UNIVERSITI PUTRA MALAYSIA

GENE EXPRESSION PROFILE, AND ANATOMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF GA INHIBITOR (PACLOBUTRAZOL) - TREATED OIL PALM (*Elaeis guineensis* Jacq.) IN NURSERY CONDITION

MOHD NASRIQ HAFIZEE ABDUL RAHMAN

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MOHD NASRIQ HAFIZEE BIN ABDUL RAHMAN

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MOHD NASRIQ HAFIZEE BIN ABDUL RAHMAN

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Chairman : Edaroyati Megat Wahab, PhD
Faculty : Agriculture

Oil palm, the major crop planted in Malaysia, becomes one of the important commodity crops with a total planted area of 5.4 million hectares in 2015. The economic value of oil palm is determined by its height, since the height of a tree will influence the cost of harvesting the fresh fruit bunch (FFB). The increase of oil palm height will increase the difficulty and may cause damages to FFB during harvesting process. Thus, this study was conducted in effort to manipulate the oil palm height (stem elongation) through the manipulation of plant growth hormones by using GA Inhibitor (paclobutrazol). The main objective of this research was to investigate the effect of paclobutrazol (PBZ) on genes expression pattern, physiological and anatomical characteristic of oil palm (*Elaeis guineensis*) in nursery condition. Oil palm in each treatment were grown under nursery condition and were treated with different concentration of PBZ ranging from 10 mgL^{-1} to 100 mgL^{-1} by using foliar spray . After six weeks of PBZ treatment, material from leaves and stem were extracted for genes expression analysis and histological observation using light microscope. Oil palm treatment with PBZ resulted in short and dwarf plant with dark green leaves and smaller stem diameter compared to control. Furthermore, leaves of treated oil palms showed an increment in photosynthesis rate and chlorophyll index with larger and shorter mesophyll palisade cell compared to control plant. PBZ treatment course parenchyma cell in the stem to grow shorter and smaller compared to control. On the other hand, basal cell showed increment in cell diameter in response to PBZ treatment, while there was no difference in term of basal cell height can be observed between PBZ treated and control plant. In addition, PBZ treated oil palm showed remarkable increment on starch granule accumulation in parenchyma cell of oil palm stem. By using *in-silico* analysis, we identified a two oil palm putative GA biosynthesis related genes which were GA2 (*EgGa2ox*) and GA20-oxidase (*EgGa20ox*). The data revealed that, PBZ treatment does not affect the expression profiles of *EgGA2ox* and *EgGA20ox* in leaf tissues as all two putative GA oxidase genes show a consistent value of relative band intensity (RBI) compared to the controls. On the other hand, the expressions of *EgGA2ox* and *EgGA20ox* slightly drop in PBZ treated oil palm stem. These results suggest that PBZ may have various effects on the GA oxidase levels from different

parts of oil palm tissues. In summary, PBZ treated oil palm had modified genes expression pattern, physiological and anatomical characteristic of the plant depending on PBZ concentration applied and plant parts. In addition, PBZ treatment also facilitates the enhancement of net photosynthesis rate, chlorophyll content and starch accumulation in oil palm tissues.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PROFIL EKSPRESI GEN SERTA CIRI ANATOMIKAL DAN FISIOLOGIKAL KELAPA SAWIT (*Elaeis guineensis* Jacq.) YANG DIRAWAT MENGGUNAKAN PERENCAT GA (PACLOBUTRAZOL) DALAM PERINGKAT SEMAIAN

