Faculty of Resource Science and Technology

NUCLEOTIDE POLYMORPHISM OF SUCROSE SYNTHASE (SUSY) FROM KELAMPAYAN (*NEOLAMARCKIA CADAMBA*)

Chong Hui Nei

Bachelor of Science with Honours
(Resource Biotechnology)
2013

NUCLEOTIDE POLYMORPHISM OF SUCROSE SYNTHASE (SUSY) FROM KELAMPAYAN (*NEOLAMARCKIA CADAMBA*)

Chong Hui Nei

A thesis submitted in partial fulfillment of the
Final Year Project 2 (STF 3015)
Bachelor of Science with Honours Resource Biotechnology

Supervisor: Dr. Ho Wei Seng
Co-Supervisor: Dr. Pang Shek Ling

Resource Biotechnology

Department of Molecular Biology

Faculty of Resource Science and Technology

University Malaysia Sarawak

2013

ACKNOWLEDGEMENTS

The completion of this FYP project required a lot of help, support, guidance and encouragement from many people. Hence, I am able to complete my research and thesis on time.

First and foremost, I would like to express my deepest appreciation and gratitude to my supervisor, Dr. Ho Wei Seng, Faculty of Resource Science and Technology, UNIMAS, for gave me an opportunity to carry out my FYP research in Forest Genomics Laboratory and for his valuable advice and encouragement till the completion of my project.

Next, I would like to express my sincere thanks to my co-supervisor, Dr. Pang Shek Ling, researcher from SARAWAK FORESTRY Corporation, for her guidance and advice during the research is carried out. I also appreciate her hard work in taught me the way to use various type of software and analyzed result.

I also would like to take this opportunity to record my sincere thanks and appreciation to all postgraduates in Forest Genomics Laboratory, UNIMAS especially Ms. Grace Ting Jen Ching and Ms. Natalie Gali for their hard work in taught me the proper way to operate various types of equipment and prepared chemicals for me to conduct experiment.

Lastly, I would like to express my sincere gratitude to my family, lab-mates and friends for gave me moral support, encouragement and help in order to complete my research and thesis.

DECLARATION

With this, I hereby declare that this thesis is my original work except for quotations and citations, all of which have been duly acknowledged. Apart from that, I would also like to declare that it has not been previously or concurrently submitted for any other degree at UNIMAS or other institutions.

_____

Chong Hui Nei

Resource Biotechnology Programme

Department of Molecular Biology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADPG	ADP-glucose
CAD	*Cinnamyl Alcohol Dehydrogenase*
CIA	Chloroform-Isoamyl Alcohol
CTAB	Cetyltrimenthylammonium Bromide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
Fru	Fructose
GAS	Gene Assisted Selection
InDel	Insertion-Deletion
NaCl	Sodium Chloride
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PVP	Polyvinylpyrrolidone
QTL	Quantitative Trait Loci
SNP	Single Nucleotide Polymorphisms
Suc	Sucrose
SuS	Sucrose Synthase

SuSy	Sucrose Synthase
UDP	Uridine Diphosphate
UDPG	UDP-glucose
UGPase	UDP-glucose pyrophosphorylase
UV	Ultraviolet

LIST OF TABLES

LIST OF FIGURES

Nucleotide Polymorphism of Sucrose Synthase (SuSy) from Kelampayan (*Neolamarckia cadamba*)

Chong Hui Nei

Resource Biotechnology Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTARCT

Neolamarckia cadamba has high commercial value as its timber used as raw materials for production of plywood, pulp and paper, boxes and crates, light construction, dug-out canoes and furniture. Sucrose synthase (SuSy) catalyzes reversible formation of sucrose (Suc) and uridine diphosphate (UDP) into fructose (fru) and UDP-glucose (UDPG). UDPG is a major glycosyl donor for biosynthesis of cellulose, callose and starch. Single nucleotide polymorphism (SNP) is an important marker for selective breeding in the selection of desirable traits without growing plant until mature to observe phenotypic traits. The aim of this study was to identify the presence of SNP(s) in *SuSy* partial genomic sequence of Kelampayan. Targeted *SuSy* sequences were amplified by polymerase chain reaction (PCR). The ~800bp of *SuSy* partial genomic sequences was subjected to BLASTn analysis to search for sequence homology in NCBI. Sequences were then aligned by using CLC Sequence Viewer 6 software to identify SNP(s). There were 37 SNPs and 2 InDel polymorphisms detected in partial sequences of *sucrose synthase*.

