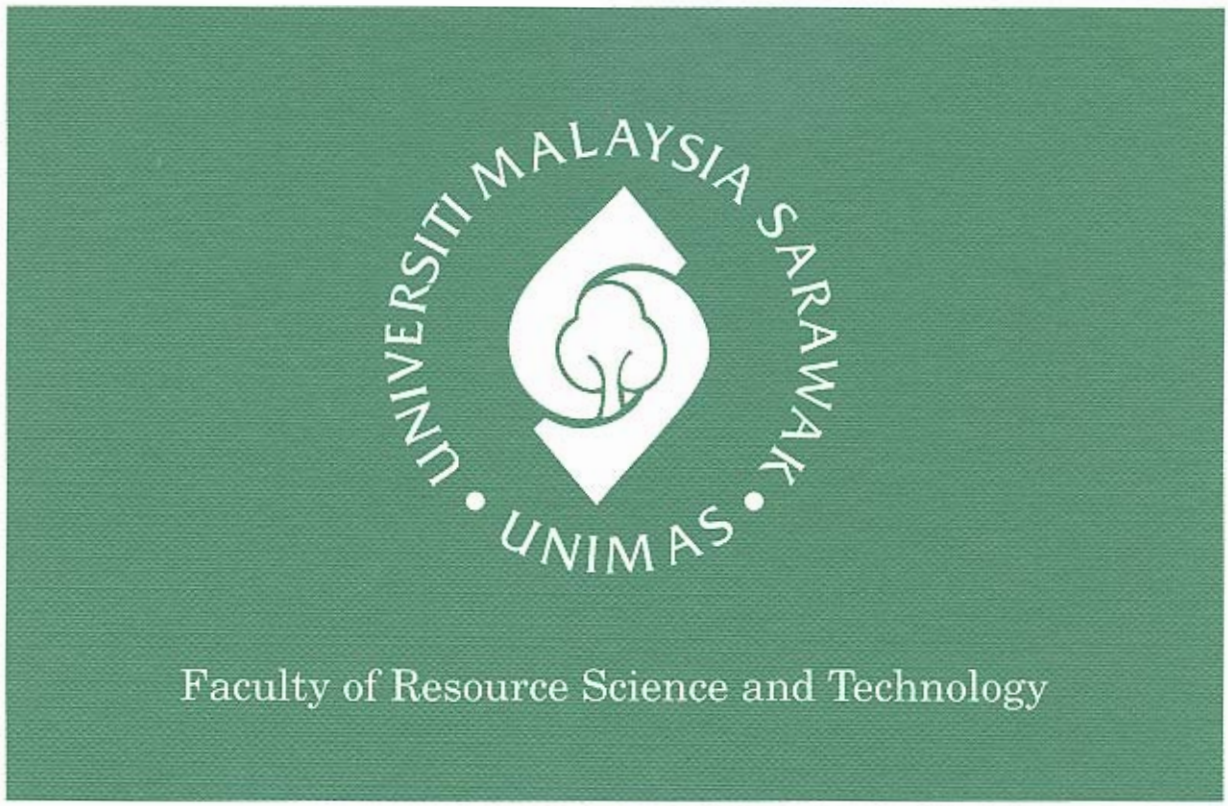

RESTRICTION DIGESTION IN SAGO USING COMMON ENZYMES

Mohammad Yazid Bin Yaman

Bachelor of Science With Honours
(Resource Biotechnology)
2004

RESTRICTION DIGESTION IN SAGO USING COMMON ENZYMES

MOHAMMAD YAZID BIN YAMAN

Resource Biotechnology Program
Faculty of Resource Science and Technology
UNIVERSITY MALAYSIA SARAWAK
March 2004

ABSTRACT

Sago is one of the most important food plants in Asia. The most popular sago palms in Malaysia normally refer to *Metroxylon sp.* such as *Metroxylon rumphii* (thorny) and *Metroxylon sago* (thornless). Sago is the cheapest source of starch in the food industries. CTAB extraction method was used to isolate high quality genomic DNA from sago plant. Extracted DNA then was digested by randomly selected restriction enzymes to investigate the recognition sites exist in sago plant. Electrophoresis was run to determine the quality and amount of DNA that has been extracted to analyze the restricted DNA digested. The result shows that, genomic DNA from sago plant has been successfully extracted using CTAB extraction method. Samples from sago plant were successfully digested using common selected enzyme, which are *Sma* I and *Hind* III.

