UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND TRANSCRIPT ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES FROM FLORAL ORGANS OF PIGEON ORCHID (*DENDROBIUM CRUMENATUM*)

NG BOON ZEAN

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IDENTIFICATION AND TRANSCRIPT ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES FROM FLORAL ORGANS OF PIGEON ORCHID (*DENDROBIUM CRUMENATUM*)

By

NG BOON ZEAN

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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of the requirement for the degree of Master of Science

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Chairman : Faridah Qamaruz Zaman, PhD

Institute : Institute of Bioscience

Dendrobium is a member of the Orchidaceae, which is one of the largest families of flowering plants. Over the centuries, orchid flowers have evolved with myriad forms and devices to attract particular pollinators. This led to the development of highly modified organs which not only contributed to the morphological diversity of orchid flowers, the success of pollination for some, but it also provided opportunities for accessing gene functions. Plant reproductive biology broadly comprises a network of biological events that represents a continuum of developmental processes beginning with the development and eventually culminating in the death of flowers. Due to the complex processes and biological mechanisms involved, analyses using molecular tools provided an opportunity to study the molecular elements that underlies the reproductive biology of orchids. This preliminary study was initiated to identify genes that are differentially expressed and putatively involved in the reproductive biology of the Pigeon orchid. Genes showing differential expression among the sepal, petal, lip and column of the Pigeon orchid were targeted using a derivative of the differential display technique known as GeneFishing$^{\text{TM}}$ technology. Ten differentially expressed transcripts were identified where sequence analyses revealed most of the transcripts include genes that were previously uncharacterized in the orchid system. Three partial cDNA clones which encode for small heat shock protein (A1C1-8), pectin methylesterase enzyme (A3C1-1) and 14-3-3 protein (A8C1-9) were selected for expression studies. Comparative expression profiling of these clones in other organs of the Pigeon orchid such as roots, stems and developing flower buds 3-4 days before anthesis via quantitative real-time RT-PCR revealed all three clones may putatively exhibit flower-specific expression. The results from real-time RT-PCR strongly suggest that these transcripts may possibly be involved in reproduction-related processes based on its localization in the column of the Pigeon orchid flowers.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Master Sains

IDENTIFIKASI DAN ANALISA TRANSKRIP GEN-GEN YANG MEMPUNYAI EKSPRESI BERBEZA DALAM ORGAN BUNGA ORKID MERPATI (*DENDROBIUM CRUMENATUM*)

Oleh

NG BOON ZEAN

Disember 2007

Pengerusi : Faridah Qamaruz Zaman, PhD

Institut : Institut Biosains

Dendrobium merupakan salah satu genus dalam Orchidaceae, yang merupakan famili terbesar dalam tumbuhan berbunga. Sejak berabad-abad yang lalu, bunga orkid telah mengalami evolusi dengan kewujudan pelbagai cara untuk menarik agen pendebungaan yang khusus. Perkembangan ini telah menyebabkan modifikasi organ yang bukan sahaja menyumbang kepada kepelbagaian morfologi bunga orkid dan kejayaan pendebungaan untuk sesetengah spesies, malah ia turut membuka peluang untuk mengkaji fungsi-fungsi gen. Biologi reproduksi tumbuhan merangkumi satu rangkaian proses-proses biologi yang bersinambungan, bermula dengan pembentukan dan berakhir dengan kelayuan bunga. Disebabkan proses-proses dan mekanisme biologi kompleks yang terlibat, analisa menggunakan teknik biologi molekul mewujudkan peluang untuk mengesan unsur-unsur genetik yang terlibat dalam biologi reproduksi orkid. Kajian in diasaskan untuk mengesan gen-gen yang mempunyai ekspresi berbeza dan kemungkinan terlibat dalam biologi reproduksi orkid merpati. Gen-gen yang mempunyai ekspresi yang berbeza dalam sepal, petal, lip dan column orkid merpati dikenalpasti dengan menggunakan salah satu cara pameran pembezaan (differential display) iaitu teknologi GeneFishing$^{\text{TM}}$. Sepuluh transkrip gen yang mempamerkan expresi berbeza berjaya dikenalpasti dan analisa jujukan gen menunjukkan kebanyakan transkrip tersebut belum pernah dikaji dalam sistem orkid. Tiga klon cDNA yang mengandungi jujukan gen yang mengkodkan protein heat-shock kecil (A1C1-8), enzim pektin metilesterase (A3C1-1) dan protein 14-3-3 (A8C1-9) dipilih untuk pencirian ekspresi. Perbandingan profil ekspresi klon-klon tersebut dalam organ-organ lain seperti akar, batang dan putik bunga 3-4 hari sebelum bunga mekar yang diselidik dengan menggunakan analisa kuantitatif real-time RT-PCR menunjukkan ketiga-tiga klon mempunyai ekspresi spesifik pada bunga. Kesimpulan daripada analisa real-time RT-PCR berdasarkan ekspresi gen-gen tersebut dalam column orkid merpati mengesyorkan gen-gen tersebut berkemungkinan besar terlibat dalam proses berkaitan dengan reproduksi.

