.) adalah tumbuhan yang paling cekap dalam penghasilan minyak di dunia. Tekanan persekitaran mempunyai kesan yang amat besar terhadap penghasilan minyak kelapa sawit, terutamanya dari segi perkembangan tumbuhan, fisiologi dan hasil minyak. Tetapi, fungsi biologi RBPs sebagai pengawalatur ekpresi gen di peringkat pasca transkripsi terhadap tekanan abiotik pokok kelapa sawit masih tidak diketahui. Kajian ini bertujuan untuk memahami pengawalatur RBP daripada kelapa sawit terhadap transkrip sasaran di peringkat pasca transkripsi, faktor-faktor pengawalatur yang berkaitan dengan RBP dalam kompleks ribonucleoprotein dan juga penglibatan RBP dalam mekanisme RNA di peringkat pasca transkripsi dan tindakbalasnya terhadap rangsangan alam sekitar di dalam kelapa sawit. Dalam kajian ini, gen yang dinamakan sebagai *EgRBP42*, mengekodkan ribonucleoprotein nuklear heterogen telah dipencilkan dan diklonkan daripada kelapa sawit. *EgRBP42* didapati daripada tag jujukan terekspres (EL684239) bunga betina kelapa sawit. EgRBP42 mengandungi dua motif pengenalan RNA di pangkalan-N dan satu domain kaya dengan glisin di pangkalan-C. Rantau penganjur *EgRBP42* mengandungi pelbagai elemen pengawalaturan yang berkaitan dengan tindakbalas terhadap cahaya, tindakbalas terhadap tekanan dan perkembangan bunga. Analisis RT-PCR masa nyata menunjukkan bahawa *EgRBP42* digekspres dalam semua tisu-tisu kelapa sawit yang diuji, termasuk daun, pucuk meristem apikal, akar, bunga betina, bunga jantan dan mesokarp dengan aras transkrip yang paling rendah di dalam akar. Protein EgRBP42 berinteraksi

dengan transkrip yang berkaitan dengan proses tindakbalas terhadap tekanan, transkripsi dan terjemahan protein. Pengesahan jujukan konsensus pada transkrip berinteraktif kepada protein EgRBP42 menunjukkan bahawa EgRBP42 terikat kepada transkrip berinteraktif yang mempunyai urutan nukleotida yang kaya dengan adenine dan guanine. Tiga varian *EgRBP42* pada ORNDVL ¶ \DQJ WLGDN GLWHUMHPDK akan, telah dikenalpasti daripada tisu daun kelapa sawit. Ekspresi *EgRBP42*, transkripsi yang berinteraksi dengan protein EgRBP42 dan transkripsi varian *EgRBP42 GL ORNDVL ¶ \DQJ WLGDN GLW* ditingkatkan (> 2 kali ganda) oleh tekanan abiotik, termasuk kemasinan, kemarau, banjir, kesejukan dan kepanasan di cakera daun (rawatan tekanan jangka masa pendek selama 30 min sehingga 28 jam) dan daun anak benih kelapa sawit (rawatan tekanan jangka masa panjang selama 7 hari). Kajian ko-pengendapan imun dan interaksi yis II hibrid menunjukkan bahawa protein EgRBP42 berinteraksi dengan pelbagai faktor pengawalaturan yang terlibat dalam tindakan transkripsi, pengangkutan nukleus-sitoplasma, degradasi mRNA dan terjemahan mRNA. Ekspresi protein EgRBP42 telah ditingkatkan (> 2 kali ganda) dalam anak benih kelapa sawit yang terdedah kepada kepanasan, kesejukan, kemarau dan kemasinan dalam rawatan tekanan jangka masa panjang selama 7 hari. Secara keseluruhan, data yang diperolehi mencadangkan bahawa protein EgRBP42 bertindakbalas terhadap pelbagai tekanan abiotik dan ia berpotensi sebagai pengangkut nukleus-sitoplasma kepada mRNA yang responsif terhadap tekanan alam sekitar supaya penerjemahan cepat akan dijalankan sebagai tindakbalas terhadap kepanasan, kesejukan dan kemarau. Kajian ini memberi maklumat berguna mengenai mekanisma pasca transkripsi yang dikawalatur oleh EgRBP42 di dalam kelapa sawit. Oleh itu, EgRBP42 mungkin memainkan peranan yang penting dalam kejuruteraan genetik kelapa sawit untuk meningkatkan tahap ketahanan terhadap tekanan persekitaran.