Keyword: *Metroxylon sago,* CTAB extraction method, restriction enzyme.

ABSTRAK

Sagu merupakan salah satu tumbuhan tanaman yang penting di Asia. Pokok sagu yang paling terkenal di Malaysia merujuk kepada Metroxylon species seperti Metroxylon rumphii yang berduri dan Metroxylon sagu yang tidak berduri. Sagu merupakan sumber kanji yang paling murah dalam industri pemakanan. Kaedah pengekstrakan CTAB digunakan untuk mendapatkat hasil genomik DNA yang berkualiti dari pokok sagu. DNA yang telah diekstrak akan dicernakan menggunakan enzim penghad yang terpilih. Ini bertujuan untuk mengenalpasti tapak pencernaan spesifik DNA pokok sagu. Elektroforesis kemudiannya dilakukan untuk menentukan kualiti dan jumlah DNA yang telah diekstrak dan menganalisa DNA yang telah dicernakan. Keputusan menunjukkan bahawa genomik DNA pokok sagu berjaya dipencilkan menggunakan kaedah pemencilan CTAB. Sample pokok sagu juga telah berjaya dicernakan menggunakan enzim yang terpilih iaitu ***Sma*** **I,** dan ***Hind*** **III.**

Kata kunci: *Metroxylon sago*, kaedah pengekstrakan CTAB, enzim penghad

1.0 INTRODUCTION

1.1 SAGO

The Sago palm, commonly found in Asian countries, includes the swamp palm (*Metroxylon sp.)* and the hilly palm (species of *Arenga, Borassus, Caryota, Corypha* and *Eugeissona*) (Tan, 1983). *Metroxylon species* is a palm species and belong to the order *Arecales*, family *Palmae*, subfamily *Calamoideae*, tribe *Calameae*, subtribe *Metroxylinae,* and genus *Meroxylon*. Sago Palm is a hapaxantic and soboliferous feather-leaf palm that accumulates starch in the stem (Kiew, 1977). The most popular sago palms in Malaysia normally refer to *Metroxylon sp.* such as *Metroxylon rumphii* and *Metroxylon sago.* Sago palms can grow in fresh water swamp, mineral soil and even in peat soils which have extremely low agricultural capability. Sago is the cheapest source of starch in the food industries. These industries include high fructose syrups, glucose-maltose, dextrose, animal feed and monosodium glutamate industries (Zulpilip *et al.*, 1991).

Sago can grow well in swampy lowlands with minimal care and can maintain high productivity throughout the year. Harvesting can even be delayed without loss of yield for up to 3 years (Stanton, 1991). Thus, in 1986, under the Fifth Malaysia Plan, the first sago plantation in the world was established in Sarawak as there are great peat swamps spread over some 1.7 million ha or 12 percent of the land area of the state (Shim, 1992). The State government recognizes sago as the gold mine of the 21st century and has taken action towards its development (Anon, 1995).

Metroxylon sago or locally known as 'Balau' was exploited both as a staple and cash crop in Sarawak. It has been identified as a suitable crop for cultivation in Sarawak. Sarawak has total area of 19,720 hectares of sago palm yielding in an annual export of 43,000 tonnes of premium quality flour (Chew & Shim, 1991). Sarawak is the main exporter of sago starch to the world market such as Singapore and Japan (Zulpilip *et al.*, 1991). In 1994, sago ranks as the fifth highest revenue by earning RM 24.4 million for the state.

The objective of this study is to extract the total genomic DNA from sago plant tissue using CTAB extraction method and therefore, the difference in genomic DNA between samples taken from various locations can be determined. Also to determine common enzymes that can be used in restriction.

