



UNIVERSITI PUTRA MALAYSIA

**DEVELOPMENT OF FLORAL EXPRESSED SEQUENCE TAG
RESOURCE FROM AND CHARACTERIZATION OF FRAGRANCE-
RELATED GENE TRANSCRIPTS IN *VANDA MIMI PALMER***

TEH SEOW LING

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**DEVELOPMENT OF FLORAL EXPRESSED SEQUENCE TAG RESOURCE
FROM AND CHARACTERIZATION OF FRAGRANCE-RELATED GENE
TRANSCRIPTS IN VANDA MIMI PALMER**

By
TEH SEOW LING

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DEVELOPMENT OF FLORAL EXPRESSED SEQUENCE TAG RESOURCE FROM AND CHARACTERIZATION OF FRAGRANCE-RELATED GENE TRANSCRIPTS IN VANDA MIMI PALMER

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Chairman: Janna Ong Abdullah, PhD

Faculty: Biotechnology and Biomolecular Sciences

Vanda Mimi Palmer (VMP) is a highly sought after fragrant-orchid hybrid in Malaysia. It is economically important in cosmetic and beauty industries and is also a famous potted ornamental plant. To date, no work corresponding to fragrance-related genes of vandaceous orchids has been reported and very limited molecular information on fragrance from other plants despite extensive analyses of floral fragrance or volatiles been studied. In fact, the biosynthesis pathways of flower fragrance are still incomplete. The aims of this study were to develop a floral expressed sequence tags (EST) resource, as well as to identify and characterize potential fragrance-related transcripts in this orchid hybrid. A previously constructed floral cDNA library of VMP representing transcripts of fragrance-associated genes and floral developmental genes was used to generate 2,132 ESTs. Clustering, annotation and assembling of the ESTs identified 1,196 unigenes which defined 966 singletons and 230 contigs. The VMP dbEST were functionally classified by Gene Ontology (GO) into three groups: Molecular Functions (51.2%), Cellular Component (16.4%) and Biological Processes

(24.6%) while the remaining 7.8% showed no hits with GO identifier. A total of 112 EST-SSR (9.4%) was mined. Five fragrance-related transcripts were selected for full-length isolation and expression analysis using real-time quantitative RT-PCR. They were *acetyl-CoA acetyltransferase (VMPACA)*, *3-hydroxy-3-methylglutaryl-coenzyme A reductase (VMPHMGR)*, *1-deoxy-D-xylulose 5-phosphate synthase (VMPDXPS)*, *linalool synthase (VMPLis)* and *lipoxygenase (VMPLox)*. Those transcripts were developmentally regulated. Three of them were highly expressed in full-bloom stage, and sepals and petals were found to be the tissues with the highest expression levels. Full-length cDNA have been obtained for two of the fragrance-related transcripts (VMPACA and VMPHMGR). Cloning and over-expression of three of the full length cDNAs [VMPACA, VMPHMGR, and a *sesquiterpene synthase (VMPSTS)* isolated from a previous study] were performed in *Escherichia coli* BL21(DE3)pLysS strain. The expression of those transcripts, fused to N-terminal thioredoxin (Trx·Tag), S·Tag and His·Tag fusion proteins in pET32(a), yielded recombinants VMPSTS and VMPHMGR which were only partially soluble while VMPACA was present as an insoluble protein. Functional enzymatic assays were carried out to analyse the functionality of the potential products produced from the catalytic activities of VMPSTS and VMPHMGR, respectively. VMPSTS was expressed as a functionally inactive recombinant protein with no sesquiterpene synthase compounds being detected. VMPHMGR, however, successfully catalyzed the conversion of HMG-CoA to mevalonate lactone. Dehydromevalonic lactone and pantolactone, derivatives of mevalonate lactone were detected from the catalytic reaction of VMPHMGR using GC-MS analysis. The development of a *Vanda Mimi Palmer* expressed sequence tags (VMPESTs) database will enhance the

understanding of the molecular biology of fragrance biosynthesis pathways in vandaceous orchids and facilitate the identification of novel fragrance-related transcripts in other scented flowers. The successful expression of the cloned products may prove to be a useful asset for applications in the perfumery industry for the generation of custom-made fragrance products.



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

**PEMBANGUNAN PANGKALAN DATA ‘EXPRESSED SEQUENCE TAG’
DARIPADA DAN PENCIRIAN GEN TRANSKRIP BERKAITAN BAU
WANGI DALAM BUNGA VANDA MIMI PALMER**