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I certify that a Thesis Examination Committee has met on 21 May 2015 to conduct the final examination of Yeap Wan Chin on her thesis entitled "Isolation and Characterization of a Novel Transcript Encoding a RNA-Binding Protein from Oil Palm (*Elaeis guineensis* Jacq.)" 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 Doctor of Philosophy.

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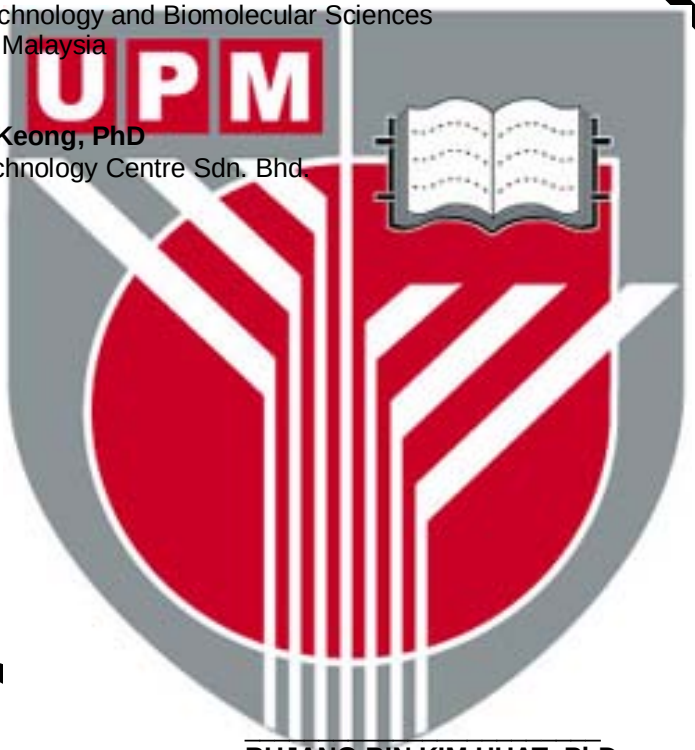

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LIST OF ABBREVIATIONS

.	alpha
	beta
	gamma
/	delta
%	percentage
°C	degree Centigrade
AAPK	ABA-activated protein kinase
AbA	Aureobasidin A
ABA	Abscisic acid
AD	activation domain
Ade	adenine
AG	AGAMOUS
AKIP1	AAPK-interacting protein 1
ARE	adenylate/uridylate-rich element
ATP	adenosine triphosphate
AtTRN1	transportin 1
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
bp	base pair
BSA	bovine serum albumin
CaCl2	calcium chloride
CBC	cap-binding complex
C-terminal	carboxyl terminal
cDNA	complementary DNA
cm	centimetre
CPL1	C-terminal domain phosphatase-like 1
cpRNP	chloroplastid ribonucleoproteins
CPSF	cleavage and polyadenylation specific factor
DNA	deoxyribonucleic acid
DNase I	deoxyribonuclease
dNTP	deoxynucleotides
ds-cDNA	double stranded cDNA
DEPC	diethyl pyrocarbonate
DMSO	dimethylsulphonyl oxide
DTT	dithiothreitol
EtBr	ethidium bromide
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol bis- -aminoethyle ether)
EgRBP42	*Elaeis guineensis* RNA-binding protein 42 kDa
eIF	translation initiation factors
EJC	exon junction complex
EMSA	electrophoretic mobility shift assay
FCA	flowering control locus A
FLC	FLOWERING LOCUS C
FLK	flowering locus K
FPA	flowering time control protein A
FY	flowering locus Y
GA	giberrellin acid

