



UNIVERSITI PUTRA MALAYSIA

**IDENTIFICATION OF A PUTATIVE MONOLIGNOL TRANSPORTER
GENE HOMOLOG III IN *ORYZA SATIVA***

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FBSB 2015 137

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*SATIVA***

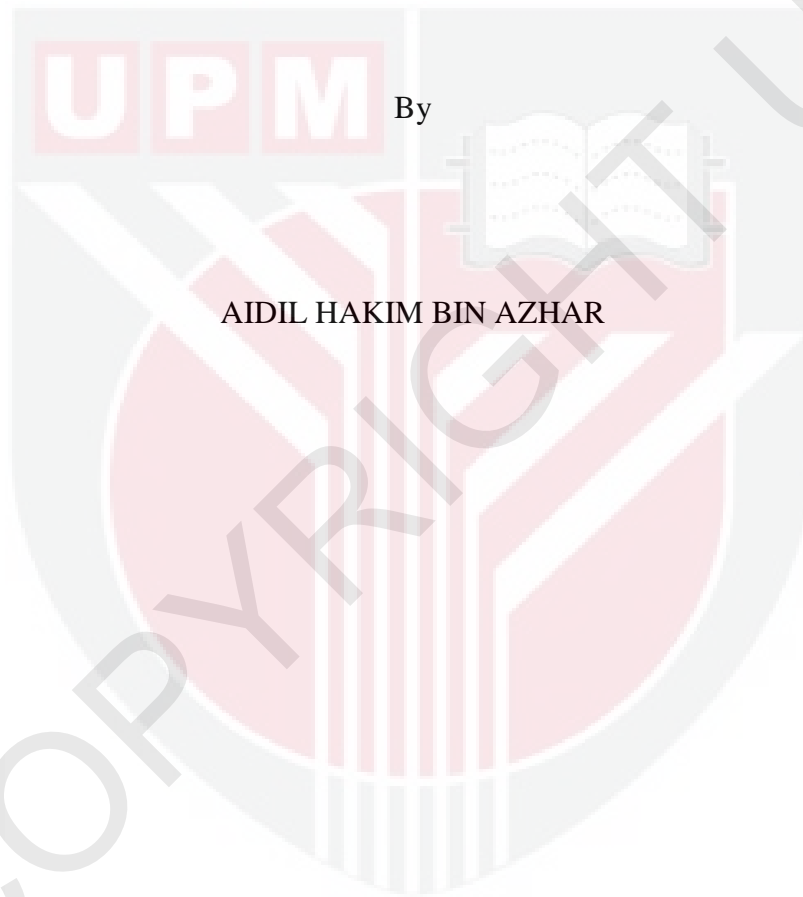
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BACHELOR OF SCIENCE (HONS)

UNIVERSITI PUTRA MALAYSIA

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HOMOLOG III IN *ORYZA SATIVA***



Thesis Submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology
and Biomolecular Sciences, Universiti Putra Malaysia, in Fulfillment of the Requirement for the

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Approval

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfillment of the requirement for the degree of Bachelor of Science (Hons). The member of the Supervisory Committee was as follows:

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Declaration

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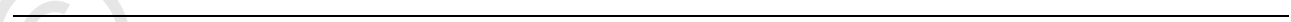
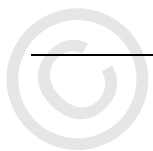
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Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement for the degree of Bachelor of Science (Hons)

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By

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JUNE 2015

Chair: Dhilia Udie Lamasudin, PhD
Faculty: Biotechnology and Biomolecular Sciences.

Lignin is important to plant cell as it gives a strong structure and protects the plants from biotic and abiotic stresses. Lignin is made up from monomers called monolignols. There are three types of monolignols which are p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Monolignols are synthesized in the cytoplasm of the cell. These monolignols will be transported through the plasma membrane and polymerized at the cell wall of the plant. The mechanism of monolignol transport is still unclear. One previous study has shown that *AtABCG29* is involved in transporting p-coumaryl alcohol across plasma membrane in *Arabidopsis thaliana*. *AtABCG29* gene encodes for protein that is localized at the plasma membrane and is called monolignol transporter. The aim of this study is to identify the gene that encodes for the protein that is involved in transporting monolignol across the plasma membrane in *Oryza sativa*. The gene of hypothetical protein OsI_23352 from *Oryza sativa* has shown to have a high percentage of identity and homologous to *AtABCG29* in *Arabidopsis thaliana*. Therefore it was chosen as candidate to find the monolignol transporter gene homolog III in *Oryza sativa*.

