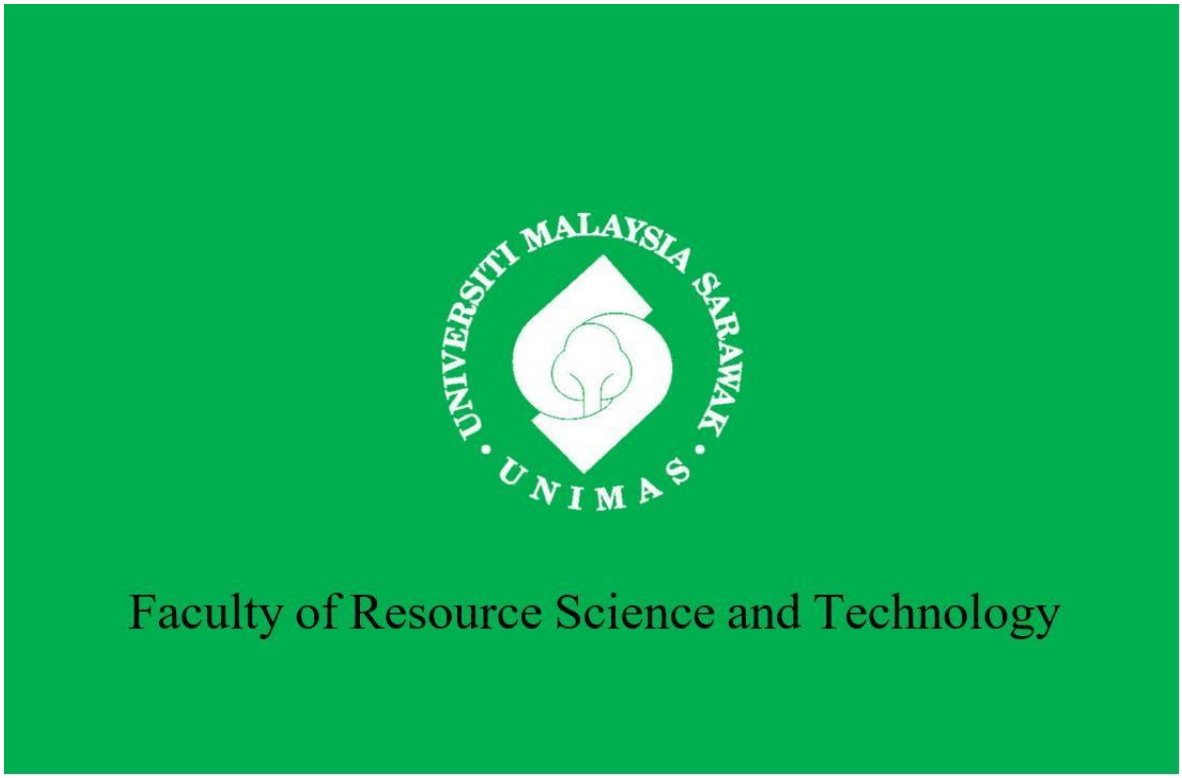

Expression Analysis of Differentiated Sago Palm Gene (Sulfate Transporter and Boron Transporter) via Amplified cDNA

Samuel Ng Chun Keat (71476)

Bachelor of Science with Honours
(Resource Biotechonology)
2022

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Samuel Ng Chun Keat

A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours

(Resource Biotechnology)

SUPERVISOR: PROFESSOR DR. MOHD HASNAIN BIN MD HUSSAIN

Programme of Resource Biotechnology
Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK

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Expression Analysis of Differentiated Sago Palm Gene (Sulfate Transporter and Boron Transporter gene) via Amplified cDNA

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ABSTRACT

The scientific name of the sago palm is *Metroxylon sagu*. It is a species that can be found in wetland areas of Malaysia. The present study looks on the gene expression level of sulfate transporter and boron transporter gene by using trunking and non-trunking sago palm leaves. Liquid nitrogen was used to deactivate the nucleases in the leaves. CTAB extraction method was used for RNA extraction. DNase treatment was carried out to increase the yield of RNA. Primers were designed for targeting sulfate transporter gene and boron transporter gene. Gel electrophoresis was done for visualize the RNA, fragments then convert to cDNA and undergo amplification. The expression level of sulfate transporter gene and boron transporter gene was found to be similar in trunking and non-trunking sago trees. Thebands in sulfate transporter gene were more fainted when compared to boron transporter gene. Further research on the gene expression level of both gene should be conducted in order to understand the formation of non-trunking sago palm trees and due to its high potential in economic application.