Key Words: *Neolamarckia cadamba*, Polymerase Chain Reaction (PCR), Single Nucleotide Polymorphism (SNP), Sucrose Synthase (SuSy), UDP-glucose (UDPG)

ABSTRAK

Neolamarckia cadamba memiliki nilai komersial yang tinggi kerana kayunya boleh digunakkan untuk menghasilkan papan lapis, pulpa, kertas, kotak, kanu dan perabot. Sukrosa sintase (Susy) memangkinkan pembentukan berbalik sukrosa (Suc) dan uridine difosfat (UDP) ke fruktosa (Fru) dan UDP-glukosa (UDPG). UDPG ialah penderma utama bagi glycosyl untuk menghasilkan selulosa, callose dan kanji. Poilorfisme nukleotida tunggal (SNP) ialah penanda yang penting untuk digunakkan dalam pembiakan selektif bagi memilih tumbuhan yang mempunyai ciri-ciri yang diingini tanpa menanam tumbuhan tersebut sehingga matang demi melihat ciri-ciri fenotip. Tujuan kajian ini adalah mengenal pasti kehadiran SNP (s) dalam urutan genomik separa SuSy dalam Kelampayan. Reaksi berantai polymerase (PCR) menghasilkan urutan Susy yang specifik. Urutan genomik separa Susy yang bersaiz ~ 800bp dianalisis dengan BLASTn untuk mencari homologi urutan dalam NCBI. Kemudian, urutan disusun dengan menggunakan CLC Sequence Viewer 6 untuk mengenal pasti SNP (s). Sebanyak 37 SNPs dan 2 InDel polimorfisme teleh dikenal pasti dalam urutan sukrosa sintase gen.

Kata kunci: *Neolamarckia cadamba*, Reaksi berantai polymerase (PCR), Polimorfisme nukleotida tunggal (SNP), Sukrosa sintase (SuSy), UDP-glukosa (UDPG)

CHAPTER I

INTRODUCTION

Forest plantation is the process of tree replanting that can produce higher volume of timber per unit area to ensure adequate supply of timber. It is estimated that planted forest can produce 15 million cubic metres of timber per year (PERKASA, 2012). Timber and timber products such as furniture is the fourth largest income contributor to our country economy after oil, gas and palm oil (PERKASA, 2012). Therefore, timber-based industry is recognized as one of the ten priority industries in Sarawak Corridor of Renewable Energy (SCORE) plan.

Sarawak State Government aspired to establish 1 million hectares planted forests by the year 2020 (PERKASA, 2012). Hence, high quality and fast growing forest trees must be planted in order to achieve Sarawak forest planted aim which are ensure continuous supply of raw materials for local timber-based industries; contribute raw materials alternative sources; rehabilitate the logged and shifting cultivation areas; and provide business and employment opportunity for local peoples (Boerjan, 2005; PERKASA, 2012). However, until June 2012, only 297, 052 hectares or 30% of forest had been planted (PERKASA, 2012). To make the aspiration to realized, government increases annual planting rate to 78, 909 hectares per year from 2012 to 2020 (PERKASA, 2012).

There are many species planted in forest plantation area including *Neolamarkia cadamba. N. cadamba* is one of the fast growing hardwood species which suitable for forest plantation programme. Therefore, the Sarawak Government has identified *N. cadamba* as one of the ten priority tree species for large-scale tree plantations (Jugi and Joe, 2008).

