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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES RELATED TO HEIGHT INCREMENT IN OIL PALM (Elaeis guineensis Jacq.)

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June 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the degree of Master of Science

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Chair : Assoc. Prof. Parameswari A/P Namasivayam - PhD
Faculty : Biotechnology and Biomolecular Sciences

The effort towards developing dwarf palm population with novel traits has great importance to the oil palm industry, mainly due to the high cost of harvesting fruits from tall palms and crop improvements. Reducing palm height not only brings positive effect on harvesting cost, it will significantly extent the economic cropping cycle. Through the advancement in molecular technologies, identification of potential candidate genes that regulate in dwarfism can be achieved. In this study, six subtracted cDNA libraries were constructed by the Suppression Subtractive Hybridization (SSH) method using spear leaf tissue samples from MPOB Planting Series 1 (PS1) and FELDA P.P.P. Tun Razak (BACKCROSS, AG1) breeding lines. A total of 973 sequences (forward and reverse) were generated from six subtracted libraries. The similarity searches using BLASTX revealed that six clones were identified to be involved in dwarfism based on its putative functions. The gene transcripts encoding for: brassinosteroid biosynthesis-like protein (DWF1), BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative (BRI1), late elongated hypocotyl protein (LHY), gibberellin receptor GID1, putative (GID1), sterol 24-methyltransferase 1 (SMT1) and E3 ubiquitin-protein ligase MARCH6 (E3Ub). These candidate dwarfing genes were reported to be involved in various stages of brassinosteroids (BRs) and gibberellins (GAs) biosynthesis and signaling pathways for plants growth and development. BRs are plant steroids that present in vegetative tissues such as shoot, leaves and stems; pollen grains, anthers and seeds. BRs control diverse physiological processes including cell division and elongation, embryogenesis, fertility, delayed senescence and vascular differentiation. GAs stimulate critical stages in plant growth and development such as plant height, cell wall modification, seed germination, flowering and leaf expansion. Gene validation analysis via qRT-PCR has revealed the expression levels of each potential candidate dwarfing genes in all tested samples, normalized by two most stable reference genes, manganese superoxide dismutase-like protein (PD569) and hypothetical protein (EA1332). Based on the analysis, higher expression level of BRI1, LHY and SMT1 genes were observed in dwarf palms compared to standard palms with normalized fold-difference of 2.3285, 1.5620 and 4.9044, respectively. However, lower expression of DWF1, GID1 and

E3Ub were observed in dwarf palms compared to standard palms with normalized fold-difference of 0.8378, 0.7003 and 0.9631, respectively. Statistical analysis using Paired Samples T-Test showed that the expression levels of DWF1, BRI1, LHY, GID1 and E3Ub were not significantly expressed in dwarf palms. However, the SMT1 expression level was highly significant in dwarf palm, AG1-22. The expression profile of SMT1 in all tested samples was carried out using AG1-22 as the control baseline (1.0000 expression levels), where the GOI expression level below 1.0000 indicates as down-regulated; and above 1.0000 is up-regulated. The result showed that the dwarf palm, AG1-12 was up-regulated with 1.3161 expression value. Therefore, SMT1 gene may be potentially useful molecular marker for the screening of dwarf palm planting materials.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGENALPASTIAN GEN EKSPRESI TERBEZA BERKAITAN PENINGKATAN KETINGGIAN KELAPA SAWIT

