UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERIZATION OF AVNA LIBRARY FROM THE FLESH OF DURIAN (DURIO ZIBETHINUS MURR.) CLONE D24

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Dedicated to my parents

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfilment of the requirement for the degree of Master of Science

MOLECULAR CHARACTERIZATION OF cDNA LIBRARY FROM THE FLESH OF DURIAN (*Durio zibethinus* Murr.) CLONE D24

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April 2005

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The genus *Durio* is originated from Malaysia. Out of 198 registered clones, D24 is known as the best variety not only in Malaysia but also in the world. However, molecular genetic study on durian is still limited. This study was one of the initial molecular work which could contribute to the database of clone D24 durian. Firstly, we have compared three conventional methods for total RNA isolation from different types of durian tissues. The results showed that the CTAB procedure produced the best yield and high quality of total RNA from 4-month old durian flesh. The yield ranged from 75.8 μg to 272.5 μg per gram of frozen durian tissue with an average purity ratio A_{260}/A_{280} of 1.78. The RNA from 4-month old durian flesh of clone D24 was successfully used for the construction of a cDNA library.

The titer of the primary cDNA library was 5.1×10^6 pfu/ml. The percentage of recombinant plaques was 99% which indicated a sufficient cDNA library

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quality for full-length cDNA screening. A total of 30 randomly collected clones were generated from the cDNA library. Based on the comparison of these sequences with the GENEBANK, the sequences were classified into 7 groups according to their putative functions i.e. general metabolism (10 sequences); DNA and protein synthesis (4 sequences); glycosylation and transport proteins (1 sequence); respiration chain and photosynthesis (1 sequence); regulation mechanism (6 sequences); immunology (1 sequence); and novel genes (7 sequences). The initial molecular biology information of durian will also support further basic molecular work for genetic engineering and crop improvement application in the future.

Consequently, two interesting clones, putative FKBP12 and quinone reductase, were subjected to further characterization. These genes were found to be present in the genome as single copy genes and expressed not only in the 4-month old durian flesh but also in other tissues such as the leaves and young flower buds. However, these genes were not found to be expressed in ripening flesh tissues. The former, FKBP12 has a main function of dissecting higher plants Ca^{2+}-dependent signal pathway. The latter, quinone reductase plays an important role in xenobiotic detoxification.

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PERPUSTAKAAN SULTAN ABDUL SAMAD
UNIVERSITI PUTRA MALAYSIA

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia
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PENCIRIAN MOLEKUL PERPUSTAKAAN ISI DURIAN KLON D24

Oleh

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Pengerusi : Profesor Madya Suhaimi bin Napis, Ph.D.

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Genus Durio (*Durio zibethinus Murr.*) adalah berasal dari Malaysia. Daripada 198 klon durian yang telah didaftarkan, klon D24 merupakan klon yang terbaik di Malaysia malahan di seluruh dunia. Walaubagaimana pun, analisis genetik molekular ke atas durian masih terhad. Kajian yang telah dijalankan ini merupakan langkah awal dalam kajian molekular ke arah menyumbangkan data molekular bagi durian klon D24. Pada peringkat pertama kajian, perbandingan terhadap tiga kaedah pengekstrakan RNA telah dikaji dengan menggunakan pelbagai jenis sampel tisu durian. Keputusan menunjukkan kaedah CTAB memberikan hasil RNA yang paling memuaskan dari segi kualiti dan kuantiti berbanding dengan dua kaedah yang lain. Hasil RNA yang diperoleh adalah dalam lingkungan 75.80 ug hingga 272.5 ug daripada setiap gram sampel tisu durian yang digunakan. Hasil tersebut memberikan purata nisbah ketulenan A_{260}/A_{280} sebanyak 1.78. Perpustakaan cDNA telah berjaya dibina dengan menggunakan sampel RNA daripada isi durian yang berusia empat bulan.

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Titer bagi perpustakaan cDNA primer adalah berjumlah $5.1x10^6$ pfu/ml. Peratusan plak rekombinan yang terhasil adalah 99%, menunjukkan kualiti pupustakaen cDNA yang mencukupi untuk penyaringan. Sebanyak 30 klon telah dipencilkan daripada perpustakaan cDNA ini. Berdasarkan perbandingan homologi antara klon dan GENEBANK, jujukan-jujukan tersebut boleh dikelaskan kepada tujuh kumpulan berdasarkan kepada anggapan fungsi iaitu metabolisme umum (10); DNA dan sintesis protein (4); glikosilasi dan protein pengangkutan (1); respirasi dan fotosintesis (1); mekanisme regulasi (6); imunologi (1) dan gen novel (7). Adalah diharapkan maklumat biologi molekul durian ini pada masa depan dapat menyokong kajian molekul asas lain untuk kerja-kerja kejuruteraan genetik dan peningkatan hasil durian.