Oleh

TEH SEOW LING

Ogos 2011

Pengerusi: Janna Ong Abdullah, PhD

Fakulti: Bioteknologi and Sains Biomolekul

Kewangian *Vanda Mimi Palmer* (VMP) merupakan satu aset yang penting dari segi ekonomi terutamanya dalam bidang kosmetik dan kecantikan. Permintaan tinggi terhadap bunga wangian telah menyumbangkan kepada popularity VMP sebagai pokok hiasan yang digemari di Malaysia. Malangnya, sehingga kini, tiada kajian biologi molekul tentang gen penghasilan wangian daripada VMP dilaporkan dan maklumat tersebut daripada tumbuhan lain juga masih terhad. Walaupun penyelidikan telah dijalankan secara berleluase dari segi biokimia, tapak jalan biokimia penghasilan wangian bagi bunga wangi masih tidak lengkap. Tujuan penyelidikan ini adalah untuk mengkaji wangian VMP terutamanya dalam aspek biologi molekul. Satu pangkalan data yang dinamakan Expressed Sequence Tag (EST) telah dibangunkan untuk mengenalpasti dan mencirikan transkrip yang berpotensi bagi penghasilan wangian. Satu perpustakaan cDNA (*cDNA library*) untuk bunga VMP yang dihasilkan sebelum ini telah digunakan dengan penghasilan sejumlah 2,132 EST diperolehi. Penghasilan kelompok EST tersebut mengenalpastikan 1,196 ‘unigenes’ di mana 966 adalah ‘singletons’ dan

230 adalah 'contigs'. Kumpulan EST tersebut diatursusun dengan menggunakan kaedah 'Gene Ontology' (GO) kepada tiga kategori berdasarkan fungsi masing-masing: Fungsi Molekul (51.2%), Komponen Sel (16.4%) dan Proses Biologi (24.6%) dan selebihnya (7.8%) tidak menunjukkan persamaan dengan identifier GO. Sebanyak 112 'EST-SSR' telah dilombong. Lima EST dikenalpasti sebagai transkrip cDNA yang terlibat dalam penghasilan wangian telah dipilih untuk pemencilan gen lengkap dan pencirian dengan menggunakan tindakbalas rantaian polymerase masa nyata (*real-time* RT-PCR). Kelima-lima transkrip tersebut ialah 'acetyl-CoA acetyltransferase' (VMPACA), '3-hydroxy-3-methylglutaryl-coenzyme A reductase' (VMPHMGR), '1-deoxy-D-xylulose 5-phosphate synthase' (VMPDXPS), 'linalool synthase' (VMPLis) and 'lipoxygenase' (VMPLox). Kelima-lima transkrip tersebut menunjukkan ekspresi yang berlainan dalam peringkat perkembangan bunga VMP yang tentu. Pada keseluruhannya, ekspresi tinggi diperolehi bagi bunga yang telah berkembang penuh manakala *sepal* dan *petal* merupakan tisu bunga yang mengekspres secara dominan. Jujukan lengkap bagi gen transkrip yang berkaitan bau wangi (VMPACA dan VMPHMGR) telah diperolehi. Pengklonan dan ekspresi secara berlebihan telah dilakukan ke atas ketiga-tiga cDNAs yang lengkap jujukan iaitu [VMPACA, VMPHMGR, dan *sesquiterpenes synthase* (VMPSTS) yang dipencil dalam kajian lepas] di dalam strain 'Escherichia coli BL21(DE3)pLysS'. Pengekspresian ketiga-tiga transkrip bergabung dengan 'N-terminal thioredoxin (Trx-Tag)', 'S-Tag' dan 'His-Tag' protein di dalam pET32(a) telah menghasilkan rekombinan VMPSTS dan VMPHMGR yang separuh larut dan rekombinan VMPACA yang tidak larut langsung. Esei enzim telah dijalankan untuk pengenalpastian fungsi produk yang dihasilkan daripada aktiviti pemangkinan VMPSTS dan

VMPHMGR. VMPSTS didapati telah diekspres sebagai protein rekombinan yang tidak aktif sebab tiada hasilan sebatian kimia wangian yang dapat dikesan menggunakan alat kromatografi gas-spektrometrik jisim (GC-MS). Akan tetapi, VMPHMGR berjaya menukarkan substrat HMG-CoA kepada mevalonat lakton, dehidromevalonike lakton dan pantolakton sepertimana yang telah dikesan menggunakan GC-MS. Kesimpulannya, pembangunan pangkalan data 'Vanda Mimi Palmer Expressed Sequence Tags' (VMPESTs) adalah penting untuk meningkatkan kefahaman di peringkat biologi molekul berkaitan dengan tapak laluan penghasilan wangian secara lebih terperinci dalam orkid vanda dan memudahkan pengenalan transkrip yang berkaitan dengan penghasilan wangian daripada bunga wangi yang lain. Pengekspresian produk klon yang berjaya dalam kajian kini mungkin dapat diaplikasikan untuk menghasilkan produk wangian yang mengikut citarasa tertentu.

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I certified that a Thesis Examination Committee has met on **25 August 2011** to conduct the final examination of Teh Seow Ling on her Master of Science thesis entitled 'Development of floral Expressed Sequence Tag resource from and characterization of fragrance-related gene transcripts in *Vanda Mimi Palmer*' in accordance with the University and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the thesis Examination Committee were as follows:

Sieo Chin Chin, PHD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

Norazizah binti Shafee, PHD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Internal Examiner)

Rozi bt. Mohamed, PHD

Lecturer

Faculty of Forestry

Universiti Putra Malaysia

(Internal Examiner)

Sharifah Shahrul Rabiah Syed Alwi, PHD

Felda Biotechnology Centre

(External Examiner)

NORITAH OMAR, PHD

Associate 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 requirement 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)

Parameswari Namasivayam, PHD

Associate Professor

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:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

TEH SEOW LING

Date: 25 August 2011



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