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I certify that an Examination Committee has met on 3rd December 2007 to conduct the final examination of Ng Boon Zean on her Master of Science thesis entitled "Identification and Transcript Analysis of Differentially Expressed Genes from Floral Organs of Pigeon Orchid (*Dendrobium crumenatum*)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were as follows:

Maziah Mahmood, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Ho Chai Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Parameswari Namasivayam, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Rofina Yasmin Othman, PhD
Professor
Institute of Biological Sciences
Universiti Malaya
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 1 April 2008

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Faridah Qamaruz Zaman, PhD
Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Raha Hj. Abdul Rahim, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Umi Kalsom Abu Bakar, PhD
Malaysian Agricultural Research and Development Institute (MARDI)
(Member)

AINI IDERIS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 April 2008

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

NG BOON ZEAN

Date: 29 February 2008

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
λ	Lambda
µg	Microgram
µl	Microlitre
µM	Micromolar
%	Percentage
ºC	Degree celcius
2-ME	β-mercaptoethanol
ABA	Abscisic acid
ACP	Annealing control primer
BLAST	Basic Local Alignment Search Tool
bp	Basepair
CBD	Convention on Biodiversity
cDNA	Complementary deoxyribonucleic acid
CDS	Coding region
CITES	Convention on International Trade in Endangered Species
CTAB	Hexadecyl (or cetyl) trimethyl ammonium bromide
dATP	Deoxyadenine triphosphate
dCTP	Deoxycytosine triphosphate
DDRT-PCR	Differential display reverse transcription PCR
DEAE	Diethylamino ethanol
DEG	Differentially expressed gene
DEPC	Diethyl pyrocarbonate

dGTP	Deoxyguanine triphosphate
dH_2O	Distilled water
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
dTTP	Deoxythymine triphosphate
ECM	Extracellular matrix
EDTA	Ethylene diamine tetracetate
EST	Expressed sequence tag
EtBr	Ethidium bromide
F buffer	Formaldehyde buffer
FRIM	Forest Research Institute of Malaysia
g	Gram
gDNA	Genomic deoxyribonucleic acid
HCl	Hydrochloric acid
HSP	Heat shock protein
kb	Kilobasepair
kDa	Kilodalton
l	Litre
LB	Luria-Bertani
LiCl	Lithium chloride
M	Molar / molarity
mg	Milligram
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate

min	Minutes
ml	Milliliter
mM	Millimolar
MMLV	Maurine Moloney Leukaemia Virus
MOPS	3-(N-morpholindo) propane-sulphonic acid
mRNA	Messenger ribonucleic acid
M13F	M13 forward
M13R	M13 reverse
N	Normality
NaCl	Sodium chloride
NaOAc	Sodium acetate
NaOH	Sodium hydroxide
NBT	Nitroblue tetrazolium chloride
NCBI	National Centre for Biotechnology Information
ng	Nanogram
NH_4OAc	Ammonium acetate
nm	Nanometer
nmol	Nanomole
OD	Optical density
PCR	Polymerase Chain Reaction
PR	Pathogenesis-related
PVP	Polivinylpyrrolidone
RAP-PCR	RNA arbitrarily primed PCR
RNA	Ribonucleic acid
RNase	Ribonuclease

rpm	Revolution per minute
rRNA	Ribosomal ribonucleic acid
RT	Reverse Transcriptase
SAAP	Streptavidin-alkaline phosphatase
SAM	Shoot apical meristem
SDS	Sodium dodecyl sulphate / sodium lauryl sulphate
Sec	Second
SSC	Standard saline citrate
T_a	Annealing temperature
T_m	Melting temperature
TAE	Tris-acetate-EDTA buffer
TE	Tris-EDTA buffer
TM	Trademark
Tris	Tris[hydroxymethyl]aminomethane
Tris-HCl	Tris hydrochloride
U	Unit
UKBAP	UK Biodiversity Action Plan
UTR	Untranslated region
UV	Ultraviolet
V	Volt
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
x	Times

CHAPTER 1

INTRODUCTION

Flowering plants or Angiosperms made their evolutionary debut more than 140 million years ago and had since become the most successful and diverse group of plants in the history of the earth. Despite its diversity, at the heart of what defines the Angiosperms is the flower. The diversity of Angiosperm flowers are overwhelming, for example, the flowers of Orchidaceae and Liliaceae have three organs (sepals, petals or tepals) in their respective floral whorls while the flowers of Brassicaceae have four and Rosaceae, five. One may wonder what function would such variations serve? The immediate function that springs to mind is obviously pollination and reproduction, since flower cradles the reproductive organs of plants. Plant reproductive systems are comprised of a network of developmental processes manifested by several events, such as formation of floral organs, pollination, fertilization, embryogenesis, and seed and fruit development to flower senescence. Since all these events are inter-related, a better understanding of the plant reproductive system can only be achieved through research in the various developmental processes regulating each event.

Naturally, reproduction in Angiosperms would never be possible without the existence of the flower or more specifically, the sexual organs within flowers. Although it is undeniable pollinators that are drawn to the attractive display of floral organs play an equally important role in plants that rely on external assistance for reproduction. Since attracting pollinators can be a really competitive business, certain plants had evolved with modifications to their flower morphology to stay ahead. Orchids are one family that features such adaptations in floral architecture as well as in colour patterns and fragrances. Flower development is the initial step of reproductive organ construction and a prerequisite for seed development. Flower development in orchids thus becomes an important field of research, which holds major evolutionary as well as economic importance as it can potentially affect the other plant processes such as pollination and gene flow, as well as fruit production and seed dispersal (Soltis, 2002).

Dendrobium belongs to the Orchidaceae and like all flowers in this family it diverges from the structure of conventional flowers, where fusion of the androecium and gynoecium gave rise to the structure called the gynostemium or commonly referred to as the column while modifications of the petal formed the labellum or lip. The highly evolved flowers of the Orchidaceae are believed to be products of co-evolution with specific pollinators. Such morphological variations provide unique opportunities for assessing various gene functions. Orchid molecular biology however, is a young field where the availability of information on gene expression is still rather limited. It is hoped that a better understanding of the orchid reproduction system can be established by isolating the differentially expressed genes involved in developmental processes such as those related to flower development, pollination, fertilization, embryogenesis, seed and fruit formation and flower senescence.

This study therefore aims to isolate and characterize genes that are differentially expressed in the floral organs of the Pigeon orchid (*Dendrobium crumenatum*). Genes with specific, up-regulated or down-regulated expression in a certain floral organ of the Pigeon orchid were targeted using a modified version of the differential display technique known as GeneFishing^{TM}. It is hoped that the genes isolated in this study would serve as a starting point from which further information can be gathered to shed light on the reproductive system of the Pigeon orchid.

Objectives:

1) To isolate and identify differentially expressed genes from floral organs of the Pigeon orchid.
2) To characterize the expression profile of putative floral-organ specific transcripts from the Pigeon orchid.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Orchids

The name “Orchids” originated from the Greek word *orchis*, which means "testicle", from the appearance of subterranean tuberoids of the genus *Orchis*. It was first coined by Theophrastos (372/371 – 287/286 BC), a student of Aristotle who was later on considered as the father of botany and ecology. The word "orchis" made its first debut in the book, *De historia plantarum* (*The natural history of plants*) (Anonymous, 2006).

Orchids are generally classified in the plant kingdom under the division Magnoliophyta and further grouped under the class of Liliopsida. The family, Orchidaceae is placed under the order Asparagales and can be further divided into five subfamilies namely the *Apostasioideae*, *Cypripedioideae*, *Epidendroideae*, *Orchidoideae* and *Spiranthoideae* (Dressler, 1993). Together with the grasses, palms and lilies, they form the monocotyledons, the smaller of the two major units into which the flowering plants (Angiosperms) are divided. This family of plants has attracted more curiosity from the scientific community as well as the general public than the other plant groups. The interest shown on the Orchidaceae is reflected in the considerable amount of literature published each year, especially on the floristic characters and taxonomic treatment (Kurzweil and Kocyan, 2002).