g	gram
GR-RBP	glycine-rich RNA-binding protein
GST-His6	glutathione-S transferase-hexsa histadine
HCl	hydrochloric acid
His	histidine
HEPES	N-2-hydroxyethylpiperazine-N' -2-ethanesulfonic acid
hr	hours
hnRNA	heterogeneous nuclear ribonucleic acid
hnRNP	heterogeneous nuclear ribonucleoprotein
IPTG	isopropyl- -D-thioga1actoside
k	kilo
kb	kilo base pair
KCl	potassium chloride
kDa	kilot Dalton
KH	K homology
KOAc	potassium acetate
L	liter
Leu	leucine
LHP1	LIKE HETEROCHROMATIN PROTEIN1
LIF2	LHP1 ±nteracting Factor 2
LiCl	lithium chloride
M	molar
M9	M9 nucleocytoplasmic export signal
MBE	Musashi binding element
mg	milligram
MgCl2	magnesium chloride
MgSO4	magnesium sulphate
min	minutes
miRNA	microribonucleic acid
mL	millilitre
/	microlitre
mm	millimeter
mM	millimolar
MOPS	3-(N-morpholino) propane-sulphonic acid
mRNA	messenger ribonucleic acid
mRN	messenger ribonucleoprotein
NaC	sodium chloride
NaO c	sodium acetate
NaOH	sodium hydroxide
NBT	nitro blue tetrazolium
NIB	nuclear isolation buffer
ng	nanogram
N-terminal	amino terminal
NTFs	nuclear transport factors
NPCs	nuclear pore complexes
npcRNA	non-protein coding ribonucleic acid
OD	optical density
ORF	open reading frame
P bodies	processing bodies
PABP	polyadenylation-binding protein

PAGE	polyacrylamide gel electrophoresis
PABPII	polyadenylation binding protein II
PAP	polyadenylation polymerase
PBS	phosphate buffer saline
PCR	polymerase chain reaction
pI	isoelectric point
PMSF	phenylmethyl-sufonyl fluoride
poly (A)	polyadenylation
pre-mRNA	precursor messenger ribonucleic acid
PTB	polypyrimidine-tract binding
PVDF	polyvinylidene difluoride
PVPP	polypolyvinylpyrrolidone
qRT-PCR	quantitative real-time RT-PCR
RBD	RNA-binding domain
RBP	RNA-binding protein
RGG	arginine-glycine-glycine
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
RNAPII	RNA polymerase II
RNase	ribonuclease
RNP	ribonucleoprotein
rpm	revolutions per minute
RRM	RNA recognition motif
RT	reverse transcriptase
sec	second
SD	synthetic dropout
SDS	sodium dodecyl sulphate
snRNP	small ribonucleoprotein particles
SR	serine/arginine rich
TAE	tris acetate EDTA
TCA	trichloro-acetic acid
TE	tris-EDTA
TEMED	N,N,N',N'-tetrametylethylenediamine
Tris	tris[hydroxymethyl]aminomethane
Tris-HCl	tris[hydroxymethyl]aminomethane hydrochloride
Trp	tryptophan
U	unit
UTR	untranslated region
µl	microliter
µg	microgram
µm	micrometer
v/v	volume per volume
WAP	weeks after pollination
w/v	weight per volume
x g	relative centrifugal force
X-gal	5-bromo-4-chloro-3-indolyl- -D-galactopyronoside
X- .-Gal	5-bromo-4-chloro-3-indolyl alpha-D-galactopyranoside

CHAPTER 1

INTRODUCTION

Gene expression during growth and development is governed by both transcription and post-transcription regulation of mRNAs. Transcription regulation affects the expression of genes. However, the discordance between the mRNA and protein levels in eukaryotes is mainly due to post-transcriptional processing and regulation. This regulation can be achieved either directly by RNA binding proteins (RBPs) or indirectly via modulation of other regulatory factors in eukaryotes (Lorkovic, 2009). Gene encoding RBPs with RNA recognition motifs (RRMs) have received more attention in plant research recently. These RBPs are emerging as multifunctional cellular regulatory proteins involved in RNA metabolism including the regulation of transcriptional processes such as RNA synthesis, pre-mRNA splicing, capping, polyadenylation, exporting RNA from nucleus, pre-rRNA complex formation, mRNA stability and degradation. Besides that, RBPs participate in all aspects of translational processes whereby they regulate translation of functional mRNAs and storage of non-translated mRNAs. Some RBPs are also involved in chromosome structuring such as telomere maintenance that is important for chromosome stability and integrity (Chen and Varani, 2005; Glisovic *et al.*, 2008).