N. cadamba or Kelampayan is from the family of Rubiaceae. It is widely distributed in Australia, China, India, Indonesia, Malaysia, Papua New Guinea, Philippines, Singapore and Vietnam (Joker, 2000). *N. cadamba* has many economic values such as in the production of plywood, pulp and paper, boxes and crates, light construction, dug-out canoes and furniture. Besides that, *N. cadamba* is also used to relieve fever, used as tonic and for the production of yellow dye and essential oil (Krisnawati *et al*., 2011).

Sucrose synthase (SuSy) is an enzyme that catalyzes the reversible conversion of sucrose (Suc) and uridine diphosphate (UDP) into fructose (fru) and UDP-glucose (UDPG). UDPG is the major glycosyl donor for polysaccharides synthesis in all organisms especially plants (Kleczkowski, 1994). Therefore, UDPG acts as precursor or substrate for biosynthesis of cellulose, callose and starch (Kleczkowski, 1994; Amor *et al.*, 1995; Kleczkowski *et al.*, 2010).

The detection of SNP in *SuSy* partial genomic sequence is very important because SNP act as DNA marker in selective breeding. Plant breeder can select the plant with desirable characters based on marker without growing the plant until mature to observe the phenotypic traits. Before the advent of molecular markers such as SNP, plant breeder use phenotypic marker (morphological marker) to select plant with desirable traits based on the observable phenotypic traits such as plant height and grain yields (Mahajan and Gupta, 2012). This conventional method waste a lot of time to get the desired characteristics after went through several cycles of breeding and backcrossing (Mahajan and Gupta, 2012). Thus, with the advent of gene-assisted selection (GAS), the time of breeding cycle and costs for field testing are reduced while the level of selection intensity and efficiency of selection on low-heritability traits are increased (Neale and Kremer, 2011).

If SNP(s) can be identified in *sucrose synthase (SuSy)* partial genomic sequence and show association with specific phenotypic traits, it might acts as tools in gene-assisted selection (GAS) for plant breeder to select Kelampayan with desired characteristics for large scale forest plantation. As a result, it can compensate the demand of timber for various purposes. Hence, the objective of this study was to detect SNP(s) in *sucrose synthase (SuSy)* partial genomic sequence from *Neolamarckia cadamba*.

CHAPTER II

LITERATURE REVIEW

2.1 *Neolamarckia cadamba*

N. cadamba or Kelampayan is widely distributed in Australia, China, India, Indonesia, Malaysia, Papua New Guinea, Philippines, Singapore and Vietnam (Joker, 2000). The taxonomy of the *N. cadamba* is shows in the following (Dubey *et al.*, 2011):

Kingdom	: Plantae
Class	: Magnoliopsida
Order	: Rubiales
Family	: Rubiaceae
Genus	: *Neolamarckia*
Species	: *Neolamarckia cadamba*

N. cadamba can grow up to 45m tall and the trunk can grow to diameter of 100 to 160cm. The tree has broad crown and straight cylindrical bole. The shape of the leaves is ovate to elliptical with the size of 15 to 50 cm x 8 to 25 cm in green colour. The seed is in yellow or orange coloured infructescence (Joker, 2000). Figure 2.1 (a), (b), (c) and (d) show the Kelampayan tree, trunk, leaf and seed.

Every part of the *N. cadamba* has it own uses and values. For example, the timber of *N. cadamba* is used for plywood, pulp and paper, boxes and crates, light construction, dug-out canoes and furniture. Moreover, the dried bark of *N. cadamba* is used to relieve fever and use as a tonic. Besides that, the root bark is used for yellow dye production. Furthermore, the flower of the *N. cadamba* is used for production of essential oil such as Indian perfumes with sandalwood base (Krisnawati *et al.*, 2011).

