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FUNCTIONAL CHARACTERIZATION OF GIBBERELLIC ACID RELATED GENES FROM OIL PALM IN Arabidopsis thaliana FOR POTENTIAL ROLE HEIGHT REGULATION

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By

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

FUNCTIONAL CHARACTERIZATION OF GIBBERELLIC ACID RELATED GENES FROM OIL PALM IN *Arabidopsis thaliana* FOR POTENTIAL ROLE IN HEIGHT REGULATION

By

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Chairman : Noor Azmi Shaharuddin, PhD
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Oil palm is the most important commodity crop in Malaysia with a total planted area of 5.74 million hectares. In general, the economic life of the oil palm is associated with the stature of the tree. At maturity, oil palm trees commonly reach over 15 meters in height making harvesting a challenge as fruit bunches may weigh over 20 kg and become damage as the bunches fall to the ground hence reduce the quality of fresh fruit bunch (FFB). Height regulation in plant is commonly associated with gibberellic acid (GA), therefore study of genes related to GA biosynthesis and signaling will improve our understanding on height regulation mechanism in oil palm. Thus, the main objective of the research was to isolate GA-related genes from the oil palm (*Elaeis guineensis*) and characterize their functions in order to study height regulation in oil palm. Due to oil palm long life cycle, it is difficult to study the gene function *in vivo,* therefore functional characterization of the genes was conducted in heterologous system using model plant *Arabidopsis thaliana*. Three GA-related genes, *EgGA20ox*, *EgGA2ox* and *EgGAI* were isolated from leaf tissue of clonal oil palm treated with paclobutrazol (GA inhibitor). *EgGA20ox* and *EgGA2ox* genes expression were affected by paclobutrazol treatment whereas *EgGAI* gene was not affected by the paclobutrazol application. *EgGA20ox* gene was constitutively expressed in most tissues tested except for kernel. *EgGA2ox* gene was highly expressed in young root of the oil palm. On the other hand, *EgGAI* gene expression was presence in most tissues except for root and kernel. Prior to genes functional study, six expression constructs were generated consisting of three overexpression (pH2OE-*EgGA20ox*, pH2OE-*EgGA2ox*, pH2OE-*EgGAI*) and three RNAi (pH7RNAi-*EgGA20ox*, pH7RNAi-*EgGA2ox*, pH7RNAi-*EgGAI*) constructs. The constructs were transformed into *Arabidopsis* via

Agrobacterium-mediated transformation using floral dip method. Phenotypic characterization analysis of the transgenic *Arabidopsis* showed that *EgGA20ox* gene promotes vegetative and reproductive growth. Up regulation of *EgGA20ox* gene increased the height of transgenic *Arabidopsis* and length of leaf, root and silique. Flower formation of this line was also improved. Down regulated of *EgGA20ox* gene reduced the height of *Arabidopsis* and the length of leaf, root and silique. In addition, less flower formation was observed. On the contrary, up regulation of *EgGA2ox* gene reduced *Arabidopsis* height, increased leaf length and delayed in flowering. It was found that there was no effect in terms of flower formation, root and silique. Down regulation of *EgGA2ox* gene generated taller transgenic *Arabidopsis,* increased root and leaf length, early flowering but produced normal flower and silique formation. Transgenic *Arabidopsis* lines carrying oil palm GA-Insensitive (*EgGAI*) gene were also affecting the vegetative and reproductive growth. Overexpressed of *EgGAI* gene in transgenic *Arabidopsis* resulted in shorter plant and reduced root and leaf length. Delayed in flowering was also observed however there was no effect on flower and silique formations. Down regulation of *EgGAI* gene increased the transgenic *Arabidopsis* height but reduced the root and leaf length. In terms of reproductive growth, fewer flowers were generated but silique length remain similar to control plant. Based on our findings, *EgGA20ox*, *EgGA2ox* and *EgGAI* genes may play an important role in the plant growth and development. This study has shown that *Arabidopsis* can be utilized for gene functional studies especially genes involve in oil palm height regulation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENCIRIAN FUNGSI GEN BERKAITAN ASID GIBERELIK DARIPADA SAWIT DALAM *Arabidopsis thaliana* UNTUK PENGATURAN KETINGGIAN SAWIT