Functional studies and RNA sequencing of plant RBPs clearly showed that a family of RBPs that are defined as heterogeneous nuclear ribonucleoprotein (hnRNP)-like proteins are also expressed in higher plants and serve specific plant functions (Lambermon *et al*., 2000; Lorkovic *et al*., 2000). In plants, these hnRNP-like proteins have been reported to influence floral induction and development, circadian rhythms, hormone signaling, differentiation, chloroplast regulation, phloem translocation, stress response and stress tolerance (Nakamura *et al*., 1999; Li *et al*., 2002; Macknight *et al*., 2002; Quesada *et al*., 2003; Simpson *et al*., 2003; Razem *et al*., 2006; Staiger *et al*., 2003; Ham *et al*., 2009; Tillich *et al*., 2009). Post-transcriptional gene regulation plays a crucial role in the response of plants towards stress stimulus. Regulatory factors such as small RNAs and RBPs have emerged as regulators of RNA mechanism in all aspects of post-transcriptional gene regulation in plant stress responses, tolerance and adaptation. RBPs respond to stress signals through the regulation of downstream stress-related genes expression and enhance tolerance of plants towards various abiotic adversities (Lee *et al.,* 2009).

Oil palm (*Elaeis guineensis* Jacq.) is the most efficient oil-yielding crop in the world. Oil palm produces 32% of global oils and fats and utilizes the least global land area for cultivation (5.5%) compared to other oilseed crops (Oil world 2013). One hectare of oil palm plantation produces up to ten times more oil (average oil yield of ~4 tonnes of oil per hectare) than other oilseed crops (Oil world 2013). Palm oil is the most consumed oil among 17 major oils and fats traded globally

and the global consumption for palm oil was 52.1 million tonnes in 2012 (Oil world 2013). The palm oil industry is one of the key economic drivers of the agricultural sector in Malaysia. Malaysia is the second largest palm oil producer utilizing 5.1 million hectares of land for oil palm cultivation, and accounted for 10% or 18.8 million tonnes of global vegetable oils and fats output in 2012 (Oil world 2013).

Environmental stresses have a major impact on oil palm production mainly plant growth, physiology and oil yield. Environmental stresses such as flood, drought, cold and heat stress have been reported to affect abortion of inflorescence, determination of inflorescence ratio and bunch ripening in matured oil palm (Corley and Donough, 1995; Gawankar *et al.*, 2003; Cha-Um *et al.*, 2010). Intermittent water stress (rainfed) reduces 91% of oil palm fresh fruit bunch production resulting in 88.46% reduction in the yield of fresh fruit bunches (Gawankar *et al.*, 2003). Under intermittent water stress, oil palm female inflorescence production is reduced by 86% while leaf production is reduced by 30% in the early growth phase and 12.5% in the later growth phase (Gawankar *et al.*, 2003).

The biological functions of RBPs in post-transcription regulation of gene expression in response to stresses are still poorly understood in oil palm. Hence, an in-depth functional analysis of RBPs mediated regulation of target RNA metabolisms will increase the understanding of the roles of RBPs in oil palm stress responses and adaptation that are vital for the engineering of stress-tolerant plant. The aim of this research was to understand the functional role of oil palm RBP and the network of gene regulation operating at the post-transcriptional level in response to environmental stimuli in oil palm. Through the study of messenger ribonucleoproteins (mRNPs) and the constituents of RNP complexes, networks of gene regulation and the underlying mechanism operating at the post-transcriptional level in response to environmental stimuli in oil palm can be elucidated.

The specific objectives of this study were:

1. To clone and isolate full length cDNA, variants, promoter and the gene encoding putative RBP (*EgRBP42*) from *Elaeis guineensis* Jacq.
2. To identify transcripts interacting with the EgRBP42 protein and their consensus binding site.
3. To identify the regulatory factors associated with EgRBP42 in the ribonucleoprotein complex and the functional roles of EgRBP42 in post-transcriptional regulation.
4. To profile the protein accumulation and transcript abundance of *EgRBP42* and its variants under various abiotic stress conditions in oil palm.

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