1.1 RESTRICTION DIGESTION

Restriction endonucleases are enzymes that cut DNA at specific sites based on nucleotide sequence. It cleaves DNA in a very specific fashion. There are three common types of restriction enzyme, Type I, Type II, and Type III. Type I restriction enzymes recognize a specific sequence, but cleave randomly. They contain three subunits. Specificity subunit (S) which responsible for DNA sequence recognition, a methylation subunits (M) which methylates adenine, and a restriction subunits (R) which cleaves DNA at an unmethylated restriction site. These enzymes require ATP, Mg^{2+}, and S-adenosylmethionine to cleave DNA.

Type II restriction enzymes are the best known of the restriction enzyme systems (Murray, N. 2000). This is because they produce specific fragments of DNA and are key to the production of recombinant DNAs. They consist of one protein which acts as a restriction enzyme and a second protein that acts as a methylase (Murray, N. 2000.). The restriction enzymes require only Mg^{2+} as a cofactor.

Type III restriction enzymes have properties intermediate between Type I and Type II. They are multisubunit enzymes, recognize specific sequences, but typically cleave 20 to 25 nucleotides away from that sequence and rarely give complete cleavage. The restriction activity requires ATP and is enhancing by S-adenosyl-methionine. Restriction enzymes can also differ in the way they cut the DNA molecule. Some enzymes cut in the middle of the recognition sequence, resulting in a flush or blunt end. Other enzymes cleave in a staggered fashion, resulting in DNA products that have short single-stranded overhangs (usually two or four nucleotides) at each end. These are often called cohesive ends, as these single-stranded overhangs could potentially come together again through complementary base-pairing.

In this study only *Restriction endonuclease Type II* will be use. Type II restriction enzymes are most commonly used for DNA analysis. It is the best enzyme to run this experiment because it is simple and have been commercialized. The recognition sequence is often a six base pair palindromic sequence (the top DNA strand from 5' to 3' is the same as the bottom DNA strand from 5' to 3'), but others recognize four or even eight base pair sequences.

There are two kind of restriction endonuclease will be use, which is *Hin*d III and *Sma* I. *Hin*d III restriction enzymes are isolated from virus *Heamophilus influenza*. *Hin*d III cut sequence

at 5'-A/AGCTT-3' and it heat inactivation is 65°C for 15 minutes. *Hin*d III under suboptimal reaction conditions will cleave secondary recognition sites which is called star activity. After 2 to 10 fold, *Hin*d III over digestion of 1μg λ DNA substrate, results in 100% cutting, more than 95% fragments can be ligated, 95% will recut. *Hin*d III is buffered aqueous glycerol solution is supplied with 10X restriction endonuclease Buffer.

Sma I restriction enzymes are isolated from *Serratia marcescens*. *Sma* I cut sequence at 5'-CCC/GGG-3' and it heat inactivation is 65°C for 15 minutes. *Sma* I produce blunt-ended fragments. After 2 to 10 fold, *Sma* I over digestion of 1μg λ DNA substrate, results in 100% cutting, more than 80% of fragments can be ligated, and more than 80% will recut. Half-life of Sma I at 37°C is approximately 15 minutes.

2.0 MATERIALS AND METHOD

2.1 Sampling.

Young leaves, old leaves, underground stems, and fronds from sago plant (*Metroxylon spesies)* were collected from various locations in Kuching and Kota Samarahan. Collected plant tissue is very perishable, requiring special handling to ensure that no loss in dry weight occurs as decomposition will reduce the dry weight, which in turn will significantly affect the plant analysis result (Lockman, 1970). Two individuals and four parts had been taken from each location. The parts were young leaves, old leaves, underground stems, and fronds. The reason why four parts

used is, to determine which parts is DNA available most. The fresh plant samples then were kept in cool environment until proper use. Samples were stored at -20°C prior to use, to preserve the quality of the DNA. The samples were labeled according to the location and parts as shown in Table 1:

Table 1: Labeling for samples

SYMBOL	Description
K	Muara Tabuan Kuching
U	UNIMAS
S	Kampung Sungai Bandung Serian
KS	Kampung Baru Kota Samarahan
YL	Young leaf
OL	Old leaf
US	Underground stem
F	Fronds

Example: KS2YL= sample taken from Kota Samarahan, individual number 2, young leaf.