Oleh

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Sawit merupakan tanaman komoditi penting di Malaysia yang meliputi kawasan seluas 5.74 juta hektar. Umumnya, jangka hayat ekonomi sawit berkait rapat dengan ketinggian pokok. Sawit yang matang mampu mencapai ketinggian lebih daripada 15 meter menyebabkan proses penuaian mencabar kerana berat buah sawit boleh mencecah 20 kg dan rosak apabila jatuh ke tanah seterusnya mengurangkan kualiti buah sawit. Pengaturan ketinggian pokok sering dikaitkan dengan asid giberelik (AG), oleh hal yang demikian kajian gen berkaitan biosintesis dan pengisyaratan AG akan meningkatkan kefahaman terhadap mekanisme pengaturan ketinggian sawit. Sehubungan dengan itu, kajian ini dijalankan untuk mengkaji gen yang berkaitan dengan pengaturan ketinggian sawit. Tujuan utama penyelidikan ini adalah untuk memencilkan gen berkaitan AG daripada sawit (*Elaeis guineensis*) dan mencirikan fungsinya. Disebabkan hayat sawit yang panjang, kajian tentang fungsi gen secara *in vivo* adalah sukar, oleh hal yang demikian, pencirian fungsi gen telah dijalankan dalam sistem heterologus menggunakan pokok model *Arabidopsis thaliana*. Tiga gen berkaitan AG, *EgGA20ox*, *EgGA2ox* dan *EgGAI* telah dipencilkan daripada genom sawit. Gen tersebut dipencilkan daripada tisu daun yang dirawat paclobutrazol (perencat AG). Ekspresi gen *EgGA20ox* dan *EgGA2ox* dipengaruhi oleh rawatan paclobutrazol. Namun, ekspresi gen *EgGAI* tidak dipengaruhi oleh kehadiran paclobutrazol. Gen *EgGA20ox* diekspres dalam hampir kesemua tisu yang diuji kecuali tisu isirung. Gen *EgGA2ox* diekspres tinggi dalam tisu akar muda sawit. Manakala, ekspresi gen *EgGAI* ditemui dalam hampir kesemua tisu kecuali tisu akar dan isirung. Lanjutan dari itu, enam konstruk ekspresi telah dihasilkan mengandungi tiga konstruk ekspresi melampau (pH2OE-*EgGA20ox*, pH2OE-*EgGA2ox*, pH2OE-*EgGAI*) dan tiga

konstruk RNAi (pH7RNAi-*EgGA20ox*, pH7RNAi-*EgGA2ox*, pH7RNAi-*EgGAI*). Konstruk tersebut ditransform dalam *Arabidopsis* melalui perantaraan *Agrobacterium* menggunakan kaedah rendaman bunga. Analisis pencirian fenotip pokok *Arabidopsis* transgenik menunjukkan gen *EgGA20ox* menggalakkan pertumbuhan vegetatif dan reproduktif. Pengawalaturan tinggi gen *EgGA20ox* menambah ketinggian *Arabidopsis* transgenik dan kepanjangan daun, akar dan silikua. Perkembangan bunga juga dipertingkatkan. Pengawalaturan rendah *EgGA20ox* mengurangkan ketinggian *Arabidopsis* dan kepanjangan daun, akar dan silikua. Tambahan pula, pertumbuhan bunga berkurangan. Berbeza dengan *EgGA2ox*, pengawalaturan tinggi merendahkan *Arabidopsis,* memanjangkan daun dan melewatkan pengeluaran bunga. Namun demikian, tidak mempengaruhi perkembangan bunga, akar dan silikua. Pengawalaturan rendah gen *EgGA2ox* menghasilkan *Arabidopsis* transgenik yang tinggi, memanjangkan akar dan daun, mempercepatkan pembungaan namun menghasilkan bunga dan silikua yang normal. *Arabidopsis* transgenik melibatkan gen *EgGAI* mempengaruhi perkembangan vegetatif dan reproduktif. Pengawalaturan tinggi gen *EgGAI* merendahkan *Arabidopsis* transgenik dan mengurangkan kepanjangan akar dan daun. Pembungaan menjadi lewat namun tiada perubahan terhadap perkembangan bunga dan silikua. Pengawalaturan rendah gen *EgGAI* menambah ketinggian *Arabidopsis* transgenik tetapi mengurangkan kepanjangan akar dan daun. Tambahan pula, penghasilan bunga berkurang namun kepanjangan silikua adalah sama seperti pokok kawalan. Sebagai rumusan, gen *EgGA20ox*, *EgGA2ox* dan *EgGAI* berperanan penting dalam pertumbuhan dan perkembangan pokok. Kajian ini menunjukkan bahawa *Arabidopsis* boleh diolah untuk kajian fungsi gen terutama gen yang terlibat dalam pengaturan ketinggian sawit.

