UNIVERSITI PUTRA MALAYSIA

TOWARDS THE DEVELOPMENT OF SALT TOLERANT RICE VARIETIES BY OVEREXPRESSING cDNAs FROM A MANGROVE PLANT Acanthus ebracteatus Vahl

SHAHANAZ SULTANA

FBSB 2010 10

SHAHANAZ SULTANA DOCTOR OF PHILOSOPHY 2010

TOWARDS THE DEVELOPMENT OF SALT TOLERANT RICE VARIETIES BY OVEREXPRESSING cDNAS FROM A MANGROVE PLANT *Acanthus ebracteatus Vahl*

SHAHANAZ SULTANA

DOCTOR OF PHILOSOPHY
UNIVERSITY PUTRA MALAYSIA
2010

Nama penuh/Full name: Shahanaz Sultana
No. matrik (matric No.): GS16179
Program (Programme): Doctor of Philosophy
Bidang Pengajian (Field of Study): Molecular Biology and Genetic Enginnering
Fakulti/Institut (Faculti/Institute): Biotechnology and Bio-Molecular Sciences
Tajuk Thesis Muktamad (Final Title of Thesis): Towards the Development of Salt Tolerant Rice Varieties by Overexpressing cDNAs From A Mangrove Plant *Acanthus ebracteatus Vahl*
Tarikh Kelulusan Senate (Senate Approved Date): 21 October 2010

TOWARDS THE DEVELOPMENT OF SALT TOLERANT RICE VARIETIES BY OVEREXPRESSING cDNAs FROM A MANGROVE PLANT *Acanthus ebracteatus Vahl*

By

SHAHANAZ SULTANA

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

June 2010

This thesis is dedicated to

my Late Father Md. Shamsul Haque;

my Mother Kazi Sadequn Naher;

my Husband Md. Mahbub Morshed and

also to my beloved daughter Maleeha Muniyat

for their endless love and sacrifices

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

TOWARDS THE DEVELOPMENT OF SALT TOLERANT RICE VARIETIES BY OVEREXPRESSING cDNAs FROM A MANGROVE PLANT *Acanthus ebracteatus Vahl*

By

SHAHANAZ SULTANA

June 2010

Chairman : Assoc. Prof. Suhaimi Napis, PhD

Faculty : Biotechnology and Biomolecular Sciences

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. Environmental stresses such as salinity are important limiting factors for crop growth and yield. Rice is a salt-sensitive crop that suffers salinity stress from germination to maturity, resulting in very poor yield. Rice varieties with high yield and tolerance to salt have to be developed to enable cultivation of rice plant on saline area. Two cDNAs encoding monodehydroascorbate reductase (AcMDHAR) and 9-cis-epoxycarotenoid dioxygenase (AcNCED) were previously isolated from a mangrove plant, *Acanthus ebracteatus*. These enzymes were reported to be involved in different salt tolerance mechanisms in plants. Monodehydroascorbate reductase (MDHAR), an important enzyme in the ascorbate-glutathione cycle, is involved in the salt tolerance mechanism of plants through scavenging of reactive oxygen species (ROS). While NCED is an important enzyme in the oxidative cleavage reaction of abscisic acid (ABA), a plant

hormone which plays a vital role in stress tolerance. In this study, two overexpression vectors, pEXP32-AcMDHAR and pEXP32-AcNCED; were constructed using Gateway® technology and transformed into Taipei 309 and BRRI dhan29 rice varieties through *Agrobacterium*-mediated transformation, respectively. The putative transformants overexpressing AcMDHAR and AcNCED were selected *in vitro* using hygromycin and confirmed by PCR screening. The copy number of AcMDHAR in transgenic rice plants, ranged from single to multiple copies which was determined by real time PCR. The abundance of transcripts in transgenic rice plants was also analyzed by real time PCR. Transgenic rice plants overexpressing AcMDHAR treated at 100 mM NaCl showed significant increase in MDHAR enzyme activity compared to untransformed (UT) plants and showed tolerance to salt at germination, seedling and reproductive stages. These results implied that the overexpression of AcMDHAR in rice can enhance tolerance to salt through the increase of MDHAR enzyme activity. None of the rice plants overexpressing AcNCED showed tolerance to salt although expression was detected by real time PCR. The levels of ABA, in transgenic rice plants overexpressing AcNCED treated with 100 mM NaCl for 24 hours were similar to those in NaCl - treated UT plants. In contrast, higher levels of phaseic acid (PA), dihydrophaseic acid (DPA) and ABA- glucose ester (ABA-GE) were observed in rice plants overexpressing AcNCED compared to UT plants, indicating that ABA has been converted to PA, DPA and ABA-GE rapidly in the transgenic rice plants. These results suggested that the rapid degradation of excess ABA through self regulatory mechanisms may have caused the failure of these transgenic rice plants to exhibit salinity tolerance.

The findings of this study have provided important information towards the gene manipulation and the development of salt tolerant transgenic rice.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KE ARAH PENGHASILAN VARIETI PADI YANG TAHAN TERHADAP KEMASINAN DENGAN MENZAHIRKAN cDNA DARIPADA POKOK BAKAU *Acanthus ebracteatus Vahl*

Oleh

SHAHANAZ SULTANA

June 2010

Pengerusi : Assoc. Prof. Suhaimi Napis, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Padi (*Oryza sativa* L.) adalah salah satu daripada tanaman bijirin yang paling penting di dunia. Tekanan persekitaran seperti kemasinan adalah faktor penghad yang penting bagi perkembangan dan hasil tanaman. Padi adalah tanaman yang sensitif terhadap kemasinan dari peringkat percambahan hingga kematangan, mengakibatkan penghasilan yang sangat rendah. Varieti padi yang mempunyai hasil yang tinggi dan kerintangan terhadap kemasinan perlu dihasilkan untuk membolehkan penanaman pokok padi di kawasan yang bergaram. Dua cDNA yang mengkodkan monodehydroascorbate reductase (AcMDHAR) dan 9-cis-epoxycarotenoid dioxygenase (AcNCED) telah dipencilkan daripada pokok bakau, *Acanthus ebracteatus* sebelum ini. Enzim-enzim tersebut telah dilaporkan terlibat dalam mekanisma-mekanisma toleransi terhadap kemasinan yang berlainan dalam tumbuhan. Monodehydroascorbate reductase (MDHAR), satu enzim yang penting dalam kitar askorbat-glutathione, terlibat dalam

mekanisma toleransi terhadap kemasinan melalui penyingkiran spesies oksigen reaktif (ROS). Manakala NCED adalah satu enzim yang penting dalam reaksi pemotongan oksidatif asid absisik (ABA), satu hormon tumbuhan yang memainkan peranan penting dalam toleransi terhadap tekanan. Dalam kajian ini, dua vektor penzahiran, pEXP32-AcMDHAR dan pEXP32-AcNCED; telah dibina menggunakan teknologi Gateway® dan ditransformasikan ke dalam padi varieti Taipei 309 dan BRRI dhan29 menggunakan transfomasi berpengantara *Agrobacterium*. Transfoman putatif yang menzahirkan AcMDHAR dan AcNCED berlebihan telah dipilih secara *in vitro* menggunakan higromisin dan disahkan menggunakan penyaringan PCR. Jumlah salinan transgen AcMDHAR dalam padi transgenik adalah dalam lingkungan 1 hingga 22 seperti yang ditentukan menggunakan *PCR* masa-nyata. Kuantiti transkrip dalam padi transgenik ini juga dianalisa dengan PCR masa-nyata. Pokok padi transgenik yang menzahir AcMDHAR berlebihan yang dirawat dengan 100 mM NaCl menunjukkan peningkatan aktiviti enzim AcMDHAR yang signifikan berbanding dengan pokok bukan transfoman (UT) dan menunjukkan toleransi terhadap kemasinan pada peringkat percambahan, anak benih dan reproduktif. Keputusan ini menunjukkan bahawa penzahiran AcMDHAR dalam padi boleh meningkatkan toleransi terhadap kemasinan melalui peningkatan aktiviti enzim MDHAR. Tiada pokok padi yang menzahirkan AcNCED berlebihan menunjukkan toleransi terhadap kemasinan walaupun ditunjukkan oleh PCR masa-nyata. Tahap ABA dalam padi transgenik yang menzahir AcNCED dan dirawat dengan 100 mM NaCl selama 24 jam adalah sama dengan pokok UT yang dirawat dengan NaCl. Sebaliknya, tahap asid faseik, asid dihidrofaseik (DPA) dan ABA-glukos ester (ABA-GE) yang lebih tinggi diperhatikan dalam pokok padi yang menzahir AcNCED

