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

**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:

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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:

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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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| | |
|---|---|
| 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 |