Oleh

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Usaha ke arah pembiakan dan pemilihan baka sawit yang mengandungi ciri-ciri komersial mempunyai kepentingan yang besar kepada industri kelapa sawit di Malaysia. Antara objektif utama program pembiakbakaan adalah untuk mengurangkan kadar ketinggian pokok disebabkan oleh kos penuaian buah kelapa sawit yang tinggi bagi pokok kelapa sawit yang tinggi. Pembiakan benih sawit kerdil membawa kesan penting dalam pengurangan kos penuaian serta memperluaskan keupayaan penanaman. Melalui kaedah-kaedah penyelidikan berasaskan teknologi molekul yang terkini, pemilihan calon bagi gen-gen yang berpotensi untuk menghasilkan pokok sawit kerdil dan bermutu tinggi dapat dikenalpasti. Berdasarkan daripada hasil penyelidikan ini, sebanyak enam kelompok cDNA tersubtraksi telah dihasilkan melalui kaedah hibridisasi subtraksi penindasan (SSH) dengan menggunakan sampel-sampel daun muda pokok kelapa sawit kerdil dan standard daripada populasi MPOB Planting Series 1 (PS1) and FELDA P.P.P. Tun Razak (BACKCROSS, AG1). Sebanyak 973 jujukan DNA (dari kedua-dua hala) terhasil daripada enam kelompok cDNA tersubtraksi tersebut. Berdasarkan analysis jujukan melalui BLASTX, enam calon gen yang mengkodkan: brassinosteroid biosynthesis-like protein (DWF1), BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative (BRI1), late elongated hypocotyl protein (LHY), gibberellin receptor GID1, putative (GID1), sterol 24-methyltransferase 1 (SMT1) dan E3 ubiquitin-protein ligase MARCH6 (E3Ub) yang diekspres terbeza dalam pokok sawit kerdil telah dikenalpasti berkait dengan pembentukan ciri-ciri kerdil berdasarkan persamaan yang signifikan dengan jujukan dalam pengkalan data GeneBank. Gen-gen ini telah dilaporkan terlibat di dalam proses-proses penghasilan hormon seperti brassinosteroids (BRs) dan gibberellins (GAs) bagi meningkatkan pertumbuhan dan perkembangan tumbuh-tumbuhan. BRs ialah steroid tumbuhan yang terdapat pada tisu-tisu vegetatif seperti pucuk, daun dan batang; butir debunga, cepu debunga dan biji benih. BRs mengawal pelbagai proses psikologi tumbuhan termasuk pembahagian sel dan pemanjangan, embriogenesis, kesuburan, penuaan lewat dan pembezaan vaskular. GAs merangsang beberapa tahap kritikal pada pertumbuhan pokok dan pembangunan seperti ketinggian pokok, pengubahsuaian dinding sel, percambahan biji benih, pendebungaan dan pengembangan daun. Analisa pengesahan gen melalui kaedah PCR nyata-masa telah berjaya mengesan

calon-calon gen kerdil di dalam setiap sampel pokok sawit kerdil dan standard berdasarkan dua gen rujukan paling stabil iaitu manganese superoxide dismutase-like protein (PD569) dan hipotetikal protein (EA1332). Berdasarkan analisis, tahap ekspresi BRI1, LHY dan SMT1 adalah lebih tinggi pada pokok-pokok sawit kerdil berbanding dengan pokok-pokok sawit standard dengan perbezaan lipatan ternormal sebanyak 2.3285, 1.5620 dan 4.9044, masing-masing. Walaubagaimanapun, tahap ekspresi DWF1, GID1 and E3Ub adalah lebih rendah pada pokok-pokok sawit kerdil berbanding dengan pokok-pokok sawit standard dengan perbezaan lipatan ternormal sebanyak 0.8378, 0.7003 dan 0.9631, masing-masing. Seterusnya, ujian statistik menggunakan kaedah sampel berpasangan menunjukkan bahawa tahap ekspresi SMT1 adalah signifikan pada pokok sawit kerdil, manakala tahap ekspresi DWF1, BRI1, LHY, GID1 dan E3Ub adalah tidak signifikan. Walaubagaimanapun, tahap ekspresi SMT1 adalah signifikan pada pokok sawit kerdil, AG1-22. Profil expresi SMT1 pada kesemua sampel yang diuji telah dijalankan dengan menggunakan AG1-22 sebagai garis tapak kawalan (1.0000 tahap ekspresi), di mana tahap ekspresi calon-calon gen di bawah 1.0000 menunjukkan ‘down-regulated’; dan di atas 1.0000 ‘up-regulated’. Keputusan menunjukkan bahawa pokok sawit kerdil, AG1-12 adalah ‘up-regulated’ dengan nilai tahap expresi 1.3161. Oleh itu, SMT1 berpotensi untuk digunakan sebagai antara penanda molekul bagi pemilihan baka tanaman sawit kerdil.

