



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERIZATION OF DISEASE AND STRESS
RELATED GENES FROM MUSKMELON (*CUCUMIS MELOL.*)**

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FSMB 2002 10

**ISOLATION AND CHARACTERIZATION OF DISEASE AND STRESS
RELATED GENES FROM MUSKMELON (*CUCUMIS MELO* L.)**

By

LEE WENG WAH

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of Requirement for the Degree of Master of Science**

JANUARY 2002

**Specially dedicated to my parents, sister,
friends and relatives.**

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

ISOLATION AND CHARACTERIZATION OF DISEASE AND STRESS RELATED GENES FROM MUSKMELON (*CUCUMIS MELO L.*)

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JANUARY 2002

Chairman : Tan Siang Hee, Ph.D.

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Soil salinity and attack by pathogen are the major abiotic stresses in plant agriculture worldwide. Past efforts to improve plant tolerance to osmotic stress and pathogen attack through breeding and genetic engineering have shown limited success owing to the genetic complexity of stress responses.

Large-scale partial sequencing of expressed sequence tags (ESTs) towards cataloging and categorizing genetically abiotic stress responses can assist a means for the rapid discovery of stress-specific genes. A combination of two types of abiotic stresses (200mM of NaCl and 14 min of 254nm UV-irradiation) were applied to muskmelon seedlings prior to the isolation of RNA for cDNA library construction. Cold plaque hybridisation using non-induced cDNA as probe to screen the stress-induced cDNA library for stress and disease-related genes was performed. A total of 550 ESTs were generated from a stress-induced cDNA library with a cDNA insert size ranging between 500-2500bp.

Classification of the ESTs based on their designated functions showed that the majority of isolated genes were involved in salt tolerance and disease resistance. The results showed that the generation of stress-induced ESTs by partial sequencing of random cDNA clones along with expression analysis are efficient approach to identify isolated genes that are responsible for salt tolerance and disease resistance on a large scale. We believe our dbEST and the associated DNA materials would be a useful resource to scientists engaged in studies on stress-tolerance and disease resistance.

MYB proteins are known to regulate different branches of flavonoid metabolism in plants in response to wounding, UV irradiation and ethephon treatment. Their role as transcriptional regulators in response to stress treatment and pathogen attack also has been reported (Urao *et al.*, 1993; Abe *et al.*, 1997). A 279bp partial MYB-related protein was also isolated by an RT-PCR method using the degenerate primers. At the same time, the double induced stress-related muskmelon cDNA library of salt and UV irradiation provide a good source to study the gene expression and regulatory of MYB gene in relation to the stress and UV treatment. Due to the high similarity of the partial MYB sequence to the cloning vector, pBluescript SK(+), screening of the full length MYB gene was unsuccessful.

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

**PENGASINGAN DAN PENCIRIAN GEN-GEN BERKAITAN DENGAN
PENYAKIT DAN TEKANAN DARI TEMBIKAI WANGI (*CUCUMIS MELO L.*)**

Oleh

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Kemasinan tanah dan serangan penyakit merupakan tekanan abiotik tumbuhan yang utama dalam pertanian sedunia. Usaha-usaha untuk mempertingkatkan toleransi tumbuhan terhadap tekanan osmotik dan serangan penyakit melalui teknik pembiak-bakaan dan kejuruteraan genetik sebelum ini hanya menghasilkan sedikit kejayaan kerana ia melibatkan genetik yang kompleks dalam tindak balas terhadap tekanan.