Key words: *Metroxylon sagu*, cDNA, gel electrophoresis

ABSTRAK

Metroxylon sagu, nama saintifik untuk pokok sagu. Pokok sagu dapat dicari pada kawasan tanah lembap yang berada di Malaysia. Dalam kajian tahap ekspresi gen ke atas gen pengangkut sulfat dan gen pengangkut boron dalam daun. Cecair nitrogen telah digunakan untuk nyahaktifkan nuclease yang berada dalam daun. Larutan CTAB digunakan untukmengekstrakkan RNA. Rawatan DNase dilakukan untukmenambahkan hasil RNA. Penciptaan primer telah dilakukan untuk mensasarkan gen pengangkut sulfat dan gen pengangkut boron. Teknik makmal elektroforesis gel dilakukan untuk memvisualisasikan serpihan RNA dan mengubah kepada cDNA. Selepas itu, cDNA digunakan untuk menjalani proses amplifikasi. Tahap ekspresi daripada gen pengangkut sulfat dan pengangkut boronhampir serupa dalam pokok sagu batang dan tidakberbatang. Band dalam gen pengangkut boron lebih terang daripada gen pengangkut sulfate.Memandangkan metroxylon sagu berpotensi tinggi dalam bidang ekonomi, maka kajian yang lebih meluas terhadap kedua-dua gen ini harus dilaksanakan bagi mengetahui lebih lanjut tentang pembentukan pokok sagu yang tidak berbatang.

***Kata kunci**: Metroxylon sagu, cDNA, electroforesis gel*

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LIST OF ABBREVIATIONS

Abbreviation	Explanation
$CaCl_2$	Calcium chloride
cDNA	Complementary deoxyribonucleic acid
CTAB	Cetyltrimethylammonium bromide
dNTP	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
$MgSO_4$	Magnesium sulphate
PCR	Polymerase chain reaction
PVPP	Polyvinylpolypyrrolidone
RNA	Ribonucleic acid
TE	Tris-Cl contain EDTA
TBE	Tris-Borate EDTA
Tris	Trisaminomethane

CHAPTER 1

INTRODUCTION

1.1 Study Background

The scientific name of sago palm is *Metroxylon sagu* belongs to the Palmae family which is a species that can be found in wetland areas of Malaysia, Indonesia, and so on (Lal, 2003). Among other crops which can produce starch, *Metroxylon sagu* has the highest yield of starch. Before reaching the flowering stage, the stored nutrients will convert to starch and accumulate in its huge trunk (Singhal, 2008). Due to its high starch yield, the height of the sago palm tree can reach 9-15 m during maturation (Bergthaller, 2007). Thus, sago is famous to the natives as a staple food before the rice is being introduced and it is important in economic value because various fields need starch as the raw material in processing products.

1.2 Problem Statement

The starch that produced from sago palm trees is used in food products and non-food products such as bioethanol production after sago starch is converted to sago sugar, coating agent for biodegradable film, cosmetics dusting powder, and so on. In real life, there is always an exception that occurred which is the presence of a non-trunking sago palm tree. This condition exists in the plantation area of the sago palm. Several factors such as environmental factors include stress factors and insufficient nutrients in the soil may cause the sago palm trees to become non-trunking trees. Due to these several factors, non-trunking

sago palm trees may cause a decrement in starch production. Besides, these factors can cause the elevation of a specific enzyme in non-trunking sago palm trees such as enolase and phosphoglycerate kinase which will stimulate glycolysis and increment in metabolism rate (Hussain, 2019) which causing the decrement in starch production. Furthermore, due to the increment of demands of bioethanol production nowadays, the starch demand will continue to increase. Therefore, it is an interesting and fundamental need to study the analysis of gene expression of differentiated sago palm genes.

1.3 Objectives

In this study, the level of expression of differentiated sago palm genes via amplified cDNA was analysed. The objectives were as follows:

1. To study the extraction of RNA.
2. To investigate the conversion of cDNA from RNA.
3. To do expression study of sulfate transporter gene and boron transporter gene.

