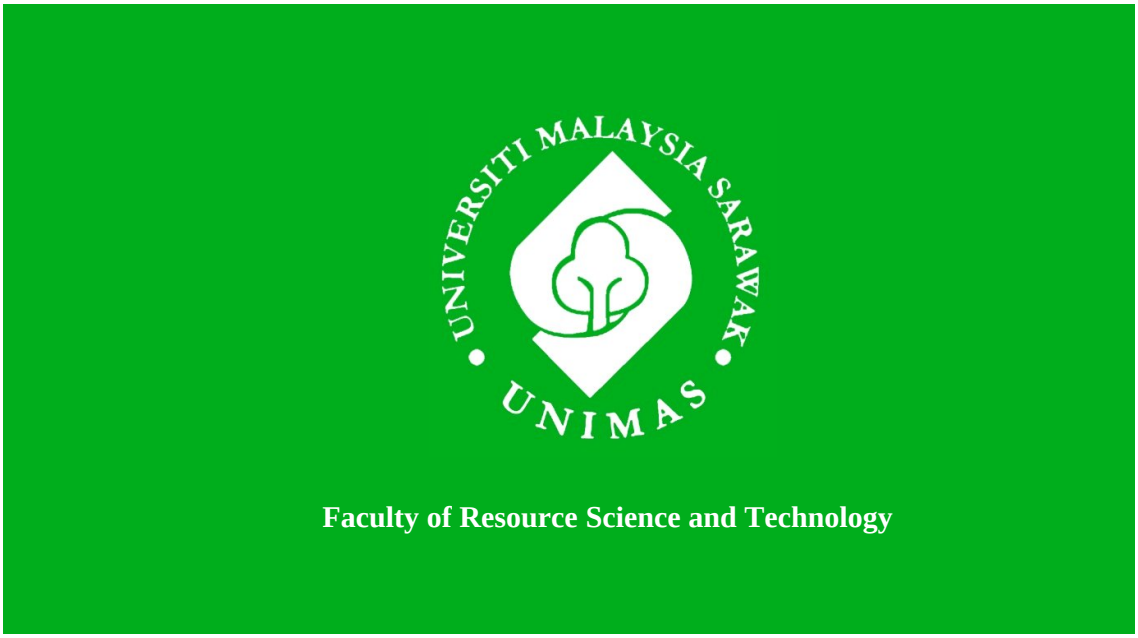


# Random Amplified Polymorphic DNA (RAPD) Profiling of Planted Sago Palm

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**Bachelor of Science with Honours**
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This project is submitted in partial fulfilment of the Final Year Project 2 (STF 3015) course

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## DECLARATION

I hereby declare that no portion of the work referred in this project has been submitted in support of application for another degree qualification of this or any other universities or institution of higher learning.

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Allysya Sylvinessa Anak Alvin

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## List of Abbreviations

| | |
|---|---|
| **bp** | base pair(s) |
| **BSA** | Bovine serum albumin |
| **CIA** | Chloroform-isoamyl alcohol |
| **CTAB** | hexadecyltrimethylammonium bromide |
| **dNTP** | deoxyribonucleoside 5'-triphosphate |
| **EtBr** | ethidium bromide |
| **ha** | hectar(es) |
| **Kbp** | Kilobase pair(s) |
| **$MgCl_2$** | magnesium chloride |
| **PCR** | polymerase chain reaction |
| **PVP** | Polyvinylpyrrolidone |
| **RAPD** | random amplified polymeric DNA |
| **Sterile $dH_2O$** | sterile distilled water |
| ***Taq*** | *Thermus aquatics* |
| **TBE** | Tris-borate-EDTA buffer |
| **TE buffer** | Tris-EDTA buffer |
| **V** | Voltage |

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## *ABSTRACT*

Sago palm (*Metroxylon sagu*) is one of the commercially important palm species in Sarawak. They can occupy a diversity of habitats at different altitudes but they show dominancy for wet habitats. Sago palm is widely planted in Sarawak due to its commercial value. The objective of study is to isolate genomic DNA from planted sago palm and to obtain DNA band profiles of the sago palm using the RAPD method. The DNA extraction of the sago palm was conducted using common CTAB extraction method. This CTAB method for DNA extraction of fresh tissue has been successfully used in laboratory for wide variety of plant families. RAPD-PCR is a method which randomly amplifies genomic DNA utilising single, short and arbitrary oligonucleotide primers. It is a fast and easy to perform since no prior knowledge on the target sequence is needed. The DNA band profiles were observed via agarose gel electrophoresis analysis. In this study, a good quality and yield of sago palm genomic DNA was extracted. Also, DNA band profiles for sago palm were successfully generated using RAPD commercial decamer primers.