Dua klon yang menarik, iaitu gen FKBP12 dan gen bagi quinone reductase telah didalami dalam kajian yang seterusnya. Gen-gen ini adalah didapati sebagai gen tunggal didalam genom dan diekspresi bukan sahaja didalam isi durian berusia empat bulan, malahan beberapa tisu durian lain seperti daun dan putik bunga muda. Walau bagaimanapun, gen-gen ini tidak dieskpresi oleh isi durian yang masak. FKBP12 berfungsi dalam laluan isyarat penggantungan ion kalsium bagi tumbuhan peringkat tinggi. Bagi quinone reductase pula, ia memainkan peranan yang penting dalam detoksifikasi xenobiotik.

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AP-PCR	Abitrarily primer polymerase chain reaction
ATP	Adenosine triphosphate
BLAST	Basic local alignment search tool
BSA	Bovine serum albumine
Ca	Calcium
CAPS	Cleaved amplified polymorphic sequences
cDNA	Complementary deoxyribonucleic acid
cpDNA	Chloroplast deoxyribonucleic acid
CTAB	Hexadecyl-trimethyl ammonium bromide
DAF	DNA amplification fingerprinting
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
DTT	Dithiothreitol
EB	Elution buffer
EDTA	Ethylene-diamine-tetra acetic acid
EST	Expressed sequence tag
EtBr	Ethidium bromide
F buffer	Formaldehyde buffer
GTTC	Guanidine thiocynate
HCl	Hydrochloric acid

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IPTG	Isopropyl β-D-thiogalactopyranoside
ITS	Internal sequence region
LB	Luria bertaini
LB-Broth	Luria bertaini broth
LiCl	Lithium chloride
$MgSO_4$	Magnesium sulphate
MOPS	3-[N-morpholino] propanesulfonic acid
mRNA	Messenger ribonucleic acid
NaOC	Sodium acetate
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
NZY	Z-amine – Yeast extract
PABP	Polyadenylate-binding protein
PCI	Phenol: chloroform:isoamyl alcohol
PCR	Polymerase chain reaction
PVP	Polyvinyl polypyrrolidone
RAF	Randomly amplified DNA fingerprinting
RAPD	Randomly amplified polymorphic DNA
rDNA	Ribosome deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
RT	Reverse transcriptase
SDS	Sodium dodecyl sulfate
SM	Sodium chloride-Magnesium sulfate buffer

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SNP	Single nucleotide polymorphism
SSC	Sodium saline citrate
SSR	Microsatellite
TAE	Tris- acetate- EDTA buffer
TE	Tris-EDTA buffer
UV	Ultraviolet
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside

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LIST OF SYMBOLS AND UNITS

α	Alpha
bp	Base pair
β	Beta
cm	Centimeter
°C	Degree celsius
U	Enzyme unit
γ	gamma
g	Gram
g	Gravity (relative centrifuge force)
kb	Kilo-base pair
kDa	Kilodalton
kg	Kilogram
µg	Microgram
µL	Microliter
mg	Milligram
mL	Milliliter
mM	Millimolar
M	Molar
ng	Nanogram
ODx	Optical density at wavelength x nanometer
%	Percent
pmol	Picomole
pfu/mL	Plaque forming units per milliliter

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rpm	Round per minute
$	United States Dollar
V	Voltage
Vol	Volume
v/v	Volume per volume
w/v	Weight per volume

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CHAPTER 1

INTRODUCTION

Durio zibethinus Murr., one of the most well known tropical fruits in the world, is native to South East Asia with its center of diversity in Borneo. It is considered as highly priced fruit in culturally, and economically aspects in South-East Asia and sometimes referred to as 'The King of Fruits' (Zappala et al., 2002). However, westerners often quickly retreat or decline any invitation to try the unique taste experience of the King of Fruits. On the other hand, Alfred Russel Wallace said "to eat Durian is a new sensation, worth a visit to the East to experience" and "the more you eat of it, the less you feel inclined to stop". They also compared the role of Durian in South-East Asian societies as to Champagne in the West (Piper, 1989).