CHAPTER 2

LITERATURE REVIEW

The gene expression analysis of sulfate transporter and boron transporter of sago palm gene via cDNA amplification is investigated in this study. The differences between sulfate transporter, boron transporter and housekeeping gene will be compared and identified in the level of gene expression and gel intensity. This study also examined the sulfate transporter and boron transporter as one of the reasons leading to the presence of non-trunking sago palm trees.

Several previous studies and discussions on the expression analysis of the sulfate transporter and boron transporter can be found in San Segundo-Val & Sanz-Lozano (2016), Kim, Zakkharkin, & Allison (2010), Rodriguez-Esteban & Jiang (2017), Lim *et al* (2019), Edward (2013), Gigolashvili & Kopriva (2014), Smith *et al* (1995), Takahashi (2019), Reid (2014), and Onuh and Miwa (2021).

2.1 Gene Expression Analysis

Robert William Holley who made the very first gene sequencing in 1964. Now, Sanger sequencing was introduced by low radioactivity and high efficiency of work which become the first-generation of sequencing and able to sequence 2.88 Mb per day (San Segundo-Val, 2016).

The gene expression analysis is a sensitive biological tools or indicator that able to analyse the formation of the gene products from the corresponding source which is mRNA (Trapnell et al, 2012). The study of the expression analysis of gene by using RNA as a base

is derived from the northern blot in 1977 and during the expression analysis of the expression level of the mRNA will be quantified (Segundo-Val & Sanz-Lozano, 2016). The gene expression analysis is important because it provides the critical illustration of the gene expression to understand more regarding the molecular mechanisms of the disorder such as cancer and metabolic disorder (Kim, Zakkharkin, & Allison, 2010). Besides, the differential gene expression able to illustrate the overview of the biological difference between both normal and defective or mutated organisms (Rodriguez-Esteban & Jiang, 2017). Furthermore, in medical field, the gene expression analysis also can be used to investigate the cancer which causes by the under expressed or overexpressed of the gene by conducting a microarrays expression analysis (Kim, Zakkharkin, & Allison, 2010).

2.2 Differential Omics in Trunking and Non-trunking Sago Palm

In sago palm, there is presence of trunking and non-trunking. Non-trunking sago palm tree is the tree which faced the stunted growth which influenced by other factors (Lim et al, 2019). Trunking sago palm tree is the normal and without having any deflect of sago palm tree. In recent research, metabolize that expressed by non-trunking sago palm tree such as haloalkanesand phosphonate shows the differences when it is compared with the trunking sago palm tree (Lim et al, 2019). Furthermore, the ACP (acyl carrier protein) which is a cofactor for biosynthesis of fatty acid also differently expressed when compared with non-trunking and trunking sago palm tree (Edward, 2013).

2.3 Sulfate Transporter

In plant, sulfur is one of the essential macronutrients for biosynthesis of essential amino acids which is S-containing such as methionine, and responsible in plant growth and development. (Gigolashvili and Kopriva, 2014). The nutrient called sulfur can be obtained in the form of sulfate with the help of sulfate transporter that locate in the root. Thus, sulfate transporter functions as uptake of sulfur from surrounding soil area via root into plant and it can be found in various of plant. H^+/ SULTRs only can be found in higher plant. In sulfate transporter, there is two components will be encoded used for adapt in different environment which is high affinity component and low affinity component (Smith et al, 1995). These two protein components are called H^+/sulfate co-transporter and has a relationship with SULTRs in phylogenetic tree (Gigolashvili and Kopriva, 2014).

In *Arabidopsis thaliana*, SULTR1;1 and SULTR1;2 is sulfate transporter whereas SHST1 and SHST2 can be found in *Stylosanthes hamata*, and HVST1 can be found in *Hordeum vulgare* (Takahashi, 2019). When low concentration of sulfate in surrounding soil, the gene will encode for high affinity component to uptake of sulfate while low affinity component encodes for internal transport of sulfate from root to other parts of plant (Smith et al, 1995). In *Stylosanthes hamata*, SHST1 and SHST2 will encodes for high affinity component in sulfate transporter for uptake of sulfate in soil while there is another gene which is SHST3 can be found in *Stylosanthes hamata* for sulfate internal transport. In an experiment, sulfate transporter (SHST1 and SHST2) activity affected by external environment which is the high concentration of sulfate in surrounding area while SHST3 sensitive to high external pH (Smith et al, 1995). In nature, there always has a defect on gene. For example, there is a deficiency of double mutant lines in *Arabidopsis thaliana*, causes the redundancy in SULTR1;1 and SULTR1;2 which both of it is high affinity

transporters and leads to stunted growth (Takahashi, 2019). Since, there is few research done on sulfate transporter in sago palm tree, thus it is interesting to find out whether sulfate transporter is one of the factors causing presence of non-trunking.