Key Words: DNA profiles, RAPD-PCR, Sago palm



## *ABSTRAK*

*Sagu* (Metroxylon sagu) *adalah salah satu daripada spesis palma komersial yang penting di Sarawak. Mereka boleh tumbuh di pelbagai habitat pada ketinggian yang berbeza tetapi mereka menunjukkan dominasi di habitat yang lembap. Sagu ditanam secara meluas di Sarawak kerana nilai komersialnya. Objektif kajian ini adalah untuk mengasingkan DNA daripada sagu yang diusahakan secara ladang dan untuk mendapatkan profil jaluran DNA untuk sagu menggunakan kaedah RAPD. Pengekstrakan DNA sagu ini telah dijalankan menggunakan kaedah CTAB yang biasa digunakan. Kaedah CTAB untuk pengekstrakan DNA tisu segar telah berjaya digunakan di dalam makmal untuk pelbagai jenis keluarga tumbuhan. RAPD-PCR adalah kaedah mengamplikasi DNA secara rawak menggunakan primers oligonukleotida yang tunggal, pendek dan secara rawak. Keadah ini dapat dilaksanakan secara cepat dan mudah kerana pengetahuan mengenai urutan DNA yang disasarkan adalah tidak diperlukan terlebih dahulu. Profil jaluran DNA telah dapat dilihat melalui gel agarose elektroforesis. Dalam kajian ini, DNA genomik berkualiti dan kuantiti yang baik telah berjaya diesktrak daripada sagu dan juga profil jaluran DNA bagi sagu telah berjaya dihasilkan menggunakan RAPD primer dekamer komersial.*



*Kata Kunci: Profil jaluran DNA, RAPD-PCR, Palma sagu*

## 1.0 Introduction

In Sarawak, there are more than 200 species of palms which is native to the state (Pearce, 1991). One of them is *Metroxylon sagu* (sago palm). The sago palm usually grows in tropical regions which it can tolerate wet growing ecology, such as lowland tropical forests and freshwater swamp. Also, sago palm is considered as one of the commercially important introduced palm species in Sarawak (Pearce, 1991). It is an increasingly socio-economically important crop in South-East Asia (Kjaer *et al.,* 2004).

Sago palm is grown commercially in plantations in Malaysia and Indonesia (Kjaer *et al.,* 2004). It is widely planted in Sarawak due to its commercial value and other local uses. Commercial products from sago palm are such as sago flour and sago starches (Sarawak Tribune, 1989 as cited in Pearce, 1991). Apart from starch production, it also contributes economically to the people and important export for the state of Sarawak (Wee & Roslan, 2012). Currently, Sarawak is the world biggest exporter of sago starch, exporting 44,700 tonnes of sago starch in 2007 to Japan, Taiwan, Singapore and other countries which cost around US$ 3.4 million to US$10.8 million (Department Of Agriculture, Sarawak as cited in Wee & Roslan, 2012).

Initially, researches that were conducted on sago palm were focused to improve the growth and yield of the sago palm but later emphasis more on to the application of the sago starch (Jamel *et al*,2011). Scientists are increasingly aware that the study on palms can help to develop the economy of tropical countries (Tomlinson, 1990). However, not many molecular studies have been conducted on sago palm (Wee & Roslan, 2012). Hence, this study was conducted.

Many disease problems of economically important palms remains unsolved because lack of understanding of the genetics of the palms (Tomlinson, 1990). In order to overcome this problem, molecular study on genetic improvement of palms should be considered. In this study, RAPD method was used to generate RAPD markers which can be useful for crop improvement programs. RAPD technique is one of the widely used applications to identify marker linked to traits of interest without the necessity for mapping the entire genome (Bardacki, 2000). In the future, these generated RAPD markers can be used as genetic information that may be used for indirect selection of traits to which the markers are linked (Ranade *et al*., 2001). Most importantly, these markers can lead us to the commercially important genetic trait such as disease resistance genes (Bardakci, 2000).

RAPD-PCR is a DNA polymorphism based on PCR to amplify random DNA sequence with single primers of arbitrary nucleotide sequence (Ghany & Zaki, 2003). In RAPD, prior knowledge of the DNA sequence for the targeted gene is not needed, as the primers will bind randomly in the template sequence although exact location is unknown (Premkrishnan & Arunachalam, 2012). Not only that, the RAPD method is relatively fast, cheap and easy to perform in comparison to other methods that have used DNA markers (Bardakci, 2000). Due to it simplicity and applicability, RAPD-PCR have captivated many scientist interest (Bardakci, 2000).