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

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°C	degree Celsius
L	litre
µl	microlitre
ml	millilitre
mg	milligram
cm	centimetre
mm	millimetre
kg	kilogram
WAT	Week after treatment
FFB	Fresh fruit bunches
PBZ	Paclobutrazol
PCR	Polymerase chain reaction
GA	Gibbellin
ox	oxidase
IPP	Isopentenyl diphosphate
GGPP	Geranylgeranyl pyrophosphate
CaMV	Cauliflower mosaic virus
CPP	Copalyl diphosphate
CPS	*ent*-copalyl diphosphate synthase
KS	*ent*-kaurene synthase
g	gravity
bp	Base pair
kb	Kilo base
cDNA	Complementary DNA
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
dNTP	Deoxyribonucleiotide triphosphate
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcription - polymerase chain reaction
qPCR	Real-time polymerase chain reaction
rpm	Rotatation per minute
EB	Elution buffer
TAE	Tris-acetate-EDTA
MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Council

CHAPTER 1

INTRODUCTION

1.1 Research background

Palm oil is the most traded oil in the world. The demand of the palm oil from the world's oils and fats market has been steadily increasing over the years (MPOB, 2017). Malaysia as one of the palm oil producing countries accounting for over one third of the total palm oil production in world export trade. The oil palm planted area across the nation covers almost 5.74 million hectares in 2016 (MPOB, 2017).

Despite vast plantation of the oil palm, the industry in Malaysia is facing the falling of fresh fruit bunches (FFB) productivity. The decrease of the yield and productivity are partly determined by the height of the oil palm tree. Management of tall oil palm tree is costly and will potentially damage the quality of the fruit upon harvesting. Current oil palm planting materials increase at the rate of 40-75 cm/year (Kushairi *et al*., 1999). The palms will be too tall thus replanting programs after 25 to 30 years is required.

Plant height is often associated with gibberellic acid (GA). GA is one of the phytohormones that regulates many vital plant growth and developmental processes which includes seed germination, leaf expansion, induction of flowering and plant height (Yamaguchi, 2008). Previous studies showed that regulating gibberellins biosynthesis and signaling altered the height of many plants species (Li *et al*., 2016; Liang *et al.*, 2014). Therefore, regulating plant height especially in oil palm by manipulating the gibberellins biosynthesis and signaling can be significant.

1.2 Problem Statements and Objectives

Numerous efforts have been done to study the height regulation in many plant species. Previous studies reported that manipulating GA-related genes affected the stature of various species of plant such as *Triticum aestivum* (Pearce *et al.,* 2015), *Oryza sativa* (Gebre *et al.*, 2013), *Solanum lycopersicum* (Chen *et al*., 2016) and *Panicum virgatum* (Wuddineh *et al*., 2015). Genes that are involved in height regulation in GA biosynthesis and signaling from the oil palm are yet to be revealed. Comprehensive researches which are related to the

characterization of the genes functions associated with GA is important to improve our understanding on height regulation mechanism in oil palm.

In planta characterization of genes function is often limited by time and cost constraints. Therefore, characterization of genes functions using heterologous system, *Arabidopsis thaliana* was opted to observe and evaluate the functions of the genes. The model plant *Arabidopsis* provides a convenient *in vivo* system for performing functional analysis of genes as better approach for cost and time efficiencies (Zubaidah *et al*., 2017).

Thus, this research was conducted to study genes that are related to height regulation in oil palm. The main objective of the research was to isolate GA-related genes from the oil palm (*Elaeis guineensis*) and characterize their functions.

Therefore, the objectives of this study are:

1. To study the effects of PBZ on the growth pattern of clonal oil palm physiologically and morphologically.
2. To isolate and perform *in-silico* characterization of gibberellic acids related genes from clonal oil palm.
3. To profile the expression of gibberellic acids related genes in different oil palm tissues using RT-PCR and PBZ treated leaf tissue using qPCR.
4. To generate transgenic *Arabidopsis* lines (overexpression and RNAi) carrying GA-related genes and perform functional charaterization analysis.

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