Penjujukan bahagian daripada teg jujukan ekspresi (ESTs) secara bersaiz besar ke arah mengkatalog dan mengkategorikan tindak balas genetik terhadap tekanan abiotik menghasilkan penemuan gen-gen yang spesifik terhadap rangsangan tekanan yang pantas. Kombinasi dua jenis tekanan abiotik (200mM NaCl dan 254nm UV radiasi selama 14 min) telah diaplikasikan terhadap anak benih tembakai wangi sebelum pengasingan RNA untuk proses penghasilan perpustakaan cDNA. Penghibridan plak sejuk dilakukan dengan menggunakan cDNA normal sebagai penyiasat untuk menskrin gen-gen yang bertanggungjawab dalam rangsangan tekanan dan

serangan penyakit dengan lebih ketara daripada perpustakaan cDNA rangsangan tekanan ini. Sejumlah 550 ESTs telah dihasilkan daripada perpustakaan cDNA rangsangan tekanan ini dengan saiz cDNA yang berjalat di antara 500-2500bp.

Klasifikasi ESTs berpandukan fungsi-fungsi mereka jelas menunjukkan bahawa majoriti daripada gen-gen ini terlibat dalam kawalan kemasinan dan mekanisme pertahanan tumbuhan dalam translasi mRNA. Hasilnya jelas menunjukkan bahawa penjana rangsangan tekanan ESTs daripada klon penjujukan bahagian secara rawak dan analisa ekspresi merupakan pendekatan yang berkesan untuk mengasingkan gen-gen yang bertanggungjawab dalam toleransi terhadap garam dan serangan penyakit secara bersaiz besar. Kami berpercaya bahawa dbEST dan bahan DNA kami akan membekalkan sumber yang berguna kepada para saintis yang terlibat dalam kajian toleransi terhadap tekanan dan penyakit.

Protein MYB dipercayai berupaya mengawal pelbagai cabang metabolisme flavonoid dalam tumbuhan di bawah rangsangan luka, radiasi UV dan rawatan ethephon. Di samping itu, peranan mereka sebagai kawalan transkripsi terhadap rangsangan tekanan and serangan penyakit turut dilaporkan. 279bp daripada faktor transkripsi MYB berjaya diasingkan daripada RT-PCR dengan menggunakan primer degenerasi. Pada masa yang sama, perpustakaan cDNA yang dijana merupakan sumber yang baik untuk mengkaji ekspresi dan kawalan gen terhadap rangsangan tekanan and rawatan UV. Pengskrinan gen MYB sepenuhnya tidak berjaya disebabkan

oleh persamaan jujukan yang tinggi di antara bahagian MYB yang diasingkan dengan vektor pengklonan iaitu pBluescript SK(+).

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I certify that an Examination Committee met on 25th January 2002 to conduct the final examination of Lee Weng Wah on his Master of Science thesis entitled "Isolation and Characterization of Disease and Stress Related Genes from Muskmelon (*Cucumis melo* L.)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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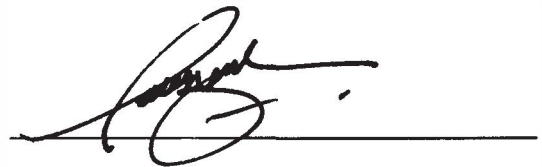


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DECLARATION

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.

A handwritten signature in black ink, appearing to read 'Lee Weng Wah', is written over a horizontal line.

(LEE WENG WAH)

Date: 22 March 2007

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CHAPTER I

INTRODUCTION

Today, about 20% of the world's-cultivated land and nearly half of all irrigated lands are affected by abiotic stresses e.g. high salinity (Rhoades and Lovedays, 1990). Environmental factors that impose water-deficit stresses, such as drought, high salinity and extreme temperature, place major limits on plant productivity. Numerous diseases caused by various sources of pathogen have been reported in various crops. Some of the infections may occur sporadically but may cause economic losses, especially in new regions where precautions are neglected (Turner, 1989). Two major strategies for controlling diseases currently employed are the use of chemical protection and breeding for resistant crops (Ou, 1972) leading to an increased usage of chemicals for pest management at an unprecedented rate (Mew, 1988) in crops plants e.g. muskmelon.

