UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION, CLONING AND CHARACTERIZATION OF SELECTED FULL-LENGTH FRAGRANCE-RELATED TRANSCRIPTS FROM ORCHID (*VANDA* MIMI PALMER)

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MASTER OF SCIENCE
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IDENTIFICATION, CLONING AND CHARACTERIZATION OF SELECTED FULL-LENGTH FRAGRANCE-RELATED TRANSCRIPTS FROM ORCHID (*VANDA* MIMI PALMER)

By

CHAN WAI SUN

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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IDENTIFICATION, CLONING AND CHARACTERIZATION OF SELECTED FULL-LENGTH FRAGRANCE-RELATED TRANSCRIPTS FROM ORCHID (*VANDA* MIMI PALMER)

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August 2009

Chairman: Janna Ong Abdullah, PhD

Faculty: Biotechnology and Biomolecular Sciences

Floral fragrance has important economical value in ornamental plants, crops and industries related to essential oils. However, the understanding of the molecular mechanisms underlying the biosynthesis of floral fragrance in monocotyledonous plants; in particular orchids, is still in its infancy. This study aimed to isolate and characterize fragrance-related genes from *Vanda* Mimi Palmer in order to enhance understanding of the molecular biology of fragrance in vandaceous orchid. *Vanda* Mimi Palmer is a tropical scented orchid with high economical value. In the effort to identify potential fragrance-related genes in *Vanda* Mimi Palmer, a floral cDNA library and a subtracted cDNA library were constructed. A total of 100 clones were selected from the cDNA library and their nucleotide sequences were determined, of which 83 clones showed homology to known amino acid sequences, comprising 6 contigs and 62 singletons, which were further assigned into 9 categories based on their functional roles. Two ESTs were identified as potential fragrance-related transcripts and they were 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) and lipoxygenase. From the Suppression

Subtractive Hybridization (SSH) library, 107 clones were up-regulated in the full bloom flowers of *Vanda* Mimi Palmer where 33 clones (3 singletons and 30 contigs) showed similarities to known sequences in the public database and were classified based on their putative functional roles as secondary metabolism (97%) and hypothetical proteins (3%), and 32 of the clones were transcripts encoding fragrance-related transcripts. The fragrance-related transcripts code for sesquiterpene synthase, tyrosine decarboxylase and putative alcohol acyltransferase. However, only three ESTs were selected for full-length gene isolation and characterization and they are putative alcohol acyltransferase (VMPAAT), sesquiterpene synthase (VMPSTS) and DXR (VMPDXR). Southern analyses showed that each of the isolated transcripts belongs to a large gene family, containing more than one copy in the *Vanda* Mimi Palmer genome. Real time RT-PCR indicated that VMPAAT and VMPSTS transcripts were expressed preferentially in floral tissues whereas VMPDXR was expressed differentially in different types of tissues (root, leaf, petal, sepal and column). All three clones showed higher transcript expressions in blooming and full bloom flowers compared to flower bud. VMPAAT and VMPDXR transcripts expressions showed no fluctuations whereas VMPSTS showed otherwise. In conclusion, the findings in this study have contributed to the GeneBank database resources for orchids and have opened some insights on molecular biology of fragrance in vandaceous orchids.

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

PEMENCILAN, PENGKLONAN DAN PENCIRIAN TRANSKRIP-TRANSKRIP LENGKAP BERKAITAN BAU WANGI YANG DIPILIH DARIPADA ORKID (*VANDA* MIMI PALMER)

Oleh

CHAN WAI SUN

Ogos 2009

Pengerusi: **Janna Ong Abdullah, PhD**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Bunga wangi mempunyai nilai ekonomi yang penting dalam tumbuh-tumbuhan hiasan, pertanian dan perindustrian berkaitan minyak wangi. Akan tetapi, kefahaman tentang mekanisme di peringkat molekular penghasilan bau wangi baru berkembang dalam tumbuhan monokot terutamanya orkid. Penyelidikan ini bertujuan untuk memencilkan dan mencirikan gen yang terlibat dalam penghasilan bau wangi untuk menambahkan kefahaman biologi molekul berkenaan bau wangi dalam orkid vandaceous. *Vanda* Mimi Palmer ialah sejenis orkid tropika yang berbau wangi, dikenalpasti mempunyai potensi untuk menjadi tumbuhan hiasan penting yang mempunyai nilai ekonomi yang tinggi. Untuk mengenalpasti gen yang terlibat dalam penghasilan bau wangi daripada *Vanda* Mimi Palmer, suatu perpustakaan cDNA (cDNA library) untuk bunga dan satu perpustakaan yang telah disaring (subtracted cDNA library) telah dihasilkan. Sebanyak 100 klon telah dipilih daripada bunga perpustakaan cDNA dan jujukan nukleotide ditetapkan, di mana 83 klon menunjukkan padanan yang sahih dengan jujukan-jujukan