(a)

(b)

(c)

(d)

Figure 2.1 (a): *N. cadamba* tree.
(Adapted from source: http://flickrhivemind.net/Tags/kadamba/Interesting)

Figure 2.1 (b): *N. cadamba* trunk.
(Adapted from source: http://www.bjkepu.gov.cn/shtp/Flora2009/25/tuanhua002.jpg)

Figure 2.1 (c): *N. cadamba* leaf.
(Adapted from source: http://www.delhitrees.com/2011/09/neolamarckiacadamba.html)

Figure 2.1 (d): *N. cadamba* seed.
(Adapted from source: http://flickrhivemind.net/Tags/kadamba/Interesting)

2.2 Sucrose Synthase (SuSy) (EC 2.4.1.13)

Sucrose synthase (SuSy) is also known as UDP-glucose-fructose glucosyltransferase. SuSy is an important enzyme that catalyzed the reversible conversion of sucrose (Suc) and uridine diphosphate (UDP) into fructose (Fru) and UDP-glucose (UDPG) in all plants cells growth (Koch, 2004). UDP-glucose (UDPG) is the precursor or substrate for biosynthesis of cell wall polysaccharides especially cellulose and callose. UDPG also serve as precursor for formation of starch.

Sucrose (Suc) + Uridine diphosphate (UDP) ⟷ Fructose (Fru) + UDP-glucose (UDPG)

Hardin *et al*. (2006) suggested that sucrose synthase enzyme is present in two forms which are soluble and membrane-associated form. The SuSy that present in membrane-associated form channel the products of sucrose breakdown, UDP-glucose to cellulose-synthase and callose synthase for cellulose and callose biosynthesis (Amor *et al*., 1995; Hardin *et al*., 2006). Amor *et al*. (1995) also stated that UDP-glucose is converted to cellulose and callose at high rates. Other that that, they also reported that SuSy enzyme catalyzed the formation of carbon needed for respiration and starch formation. Figure 2.2 shows the essential role of SuSy in the biosynthesis of cellulose.

Sucrose synthase (SuSy) also play a significant role in the biosynthesis of starch. Starch is the important form of carbon reserve in plants (Martin *et al*., 1995). UDP-glucose (UDPG) and fructose needed for formation of glucose-1-phosphate are form through the breakdown of sucrose by sucrose synthase (SuSy). Glucose-1-phosphate which later form ADP-glucose (ADPG) is then transfer to amyloplasts for starch synthesis. Moreover, ADP-

glucose (ADPG) is synthesis directly from sucrose by SuSy enzyme in the cytosol before transported to amyloplasts through an adenylate translocator for starch formation (Pozueta-Romero *et al*., 1991; Baroja-Fernandez *et al*., 2003). Figure 2.2 shows the roles of SuSy in the biosynthesis of cell wall such as cellulose and callose; and starch.

