UNIVERSITI PUTRA MALAYSIA

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

MUHAMAD AFIQ BIN ABDUL HALIM

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By

MUHAMAD AFIQ BIN ABDUL HALIM

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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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December 2017

Chairman : Noor Azmi Shaharuddin, PhD
Faculty : Biotechnology and Biomolecular Sciences

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 damaged 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 regulation 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.
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 dari 15 meter menyebabkan proses penuaan mencabar kerana berat buah sawit boleh mencapai 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 penelitian 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 dan dipengaruhi oleh rawatan paclobutrazol. Namun, ekspresi gen EgGAI tidak dipengaruhi oleh kehadiran paclobutrazol. Gen EgGA20ox dan EgGA2ox diuji dalam hampir kesemua tisu kecuali tisu isirung. Gen EgGA2ox diekspresi tinggi dalam tisu akar muda sawit. Manakala, ekspresi gen EgGAI ditemui dalam hampir kesemua tisu kecuali tisu akar dan isirung. Lanjutan dari itu, enam konstruktur ekspresi telah dihasilkan mengandungi tiga konstruktur ekspresi melampau (pH2OE-EgGA20ox, pH2OE-EgGA2ox, pH2OE-EgGAI) dan tiga
konstruk RNAi (pH7RNAi-EgGA20ox, pH7RNAi-EgGA2ox, pH7RNAi-EgGAI). Konstruktur 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. Tambahana 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. Tambahana 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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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>µl</td>
<td>microlitre</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>WAT</td>
<td>Week after treatment</td>
</tr>
<tr>
<td>FFB</td>
<td>Fresh fruit bunches</td>
</tr>
<tr>
<td>PBZ</td>
<td>Paclobutrazol</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>GA</td>
<td>Gibbollin</td>
</tr>
<tr>
<td>ox</td>
<td>oxidase</td>
</tr>
<tr>
<td>IPP</td>
<td>Isopentenyl diphosphate</td>
</tr>
<tr>
<td>GGPP</td>
<td>Geranylgeranyl pyrophosphate</td>
</tr>
<tr>
<td>CaMV</td>
<td>Cauliflower mosaic virus</td>
</tr>
<tr>
<td>CPP</td>
<td>Copalyl diphosphate</td>
</tr>
<tr>
<td>CPS</td>
<td><em>ent</em>-copalyl diphosphate synthase</td>
</tr>
<tr>
<td>KS</td>
<td><em>ent</em>-kaurene synthase</td>
</tr>
<tr>
<td>g</td>
<td>gravity</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>kb</td>
<td>Kilo base</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxyribonucleotide triphosphate</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcription - polymerase chain reaction</td>
</tr>
<tr>
<td>qPCR</td>
<td>Real-time polymerase chain reaction</td>
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<tr>
<td>rpm</td>
<td>Rotation per minute</td>
</tr>
<tr>
<td>EB</td>
<td>Elution buffer</td>
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<tr>
<td>TAE</td>
<td>Tris-aceetate-EDTA</td>
</tr>
<tr>
<td>MPOB</td>
<td>Malaysian Palm Oil Board</td>
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<td>MPOC</td>
<td>Malaysian Palm Oil Council</td>
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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 characterization analysis.
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