asid amino yang diketahui. Mereka terdiri daripada 6 ‘contigs’ dan 62 ‘singletons’ di mana selanjutnya dibahagikan kepada 9 kategori berdasarkan fungsi masing-masing. Dua ESTs dikenalpasti sebagai transkrip yang terlibat dalam penghasilan bau wangi dan mereka ialah 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) dan lipoxygenase. Daripada perpustakaan ‘Suppression Subtractive Hybridization’ (SSH), sebanyak 107 klon yang ‘up-regulated’ dalam bunga yang telah berkembang penuh di mana 33 klon (3 ‘singletons’ dan 30 ‘contig’) menunjukkan padanan yang sahih dengan jujukan yang diketahui dalam pengkalan data umum dan dikelaskan mengikut fungsi di mana metabolisme sekunder (97%) dan protein hipotetik (3%), dan 32 klon ialah transkrip yang terlibat penghasilan bau wangi. Transkrip-transkrip ini ialah 3 ‘ESTs’ yang mengekodkan sesquiterpene synthase, putatif acyltransferase dan tyrosine decarboxylase. Akan tetapi, hanya tiga ‘ESTs’ dipilih daripada dua perpustakaan cDNA ini untuk pemencilan gen lengkap dan pencirian dan mereka ialah putatif acyltransferase (VMPAAT), sesquiterpene synthase (VMPSTS) dan DXR (VMPDXR). Analisa penghibridan ‘Southern’ menunjukkan setiap transkrip yang terpilih mungkin berasal dari famili gen besar dan mengandungi lebih daripada satu salinan dalam *Vanda* Mimi Palmer genom. ‘Real time reverse transcriptase-polymerase chain reaction’ menunjukkan transkript-transkript VMPAAT dan VMPSTS diekspres secara predominan di dalam tisu bunga manakala pengekspresan VMPDXR adalah berlainan di dalam tisu yang berbeza (akar, daun, petal, sepal, kolum, bibir). Ketiga-tiga klon menunjukkan ekspresi yang lebih tinggi di dalam bunga yang sedang berkembang dan telah berkembang penuh berbanding dengan bunga kudup. Ekspresi bagi transkrip-transkrip VMPAAT dan VMPDXR menunjukkan pergerakan tidak menentu sementara

VMPSTS menunjukkan corak yang sebaliknya. Secara kesimpulan, data yang diperolehi dalam penyelidikan ini telah memberi sumbangan kepada pangkalan data 'GeneBank' terutamanya orkid dan menambahkan kefahaman di peringkat biologi molekul berkenaan bau wangi secara lebih terperinci di dalam orkid vandaceous.

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I certify that an Examination Committee met on 26 August 2009 to conduct the final examination of Chan Wai Sun on her Master of Science thesis entitled "Isolation and Characterization of Selected Full-length Fragrance-related Transcripts from Orchid (*Vanda* Mimi Palmer)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree.

Members of the Examination Committee are as follows:

Chairman
Associate Professor Dr. Ho Chai Ling
Faculty Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Internal I
Associate Professor Datin Dr. Siti Nor Akmar Abdullah
Faculty Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Internal II
Professor Dr. Tan Wen Siang
Faculty Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

External
Associate Professor Dr. Jennifer Ann Harikrishna
Faculty Science
Universiti Malaya
(External Examiner)

_____
BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Janna Ong Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

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

Parameswari a/p Namasivayam, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

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

Date: 10 December 2009

DECLARATION

I hereby declare that the thesis is based on my original work except for equations 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.

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CHAN WAI SUN

Date:

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LIST OF ABBREVIATIONS

AFLP	amplified fragment length polymorphism
AHCT	anthocyanin O-hydroxycinnamoyltransferase
AMV	Avian Myeloblastosis Virus
AOC	allene oxide cyclase
AOS	allene oxide synthase
bp	base pair
BAHD	first letter of first four characterized family members
BAMT	S-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase
BEAT	benzylalcohol *O*-acetyltransferase
BEBT	benzoyl-coenzyme A (CoA):benzyl alcohol benzoyl transferase
BPBT	benzoyl CoA: benzyl alcohol/ phenylethanol benzoyltransferase
BSA	bovine serum albumin
BSMT	benzoic acid/ salicylic acid methyltransferase
cDNA	complementary deoxyribonucleic acid
cDNA-RDA	cDNA-representational difference analysis
CHAT	acetyl CoA:(Z)-3-hexen-1-ol acetyltransferase
C_T	threshold cycle
CTAB	hexadecyltrimethyl-ammonium bromide
DAT	deacetylvindoline 4-*O*-acetyltransferase
DEPC	diethylpyrocarbonate
DMAPP	dimethylallyl diphosphate
DMSO	dimethyl sulphoxide