For the DNA extraction of the sago palms, the standard CTAB method was used with some modification (Weising *et al*., 2005). The advantage of this method is that it has low exposure to hazardous materials because chloroform is the only hazardous material used in this extraction (Fawley & Fawley, 2004). Most importantly, this method is reliable as it is quick, simple and inexpensive (Kit & Chandran, 2010).

The objectives of this study are as follows:

a) To isolate the genomic DNA of sago palm from mature leaves.
b) To obtain DNA band profile of sago palm by RAPD method using commercial decamer primers.

## 2.0 Literature Review

### 2.1 Sago Palm

Generally, the palm tree has an unbranched trunk with single group of leaves at the top, however, the sago palm has many trunks (Holttum, 1989). Sago palm and related species that can store many amount of starch in the trunk and can grow under severe environmental conditions are considered to be potential starch resources not only for food production but also ethanol production (Ehara, 2009). The food stored in starch act like a reserve for the palms tree, either for vegetative growth, reproductive development, fruit dispersal and seedling establishment (Tomlinson, 1990).

Sago palm can be categorised as wild palm and planted palm. There are several differences between wild and planted sago palm. The wild sago palm needs to compete with other tree surrounding them, thus it make the tree shade out the palms (Flach, 1997). Thus, resulting in the stunted growth of sago palm and their starch yield is lower (Flach, 1997). Meanwhile, the planted sago palms which grown commercially in plantation is taken care by the farmers. Flach (1997) stated that planted sago palm was grown on soil that fit for productive stands are able to produce sago trunk with more starch production.

In addition, *Metroxylon sagu* has become the important agricultural crop for the state of Sarawak, Malaysia and has the largest growing area within Malaysia (Wee & Roslan, 2012). In Sarawak, the sago palm is grown commercially in swampy areas near rice fields and dwellings (Pearce, 1990). Sarawak has about 65,000 ha of which 45,000 ha are held by smallholders and 20,000 ha consists of plantations (Jamel *et al.,* 2011). Pearce (1991) stated that palms are of great importance in Sarawak today not only because it has

variety of local uses but also it acts as staple food for the local people. Other than that, the local uses of sago palm are to make handcraft such as hats and baskets from the palm leave (Pearce, 1991).

In the trade business, the entrepreneurs point out that many palms of minor economic importance might as well be developed further to become major plantation crops, by selection, cultivation, and mechanical harvesting (Tomlinson, 1990). Sago palm also considered as important bioresources for not only sustainable agriculture but also rural development in swampy areas of the tropics (Ehara, 2009). The local value of the palms is influenced by the world market fluctuation when enter the world trade and in the case of sago palm, it never competed well with other sources of starch (Tomlinson, 1990). However, it is important as staple food for the local and trade materials (Tomlinson, 1990).

**2.2 Extraction of Genomic DNA from Sago leaves**

DNA extraction is a routine step in many biological molecular studies (Chen *et al*., 2010). To obtain pure DNA sample, any contamination needs to be removed. There are three contaminations associated with plant DNA that can disturb the PCR process: polyphenolic compounds, polysaccharides and RNA (Arif *et al*., 2010).

In order to extract the genomic DNA of the *Metroxylon sagu,* the extraction method used was CTAB method with some modification (Weising *et al*., 2005). One of the advantages of using CTAB method is low exposure to hazardous materials because chloroform is the only hazardous material used in this extraction (Fawley & Fawley, 2004).

CTAB extraction method has been proven to be able to extract genomic DNA of mature mango leaves which contain high concentrations of polysaccharides, polyphenols, proteins, and other secondary metabolites that poses problem in getting good quality of extracted DNA (Azmat *et al*., 2012). Therefore, CTAB extraction method was chosen to extract mature sago leaves in this study.

**2.3 RAPD-PCR**

RAPD, also known as arbitrarily primed PCR is the method use in detection of polymorphisms without prior knowledge of nucleotide sequence (Newton & Graham, 1997). Also, the standard RAPD method also define as utilisation of short synthetic oligonucleotide of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR (Bardakci, 2000).

RAPD is based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence and the primers detect polymorphism in the absence of information on the nucleotide sequence which then the polymorphisms are used as genetic marker (Williams *et al.,* 1990). The RAPD markers are cheap and easy to use than other types of genetic markers because they are only 10 bp in length, can be randomly chosen and can be used with unknown target sequences (Premkrishnan & Arunachalam, 2012). Since the amplification of the targeted sequence does not require prior knowledge of the sequence, makes it the best method to unveil an unknown sequence of polymorphic DNA.

