



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND EXPRESSION ANALYSIS OF ETHYLENE RECEPTOR
GENE FROM *ONCIDIUM* GOWER RAMSEY FLOWER**

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By

UMIKALSUM MOHAMED BAHARI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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December 2009

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

ISOLATION AND EXPRESSION ANALYSIS OF ETHYLENE RECEPTOR GENE FROM *ONCIDIUM* GOWER RAMSEY FLOWER

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December 2009

Chairman : Faridah Qamaruz Zaman, PhD

Institute : Institute of Bioscience

The process of flower senescence is influenced by the plant hormone, ethylene. In *Arabidopsis*, ethylene perception is controlled by a family of five genes, including *ETHYLENE RESPONSE 1 (ETR1)*, *ETHYLENE RESPONSE SENSOR 1 (ERS1)*, *ETHYLENE RESPONSE 2 (ETR2)*, *ETHYLENE RESPONSE SENSOR 2 (ERS2)* AND *ETHYLENE-INSENSITIVE 4 (EIN4)*. They fall into two subfamilies based on their sequence similarities. In flower, it has been reported that similar set of genes are also involved. This study was carried out to isolate the gene that is involved in ethylene signaling from *Oncidium* Gower Ramsey flower. Total RNA was extracted from self-pollinated *Oncidium* Gower Ramsey flowers followed with reverse transcriptase-polymerase chain reaction (RT-PCR) using two degenerate primer pairs (F1/R1 and F2/R2). These primers were designed based on the conserved regions of ethylene receptor genes of various plant species. Fragments of the expected size were yielded and sequenced. DNA sequences analysis showed a very high homology

to the full length ethylene receptor gene, *ETHYLENE RECEPTOR 2* (*ER2*) (Genebank AF276234) of *Oncidium* Gower Ramsey. From here, specific primers (F3/R3) were designed for 5' and 3' ends of the open reading frame (ORF) region of the *ER2* gene in order to clone a similar full length gene. The purified and sequenced PCR fragment had 98% DNA sequence similarity to the *ER2* gene. However, the newly amplified fragment was only 1595bp, encoded for 422 amino acids as compared to the 2389bp (631 amino acids) of the full length *ER2*. Sequence alignment of the two genes indicated two truncated regions between nucleotide number 1015 to 1643 (628bp) and 2013 to 2027 (14bp). These results demonstrated that we have cloned an ethylene gene named *ER2_s*, which encodes for a protein that has a missing region in the histidine kinase domain as compared to the sequence of ER protein for a similar orchid hybrid. In addition, expression of the isolated gene was detected by real-time RT-PCR at a very low level in the tested flower tissues, as well as in roots. Our results suggest that *ER2_s* may be involved in the development of different plant tissues.

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

**PEMENCILAN DAN ANALISA PENGEKSPRESAN GEN RESEPTOR
ETILINA DARIPADA BUNGA *ONCIDIUM* GOWER RAMSEY**

Oleh

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Proses kelayuan bunga adalah dipengaruhi oleh hormon tumbuhan, etilena. Persepsi etilena adalah dikawal oleh famili yang terdiri daripada lima gen, termasuk ‘*ETHYLENE RESPONSE 1*’ (*ETR1*), ‘*ETHYLENE RESPONSE SENSOR 1*’ (*ERS1*), ‘*ETHYLENE RESPONSE 2*’ (*ETR2*), ‘*ETHYLENE RESPONSE SENSOR 2*’ (*ERS2*) dan ‘*ETHYLENE-INSENSITIVE 4*’ (*EIN4*) dalam *Arabidopsis*. Kesemua gen ini adalah tergolong dalam dua subfamili berdasarkan kepada persamaan jujukan. Dilaporkan bahawa set gen yang sama juga terlibat dalam pokok bunga. Kajian ini adalah untuk memencilkan gen yang terlibat dengan signal etilena dalam bunga *Oncidium* Gower Ramsey. RNA jumlah telah diekstrak daripada bunga *Oncidium* Gower Ramsey yang telah didebungakan. Tindak balas berbalik polimerase berantai (RT-PCR) dijalankan menggunakan dua pasang pencetus (F1/R1 dan F2/R2) yang direka berdasarkan kawasan terabadi pada gen reseptor etilena pelbagai spesies

pokok. Cebisan DNA pada saiz jangkaan diperolehi dan diujuk. Jujukan DNA daripada kedua-dua cebisan menunjukkan persamaan yang tinggi terhadap jujukan lengkap gen reseptor etilena, 'ETHYLENE RECEPTOR 2' (*ER2*) (Genebank AF276234) *Oncidium* Gower Ramsey. Berdasarkan jujukan DNA ini, pencetus khusus (F3/R3) direka pada hujung 5' dan 3' rangka bacaan terbuka (ORF) gen *ER2* bertujuan mengklon jujukan lengkapnya. Cebisan PCR yang ditulen dan diujukkan ini mempunyai 98% persamaan jujukan DNA berbanding gen *ER2*. Walau bagaimanapun cebisan yang diamplifikasi tersebut hanya bersaiz 1595bp dan mengkod 422 asid amino berbanding 2389bp (631 asid amino) jujukan lengkap *ER2*. Kesepadanan jujukan bagi kedua gen ini menunjukkan wujudnya dua kawasan jujukan yang hilang iaitu diantara nukleotida ke 1015 sehingga 1643 (628bp) dan nukleotida ke 2013 sehingga 2027 (14bp). Ini menunjukkan bahawa gen etilena yang diklon (*ER2₅*) mempunyai satu kawasan yang tidak ditranslasikan pada domain histidine kinase. Tambahan pula, ekspresi gen tersebut dikesan menggunakan kaedah 'real-time RT-PCR' pada kadar yang rendah dari tisu bunga yang dikaji, dan juga tisu akar. Berdasarkan keputusan ini adalah berkemungkinan *ER2₅* terlibat dalam perkembangan tisu tumbuhan yang berbeza.

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I certify that an Examination Committee has meet on 7th December 2009 to conduct the final examination of Umikalsum Mohamed Bahari on her Master of Science thesis entitled “Isolation and Expression Analysis of Ethylene Receptor Gene from *Oncidium* Gower Ramsey Flowers” in accordance with Universiti Pertanian Malaysia (higher degree) Act 1980 and university 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 the 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 Universiti Putra Malaysia or other institutions.

UMIKALSUM MOHAMED BAHARI

Date: 3 May 2010



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