UNIVERSITI PUTRA MALAYSIA

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

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By

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Thesis Submitted to the Department of Cell and Molecular Biology, Biotechnology and Biomolecule Sciences, in Fulfillment of the Requirements for the Degree of Bachelor of Science

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

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Chair : Dhilia Udie Lamasudin, PhD

Faculty : Biotechnology and Biomolecule Sciences

Plant cells have sturdy shape because of the presence of cell wall made from three components; cellulose, hemicelluloses, and lignin. Lignin biosynthesis has been studied extensively by many researchers but monolignol transportation process is yet to be explored specifically. It is suggested that monolignol uptakes are dependent towards transport proteins residing in the membrane. *AtABCG29* gene of *Arabidopsis thaliana* codes for a transport protein has been characterized and proven that the protein transports *p*-coumaryl alcohol. However, the monolignol transport mechanism for *Oryza sativa* is still elusive. In this study, *Oryza sativa*'s gene with locus name *OsI_03578* was identified as a putative homologous gene of *AtABCG29. OsI_03578* was chosen due to its high sequence similarity with *AtABCG29*. This research was conducted to investigate whether *OsI_03578* gene encodes for a protein that involves in monolignol transportation in *Oryza sativa.* Total RNA was extracted from *Oryza sativa* and used to synthesize cDNA which served as a template to amplify *OsI_03578* sequence in *Oryza sativa*. The amplification of the gene sequence was performed by PCR using different sets of

primers. In this project, conventional RNA extraction protocol has been optimized and proven to give better RNA yield compared to RNA extraction using commercial kit. However, designation of new primers is necessary in order to amplify specific target region in the gene.

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel and Molekul sebagai memenuhi keperluan Ijazah Bacelor Sains

MENGENAL PASTI GEN YANG DIANGGAP SEBAGAI PENGANGKUT MONOLIGNOL HOMOLOG II DALAM *ORYZA SATIVA*

Oleh

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Sel tumbuhan mempunyai bentuk kukuh kerana kehadiran dinding sel yang diperbuat daripada tiga komponen; selulosa, hemiselulosa dan lignin. Biosintesis lignin telah dikaji secara meluas oleh ramai penyelidik tetapi proses pengangkutan monolignol masih belum diterokai secara khusus. Adalah dicadangkan bahawa pengambilan monolignol bergantung kepada protein pengangkut yang berada di membran. *AtABCG29* gen mengekod protein pengangkut bagi tumbuhan *Arabidopsis thaliana* telah dicirikan dan ianya terbukti bahawa protein yang mengangkut *p*-coumaryl alkohol. Walau bagaimanapun, mekanisme pengangkutan monolignol untuk *Oryza sativa* masih sukar difahami. Dalam kajian ini, satu gen *Oryza sativa* dengan nama locus *OsI_03578* telah dikenal pasti sebagai gen homolog daripada *AtABCG29. Gen OsI_03578* telah dipilih dalam kajian ini kerana persamaan jujukan yang tinggi dengan gen *AtABCG29*. Kajian ini telah dijalankan bagi menyiasat sama ada gen *OsI_03578* mengekod untuk protein yang

terlibat dalam pengangkutan monolignol dalam *Oryza sativa*. RNA keseluruhan telah diasingkan daripada *Oryza sativa* dan digunakan untuk mensintesis cDNA yang berkhidmat sebagai templat untuk mengenalpasti urutan *OsI_03578 Oryza sativa*. Amplifikasi jujukan gen dilakukan dengan menjalankan reaksi berantai polimerase dengan menggunakan set primer yang berbeza. Dalam projek ini, protokol pengekstrakan RNA konvensional telah dioptimumkan dan terbukti memberikan hasil RNA lebih baik berbanding dengan pengekstrakan RNA menggunakan kit komersial. Walau bagaimanapun, penetapan primers baru adalah perlu untuk menguatkan kawasan sasaran tertentu dalam gen.

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APPROVAL

This thesis was submitted to the Department 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. The member of the Supervisory Committee was as follows:

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DECLARATION

Declaration by undergraduate

I hereby confirm that:

- this thesis is my original work;
- quotations and citations which have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- written permission must be obtained from the supervisor before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials;
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Declaration by Supervisor

This is to confirm that the research conducted and the writing of this thesis was under supervision.