The discovery of novel genes has traditionally been a laborious task. Furthermore, the conventional breeding techniques to produce disease resistant and stress tolerant lines are limited, time consuming and laborious. Consequently, the development of disease resistant rice cultivars has received greater attention in the international food research programs since 1962 (Ou, 1972). The discovery of novel genes and gene expression patterns, and improved understanding of their roles in stress adaptation in response to abiotic stress has provided the basis for effective engineering

strategies, leading to enhanced stress tolerance. However, only a few new plants with improved stress resistance have been introduced for trial. No studies have been reported on defense or stress responsive gene expression in muskmelon.

Genomic approaches have revolutionized our understanding of plant tolerance to drought, diseases, high salinity and low temperature in recent years (Zhu, 2001). Progress is now anticipated through comparative genomics studies of an evolutionarily diverse set of model organisms, and through the use of techniques such as high-throughput analysis of expressed sequence tags, large-scale parallel analysis of gene expression, targeted or random mutagenesis and gain-of-function or mutant complementation.

Muskmelon (*Cucumis melo* L.) is an important vegetable crop in the temperate zone (Jelaska, 1986) but also grows very well especially in country like Malaysia. For the year 1986, the estimated cultivation area was 160 ha and the demand for this crop has increased over the past few years to serve the dessert and canned food industries (Norlia, 1986). *Cucumber Mosaic Virus* (CMV), *Watermelon Mosaic Virus* (WMV), *Fusarium oxysporum* and *Mycosphaerella melonis* are the pathogens that evoke tremendous crop losses in term of yield and quality (Whitaker and Davis, 1962). Thus, the isolation and characterization of disease resistance or stress responsive genes through genetic engineering are the initial steps in the commencement of a molecular biology study of muskmelon towards the understanding of defense and stress response mechanisms.

Therefore, it is important that effective techniques and strategies are developed in order to have a better control over diseases and to overcome any potential environmental stresses on the plants. A more suitable strategy of controlling plant diseases and environmental stresses is the use of resistant cultivars. Large-scale single-pass sequencing of randomly selected cDNA clones, gives rise to a large number of expressed sequence tags and provides a cost-effective and rapid alternative route towards the isolation of high salinity tolerance and disease resistant related genes (Ohlrogge and Benning, 2000).

The objectives of this study are to isolate and characterise disease resistant and stress related genes from stress-induced cDNA library of muskmelon using the strategy of expressed sequence tags. Northern blot analyses were carried out to study the gene expression of the isolated genes in response to stresses. Southern blot were also carried out to determine the copy number of the isolated genes in the muskmelon genome. The sequence and clones resulting from this study may provide useful information for researchers to further study the molecular mechanisms of plant salinity tolerance and disease resistance.

CHAPTER II

LITERATURE REVIEW

The Botany of Muskmelon (*Cucumis melo* L.)

The muskmelon (*Cucumis melo* L.) belongs to the *Cucurbitaceae* family which encompasses more than 130 genera and over 900 species (Jeffrey, 1980). The muskmelon originated from the tropics and subtropics of Africa (Leppik, 1966) and was first introduced to Malaysia in the late 70's or early 80's (Norlia, 1986). The cultivated varieties are tendril-climbing or prostrate annual (Purseglove, 1968).

Muskmelon has a diploid chromosome number of $2n=24$ (Whitaker, 1930; Whitaker and Bohn, 1954; Ramachandran and Seshadri, 1986) and most of them are andromonoecious producing both male flower and perfect flowers (hermaphrodite) (Munger, 1942).

The shape of the fruits varies among the varieties, they are found in spherical, oval and elongated shape. Jade Dew, Emerald Sweet, Hami Melon, Sun Lady and Milky Way are the most popular varieties in Malaysia (Norlia, 1986; Syed Mohd, 1992). These varieties are F1 hybrids that are imported from Japan and Taiwan. The other cucurbits include cucumber (*Cucumis sativus*), watermelon (*Citrullus vulgaris*), pumpkin (*Cucurbita maxima*) and gourds (*Cucurbita pepo*).