Oleh

MOHD NASRIQ HAFIZEE BIN ABDUL RAHMAN

Mac 2016

Pengerusi : Edaroyati Megat Wahab, PhD
Fakulti : Pertanian

Kelapa sawit merupakan tanaman utama di Malaysia dan menjadi salah satu tanaman komoditi penting dengan keluasan tanaman meliputi kawasan 5.4 juta hektar pada tahun 2015. Faktor ekonomi kelapa sawit ditentukan oleh ketinggian, kerana ketinggian pokok akan mempengaruhi kos penuaian tandan buah kelapa sawit. Peningkatan ketinggian kelapa sawit akan meningkatkan kesukaran dan boleh menyebabkan kerosakan tandan buah sawit semasa proses penuaian. Oleh itu, kajian ini dijalankan dalam usaha untuk memanipulasi pertumbuhan kelapa sawit (pemanjangan batang) melalui manipulasi hormon pertumbuhan dengan menggunakan perencat GA (paklobutrazol). Tujuan utama kajian ini adalah untuk mengkaji kesan paclobutrazol (PBZ) terhadap profil ekspresi gen serta perubahan ciri-ciri fisiologikal dan anatomikal kelapa sawit (*Elaeis guineensis*) pada peringkat semaian. Setiap pokok kelapa sawit dibesarkan pada peringkat semaian dan telah dirawat dengan menggunakan PBZ dengan kepekatan yang berbeza di antara 10 mgL^{-1} hingga 100 mgL^{-1} dengan menggunakan semburan foliar. Selepas enam minggu tamat sesi semburan PBZ, sampel dari daun, batang dan basal dikumpulkan untuk tujuan analisis ekspresi gen dan permerhatian histologikal menggunakan mikroskop cahaya. Dari keputusan yang diperolehi, semburan PBZ kepada kelapa sawit menyebabkan tumbuhan ini renek disamping berdaun hijau gelap dan diameter batang yang lebih kecil berbanding kelapa sawit kawalan. Tambahan pula, daun kelapa sawit dengan semburan PBZ menunjukkan kenaikan kadar fotosintesis dan relatif indek klorofil, disamping mempunyai sel mesophyll yang lebih besar dan pendek berbanding kelapa sawit kawalan. Selain itu, semburan PBZ menyebabkan pertumbuhan sel parenkima pada batang kelapa sawit menjadi renek dan kecil jika dibanding dengan kelapa sawit kawalan. Sebaliknya, sel basal menunjukkan peningkatan diameter sebagai tindak balas kepada semburan PBZ, manakala tiada sebarang perbezaan dari segi ketinggian sel boleh diperhatikan di antara kelapa sawit semburan PBZ dan kawalan. Di samping itu, kelapa sawit semburan PBZ menunjukkan peningkatan yang luar biasa pada kandungan kanji didalam sel parenkima terutamanya pada batang kelapa sawit. Dengan menggunakan analisi *in-silico*, dua gen kelapa sawit telah dikenal pasti dan dianggap berkaitan dengan GA

Data menunjukkan semburan PBZ tidak memberi sebarang kesan pada terhadap profil ekspresi bagi *EgGA2ox* dan *EgGA20ox* dalam tisu daun, ini dibuktikan dengan nilai intensiti band relatif (RBI) yang konsisten berbanding kelapa sawit kawalan. Sebaliknya, pada sel batang, ekspresi bagi *EgGA2ox* dan *EgGA20ox* mengalami sedikit penurunan berbanding kelapa sawit kawalan. Keputusan ini menunjukkan bahawa PBZ mempunyai pelbagai kesan ke atas paras kandungan GA oxidase dan ianya bergantung kepada jenis tisu dan bahagian pokok kelapa sawit tersebut. Secara ringkasnya, semburan PBZ telah mengubahsuai sebahagian corak ekspresi gen serta ciri-ciri fisiologikal dan anatomikal kelapa sawit bergantung kepada kepekatan PBZ dan bahagian tumbuhan terlibat. Di samping itu, rawatan PBZ juga membantu meningkatkan kadar fotosintesis, kandungan klorofil dan pengumpulan kanji dalam tisu kelapa sawit.

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LIST OF ABBREVIATIONS

°C	Degree celsius
µl	microlitre
µm	micrometer
$ngµl^{-1}$	Nanogram per microliter
mgL^{-1}	miligram per litre
mg	miligram
m	meter
cm	Centimetre
mmHg	millimeter of mercury
WAT	Week after treatment
FFB	Fresh fruit bunches
PBZ	Paclobutrazol
GA	Gibberellin
ox	Oxidase
IPP	Isopentenyll diphosphate
GGPP	Geranylgeranyl pyrophosphate
DMAP	Isomer dimethylallyl pyrophosphate
CPP	Copalyl diphosphate
C	Carbon
CPS	*ent*-copalyl diphosphate synthase
KS	*ent*-kaurene synthase
IAA	Indole-3-acetic acid
CRD	Complete Randomized Design
$µmolCO_2\ m^{-2}\ s^{-1}$	Photosynthersis rate
$mmolH_2O\ m^{-2}\ s^{-1}$	Transpiration rate
$molH_2O\ m^{-2}\ s^{-1}$	Stomatal conductance
%	Percentage
bp	Base pair
kb	Kilo base
cDNA	complementary DNA
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
RT-PCR	Reverse Transcription Polymerase Chain Reaction
g	gravity
EB	Elution buffer
rRNA	Ribosomal ribonucleic acid
TAE	Tris-acetate-EDTA
RBI	Relative band intensity
MPOB	Malaysia Palm Oil Board
MPOC	Malaysia Palm Oil Council
C-score	Cleavage score
S-score	Signal score
D-score	Average of C and S- score