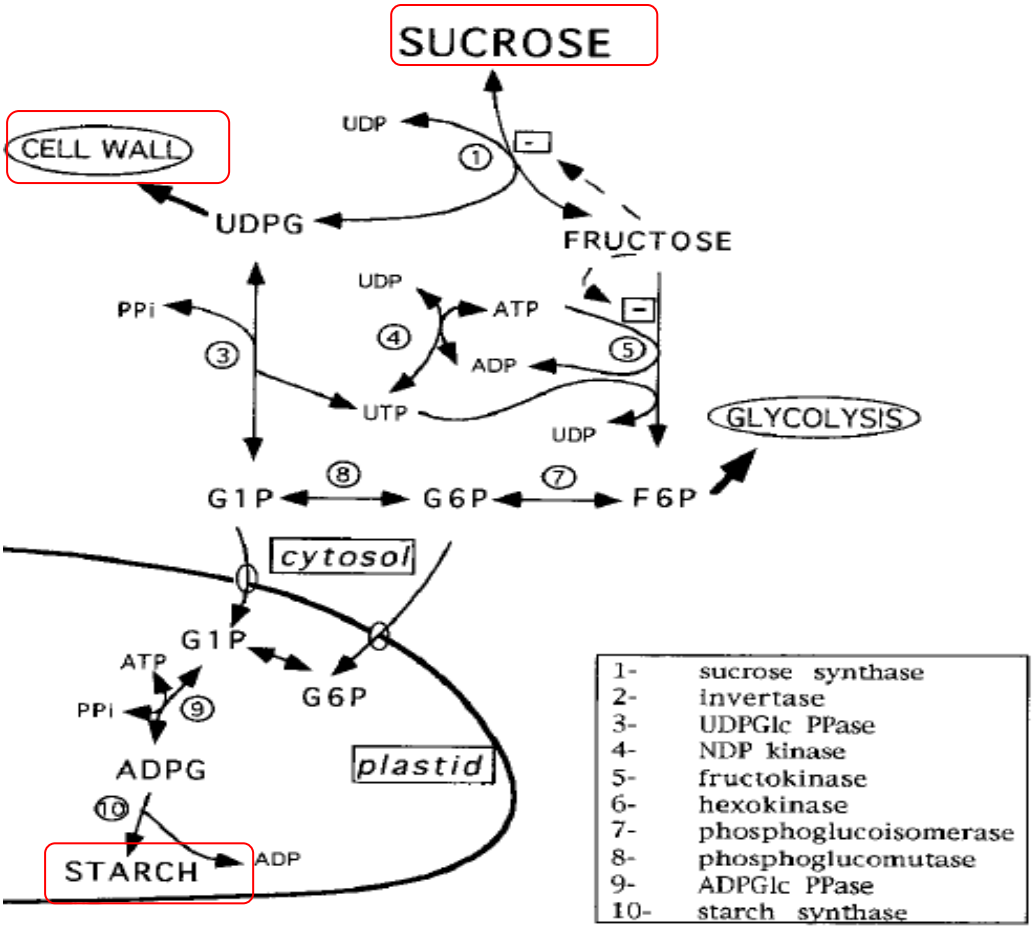

Figure 2.2: Involvement of sucrose synthase (SuSy) in the formation of cell wall and starch. (Adapted from source: http://www.plantphysiol.org/content/113/3/739.full.pdf)

Persia *et al.* (2008) proposed that SuSy functions to provide UDP-glucose for the synthesis of pectins, cellulose and callose in tobacco (*Nicotiana tabacum*) pollen tube. SuSy is present in soluble, plasma membrane, Golgi fraction and associated to the cell wall

of pollen tubes. The distribution of SuSy in different part of pollen tube is depends on the present of brefeldin A and nutrition status of pollen tube.

Sucrose synthase (*SuSy*) gene is also play an important role in cell initiation, elongation and seed development. A study was performed by Ruan *et al*. (2003) in cotton fiber through the introduction of *SuSy* suppression constructs into cotton to study the function of *SuSy* gene. Suppression of *SuSy* gene in cotton concluded that *SuSy* plays a rate-limiting role in single-celled fibers initiation and elongation process; repression in fiber development; and inhibition in endosperm and embryo development.

Xu *et al*. (2010) has conducted a study on the overexpression of aspen *sucrose synthase* gene in transgenic *Arabidopsis* plants. They transformed an overexpression vector which contains an aspen *SuS* gene (*PtrSUS1*) to study the function of *SuSy* gene. They reported that the transgenic *Arabidopsis* flowering in the early development stage, has faster growing rate and has increased tolerance to higher sucrose concentration.

2.3 Wood Formation

Plants cell wall is divided into primary cell wall and secondary cell wall (Keegstra, 2010). Secondary cell wall or wood is one of the important world trade products because it provides fuel, fibers and sawn timbers as commodities. The fuel produce from wood is important for the production of renewable energy while the fibers are important for the production of pulp, boards and paper products. Moreover, the sawn timbers are needed for building construction and furniture production (Plomion *et al.*, 2001). Due to it high commercial value, the forest tree is grow as crop in the same method of vegetables planting.