Signature : _____

Name of
Chairman of
Supervisory
Committee : _____

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LIST OF ABBREVIATIONS

µg	Microgram
µl	Microliter
4CL	4-(hydroxy)cinnamoyl CoA ligase
A_{230}	Absorbance at the wavelengths of 230 nm
A_{260}	Absorbance at the wavelengths of 260 nm
A_{280}	Absorbance at the wavelengths of 280 nm
ABC	Adenosine triphosphate-binding casette
ABCB	Adenosine triphosphate-binding casette subfamily B
ABCC	Adenosine triphosphate-binding casette subfamily C
ABCD	Adenosine triphosphate-binding casette subfamily D
ABCG	Adenosine triphosphate-binding casette subfamily G
ACT11	Actin 11 gene
ATP	Adenosine triphosphate
BLAST	Basic local alignment search tool
blastp	Protein protein BLAST
B-Me	β-mercaptoethanol
bp	Base pair
C3H	*p*-coumarate 3-hydroxylase
C4H	Cinnamate 4-hydroxylase
CAD	Cinnamyl alcohol dehydrogenase
CCoAOMT	Caffeoyl CoA O-methyltransferase
CCR	Cinnamoyl CoA reductase
cDNA	Complementary deoxyribonucleic acid
CoA	Coenzyme A
COMT	Caffeic acid/5-hydroxyferulic acid O-methyltransferase
CQT	Hydroxycinnamoyl CoA: quinatehydroxycinnamoyltransferase
CST	Hydroxycinnamoyl CoA: shikimatehydroxycinnamoyltransferase

CTAB	Cetyltrimethylammonium bromide
DEPC	Diethylpyrocarbonate
DPL	Drug, peptides and lipid export
EDTA	Ethylenediaminetetraacetic acid
EPD	Eye pigment precursors and drug export
F5H,	Ferulate 5-hydroxylase
FAE	Fatty acid export
G	Guaiacyl
gDNA	Genomic deoxyribonucleic acid
H	Hydroxy-coumaryl
IM	Integral membrane
kb	Kilobase
LiCL	Lithium chloride
MDR	Multidrug resistance
MRP	Multidrug resistance-associated protein
ml	Mililiter
NaOAc	Sodium acetate
NCBI	The National Center for Biotechnology Information
Ng	Nanogram
Nm	Nanometer
OAD	Organic anion and drug export
^{o}C	Degree celcius
Pal	Phenylalanine ammonia lyases
pCCoA3H	*p*-coumaryl CoA 3-hydroxylase
PCR	Polymerase chain reaction
PDR	Pleiotropic drug resistance
PVPP	Polyvinylpolypyrrolidone
RNA	Ribonucleic acid
rpm	Rounds per minute
RT-PCR	Reverse transcription polymerase chain reaction
S	Sinapyl

SAD	Sinapyl alcohol dehydrogenase
TAE	Tris base, acetic acid and EDTA
TAIR	The *Arabidopsis* Information Resource
Tris-HCl	Tris hydrochloride
V	Volt
v/v	Volume/volume
w/v	Weight/volume

CHAPTER 1

INTRODUCTION

The plant body is passively protected against the infection of pathogens by the plant's superficial covering layers which consist of epidermis and cuticle. Plant's cell walls play important roles in structural support and protection against several stresses. It also functions as a pressure vessel which prevents over-expansion when the surrounding environments are hypotonic towards the plant. Lignin is nature's second most abundant natural polymer, a runner up to cellulose. By genetically engineering or modifying lignin biosynthesis it may be of beneficial in agricultural and industrial field in today's modern world. Due to the significant roles of lignin biosynthesis on economy (biomass production) and play a central role in higher plant development, it became an important topic in plant biochemistry (Wang *et al.*, 2013). Many researchers have done studies to extend the knowledge on lignin biology for example is the synthesis of lignin building blocks, monolignols, in the cytosol, and polymerization of phenolic heteropolymers that is lignin.

However not much has been explored on the mechanism of the monolignols' transportation out to the cell wall after its being synthesized in cytosol. It has been suggested that, ATP-binding cassette (ABC) protein may also involve in monolignol transportation. These protein families have been shown to be involved in transporting large varieties of molecules including secondary metabolite and hormones. Alejandro *et al.* (2012) have demonstrated that an ABC protein encode by AtABCCG29, serves as a monolignol transporter in *Arabidopsis thaliana* and this transporter is shown to be

specific for transporting for only one out of three monolignols which is *p*-coumaryl alcohol. Plant lignifications are for plant like *Oryza sativa* as it support and protect the plant in overcoming biotic and abiotic stresses. Understanding this mechanism can improve *Oryza sativa*'s ability to overcome these stresses, but so far, no research has been done to identify its monolignol transport mechanism. By using the protein sequence of *AtABCG29*, basic local alignment has been performed with *Oryza sativa* proteome and *OsI_03578* was shown to have significant sequence similarity in with *AtABCG29*. This research was performed to investigate and support the hypothesis, whether *OsI_03578* protein may functions as a monolignol transporter protein for any of the monolignols synthesized in the cells of *Oryza sativa.*

Realizing the potential of *OsI_03578* gene that encodes for a monolignol transporter, an attempt was made to prove the hypothesis. The objectives of this study were;

1. To optimize RNA extraction protocol for *Oryza sativa.*
2. To identify a putative monolignol transporter gene homolog from *Oryza sativa.*

REFERENCES

Alejandro, S., Lee, Y., Tohge, T., Sudre, D., Osorio, S., Park, J., *et al*. (2012). *AtABCG29* is a monolignol transporter involved in lignin biosynthesis. *Current Biology, 22*(13), 1207-1212.