CHAPTER 1

INTRODUCTION

1.1 Research background

Oil palm (*Elaeis guineensis*) was introduced as an ornamental plant in Malaysia. After almost 40 years later, it was first cultivated commercially in Selangor, laying the foundation of oil palm plantations and industries in Malaysia (Coley and Tinker, 2003). Since then, oil palm becomes one of important commodity crops in Malaysia with a total planted area of 5.4 million hectares in 2015 (Malaysian Palm Oil Board, MPOB). Based on the 2015 report, Malaysia is one of the largest producers and exporters of palm oil (Malaysia Palm Oil Council, MPOC), accounting about 39% of world palm oil and fats production, and 44% of global exports.

To meet the healthy palm oil demand, Malaysian oil palm industry is facing the rising production cost which is partly due to the reduction of fresh fruit bunches (FFB) productivity. This is because the yield and productivity of oil palm are not constant as they are influenced by several factors such as genetic, site characteristics, and plant's structure (Coley and Tinker, 2003).

One of the key factors that determine the economic life of an oil palm is the height of the tree, since the height will influence the cost of harvesting FFB. A matured oil palm tree can grow up to 20 m tall after 5 years (Coley and Tinker, 2003) which can potentially damage the oil palm fruits during the harvesting process, thus decreasing the quality of FFB. Overall, the production cost is dictated by the net profit from FFB sells, which is based on the global prices, and also, the management and operational costs.

1.2 Problem Statement and Objectives:

Over the last decade, there have been concentrated researches to reduce the height of commercial crops. Breeding programmes have been initiated in order to select dwarf materials that can lead to the manipulation of plant growth, as well as progressively increasing the yield and productivity of many crops. Thus, this study was conducted in the effort to manipulate the oil palm growth, especially the stem elongation. As for the oil palm, since its economic life is determined by the height of the tree, the increase of height will affect the cost and the difficulty to harvest the FFB. The manipulation of the plant growth can be achieved through the manipulation of plant growth hormones, particularly gibberellins.

The comprehensive understanding of plant growth regulation system, especially the physiological process of gibberellins (GAs) and its properties, are required before manipulating GAs to regulate oil palm growth. This includes investigating the causes of GAs hormone reduction in plant tissue, and finding the type of dwarf materials that can be used to inhibit the synthesis of GAs.

Based on recent studies on plant growth regulation of several plant species, *Solanum tuberosum*, *Brunonia* and *Calandrinia sp.*(Tekalign *et at.*, 2005; Bidadi *et al.*, 2009; Wahyuni *et al.*, 2011), paclobutrazol (PBZ) was proven to be the most effective plant growth retardant that restricts the height of the aforementioned plants. However, there are still lacks of comprehensive researches related to the application of PBZ on oil palm. Hence, this study was conducted in an attempt to observe and evaluate the effects of this dwarf material on morphological and anatomical characteristic, as well as biosynthesis reactions, in oil palm tissues.

Therefore, the objectives of this study, includes:

1. To determine the optimum concentration of PBZ as stem growth inhibitor in oil palm seedlings.
2. To elucidate the morphological, anatomical, and physiological changes in oil palm seedling as affected by the PBZ application.
3. To determine the expression profile of putative GA biosynthesis related genes on PBZ treated oil palm seedlings.

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BIODATA OF STUDENT

I was born on September16, 1988 in Arau, Perlis. Throughout my primary and secondary school, I performed well in both academic and co-curricular. I finished my secondary school at Sekolah Menengah Darul Ehsan, Selangor or so-called SMKDE. Then, I continued my studied at Matriculation College in life science at Kolej Matrikulasi Negeri Sembilan and pursuing my degree level at Universiti Putra Malaysia. I graduated in the year 2010 in BSc (Hons.) Sciences- Biology. During my degree, my academic advisor, Prof. Jambari Hj Ali has encouraged me to further my study. My supervisor, Dr. Edaroyati Megat Wahab which I knew him for almost 4 years has guided me throughout my research in master program together with my co-supervisor Dr Zubaidah Ramli, Dr Noor Azmi Shaharuddin and Associate Professor Dr Nor Ashikin Psyquay. I advanced my laboratory experiences and skills under their supervision. At present, I found myself to the enthusiastic obtain knowledge regarding plant physiology, anatomy and molecular study either its basic info or in details. I really hope to become one of knowledgeable biologist so that all the knowledge can be shared and the journey of discovery in life will keep continue

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