RAPD method is ideally suited to fingerprinting application because it is fast, requires little materials and easy to conducts (Newton & Graham, 1997). Furthermore,

RAPD most suited for the study of plant breeding such as strawberries, wheat, soya bean, tomato, potato and corn (Newton & Graham, 1997).

### 2.4 RAPD analysis on Sago Palm

The use of genetic markers to enhance the efficiency of crop improvement has long been recognised (Weck & Ashri, 1996). In the early nineties, molecular marker-mediated systems had been used in breeding program of wheat, barley, maize and rice (Weck & Ashri, 1996). Since then, newer genetic markers based on PCR, including RAPD markers were available (Weck & Ashri, 1996). In order to improve the starch production of the *Metroxylon sagu,* the RAPD approach may be apply to generate RAPD markers for the sago palms breeding and selection programs. The success to generate the RAPD marker may help to improve the growth of the sago palms in the plantation.

To date, RAPD approach is more likely used by the researchers to study the DNA profile of bacteria strain such as *Salmonella*, *Staphylococcu, W. bancroftis* and many other strains. However, the RAPD approach can also be used on plant. In recent study, the RAPD markers have been used to estimate the genetic diversity in tetraploid Alfafa population for breeding purpose (Nagl *et al.,* 2011). The genetic diversity of different varieties of Alfafa was characterized based on RAPD markers and the markers successfully detected genetic variation that occurred within each variety. The markers generated from RAPD, which are PCR-based markers, are simpler to use compare to other available DNA markers (Weck & Ashri, 1996).

Until today, there are not many studies that have been done on sago palm using the RAPD approach. In 2009, the genetic relationship of sago palm in Indonesia was revealed

using the RAPD markers (Abbas *et al*., 2009). Their study found that the sago palm in Indonesia can be divided into several groups based on their pylogenetic construction.

### 2.5 Primers

Primers play an important role in RAPD-PCR. Unlike normal PCR which uses two primers, RAPD amplification only uses single primers of arbitrary sequence (Williams *et al*., 1990). Primers used are short, single-stranded DNA molecule which complementary to the end of a defined sequence of DNA molecule (Newton & Graham, 1997). In RAPD, the primers will bind somewhere in the sequence and the exact location is also unknown (Premkrishnan & Arunachalam, 2012). Meanwhile, Bardakci (2000) stated that at an appropriate temperature during thermal cycle, oligonucleotide primers of random sequence bind several priming sites on the complementary sequence in the DNA template and produce discrete DNA products as long as the priming sites are within an amplifiable distance with each other.

Generally, the length of the primer use in a RAPD method is 10 bp long. However, several studies had been conducted using different length of primers. In a study conducted on *Staphylococcus* strain by Welsh and McClelland (1990), they independently developed a similar method as RAPD technique using primers about 15 bp long with modification on amplification and electrophoresis condition and called it the arbitrarily primed polymerase chain reaction technique (AP-PCR) in genomic fingerprinting. In other study, PCR amplification using as short as 5 bp arbitrary primers has been used producing more complex DNA fingerprinting profile (Caetano-Annoles *et. al*., 1991).

In previous study, 10 bp oligonucleotide primers were used to study the genetic relationship of sago palm in Indonesia based on RAPD markers (Abbas *et al*., 2009). Their study using RAPD primers found that the sago palm in Indonesia can be divided into several groups based on their pylogenetic construction. Other than that, there also other study on plant population that conducted their RAPD technique using 10 bp primers such as study on population of *Jacaranda decurrens* Cham (Bertoni *et al.,* 2010).

## 3.0 Materials and Methods

### 3.1 Materials

#### 3.1.1 DNA extraction method

1. Liquid nitrogen
2. CTAB buffer (2% CTAB and 1% PVP)
3. CIA (24:1)
4. Cold isopropanol
5. Wash buffer (76% ethanol and 10mM ammonium acetate)
6. TE buffer (pH 8.0)
7. 0.2% β-mercaptoethanol

#### 3.1.2 Agarose gel electrophoresis

1. 1X TBE buffer
2. EtBr solution (1.5µl / 25ml gel)
3. Agarose powder
4. 6X loading dye

### 3.1.3 RAPD PCR

1. 5X PCR buffer (Promega)
2. 2 mM dNTPs
3. 25 mM $MgCl_2$
4. 5 U/µl *Taq* DNA polymerase (Fermentas)
5. Sterile $ddH_2O$
6. Template DNA
7. 10 mM primers ($1^{st}$ BASE Inc)

## 3.2 Methods

### 3.2.1 Plant samples collection

Fresh leaves of planted sago palm (*Metroxylon sagu*) in Kota Samarahan and Mukah area were used. During the collection period, the leaves were kept cool on ice to maintain the freshness of the plant tissues. Then, the leaves were cleaned and packed inside a plastic bag and then stored at -20°C freezer prior to DNA extraction.

