ISOLATION AND CHARACTERIZATION OF SALT-INDUCED GENES FROM THE HALOPHYTIC PLANT *Cakile maritima*

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2002.
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APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: Isolation and characterization of salt-induced genes from the halophytic plant *Cakile maritima*
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ABSTRACT

The halophytic plant (*Cakile maritima*) possess many unique genetic characteristics contributing to plant's ability to withstand and tolerate salinity conditions. It is a member of the *Brassicaceae* family, with a small genome size (≈ 720 Mb) and a short life cycle, making it of value for conducting genetic studies to understand molecular bases of salinity. In the present study, *C. maritima* seedlings were exposed to different salt treatments for different exposure time to identify new salinity-responsive genes. One group was directly treated with different salinity levels (0, 100, 200, 300 and 400 mM NaCl) for 4, 8, and 12 hrs.; while the second group was subjected to a gradual increase of salt concentration. Some of the transcripts that were differentially expressed in response to the salinity stress were isolated using the differential-display reverse transcription-PCR (DDRT-PCR) technique. Fourteen re-amplified fragments were selected, and were bioinformatically analyzed and annotated. The annotated fragments were categorized according to their proposed function into two major categories. The first group showed homology to genes related to cellular membranes stabilization {Rho GTPase-activating protein, β-galactosidase gene, Arabinogalactan (AGP) gene, and ATP-Binding Cassette transporter (ABC transporter) C family member 10). The second group of transcripts revealed homology to genes involved in pathways directly or indirectly involved in stabilizing cells under salinity stress {receptor 3 from Toll-like protein (TLR3), 4 fragments homologues to different Alcohol dehydrogenase (*ADH*) gene, Polybromo protein, 3-hydroxyisobutyryl-CoA hydrolase gene, MOR1 gene and 2 fragments homologue to sulfite reductase (*SiR*) gene}.The sequence of Alcohol dehydrogenase (*ADH* and sulfite reductase (*SiR*) were used to design specific primers for RACE and full length gene isolation. The 5' and the 3' ends of the two genes were determined and the full length of the *ADH* gene was isolated, with the expected size of 1200bp. The *ADH* was cloned into The pYES2 expression vector for expression in *Saccharomyces cerevisiae* . The transgenic yeast cells were assayed for salt tolerance under different salt concentrations (0.0 M, 0.5 M,1.0 M, 1.5M, 1.7M,1.8M,1.9M, 2M, 2.1M, 2.2M and 2.3M NaCl) for 7days. Yeast growth was measured at OD600nm. The transgenic cells showed high level of salinity tolerance and higher proline content than non-transgenics under the same different salt concentrations. In addition, the SiR gene was isolated, while lacking 517 bp out of the 2 kp full length.

Key words: (*Cakile maritima*), differential-display (DDRT-PCR), gene expression, *Saccharomyces cerevisiae* *ADH* gene *SiR* gene gene isolation

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LIST OF ABBREVIATIONS

Ap1	Arbitrary primer
$T_{11}A$	Anchored primer A
BLASTn	Nucleotide blast (Search a nucleotid database using a nucleotide query
NCBI	National Center for Biotechnology Information
cDNA	Complementary DNA
ddH_2O	Double-distilled water
DDRT-PCR	Differential display reverse transcription-PCR
E.coli	*Escherichia coli*
IPTG	Isopropyl β-D-1-thiogalactopyranoside
X-Gal	5-Bromo-4-Chloro-3-Indolyl β-D-Galactopyranoside
CIP	Calf Intestine Alkaline Phosphatase
PCR	Polymerase chain reaction
min	minutes
sec	seconds
rpm	Round per minute
Re-PCR	Reamplified-PCR
RACE	Rapid amplification of cDNA ends
ROS	Reactive oxygen species
SOD	Superoxide dismutase
GTPase	Guanosine triphosphatases
Arf	ADP ribosylation factor
Ras	Rat sarcoma viral oncogene homologue
Ran	Ras-related nuclear protein
Rho	Ras homologous
ADH	Alcohol dehydrogenase
SIR	Sulfite reductase
E value	Expectation valu

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