2.4 Boron Transporter

In plant, boron is one of the essential nutrients which is a trace element for plant development. Boron is needed but it has to be in an appropriate amount as high amount of boron intake is toxic to the plant. But then amount of boron in soil is limited. Thus, it also causes the plant faced boron deficiency which leads to the restriction in extension in leaves' length, elongation of root and so on (Onuh and Miwa, 2021). The functions of boron transporter are to pump the boron into the cell wall by using the efflux mechanism, transportation of boron from root to shoot and prevent the plant over intake of boron causes toxicity (Reid, 2014). The uptake of boric acid from soil, it is uncharged at favourable pH (Reid, 2014).

In the past research, Arabidopsis thaliana has two types of molecules to help boron transporter which is BORs and NIPs (nodulin-26-like intrinsic proteins) (Onuh and Miwa, 2021). The BORs functions as an exportation of borate from the plant cells while NIPs functions as ease of permeation of boric acid (Onuh and Miwa, 2021). Research found that AtNIP5;1 is a NIP II protein which can be a passage f or boric acid, hence besides Arabidopsis thaliana, crop plants also have boron transporter which is AtNIP5;1 homologs and can be found in Cucumis sativus and Oryza sativa (Onuh and Miwa, 2021).

When there is a deficiency of boron in the soil, boron transporter gene, AtBOR1 in matured endodermal cell in Arabidopsis thaliana will not undergo increment of gene expression which its activity be controlled and this situation is similar to the Oryza sativa,

OsBOR1 (Reid, 2014). In the non-trunking sago palm tree, the boron transporter may also face this situation, thus it is important to find out the reason behind non-trunking sago palm tree.

CHAPTER 3

MATERIALS AND METHODS

3.1 mRNA Extraction

Mortar and pestle were used to grind about 1.2 g of sago leaf with the help of liquid nitrogen. Extraction buffer that contained 2 % w/v CTAB reagent, 2 % v/v PVPP, 25 mM of EDTA (pH 8.0), 2 M NaCl, and 100 mM of Tris-Cl buffer (pH 8.0) with the volume 15 ml and 1 % beta-mercaptoethanol were added into the tube that contained fine powder of sago leaf. The tubes were vortexed about few seconds and incubated in room temperature about 5 minutes. There was about 15 ml of chloroform added in the Nalgene tubes. The tubes that contained sample were then vortexed about 5 minutes. Next, centrifugation was done at 13200 rpm at 4 °C about 5 minutes. The top aqueous layer was carefully displaced out into a new Nalgene tube. There was about 15 ml of chloroform added in sample tube. The tubes were then vortexed for 5 minutes. After 5 minutes, the tubes were centrifuged at 13200 rpm at 4 °C about 5 minutes. The top aqueous layer was transferred into a new Nalgene tube. The equal volume of ice-cooled isopropanol was added and the tubes were incubated for 10 minutes. The tube was centrifuged about 15 minutes at 13200 rpm at 4 °C.

After that, supernatant in the tubes had to be discarded and 1 ml of ice-cooled 70% ethanol. At this stage, ethanol was used to rinse the lining of tube to wash down pellets from the lining. The sample mixture was transferred into a new 1.5 ml of centrifuge tube. The centrifuge tubes were centrifuged at 13200 rpm about 2 minutes. The supernatant was then casted away again and about 1 ml of ice-cooled 70 % ethanol added into the tubes. The tubes were centrifuged at 13200 rpm about 2 minutes. After that, the supernatant was discarded