### 3.2.2 DNA extraction

Total genomic DNA of planted *Metroxylon sagu* was extracted using the common CTAB extraction method with some modification (Weising *et al*., 2005).

The leaf of the sample was cut into small pieces before the grinding process. Then, the small pieces of the leaf sample were ground to powder in liquid nitrogen by using mortar and pestle. Beforehand, the apparatus for the grinding process such as mortar and pestle was autoclaved to ensure the sterile condition throughout the experiment.

After the sample has been ground to fine powder it was then placed into sterile 1.5 ml centrifuge tube. Firstly, 1.0 ml of CTAB buffer was pre-heated at 65°C water bath for 10 min. After the CTAB buffer was pre-heated, 2.0 µl of β-mercaptoethanol was added to the buffer and mixed gently. After that, the mixture was incubated in a water bath at 65°C for 30 min and mixed gently every few minutes. After 30 min of incubation, remove the tube from the water bath and allowed to cool at room temperature. 200 µl of CIA was added and mixed gently

until single phase was appeared. The mixture was then centrifuge at 13, 000 rpm at 4°C for 5 min. The aqueous phase of the mixture was transferred to a new tube. Then, second CIA extraction was performed as above. For second CIA extraction, estimate the volume of transferred aqueous phases and added CIA according to the volume of the transferred aqueous phases. After the centrifugation step, the aqueous phase was transferred into a new tube and 600 ul of cold isopropanol was added and mixed gently. Next, the mixture was incubated at -20°C freezer for at least 30 min. Then, followed with centrifugation at 13, 000 rpm at 4°C for 5 min. The supernatant was discarded leaving only the pellet at the bottom of the tube. Later, 1.0 ml of wash buffer was added and mixed gently before stored the mixture in -20°C freezer for at least 30 min. Then, centrifuged again at 13, 000 rpm at 4°C for 5 min and air dry the pellet after the supernatant was discarded. The pellet was then dissolved in 20 µl of TE buffer and stored at 4°C until required.

### 3.2.3 Determination of Quality of Extracted DNA

After the extraction method, the quality of the extracted DNA was determined by agarose gel electrophoresis on a 1% (w/v) agarose gel in TBE buffer and the gel was stained using EtBr. The extracted DNA was separated at 100V for 45 min. 1kbp DNA ladder was used as size and concentration markers. The documentation of the DNA bands was performed using gel documentation system.

### 3.2.4 RAPD-PCR Analysis and Optimisation

For the RAPD-PCR analysis, seven 10-mer RAPD primers (1st BASE Inc) as listed in **Table 1** were screen to determine which primers are able to produce band. RAPD-PCR analysis was conducted using the following components: 2 µl of genomic plant DNA, 4 µl of 5X PCR buffer, 4.5 mM $MgCl_2$, 0.2 mM dNTP mix, 1U *Taq* DNA polymerase, 1.0 µM primer and sterile $ddH_2O$ in 20 µl reaction mixture. For the PCR amplification cycle, it conducted with the following conditions: 5 min initial denaturation at 95 °C and followed by 40 cycles of 30 sec denaturation at 94 °C, 1 min and 30 sec annealing at 34.5 °C, 1 min and 30 sec extension at 72 °C, and final extension of 5 min at 72 °C.

Next, optimisation on annealing temperature was conducted using the RAPD primers that give satisfactory banding pattern. The annealing temperature was conducted at 34.5°C and 37°C. The list of the primers and their sequences is summarised in **Table 1**.

**Table 1**: Sequences of seven RAPD primers that were screened before optimisation.

| Primers | Sequence |
|---|---|
| OPA 05 | 5'–AGG GGT CTT G-3' |
| OPA 07 | 5'-GAA ACG GGT G-3' |
| OPA 08 | 5'-GTG ACG TAG G-3' |
| 0PA 15 | 5'-TTC CGA ACC C-3' |
| 0PA 16 | 5'-AGC CAG CGA A-3' |
| 0PA 17 | 5'-GAC CGC TTG T-3' |
| 0PA 18 | 5'-AGG TGA CCG T-3' |

### 3.2.5 Detection of the RAPD-PCR products

The PCR products were separated using agarose gel electrophoresis on a 1.50% (w/v) agarose gel in TBE buffer at 100V. Beforehand, the gel was stained using EtBr. 1kbp DNA ladder was used to determined size of the PCR products. The documentation of the DNA bands was performed using gel documentation system.