Economical Value of Muskmelon

Muskmelon is cultivated in Malacca, Pahang and Johore (Norlia, 1986) and the cultivated area was estimated to be 160ha in year 1990 (Radziah and Ahmad, 1990). The muskmelon fruits with sweet flesh and nice aroma are economically important and are high in demand in comparison to cereals or vegetable crops and are fast becoming an export commodity (Alang *et al.*, 1990) for South East Asia, Central China and East Africa (Norlia, 1986; Jelaska, 1986; Dong *et al.*, 1990). The market demand for this crop increased tremendously over the last few years due to its higher yield, short maturity and superior fruit quality. It is exported to Singapore, Hong Kong, Brunei and a few other countries (FAMA, 1992).

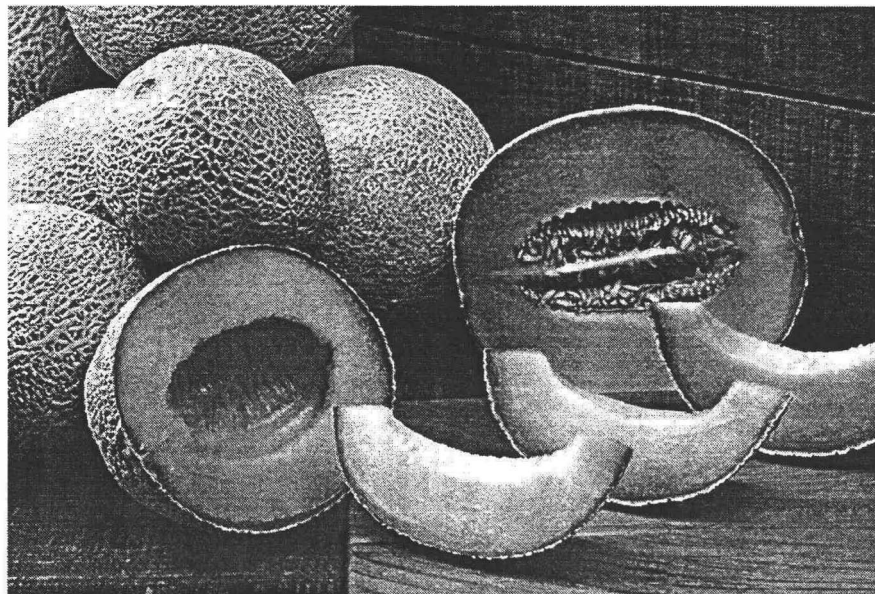


Figure 1 : Birdie melon, one of the most popular variety of cultivated muskmelon in Malaysia.

Diseases in Muskmelon

The diseases that normally attack cucurbits are numerous and widespread causing tremendous crop losses in terms of yield and quality (Known-you, 1992). Commonly known diseases are *Cucumber Mosaic Virus (CMV)*, *Watermelon Mosaic Virus (WMV)*, fusarium wilt (*Fusarium oxysporum*), downy mildew (*Pseudoperonospora cubensis*) and stem rot (*Mycosphaerella melonis*). The infections are extremely difficult to handle though control measures have been properly sought (Whitaker and Davis, 1962). In addition to the pathogens mentioned above, crops losses have also been compounded by the mono-culture of muskmelon as well as the excessive application of agricultural pesticides and improper cultivation practices.

Transcription Factors

Gene regulation in eukaryotes occurs at different levels and has been intensively studied in the area of transcription. Essential proteins involved in this process are transcription activators which influence and regulate the expression of specific target genes (Katagiri and Chua, 1992). Different plants species can vary in their responses to environmental changes, depending on the habitat in which they have evolved. The elucidation of the mechanisms through which various stresses can be managed by different plant species is very valuable. Among the different mechanisms that can affect gene expression, transcriptional regulation is presently the best characterised in