berbanding pokok UT, menunjukkan ABA telah berubah kepada PA, DPA dan ABA-GE dengan cepat dalam pokok padi transgenik. Keputusan ini mencadangkan bahawa degradasi ABA ynag cepat melalui mekanisma pengawalan sendiri mungkin menyebabkan kegagalan pokok padi transgenik untuk menunjukkan toleransi terhadap kemasinan. Hasil daripada kajian ini memberikan maklumat yang penting ke arah manipulasi gen dan penghasilan padi transgenik yang toleran terhadap kemasinan.

ACKNOWLEDGEMENTS

I would like to give thanks to the Almighty Allah for giving me strength and wisdom to complete my study. May His name be glorified and praised.

I would like to express my gratitude to Assoc. Prof. Dr. Suhaimi Napis for giving me the opportunity to pursue my Ph.D study. My sincere appreciation is extended to Assoc. Prof. Dr. Ho Chai Ling for her guidance, constructive criticisms, encouragement and support provided during the study period and thesis preparation. My sincere gratitude also to Dr. Parameswari Namasivayam for her guidance, suggestions, and advice throughout the study.

It is my greatest pleasure to acknowledge Third World Organization for Women in Science (TWOWS) for awarding me the fellowship for the study. I am also very grateful to my employer organization Bangladesh Rice Research Institute (BRRI) to grant my deputation for pursuing my study.

I wish to express my heartfelt gratitude and appreciation to my husband Md. Mahbub Morshed and my daughter Maleeha Muniyat for their endless love; sacrifices; physical and mental support to make this thesis a reality.

I wish to thank Assoc. Prof. Dr. Norihan Saleh for allowing me to use the tissue culture laboratory. I would also like to thank Dr UmaRani Sinnah, Department of Crop Science,

Faculty of Agriculture, University Putra Malaysia for allowing me to use the photosynthetic meter.

I would like to extend my gratitude to Dr. Francisco J Corpas Departamento de Bioquimica, Biología Celular y Molecular de Plantas, SPAIN for his kind technical suggestion for MDHAR enzyme assay. I also like to extend my gratitude to Dr. Se Ooi, MPOB for helping me to do the hormone quantification. I wish to express my sincere gratitude to Ky, Thuc and Samanthi for their technical knowledge and support. Many thanks to Nghia, Hasna, Laila, Tran, Chi, Chin Jit, Waisun, Kalai, Raosan, Kea tai, Yanti, Mostafa for their friendship, support and encouragement. My special thanks also to Fatheh for translating my abstract from English to Bahasa Malayu.

Last but not least, I wish to express my deepest gratitude and appreciation to my parents, siblings and family for their love and constant support through out my study.

I certify that an Examination Committee has met on 25/06/2010 to conduct the final examination of Shahanaz Sultana on her Ph D thesis entitled "Towards the Development of Salt Tolerant Rice Varieties by Overexpressing cDNAs from a Mangrove Plant" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee were as follows:

(Chairman)

(Internal Examiner)

(Internal Examiner)

(External Examiner)

Prof. Dr. Bujang Kim Huat, Ph D
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Suhaimi B Napis, Ph D
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Ho Chai Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Parameswari 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: 21 October 2010

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 other institutions.

_____

Shahanaz Sultana

Date: 29/06/10

TABLE OF CONTENTS