Abstrak yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk ijazah sarjana muda sains (kepujian)

PENGENALPASTIAN HOMOLOG GEN PUTATIVE PENGANGKUT MONOLIGNOL III DALAM *ORYZA SATIVA*

Oleh

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Lignin adalah penting bagi dinding sel tumbuhan kerana ia memberikan struktur yang kukuh dan melindungi tumbuhan daripada tekanan biotik dan abiotik. Lignin terdiri daripada monomer iaitu monolignol. Terdapat tiga jenis monolignol iaitu alkohol p-coumaryl, alkohol coniferyl dan alkohol sinapyl. Monolignol dihasilkan di dalam sitoplasma. Monolignol akan diangkut melepasi membran plasma dan menjadi polimer di dinding sel. *AtABCG29* terlibat dalam pengangkutan alkohol p-coumaryl dalam *Arabidopsis thaliana*. *AtABCG* mengkod bagi protein yang terdapat di membran plasma yang dikenali sebagai pengangkut monolignol. Protein tersebut hanya mengangkut alkohol p-coumaryl. Matlamat kajian ini adalah untuk mengenalpasti gen yang mengkod bagi protein yang terlibat dalam pengangkutan alkohol p-coumaryl, alkohol coniferyl dan alkohol sinapyl di dalam *Oryza sativa*. Gen bagi protein hipotetikal *OsI_23352* dari *Oryza sativa* telah menunjukkan ia mempunyai peratusan identiti yang tinggi kepada *AtABCG29* dalam *Arabidopsis thaliana*. Oleh itu, ia dipilih sebagai calon bagi pengenalpastian homolog gen putative pengangkut monolignol III dalam *Oryza sativa*.

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LIST OF ABBREVIATIONS

ABC	ATP-Binding Cassette
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
CTAB	Cetyltrimethylammonium bromide
cDNA	Complementary DNA
DNA	Deoxyribonucleic acid
EtBr	Ethidium bromide
EMBL-EBI	European Molecular Biology Laboratory- European Bioinformatics Institute
F5H	Ferulate 5-Hydroxylase
GT	Glucosyl transferases
GH	Glucosyl hydrolases
gDNA	Genomic DNA
MATE	Multi drug and toxic compound extrusion
mRNA	Messenger RNA
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
PAL	Phenylalanine ammonia lyase
PVP	Polyvinylpyrrolidone
RNA	Ribonucleic acid

CHAPTER 1

INTRODUCTION

ATP-Binding Cassette Transporter or also known as ABC transporter is a protein that localized at the plasma membrane of the cell. It is a transmembrane protein that helps to transport molecule across the plasma membrane. This protein utilizes energy from ATP in order to perform its function. ABC superfamily protein present in all kingdoms of living organism. ABC protein are also can be classified into seven subfamily ranging from A to G. Each subfamily has their specific function. They transport a certain type of molecule across the plasma membrane. There are a large number of ABC transporters which their function is still unknown. The substrate that they bind to or transport is remain unknown. The ABC transporter is expected to play an important role in cell detoxifications and ensure the healthy growth of the cell. Research on ABC transporter is important in order to understand the physiological role of this protein. Hence, it can be used in many applications such as biotechnology to improve the quality of crops in agriculture. It is also can be useful for application that related to human disease. In previous study, it is found that AtABCG29 protein which means ABC protein under subfamily G number 29 is involves in p-Coumaryl alcohol transport during lignin biosynthesis in *Arabidopsis thaliana* sp (Alejandro et al., 2012). During the process of lignin biosynthesis, monolignols are polymerized after being transported through the plasma membrane. It polymerizes at the secondary wall of the plant cell. The process of how they polymerizes are still elusive but a lot of studies has been done throughout the world. It involves many complex chemical reactions. Different species or type of plant has different composition of lignin. Some plant has more p-Coumaryl alcohol amount in their lignin compares to other monolignol and other plant might have sinapyl alcohol and coniferyl alcohol in higher amount compared to p-Coumaryl alcohol in

their lignin composition. (Fergus and Goring, 1970; Liu, 2012). The objectives of this study were to identify a putative monolignol transporter gene homolog III in *Oryza sativa* and to optimize the total RNA isolation from *Oryza sativa*. The candidate gene was chosen by performing Basic Local Alignment Sequence Tools (BLAST) that has the highest percent of identities against AtABCG29 gene from *Arabidopsis thaliana*. Multiple sequence alignment also done to generate phylogenetic tree diagram. Hypothetical uncharacterized protein OsI_23352 was found to be the most related to the AtABCG29. Thus it is predicted to have the similar function as monolignol transporter. The total RNA extraction by using CTAB method seems to have higher yield compared to extraction kit. Two short fragments are also amplified by using PCR.

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