In Malaysia, a total of 179 clones had been registered, and Clone D24 in particular has been a popular commercial cultivar (Chan, 2000). Some other clones (D2, D98, etc) are much sought after and sold at a higher price in the market. The value of durian exports alone accounted for over 40% of total fruit exports in 1989 (Ali, 1993). However, little research has been carried out in the past on durian.

Recently, the improvement of fruit production and quality is the main strategy of the national agro-industry, of which eight fruits have been given the priority for research and production including durian for the fresh market. Application of molecular approach to develop durian variety was emphasized in the

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National Agriculture Policy (NAP3) (Rahman, 2000). For this reason, construction of a cDNA library from durian flesh will play an important role in the future durian breeding and improvement.

A cDNA library contains DNA fragment that is derived from cellular mRNA by reverse transcriptase. The advantage of a cDNA library is that genes may be studied as they exist in the organism with control elements intact. A cDNA clone would have no introns or control elements, but if supplied with bacterial promoter, it can still be expressed. Based on the database from a cDNA library, new genes can be discovered and studied such as genes controlling odor, color or with medicinal application. Moreover, crop improvement and the molecular marker system may be applied with the valuable information from a cDNA library.

The objectives of this study were:

To evaluate effective methods for the isolation of total RNA from different types of durian tissue;

To construct a cDNA library;

To generate and analyze some clones from the cDNA library; and

To isolate and characterize some intersting genes in durian.

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CHAPTER 2

LITERATURE REVIEW

2.1 Durian

2.1.1 Taxonomic History

In the early time, the family Bombacaceae includes three sub-tribes, *Adansonieae, Matisieae*, and *Durioneae*. The divisions were classified based on differences in leaf morphology. The genus *Durio* belongs to the *Durioneae* which is characterized by simple entire penninervate leaves (Brown, 1997). The Bombacaceae actually shares more anatomical similarities with the Malvaceae (especially the genus Hibiscus) than they do with the Sterculiaceae. The difference in chromosome number is one of the evidence that can be used to distinguish Durioneae from the other Bombacaceous tribes. Chromosome counts of durian (2n=28) is lower than those of Bombacaceous species (2n=72) from other tribes but closer to those found in related Sterculiaceae, Tiliaceae and Malvaceae (Baum and Oginuma, 1994). Recently, phylogenetic relationship within *Durioneae* was studied based on sequences of chloroplast (ndhF) and nuclear ribosomal (ITS). The data suggested that *Durio* and its relatives appear to be more closely related to the tribe *Helicters* and *Reevesia* (Sterculiaceae) than to Bombacaceae (Alverson *et al.*, 1999; Nyffeler and Baum, 2000).

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The genus of *Durio* consists of 28 species. However, there are only six species with edible fruits, of which include *D. oxleyanus* Griffith, *D. graveolens* Becc., *D. dulcis* Becc., *D. grandiflorus* Becc., *D. kutejensis* Becc. and *D. zibethinus* Murr. (Kanzaki *et al.*, 1998). Among them, *Durio zibethinus* Murr. is one of the most interesting tropical fruits found in South East Asia. The species *Durio zibethinus* was attributed by Murray in 1774 (Brown, 1997).

2.1.2 Morphology

Durian tree, a tall handsome tree reaching up to 37 m in height, is usually straight, rough, with peeling trunk up to 1.2 m in diameter. The bark is grey or reddish–brown (Brown, 1997). The evergreen, alternate leaves are densely covered with golden hair on the underside. The leaves are 6-22 cm long and 2.5-8 cm wide, depending on the variety (Yaacob and Subhadrabandhu, 1995). The fruits are egg-shaped or oblong to nearly round. The shape may be short or long depending on the variety and ranges from 15 to 30 cm long from 12.5 to 50 cm wide, and up to 8 kg in weight (Morton, 1987). The yellow or yellowish skin is covered with many pointed spines about 1-2 cm long, and very hard, almost woody, protecting the flesh inside when the fruit falls from a great height. A mature tree, between 15–20 years old, can produce 100 or more fruits per year while a young tree bears about 50 fruits, depending on the variety and location (Piper, 1989). The aroma develops as the fruit ripens and reaches its peak 2-3 days after the fruit drops. At this particular time, the durian's taste is very delicious or the flavor is at its best.

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