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

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LIST OF ABBREVIATIONS

Δ	delta
∞	inifinity
β-ME	2-Mercaptoethanol
A. thaliana	*Arabidopsis thaliana*
A	Absorbance ratio
ABA	abscisic acid
ACC	aminocyclopropane-1-carboxylic acid
ACTIN	Actin-AY550991
AGL15	MADS domain protein
AMV	Avian Myeloblastosis Virus
AVROS	Algemene Vereniging van Rubberplanters ter Oostkust van Sumatera
BAK1	BRI1-Associated Receptor kinase
BL	brassinolide
BLAST	Basic Local Alignment Search Tool
bls1	brassinosteroid light and sugar1
bnaC.dwf	brassica napus dwarf mutant
bp	base pair
BP	before present
BRI1	brassinosteroid-insensitive1
BR	brassinosteroids
Ca	calcium
cbb1	cabbage1
CCC	chlormequat chloride
cDNA	complementary DNA
CIGR	chitin-inducible gibberellin-responsive protein
CO_2	carbon dioxide
cpd	constitutive photomorphogenesis and dwarfism
CR	campesterol
C_t	threshold cycle
CTAB	hexadecyl trimethyl-ammonium bromide
D x P	*Dura* x *Pisifera*
DDF	Dwarf and Delayed Flowering
DEPC	diethyl pyrocarbonate
det2	de-etiolated2
dim	diminuto
DNase 1	deoxyribonuclease 1
ds	double-stranded
dNTP	deoxynucleotide triphosphate
dwf	dwarf
DWF1	brassinosteroid biosynthesis-like protein
E.guineensis	*Elaeis guineensis*
E.odora	*Elaeis odora*
E.oleifera	*Elaeis oleifera*
E	Pfaffl efficiency
E-value	expectation value
EA1332	hypothetical protein
EDTA	ethylenediaminetetra acetic acid
EMBOSS	European Molecular Biology Open Software Suite

EtBr	ethidium bromide
FFB	fresh fruit bunch
g	relative centrifugal force
ga	GA biosynthesis mutant
GA	gibberellin
GC	guanine-cytosine
GGPP	geranylgeranyl diphosphate
GID1	Gibberellin-insensitive dwarf 1
GO	Gene Ontology
GOI	gene of interest
gsd1-1D	GA-insensitive dwarf1-1D
GUS	beta-glucuronidase
HK	reference gene
HPLC	High Performance Liquid Chromatography
H_2O_2	hydrogen peroxide
htd	high-tillering and dwarf
HvBAK1	Brassinosteroid Insensitive1-Associated Kinase1 of barley
IPTG	isopropyl β-D-1-thiogalactopyranoside
K	potassium
LB	Luria Bertani
LHY	late elongated hypocotyl
LiCl	lithium chloride
lka	brassinosteroid insensitive 1 homolog of pea
M	gene expression stability measure
Mg	magnesium
MPOB	Malaysian Palm Oil Board
mRNA	messenger RNA
N	nitrogen
NaAc	sodium acetate
NCBI	National Center for Biotechnology Information
NF	Normalization factor
nr	non-redundant
NRT	non-reverse transcription
NTC	non-template control
NTH15	KNOX homeodomain protein
ORFs	Open Reading Frames
P	phosphorus
PCR	Polymerase Chain Reaction
PD380	ribosomal protein S27-like protein
PD569	manganese superoxide dismutase-like protein
ph1	plant height 1
PNO8	N-octyl-3-nitro-2,4,6-trihydroxybenzamide
PS1	Planting Series 1
qRT-PCR	quantitative Real-Time PCR
Rht	reduced height gene
RIN	RNA Integrity Number
RNA	Ribonucleic acid
RNAi	RNA interference
RNase	ribonuclease
rRNA	ribosomal RNA

RSG	repression of shoot growth
RT	reverse transcription
S	sulfur
sd1	semi-dwarf 1
SDS	sodium dodecyl sulfate
sdw1	semi-dwarfing 1
SERK3	Somatic Embryogenesis Receptor Kinase3
SLY1	SLEEPY1
SMT1	sterol 24-C-methyltransferase 1
SSH	Suppression Subtractive Hybridization
ste	steroid
STR	stigmasterol
T_a	annealing temperature
T_m	melting temperature
Tris-HCl	Tris hydrochloride
U	units
UBIQUITIN	Polyubiquitin-EL689143.1-TransContig
USDA	United States Department of Agriculture
UV	ultraviolet
X-Gal	5-bromo-4-chloro-indolyl-β-D-galactopyranoside

CHAPTER 1

INTRODUCTION

Oil Palm (*Elaeis guineensis* Jacq.) is currently the most important oil crop in regards to its future potential to be the world's most versatile vegetable crop. In 2010, oil palm has turned out to be the highest oil-yielding crop compared to other oil-bearing crops such as soybean, cottonseed, groundnut, sunflower, rapeseed, corn, coconut, safflower, olive, castor, sesame and linseed seeds (Ramli, 2011). The annual production of oil palm has significantly increased from 1.26 million tonnes in the early 1960s to 45.59 million tonnes in 2010, with Malaysia and Indonesia as the major exporters (Oil World, 2010). Therefore, it is important to foster the increased production of oil palm at a much higher rate, as the population of much of the developing countries is continuously growing, and ultimately increase the rate of dietary fats and oils consumption.

