



UNIVERSITI PUTRA MALAYSIA

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

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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

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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

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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 mekanisme-mekanisma toleransi terhadap kemasinan yang berlainan dalam tumbuhan. Monodehydroascorbate reductase (MDHAR), satu enzim yang penting dalam kitar askorbat-glutathione, terlibat dalam



mekanisme 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 BRR1 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.



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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:

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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

	Page
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	xii
DECLARATION	xii
LIST OF TABLES	xvii
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INRODUCTION	1
2 LITERATURE REVIEW	
2.1 Rice plants	4
2.1.1 Rice variety BRR1 Dhan29	6
2.1.2 Rice variety Taipei 309	8
2.2 Rice cultivation practice	8
2.3 Importance of rice	9
2.4 Effect of salt on plants	11
2.4.1 Effects on seed germination and seedling establishment	12
2.4.2 Effects on vegetative growth	13
2.4.3 Effects on reproductive stage	14
2.4.4 Effects on photosynthesis	15
2.4.5 Effects on nutrient uptake	16
2.5 Salt tolerance mechanisms in plants	18
2.5.1 Homeostasis	18
2.5.2 Detoxification	23
2.5.3 Hormones	24
2.5.4 Other mechanisms	25
2.5.5 Mangrove	27
2.5.6 Salt tolerance genes	29
2.6 Genetic engineering and tissue culture of rice	39
2.6.1 <i>Agrobacterium tumefaciens</i> - mediated gene transformation	41
2.6.2 Gateway technology	46
2.7 Real time PCR	49
3 OVEREXPRESSION OF AcMDHAR FROM A MANGROVE PLANT IN RICE FOR SALT TOLERANCE	
3.1 Introduction	51
3.2 Materials and methods	54



3.2.1	Materials	54
3.2.2	Construct preparation for overexpression studies	54
3.2.3	<i>Agrobacterium</i> -mediated transformation into rice	66
3.2.4	Molecular analysis of transgenic rice plants	70
3.2.5	Screening of putative transformants with hygromycin B	79
3.2.6	Morphological and yield contributing characteristics analyses of T ₁ generation	80
3.2.7	Examining the salt tolerance of pEXP32- AcMDHAR transgenic plants	81
3.2.8	Leaf senescence assay for salinity stress tolerance	87
3.2.9	Statistical analysis	87
3.3	Results	
3.3.1	The Construction of overexpression vector, pEXP32- AcMDHAR	88
3.3.2	<i>Agrobacterium</i> -mediated transformation of pEXP32 AcMDHAR into rice variety <i>Oryza sativa</i> japonica variety Taipei 309	92
3.3.3	Molecular analysis of pEXP32-AcMDHAR transformants	94
3.3.4	Phenotypic change of T ₀ pEXP32-AcMDHAR plants	98
3.3.5	Segregation analysis of AcMDHAR transgene in T ₁ transgenic plants	100
3.3.6	PCR screening of pEXP32-AcMDHAR transformants at T ₁ generation	103
3.3.7	Phenotypic characteristics of pEXP32-AcMDHAR transformed plants at T ₁ generation	104
3.3.8	Examine the salt tolerance of transgenic rice plants overexpressing AcMDHAR	107
3.3.9	Leaf senescence assay	124
3.4	Discussion	
3.4.1	Production of transgenic rice by overexpressing AcMDHAR	127
3.4.2	Morphological and yield contributing characteristics of rice plants overexpressing AcMDHAR	129
3.4.3	Salt tolerance of rice plants overexpressing AcMDHAR	130
3.5	Conclusion	133
4	OVEREXPRESSION OF AcNCED FROM A MANGROVE PLANT IN RICE FOR SALT TOLERANCE	
4.1	Introduction	134
4.2	Materials and methods	136
4.2.1	Materials	136
4.2.2	Sequencing of the full length cDNA sequence of AcNCED	136
4.2.3	Construct preparation for overexpression studies	137
4.2.4	<i>Agrobacterium</i> -mediated transformation into rice	143
4.2.5	Molecular analysis of pEXP32-AcNCED transgenic rice plants	144
4.2.6	Screening of putative transformants with hygromycin B	150



4.2.6.1	Determination of MIC of hygromycin B for seeds	150
4.2.6.2	Segregation analysis of transformants	151
4.2.7	Morphological and yield contributing characteristics analysis	151
4.2.8	Examine the salt tolerance of transgenic rice plants overexpressing AcNCED	152
4.2.9	Determination of ABA, PA, DPA and ABA-GE	152
4.3	Results	
4.3.1	Sequence analysis of AcNCED transcript	154
4.3.2	The construction of overexpression vector pEXP32-AcNCED	161
4.3.3	<i>Agrobacterium</i> -mediated transformation of pEXP32-AcNCED into <i>Oryza sativa</i> indica rice variety BRRI dhan29	165
4.3.4	Molecular analysis of pEXP32-AcNCED transformants	167
4.3.5	Segregation analysis of AcNCED transgene in T ₁ transgenic plants	172
4.3.6	PCR screening of pEXP32-AcNCED transformants at T ₁ generation	174
4.3.7	Phenotypic characteristics of T ₁ pEXP32-AcNCED transformed plants	174
4.3.8	Examining the salt tolerance of pEXP32-AcNCED transgenic plants	178
4.3.9	Determination of ABA and metabolites	183
4.4	Discussion	
4.4.1	Production of transgenic rice overexpressing AcNCED	185
4.4.2	Morphological and yield contributing characteristics of rice plants overexpressing AcNCED	187
4.4.3	Salt tolerance of rice plants overexpressing AcNCED	187
4.5	Conclusion	189
5	GENERAL DISCUSSION AND RECOMMENDATIONS	
5.1	General discussion	190
5.2	Recommendations	192
6	CONCLUSIONS	193
	REFERENCES	195
	APPENDICES	226
	BIODATA OF STUDENT	246
	LIST OF PUBLICATIONS	247