Brule, S., and Smart, C. C. (2002). The plant PDR family of ABC transporter.*Planta, 216*, 95-106.

Ebrahim, S., Usha1, K., and Singh, B. (2011). Pathogenesis related (PR) proteins related to plant defense mechanism. *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances, ,* 1043-1054.

Fraser, C. M., and Chapple, C. (2011). The phenylpropanoid pathway in *Arabidopsis*. *The Arabidopsis Book,*

Garcia, O., Bouige, P., Forestier, C., andDassa, E. (2004).Inventory and comparative analysis of rice and *Arabidopsis* ATP-binding cassette (ABC) systems. *The Journal of Molecular Biology, 343*, 249-265.

Grabber, J. H. (2005). How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. *45*(3), 820-831.

Hatfield, R., and Vermerris, W. (2001). Lignin formation in plants. The dilemma of linkage specificity. *Plant Physiology, 126*, 1351-1357.

Huang, S., An, Y., McDowell, J. M., McKinney, E. C., and Meagher, R. B. (1997). The *Arabidopsis* ACT11 actin gene is strongly expressed in tissues of the emerging inflorescence, pollen, and developing ovules. *Plant Molecular Biology, 33*, 125-139.

Humphreys, J. M. and Chapple, C. (2002). Rewriting the lignin roadmap. *Current Opinion in Plant Biology, 5*, 224-229.

Jain, M., Nijhawan, A., Tyagi, A. K., andKhurana, J. P. (2006). Validation of housekeeping genes as internal control for studying gene expression in rice by quantitative real-time PCR. Biochemical and Biophysical Research Communications, 345, 646-651.

Jasinski, M., Ducos, E., Martinoia, E., andBoutry, M. (2003). The ATP-binding cassette transporters: Structure, function and gene family comparison between rice and *Arabidopsis*. *Plant Physiology, 131*, 1169-1177.

Kang, J., Park, J., Choia, H., Burlab, B., Kretzschmar, T., Lee, Y., *et al*. (2011). Plant ABC transporter. *The Arabidopsis Book, 9*, 1-25.

Liu, C. (2012). Deciphering the enigma of lignification: Precursor transport, oxidation, and the topochemistry of lignin assembly. *Molecular Plant, 5*(2), 304-317.

Miao, Y., and Liu, C. (2010). ATP-binding cassette-like transporters are involved in the transport of lignin precursors across plasma and vacuolar membranes. *The Proceedings of the National Academy of Sciences, 107*(52), 22728-22733.

Moons, A. (2003). *Ospdr9*, which encodes a PDR-type ABC transporter, is induced by heavy metals, hypoxic stress and redox perturbations in rice roots. *Federation of European Biochemical Societies Letters, 553*, 370-376.

Purification of total RNA from plant cells and tissues and filamentous fungi. (2008). *RNeasy® mini handbook* (4th ed., pp. 51-55). Germany: QIAGEN

Reverse transcription with elimination of genomic DNA for quantitative, real-time PCR. (2009). *QuantiTect® reverse transcription handbook* (pp. 11-13). Germany: QIAGEN.

Sibout, R., and Hofte, H. (2012). Plant cell biology: The ABC of monolignol transport. *Current Biology, 22*(13).

Syafiq Sharian. (2012). *Cloning, expression, and characterization of stearoyl-ACP desaturase gene from Jessenia bataua Mart. var. bataua.* Unpublished Master in Science, Universiti Putra Malaysia, Selangor, Malaysia.

Trobleshooting: 260/280 ratio. (2008). *NanoDrop 1000 spectrophotometer V3.7 User's manual* (pp. 17-11-17-13). Wilmington, USA: Thermo Fisher Scientific Inc.

Vanholme, R., Demedts, B., Morreel, K., Ralph, J., and Boerjan, W. (2010). Lignin biosynthesis and structure. *Plant Physiology, 153*, 895-905.

Wang, Y., Chantreau, M., Sibout, R., and Hawkins, S. (2013). Plant cell wall lignification and monolignol metabolism. *Frontiers in Plant Science, 4*, 1-14.

Weng, J., andChapple, C. (2010). The origin and evolution of lignin biosynthesis. *New Phytologist, 187*, 273-285.

Wong, J. H. (2011). *Functional analysis of PAL2 gene promoter in Arabidopsis thaliana (L.) heynh. during plant development exposed to biotic and abiotic stresses.* Unpublished Master of Science, Universiti Putra Malaysia, Selangor, Malaysia.

Yu, J., Hu, S., Wang, J., Wong, G., Li, S., Liu, B., *et al*. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science, 296, 79-92.

Zuraidah Zanirun. (2008). *Optimization of lignin peroxidase production from Pycnoporus sp.* Unpublished Master of Science, Universiti Putra Malaysia, Selangor, Malaysia.,