



UNIVERSITI PUTRA MALAYSIA

**IDENTIFICATION OF PUTATIVE MONOLIGNOL TRANSPORTER GENE
HOMOLOG I IN *Oryza sativa***

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**IDENTIFICATION OF PUTATIVE MONOLIGNOL TRANSPORTER
GENE HOMOLOG I IN *Oryza sativa***

By

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**Thesis Submitted to the Department of Cell and Molecular Biology
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Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement for the Bachelor of Science (Honours) Cell and Molecular Biology

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Lignin is present in plant's cell wall constitutes which mainly function to provide vital structural support to the plant. The transportation of lignin precursors, monolignols, from the membrane to the cell wall, where they are oxidized and polymerized, is facilitated by ATP-dependent transport process. This process involves ATP-binding cassette-like transporters, namely monolignol transporter. Studies have been conducted using *Arabidopsis thaliana* as an experimental model to prove this idea. Hence, this project was performed using *Oryza sativa* (cultivar MR219) to identify putative monolignol transporters based on sequence identity detection of a target gene from these two organisms. The putative target gene sequences that possessed a high identity with *AtABCG29* gene was obtained from the BLAST program (NCBI) and subjected to a specific primer designing step. A number of primer sets were designed to amplify fragment-by-fragment in isolating the whole sequence of the putative target gene, which is approximately 4.7 kb in length. Total RNA was extracted from four weeks old seedlings of *O. sativa* followed by the synthesis of cDNA and polymerase chain reaction to amplify the target gene by using the specifically designed primers. A housekeeping gene (*Actin11*) was used as a positive control in this study. Final results showed that out of eleven primer pairs designed, four of them produced a significant single band at expected size. Thus, it can be concluded that the putative target gene in *O. sativa* has a relatively high sequence homology with *AtABCG29* from *Arabidopsis*.

Keywords: monolignol ABC-transporter, sequence homology, gene identification

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk Bacelor Sains (Kepujian) Biologi Sel dan Molekul

PENGECAMAN HOMOLOG GEN PENGANGKUT MONOLIGNOL I YANG
DIDUGA DALAM *Oryza sativa*

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Lignin terdapat di dalam sel tumbuhan yang fungsi utamanya adalah untuk memberikan sokongan struktur kepada tumbuhan. Pengangkutan pelopor lignin yang dikenali sebagai monolignol dari membran sel ke dinding sel, di mana ianya teroksida dan dipolimerkan dibantu oleh proses angkutan bersandar ATP. Proses ini melibatkan kaset pengikat ATP bak pengangkut iaitu pengangkut monolignol. Kajian telah dijalankan sebelum ini dengan menggunakan *Arabidopsis thaliana* sebagai model eksperimen bagi membuktikan idea ini. Sehubungan dengan itu, projek ini dijalankan dengan menggunakan *Oryza sativa* (kultivar MR219) untuk mengenal pasti pengangkut monolignol ini berdasarkan pengesanan identiti jujukan gen sasaran daripada kedua-dua organisma. Jujukan gen sasaran yang mempunyai homologi yang tinggi dengan gen *AtABCG29* telah diperolehi daripada program BLAST daripada NCBI dan digunakan sebagai rujukan dalam mereka bentuk primer yang spesifik. Beberapa set primer telah direka untuk memperbanyakkan pecahan dalam mengasingkan seluruh jujukan gen sasaran, iaitu kira-kira 4.7 kb panjang. Keseluruhan RNA telah diekstrak daripada anak pokok *O. sativa* yang berusia empat minggu diikuti dengan sintesis cDNA dan tindak balas berantai polimerase untuk memperbanyak gen sasaran. Gen penyelenggara (Actin11) telah digunakan sebagai kawalan positif dalam kajian ini. Keputusan akhir menunjukkan bahawa daripada sebelas pasangan primer yang direka, empat daripada mereka menghasilkan jalur tunggal bererti pada saiz jangkaan. Oleh itu, dapat disimpulkan bahawa gen sasaran di *O. sativa* mempunyai jujukan homologi yang agak tinggi dengan *AtABCG29* dari *Arabidopsis*.

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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 fulfilment of the requirement for the Bachelor of Science (Honours) Cell and Molecular Biology. The member of the Supervisory Committee was as follows:

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DECLARATION

Declaration by undergraduate student

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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENTS	iv
APPROVAL	v
DECLARATION	vi
TABLE OF CONTENTS	viii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xii
CHAPTER	
1 INTRODUCTION	
1.1 Introduction	1
1.2 Objectives of study	2
2 LITERATURE REVIEW	
2.1 Biology and ecology of <i>Oryza sativa</i>	3
2.1.1 General description of <i>Oryza sativa</i>	3
2.1.2 Taxonomy and genetics	4
2.1.3 The classification of common species	4
2.2 Lignin in nature	5
2.2.1 Monolignols- Precursor of lignin	6
2.3 ATP-binding Cassette (ABC) transporter protein family	8
2.3.1 The structural characteristics of ABC transport protein	9
2.3.2 Monolignol transporter	10
3 MATERIALS AND METHOD	
3.1 Target gene(s) identification and primer designing	12
3.2 Plant materials and growth condition	12
3.3 Preparation of buffers and solutions	
3.3.1 CTAB extraction buffer	14
3.3.2 Tris-EDTA (TE) buffer for oligonucleotides storage	14
3.3.3 50xTris-Acetate (TAE) for Agarose Gel Electrophoresis	14
3.4 RNA extraction from <i>Oryza sativa</i> (cultivar MR219)	
3.4.1 Qiagen RNeasy Plant Mini Kit	15
3.4.2 CTAB RNA extraction	15
3.5 First-strand cDNA synthesis	16
3.6 Amplification of the target gene by using Polymerase Chain Reaction	17

3.7	Agarose Gel Electrophoresis (AGE)	17
4	RESULTS AND DISCUSSION	
4.1	Identification of <i>AtABCG29</i> homolog in <i>Oryza sativa</i>	18
4.2	Total RNA extraction from <i>Oryza sativa</i> (Cultivar MR219)	
4.2.1	Spectrophotometric quantification analysis of RNA samples	21
4.2.2	Visualization of ribosomal RNA subunits via electrophoresis	22
4.3	cDNA synthesis from total RNA samples	24
4.4	Amplification of target sequence via Polymerase Chain Reaction	26
5	CONCLUSION	
5.1	Conclusion	31
5.2	Recommendations for future works	31
	REFERENCES	32
	APPENDICES	34

LIST OF TABLES

Table	Description	Page
3.1	List of primer pairs and corresponding amplification targets	13
4.1	Total RNA quantity and quality by spectrophotometric determination	21



LIST OF FIGURES

Figure	Description	Page
2.1	Chemical structure of lignin precursors	7
2.2	Molecular architecture of ABC Transporters	10
4.1	Screenshot image of blastn program result of <i>atABCG29</i> genomic DNA sequence	18
4.2	Multiple sequence alignment of nucleic acid sequence of the <i>AtABCG29</i> with different monocotyledonous plants	20
4.3	Separation of ribosomal RNA subunits extracted using RNeasy plant mini kit in agarose gel electrophoresis	23
4.4	Separation of ribosomal RNA subunits extracted using CTAB based method in agarose gel electrophoresis	23
4.5	Agarose gel electrophoresis of synthesized cDNA from total RNA extracted from <i>O. sativa</i>	25
4.6	Gel image of Actin 11 amplification product	26
4.7	Illustration of primer pairs target sites	27
4.8	Gel image of non-optimized PCR products	28
4.9	Gel image of optimized PCR products	29

LIST OF ABBREVIATIONS

ABC	ATP- binding cassette
AGE	Agarose Gel Electrophoresis
ATP	Adenosine triphosphate
BLAST	Basic local alignment search tool
cDNA	Complementary deoxyribonucleic acid
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
NCBI	National Centre For Biotechnology Information
PCR	Polymerase chain reaction
PDR	Pleiotropic drug resistance
PVPP	Polyvinylpolypyrrolidone
RNA	Ribonucleic acid
TAIR	The Arabidopsis Information Resources

INTRODUCTION

1.1 Introduction

Lignin, a component of the plant cell walls gives the overall structural strength to the plant which enables it to hold itself up on the ground. Comprised of three different type of precursors, called the monolignols, these monomers specifically *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol are known to be synthesized in the cytosol. However, studies shows that these monolignols end up at various subcellular locations without any known transport mechanism until recently reported by Alejandro et al., (2012) where they proved that the transportation of one of the monolignols, *p*-coumaryl in *Arabidopsis* involves an ATP-binding cassette (ABC) transporter, *AtABCG29*.

Monolignol transporters had previously been identified in *Arabidopsis*. However, it is not yet found in monocots specifically in *Oryza sativa*. Hence, in this particular study, we aim to identify and isolate the putative gene homolog of *AtABCG29* in *Oryza sativa* specifically MR219 cultivar which is one of the major crops grown in Malaysia. Bioinformatics approaches are the well-known way of research for nucleic acid sequence analyses to find new findings thus, it is one of the major approaches used in this study. The genomic sequence of *AtABCG29* was used as the template sequence in order to identify sequence homolog in *Oryza sativa* databases. The long term overall idea of this project is to manipulate the transport system of the lignin precursors in *O. Sativa* to demonstrate potential involvement of these molecules against pathogen attack where plants use their naturally existing compound as their self-defence mechanism.

1.2 Objective of study

The aims of this study are:

- a) To isolate putative monolignol transporter gene homolog in *Oryza sativa* (cultivar MR219).
- b) To compare the efficiency of RNA extraction from *Oryza sativa* by using conventional CTAB-based and kit-based method.



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