KS1OL= sample taken from Kota Samarahan, individual number 1, old leaf.

U1US = sample taken from Unimas, individual number 1, underground stems.

2.2 Isolation of DNA from Sago.

Materials:

Parts from *Metroxylon sago* (Underground stems, young leaf, old leaf, fronds)

Liquid nitrogen

2X CTAB

Proteinase K

Chloroform: Isomyl alcohol (24:1)

Iced-cold Isopropanol

Tris EDTA (with RNase at 1 mg/ml)

4M NH_4OAC

RNAse

Modified CTAB Methods (miniprep):

Leaves were weight out 0.1 g and cut into sections to fit in a small mortar and grinded to a powder on liquid nitrogen. Hot 65 °C 2X CTAB of 1.3 ml was then added and grinded again with pestle. Then, 100 µl Proteinase K was added and grinded again. Mixture was the pipetted into a 1.5 ml microcentrifuge tube. Samples were then incubated for 30 minutes in a 65 °C waterbath and shaked every few minutes. Then, 600 µl (2/3 volume) of chloroform: Isoamyl alcohol (24:1) was added in and shaked well for 2 minutes. Mixture was then centrifuged for 15 minutes at 4 °C.

The tubes from centrifuge retrieved carefully. The aqueous layer was removed and placed in a 1.5 ml microcentrifuge tube. After that, 600 µl (2/3 volume) of ice-cold isopropanol (-20 °C) was added. Mixture was then inverted several times to mix and placed in the -20 °C freezer for at least 30 minutes. The tubes were then centrifuged for 15 minutes. The liquid were then poured off and the pellets were dried. When the pellets are dried, 36.6 µl of TE with RNAse A was added and mix to get the pellet into solution. Then, 1.0 ml of washing buffer was added and mixture was stored at -80 ^{0}C for 20 minutes. Tubes were then centrifuged for 15 minutes. The liquid was then poured off and the pellets were dried. The DNA pellets were then resuspended in 100 µl of TE.

2.2.1 Agarose gel electrophoresis

Materials

Electrophoresis Equipment

Ficol Loading Dye Buffer (6X)

Agarose powder

Standard DNA Ladder Marker (*Hind* III digest of λ phage DNA)

Sterile distilled deionized water (ddH_2O)

10 mg/ml Ethidium bromide (EtBr Stock Solution)

TBE Buffer (10X)

***Preparation of TBE (10X)**

An amount of 27 g of Tris Base and 13.75 g of Boric acid were weight and added to 175 ml of distilled deionized water. Mixture was then dissolved by stirring with magnetic stirrer and 10 ml of 5M EDTA pH 8.0 was added. The volume was adjusted to 250 ml with distilled deionized water. Mixture then autoclaved and stored at room temperature. For working solution, 100 ml of 10X TBE was diluted with 900 ml distilled deionized water to make it 1X TBE.

2.2.2. Preparation of gels

Agarose gel was prepared by added 0.9 g of agarose powder in 100 ml 1X TBE Buffer. Mixture was then heated in the microwave oven until it melted or completely dissolved. At this stage, boiling must be avoided. Appropriate gel comb size was used based upon the number of samples and then positioned in the gel tray. The melted agarose was then poured onto the gel tray and allowed to solidify about 30 minutes at room temperature.

2.2.3. Preparing samples and Running gels.

The comb was removed gently by puling evenly upward. The gel tray was positioned in the electrode chamber and covered with gel running buffer (1X TBE). When the agarose gel and the well were submerged and filled, the samples were then loaded into the wells. Samples were prepared by mixing 4 µl of DNA sample with 2 µl of Ficol 6X loading dye. The samples were