In Malaysia, oil palm is currently the most important commodity apart from rubber. The effort to increase the oil palm production has become one of the most important agenda in the National Key Economic Area (NKEA) under the Economic Transformation Programme (ETP) due to its potential to increase the Malaysian Gross Net Income (GNI) of RM230.9 billion by 2020 (ETP Annual Report, 2013).

Many strategic plans have been carried out to improve the oil palm productivity such as accelerating the re-planting and new planting of the oil palm, improving fresh fruit bunch yield, improving worker productivity, increasing the oil extraction rate, and developing biogas facilities at palm oil mills. In order to achieve these, the production of dwarf oil palm varieties with novel traits could be the starting point. Establishing dwarf palm population will significantly bring many positive effects to the industry in the future.

Dwarfism is a desirable trait for many agricultural plants such as wheat, rice, barley and maize, mainly to mechanize harvesting, reduce lodging, increase resistance to wind and rain, and increase the harvesting index (Itoh et al., 2004; Muangprom et al., 2005; Zou et al., 2005; Kovi et al., 2011). In oil palm, the effort towards developing dwarf palm population has been of great interest to researchers and oil palm breeders. The major reason is because fruits from tall palms are difficult to harvest. The un-harvested fruit clusters will detach and scatter on the ground, yielding fruits with less oil and poor in quality (Ebongue et al., 2008). This phenomenon resulted in declining overall oil palm yield and productions. Heavy labour cost of picking up loose fruit is also important to consider since the harvesters spend more time on collecting loose fruit than cutting down bunches (Gan et al., 1995). Apart from that, harvesting tall palm trees require expert foreign workers which will not be so readily available due to their temporary employment visit pass status. As a result, plantations suffer from a great loss of labour which will simultaneously affect crop productivity (Zulnasri, 2010). The costs for hiring new intake of estate labourers are much higher as the palm breeder needs to bear the costs for permit renewal, levy, training, accommodation and medical fee.

Dwarf palms are much easier to harvest and maintain. Reducing the height increment in palm trees in future will deliberately bring positive effect on harvesting cost and significantly extend the economic cropping cycle. Previous studies have proved that dwarf varieties were highly associated in yields, higher fertility, early maturity and high tillering capacity (Hedden, 2003 and Khush, 2001). Another reason of breeding palms with low height increment is to increase resistance to rain and strong winds (Corley and Tinker, 2003). To date, many efforts have been made to develop dwarf palms with superior genetic varieties. For example, the Malaysian Palm Oil Board (MPOB) has conducted a series of selections and breeding trials by utilizing seeds from different producers and origins (Corley and Tinker, 2003). The Nigerian selections are the most valuable breeding materials due to its low annual height increment characteristics (Rajanaidu and Jalani, 1994). The MPOB Planting series 1 (PS1) population used in this study was developed from crossing of Nigerian *duras* and AVROS *pisiferas*, having specific traits of interest i.e. high oil-yield and low height increment (Sharma, 1999).

To date, there is no molecular study have been established to classify genes associated with dwarfism in oil palm. The Suppression Subtractive Hybridization (SSH) procedure developed by GIAGEN, USA has provided a new strategy for the isolation of differentially expressed genes with higher successful rate compared to other method such as northern blotting and DNA fingerprinting. Identified genes associated in height will be cloned and sequenced to establish their expression profiles, and further validated using the quantitative Real-Time PCR (qRT-PCR). In future, the molecular markers for height traits isolated from this study can be utilized to screen out the oil palm seedlings to predict dwarf population at the early stage. These hybrid progenies that possess slow yearly height traits will subsequently reduce the overall production costs, increase crops productivity, ease of harvesting, and improve the quality and quantity of oil palm.

The objectives of this study were:

1. To identify differentially expressed genes that regulate dwarfism in oil palm using the SSH approach.
2. To perform gene annotation with available transcriptome sequence database.
3. To validate the differentially expressed genes using qRT-PCR and statistical analysis.

In future, these candidate genes can be used as potential molecular markers for screening of height traits in oil palm, and coordinate the differentially expressed genes candidate with oil palm genome sequence database.

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