DNA	deoxyribonucleic acid
DXR	1-deoxy-D-xylulose 5-phosphate reductoisomerase
DXS	1-deoxy-D-xylulose 5-phosphate synthase
dNTP	deoxynucleoside triphosphates
EDTA	ethylenediaminetetraacetic acid
EF	elongation factor
EST	expressed sequence tag
EtBr	ethidium bromide
FA	formaldehyde agarose
FGP	Floral Genome Project
FRET	Fluorescent Resonance Energy Transfer
GSPs	gene specific primers
HbDXR1	*Hevea basiliensis* DXR
HCBT	anthranilate N-hydroxycinnamoyl/ benzoyltransferase
13(S)-HPLA	13(S)-hydroperoxylinolenic acid
IAA	indole acetic acid
IPP	isopentenyl diphosphate
IPTG	isopropylthio-β-D-galactoside
ISSR	intersimple sequence repeats
JA	jasmonic acid
JMT	jasmonate carboxyl methyltransferase
kb	kilo base pair
LB	Luria-Bertani

LiCl	lithium chloride
LOX	lipoxygenase
M	molarity
M	gene stability measure
MADS	MCM1-AGAMOUS-DEFICIENS-SRF
MeJA	methyl jasmonic acid
MEP	2-C-methyl-D-erythritol 4-phosphate
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
MOPS	3-N-morpholino propanesulfonic acid
MTHFR	5, 10-methylene-tetrahydrofolate reductase
MVA	mevalonate
NaCl	sodium chloride
NaH_2PO_4	sodium dihydrogen phosphate
Na_2HPO_4	sodium hydrogen phosphate
NaOAc	sodium acetate
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
NH_4OAc	ammonium acetate
OD	optical density
ODO1	ODORANT1
OPDA	(9S, 13S)-oxophytodienoic acid
OPR	12-oxo-PDA reductase

ORF	open reading frame
PAL	phenylalanine ammonia-lyase
PAAS	phenylacetaldehyde synthase
PbDXR	*Phalaenopsis bellina* DXR
PbGDPS	*Phalaenopsis bellina* geranyl diphosphate synthase
PcDXR	grey poplar DXR
PCR	polymerase chain reaction
PdLOX	*Populus deltoids* lipoxygenase
PhCFAT	Petunia's acetyl CoA: coniferyl alcohol acyltransferase
PhPAAS	Petunia's phenylacetaldehyde synthase
PhBPBT	Petunia's benzoylCoA: benzyl alcohol/ phenylethanol benzoyltransferase
PhBSMT	Petunia's benzoic acid/salicylic acid carboxyl methyltransferase
PhCFAT	petunia's coniferyl alcohol acyltransferase
PhIGS1	isoeugenol synthase 1
Pfu	plaque forming unit
PVP	polyvinyl pyrrolidone
R^2	correlation coefficient
RACE	rapid amplification of cDNA ends
RAPD	random amplified polymorphic DNA
RcOMT1	rose orcinol methyltransferase 1
RcOMT2	rose orcinol methyltransferase 2
RFLP	restriction fragment length polymorphism
RhAAT	rose alcohol acetyltransferase

RhGP1	rose geranylgeranylated protein 1
RNA	ribonucleic acid
RNase	ribonuclease
rRNA	ribosomal RNA
RT-PCR	reverse transcription-polymerase chain reaction
SA	salicylic acid
SAM	S-adenosyl methionine
SDS	sodium dodecyl sulfate
SKP1	S-phase kinase-associated protein 1
SSC	standard saline citrate
SSH	subtractive suppression hybridization
SSR	simple sequence repeats
TAE	tris-acetate-EDTA
TE	tris-EDTA
T_m	melting temperature
Tris-HCl	tris-hydrochloric acid
TUC	tentative unique contig
TUG	tentative unique gene
TUS	tentative unique singleton
U	unit
Ubi	ubiquitin
UPM	universal primer A mix
UTR	untranslated region

UV	ultraviolet
V	volts
VMPAAT	alcohol acyltransferase from *Vanda* Mimi Palmer
VMPDXR	DXR from *Vanda* Mimi Palmer
VMPSTS	sesquiterpene synthase from *Vanda* Mimi Palmer
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside