



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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EXPRESSED GENES FROM FLORAL ORGANS OF PIGEON ORCHID
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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**

December 2007



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
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™ 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

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Disember 2007

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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 molekuler mewujudkan peluang untuk mengesan unsur-unsur genetik yang terlibat dalam biologi reproduksi orkid. Kajian ini 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 GeneFishingTM. Sepuluh transkrip gen yang mempamerkan ekspresi 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.

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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



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF PLATES	xv
LIST OF ABBREVIATIONS	xvii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 Introduction to Orchids	4
2.1.1 Taxonomy and Classification	6
2.1.2 Morphology and Adaptations	9
2.1.3 Floral Homologies	14
2.1.4 The Pigeon Orchid – <i>Dendrobium crumenatum</i>	17
2.1.5 Differentially Expressed Genes (DEGs) in Orchids	21
2.2 Plant Reproductive Biology	23
2.2.1 The Flowering Process	24
2.2.2 Biology of Fertilization	33
2.2.3 Post-Pollination Developments	36
2.3 Gene Isolation	41
2.3.1 GeneFishing™ Technology	47
2.3.2 Characterization of Gene Expression	51
3 MATERIALS AND METHODS	
3.1 Plant Materials	54
3.2 RNA Extraction	56
3.3 GeneFishing™ for DEGs	62
3.3.1 First-Strand cDNA Synthesis	63
3.3.2 GeneFishing™ PCR Amplification with Arbitrary ACP™ Primers	64
3.4 Cloning and Sequencing of DEGs	66
3.4.1 TA Cloning	66
3.4.2 Plasmid Miniprep	68
3.4.3 Analyzing Plasmid Clones by PCR	68
3.4.4 Sequence Analysis	69
3.4.5 Preparation of DMSO Stock	70
3.5 PCR Cloning of Housekeeping Gene	70
3.5.1 First-strand cDNA Synthesis	71
3.5.2 Primer Design	71



3.5.3	RT-PCR Cloning	72
3.5.4	Sequencing	73
3.6	Expression Analysis	73
3.6.1	Semi-Quantitative RT-PCR Analysis	73
3.6.2	Real-Time Reverse Transcription (RT) PCR	82
4	RESULTS AND DISCUSSION	
4.1	Plant Materials	90
4.2	Optimization of Total RNA Isolation	92
4.3	Identification and Cloning of DEGs	99
4.3.1	DEGs Amplified with Arbitrary ACP1 and dT-ACP2	101
4.3.2	DEGs Amplified with Arbitrary ACP2 and dT-ACP2	104
4.3.3	DEGs Amplified with Arbitrary ACP3 and dT-ACP2	106
4.3.4	DEGs Amplified with Arbitrary ACP8 and dT-ACP2	106
4.4	Sequence Analysis on DEGs	109
4.4.1	Sequence Analysis of Clone A1C1-8	112
4.4.2	Sequence Analysis of Clone A3C1-1	117
4.4.3	Sequence Analysis of Clone A8C1-9	121
4.5	RT-PCR Cloning of <i>D. crumenatum</i> Housekeeping Gene	125
4.6	Expression Studies	130
4.6.1	Semi-quantitative RT-PCR	130
4.6.2	Confirmation of DEG Expression using Real-Time RT-PCR	144
4.7	The Significance of DEGs from Floral Organs of <i>D. crumenatum</i>	169
5	CONCLUSION	174
	BIBLIOGRAPHY	176
	APPENDICES	198
	BIODATA OF THE AUTHOR	208



LIST OF TABLES

Table		Page
1	Comparisons of RNA purity and yield from the organs of <i>Dendrobium crumenatum</i> using selected RNA extraction methods	95
2	Summary of DEG clones from Pigeon orchid with significant homology matches	111
3	Putative clones isolated from the column of <i>Dendrobium crumenatum</i> with significant homology matches to Cyclophilin 128	128
4	Average cDNA transcript amounts of target genes in the respective organs	164



LIST OF FIGURES

Figure		Page
1	Sections of an orchid flower depicted by Darwin	16
2	Classification of the Pigeon orchid	19
3	Pigeon orchid (<i>Dendrobium crumenatum</i>) flower	19
4	Model suggested by Coen and Meyerowitz (1991) on the domain action of the three classes of organ homeotic genes (illustrated as A, B and C) on the organ formation of the 4-whorl region of a floral primordium	30
5	Gene structure in MIKC-type MADS-box genes commonly found in plants	30
6	Floral organs of the Pigeon orchid flower	55
7	Experimental procedures involved in identifying and cloning of DEGs from <i>Dendrobium crumenatum</i> floral organs and the subsequent characterization studies on putative clones	65
8	Developing bud of the Pigeon orchid flower	91
9	Pigeon orchid plant	91
10	The nucleotide sequence and translated amino acid sequence of clone A1C1-8	114
11	Amino acid alignment of clone A1C1-8 with small heat shock proteins from other plants	116
12	The nucleotide and translated amino acid sequence of clone A3C1-1	119
13	Amino acid alignment of clone A3C1-1 with other pectin methylesterases	120
14	The nucleotide and translated amino acid sequence of clone A8C1-9	123
15	Amino acid alignment of clone A8C1-9 with closely linked 14-3-3 proteins	124
16	The nucleotide sequence and translated amino acid sequence of clone dCyp2	128



17	Amino acid alignment of clone dCyp2 with other Cyclophilin	129
18	The nucleotide sequence, translated amino acid and 3' untranslated region (UTR) of clone A1C1-8	136
19	The nucleotide sequence, translated amino acid and 3' untranslated region (UTR) of clone A3C1-1	137
20	The nucleotide sequence, translated amino acid and 3' untranslated region (UTR) of clone A8C1-9	138
21	The nucleotide sequence, translated amino acid and 3' untranslated region (UTR) of clone A1C1-8 for real-time RT-PCR primer design	149
22	The partial cDNA nucleotide sequence, translated amino acid and the amplified region (underline) of clone dCyp2 with specific primers, Cyp2S and Cyp2AS, during real-time RT-PCR	150
23	Relative efficiency plot of ΔC_T versus log cDNA dilution	159
24	Amplification plot of cDNA dilutions (standards) for various cDNA transcripts detected during real-time RT-PCR amplification	162
25	Standard curves for quantification of various transcript amounts in the eight sample organs	163
26	Relative expression level of DEGs in various orchid organs quantified by real-time RT PCR using the standard curve method	168



LIST OF PLATES

Plate		Page
1	RNA isolated from orchid organs using various protocols on 1.2% (w/v) denaturing formaldehyde agarose gel	97
2	Gel electrophoresis of PCR products from dorsal sepal, lateral sepal, petal, lip and whole column with arbitrary ACP1 and dT-ACP2 primers on 1.5% (w/v) agarose gel	102
3	PCR screening on plasmid clones of A1D1 and A1C1 with M13 forward and reverse primers and plasmid concentration check on 1.2% (w/v) agarose gel.	102
4	Gel electrophoresis of PCR products from dorsal sepal, lateral sepal, petal, lip and whole column with arbitrary ACP2 and dT-ACP2 primers on 1.5% (w/v) agarose gel	105
5	PCR screening on plasmid clones of A2L1 and A2C1 with M13 forward and reverse primers and plasmid concentration check on 1.2% (w/v) agarose gel	105
6	Gel electrophoresis of PCR products from dorsal sepal, lateral sepal, petal, lip and whole column with arbitrary ACP3 and dT-ACP2 primers, on 1.5% (w/v) agarose gel	107
7	PCR screening on plasmid clones of A3C1 with M13 forward and reverse primers and plasmid concentration check on 1.2% (w/v) agarose gel	107
8	Gel electrophoresis of PCR products from dorsal sepal, lateral sepal, petal, lip and whole column (WC) with arbitrary ACP8 and dT-ACP2 primers, on 1.5% (w/v) agarose gel	108
9	PCR screening on plasmid clones of A8C1 with M13 forward and reverse primers and plasmid concentration check on 1.2% (w/v) agarose gel	108
10	Isolation of partial Cyclophilin sequence of <i>Dendrobium crumenatum</i>	127
11	Total RNA from five floral organs of <i>Dendrobium crumenatum</i> used to synthesize first-strand cDNAs	133
12	First-strand cDNAs of five floral organs synthesized using the Quantitect Reverse Transcription Kit (Qiagen)	133



13	Optimization of cycle number for semi-quantitative RT-PCR analysis	140
14	Optimization of annealing temperature for semi-quantitative RT-PCR analysis	140
15	Expression of A1C1-8, A3C1-1 and A8C1-9 in various floral organs, characterized by semi-quantitative RT-PCR analysis, on 1.5% (w/v) agarose gel	142
16	Confirmation of amplification specificity in semi-quantitative RT-PCR analysis via Southern hybridization with biotin-labelled probes	142
17	Various template normalization procedures tested on the eight sample organs of <i>Dendrobium crumenatum</i>	147
18	Optimization of annealing temperatures using gradient RT-PCR, for three candidate housekeeping genes	152
19	Preliminary RT-PCR amplification on first-strand cDNAs with Cyp2S and Cyp2AS primers to determine normalization of PCR templates, on 1.5% (w/v) TAE agarose gel	154
20	Selection of <i>Dendrobium crumenatum</i> candidate housekeeping genes (endogenous controls) in eight test organs amplified with RT-PCR, on 1.5% (w/v) TAE agarose gel	156



LIST OF ABBREVIATIONS

α	Alpha
β	Beta
λ	Lambda
μg	Microgram
μl	Microlitre
μM	Micromolar
%	Percentage
$^{\circ}\text{C}$	Degree celcius
2-ME	β -mercaptoethanol
ABA	Absciscic 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 ₂ O	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-morpholino) 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 ₄ OAc	Ammonium acetate
nm	Nanometer
nmol	Nanomole
OD	Optical density
PCR	Polymerase Chain Reaction
PR	Pathogenesis-related
PVP	Polivinylypyrrolidone
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 GeneFishingTM. 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 tuberoles of the genus *Orchis*. It was first coined by Theophrastus (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 *Spiranθοideae* (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).