and 1 ml of Tris-HCl buffer which also contained $MgSO_4$, and $CaCl_2$ was added followed by adding 2 µl of RNase-free DNase. At this stage, the mixture was pipetted mix to avoid any air bubble. The tubes were then incubated at room temperature for 30 minutes. After 30 minutes, 100 µl of EDTA (pH 8.4) was then added, pipette mixed and incubated on ice for another 2 minutes. The tubes then centrifuged at 13200 rpm about 15 minutes. At this stage, the top aqueous layer of mixture was then separated into 2 new centrifuge tubes with volume 540 µl for each tube. There was about 540 µl of ice-cooled isopropanol was added into 2 new tubes and mixed gently by using pipette. The tubes were incubated 5 minutes at room temperature, and the next 5 minutes on ice. The tubes were centrifuged at 13200 rpm about 15 minutes. After 15 minutes, the supernatant was discarded and 1 ml of ice-cooled 70 % ethanol was added into new tubes. The tubes were centrifuged again at 13200 rpm about 2 minutes and the supernatant was discarded. 1 ml of ice-cooled 7 % ethanol was added into tubes. Centrifugation was done at 13200 rpm about 2 minutes. The supernatant was discarded and air dry for 10 minutes. The pellets were obtained after airdried.

3.2 RNA Integrity

The obtained pellet from each tube was dissolved into 100 µl of TE buffer. 1 µl of distilled water loaded into nanodrop machine and waited for 1 minute. Then, 1 µl of TE buffer loaded into nanodrop machine as the blank solution. 1 µl of the sample was used to undergo RNA integrity check by using nanodrop machine. The three wavelengths were detected which is A260, 260/230, and 260/280 and the amount of RNA was determined.

3.3 Synthesis of cDNA

There was about 10 µl of Master Mix of 2x Reverse Transcription that contained 4.2 µl of nuclease-free water, 2.0 µl of 10x RT buffer, 0.8 µl of 100 mM, 25x dNTP Mix, 2.0 µl of 10x Anchored Oligo (dT) primer, and 1.0 µl of MultiScribe™ Reverse Transcriptase was added into tube that contained 10 µl of sample. After that, PCR was done with preset parameters and 35 cycles were done.

3.4 Primer Preparation and Design

The primer was designed by using primer3 software with preset parameters. The forward and reverse primers were designated for targeting sulfate transporter gene and boron transporter gene. Primers then checked by the software called Primer Stats. Details can be checked at Appendix B.

3.5 Amplification of cDNA

GoTaq DNA Polymerase master mix contained 3 µl of GoTaq 5X Green, 0.3 µl of dNTP, 0.45 µl of designed forward primer and reverse primer, 1.13 µl of 1.25 u GoTaq DNA Polymerase, 0.72 µl of 25 mM $MgCl_2$, and 7.95 µl of nuclease-free water. 14 µl of the master mix was then added into tube that contained 1.0 µl of the sample. PCR was done with preset parameters.

3.6 Gel Electrophoresis

There was about 0.25 g of agarose powder, and 25 ml of 1x TBE buffer were added into a microwavable flask to prepare 1 % agarose gel for gel electrophoresis. The mixture was then heated by using microwave for few minutes until it boils. Then, the flask that contained mixture was run under tap water until bubbles were formed. After that, 6.6 µl of ethidium bromide added into the Erlenmeyer flask and swirled gently. The mixture was poured gently on casting tray. The comb was put gently on the designated place and wait for gel to completely solidify for 30 minutes. After 30 minutes, the comb and buffer dams were slowly be removed and put into the casting tray. 1x TBE buffer poured into the tray until the buffer covered the whole gel. 5 µl of 1 kb DNA ladder and 5 µl of amplified cDNA samples were slowly loaded into the lane. The lid was then closed after sample loaded. The power supply was set to 140 V with 25 minutes. For mRNA extraction, gel electrophoresis also been done with the same voltage and time.

3.7 Analytical Methods

3.7.1 Comparison Within the Transporter Genes and Housekeeping Gene

The sulfate transporter gene, boron transporter gene, and housekeeping gene were compared by using the results produced from gel electrophoresis which was in the gene expression level. After that, the location of bands of both genes that were not similar to the housekeeping gene was determined and compared with the housekeeping gene.

3.7.2 Gel Intensity Identification

To identify the gel intensity of the three samples, the results from the gel electrophoresis were compared. Both sulfate transporter gene and boron transporter gene were compared with the housekeeping gene to identify the diff erences between both genes and the housekeeping gene. The size of bands and thickness of bands were compared within sulfate transporter gene and boron transporter gene from trunking and non-trunking sago palm. ImageJ software was used for analysing band intensity.