According to Demura and Fukuda (2006), wood formation involves several steps which are cell division, cell expansion, cell wall thickening, programmed cell death and heartwood (HW) formation. Firstly, procambial cells and cambial daughter cells are divided to form xylem on the inner side and phloem on the outer side. The two main types of xylem cells are xylem fibres and tracheary elements. Xylem fibres are used to support the plant bodies mechanically. Tracheary elements comprise of tracheids, metaxylem vessels and protoxylem vessels which function to transport water and solutes. Then, primary cell wall formation and modification are occurred through expansion of procambial cells and cambial daughter cells. Next, cell wall thickening is occurs to synthesis and deposite cellulose, hemicelluloses, cell wall protein and lignin (Plomion *et al.*, 2001). Lastly, programmed cell death happens to form an empty tube with secondary cell walls and heartwood. Figure 2.3 shows the wood formation schematic.

Secondary cell wall thickening is one of the most important stages in the formation of wood. The major components of wood are cellulose (40 to 50%), hemicelluloses (25%), lignin (25% to 35%), pectin and cell wall protein (Plomion *et al.*, 2001). These cell wall polysaccharides are formed from the UDP-glucose pyrophosphorylase (UGPase) product, UDP-glucose that acts as a precursor. Another enzyme which catalyzed the formation of UDP-glucose is sucrose synthase (SuSy). Thus, SuSy is one of the importance enzymes that involve in the biosynthesis of cell wall polysaccharides such as cellulose and callose.

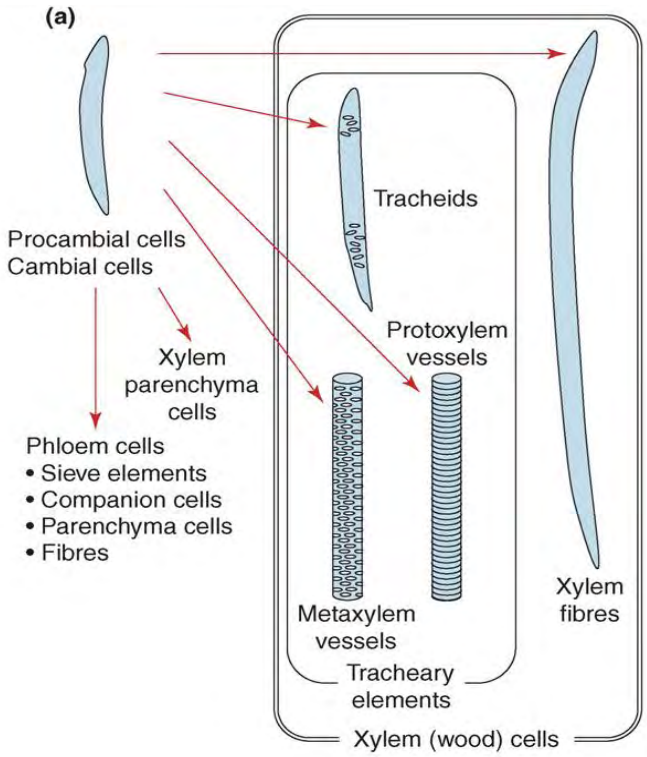

Figure 2.3: Wood formation (xylem) schematic model.
(Adapted from source: http://www.sciencedirect.com/science/article/pii/S136013850700012X)

2.4 Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms (SNPs) are also pronounced as 'snip'. SNPs are nucleotide variation in the DNA sequence of the same species. SNPs are cause by two types of nucleotide base substitution which are transition substitution and transversion substitution. A transition substitution occurs when the substitution happens in between purines (A, G) and pyrimidines (C, T) while a tranversion substitution occurs in between a purine and a pyrimidine.

SNPs can occur within coding regions (exon) and non-coding regions (intron). SNPs that occur in coding region can be divided into synonymous mutation and non-synonymous mutation. Synonymous mutation is also known as silent mutation does not

cause changes on the amino acid. As a result, the function or structure of the encoded protein will not change. Non-synonymous mutation occurs when the encoded amino acid is alters. Hence, the function or structure of the resulted protein is change. This alteration is causes by missense mutation or nonsense mutation.

The application of SNPs is act as molecular markers in human disease genetics, pharmacogenetics, breeding, bioprocess and Quantitative Traits Loci (QTL), population genetics and evolution (Liao and Lee, 2010).

In the study of population genetics and evolutionary, SNPs are useful to detect the unknown polymorphisms of DNA sequences and detect the individuals for known polymorphisms. SNP detection technologies are highly automated, efficient and inexpensive methods (Kwok and Chen, 2003).

Gene-assisted selection (GAS) is uses to select animals or plants with desirable traits without growing to maturity. This application helps to save a lot of time and money as compare to conventional method. SNPs are evolutionarily conserved and stable after went through several generations and thus suitable for marker-assisted breeding programs.

Quantitative Traits Loci (QTL) is defined as loci contain the gene which control continuous traits such as plant height and skin color. SNPs provide genotypic data for the quantitative traits loci analysis. QTL analysis is a statistical method which analyzes the association between genotype and continuous traits based on phenotypic data and genotypic data.

2.5 Application of Single Nucleotide Polymorphisms

Single nucleotide polymorphisms can be applied in marker-assisted breeding. Tchin *et al.* (2011) stated that *cinnamyl alcohol dehydrogenase (CAD)* gene has an association with the wood properties which are wood density, specific gravity and cell wall thickness of *Acacia mangium* Superbulk. There are five SNPs detected in the *CAD* exons whereas two SNPs were found in the *CAD* introns. The detected SNPs caused mutation to occur. This mutation affects the structural, functional and biochemical properties of the resulted enzyme. Consequently, biosynthesis of lignin is affected and thus the phenotypic traits of *A. mangium* Superbulk are changed.

From the study done by Garg *et al.* (2012), they mentioned that transition SNP in *TaMYB2* is associated with the level of dehydration tolerance in wheat. They had studied the level of dehydration tolerance on 28 wheats (*Triticum aestivum* L.). Their results concluded that 18 wheats were dehydration tolerance and 10 wheats were dehydration sensitive. Dehydration tolerance wheats had single nucleotide different on *TaMYB2* with the dehydration sensitive wheats. Thus, dehydration tolerance wheats had nucleotide adenine while dehydration sensitive wheats had nucleotide guanine. This variation in sequence of *TaMYB2* affects the level of dehydration tolerance.

CHAPTER III

MATERIALS AND METHODS

3.1 Collection of Leaf Samples and Inner Bark DNA

The leaf samples and inner bark DNA of *Neolamarckia cadamba* were obtained from Forest Genomics and Informatics Laboratory, FRST, UNIMAS.

3.2 DNA Isolation and Purification

3.2.1 Chemical and Reagents

CTAB extraction buffer that contains 100 mM Tris HCI (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2% Cetlytrimethyl ammonium bromide (CTAB), 1% PVP (Polyvinylpyrrolidone); liquid nitrogen, 2% (v/v) β-mercaptoethanol, chloroform/isoamyl alcohol (24:1 v/v), isopropanol and wash buffer.

3.2.2 DNA Isolation

CTAB DNA isolation method that used in this project was modified from Doyle and Doyle protocol (1990). First of all, the mixture of six mililitres of CTAB isolation buffer and 2% β-mercaptoethanol in 15 ml Falcon tube was preheated at 60ºC in the water bath for 30 minutes. The leaf samples were rinsed with ddH_2O before grinding. One gram of fresh leaf was ground into fine powder with liquid nitrogen in the pre-chilled mortar. The fine powder was transferred into the Falcon tube that contains preheated CTAB isolation buffer by using spatula. Next, the mixture of fine powder and CTAB isolation buffer was mixed gently by inverting for ten times. After that, the mixture was incubated at 60ºC for 2 hours.