ISOLATION AND CHARACTERIZATION OF ENVIRONMENTAL STRESS RELATED GENES FROM SOME WILD PLANTS

By

HAYTHAM MOHAMED ABD ELHALIM
B.Sc. Agric. Sc. (Genetics), Ain Shams University, 2003

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ABSTRACT

Haytham Mohamed Abd-Elhalim Ismail: Isolation and Characterization of Environmental Stress Related Genes from Some Wild Plants. Unpublished M.Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2010.

Proline is the most common organic solutes that play an active role in the mechanism of plant salt tolerance. Ornithine δ-aminotransferase (δ-OAT) is a key enzyme in proline biosynthetic pathway and is implicated in salt tolerance in higher plants. In this study full-length coding sequence encoding the Ornithine δ-aminotransferase enzyme was isolated from legume *Vicia villosa* by RT-PCR approach. *In-silico* mapping of *Vicia villosa* Ornithine δ-aminotransferase (VvOAT) revealed that this gene is located on barrel medic chromosome 3, lotus japonicus chromosome 1, soybean chromosome 4 and common bean chromosome 1. Docking generated 3D model of the gene with its known substrate and inhibitors was performed, the results indicated that PHE170 and ASN171 in VvOAT are the important determinant residues in binding substrate and inhibitors. Obtained protein from gene expression showed a molecular mass compatible with the predicted mass, confirming that the protein is fully intact and not truncated. After gene delivery into *E. coli* host HB101, VvOAT gene enhanced its salt tolerance.

Key words: *Vicia villosa* - Salt tolerance – Osmoprotectants - Ornithine δ-aminotransferase - RT-PCR

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LIST OF ABBREVIATION

ABA	Abscisic acid
ACP	Annealing-control-primer
APX	Ascorbate peroxidase
Blast	Basic local alignment search tool
CBF	C-repeat binding factors
Cd	Conserved domain
DEGs	Differentially expressed genes
DREB	Dehydration response element binding protein
EST	Expression sequence tags
FAO	Food and Agriculture Organization
GSA	Glutamate semialdehyde
Hsps	Heat-shock proteins
IAA	Indole-acetic acid
LEA	Late embryogenesis abundant
MAPK	Mitogen activated protein kinase
NJ	Neighbor-joining
OAT	Ornithine aminotransferase
ORF	Open reading frame
Orn	Ornithine
P5CDH	P5C dehydrogenase
P5CR	Pyrroline-5-carboxylate reductase
P5CS	Pyrroline-5-carboxylate synthase

PAGE	Polyacrylamid gel elecrtophresies
PCR-SSH	PCR-based suppression subtractive hybridization
PDB	Protein Data Bank
PDH	Pro dehydrogenase
PLP	Pyridoxal-5-phosphate
PRE	Proline responsive elements
Pro	Prolin
RACE	Rapid amplification of cDNA ends
RT-PCR	Reverse transcriptase-polymerase chain reaction
ROS	Reactive Oxygen Specie
SOD	Superoxide dismutase
SOS	Salt overlay salinity
TFs	Transcription factors

I. INTRODUCTION

Early communities were situated near reliable water sources with the consequence that crops became gradually adapted/selected for growth under near optimal conditions of water supply **(Bressan *et al.,* 2009)**, they established agrarian societies in order to give rise to the civilization.

One of the major obstacles encountering the development of agrarian societies is abiotic stress, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress such stresses are serious threats to agriculture and result in the deterioration of the environment. Abiotic stresses are considered as the primary causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% **(Boyer, 1982; Bray *et al.,* 2000)**.

Salt stress is one of the abiotic stresses that impose a major environmental threat to agriculture. Its adverse impacts are getting more serious problem in regions where saline water is used for irrigation (**Türkana and Demiral, 2009)**. The increased salinization of arable land showed that it is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 **(Wang *et al.,* 2003)**.

Salinity affects all the major processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism **(Parida and Das, 2005)**.

The final effect is estimated as death of plants or decrease in productivity. Suppression of growth occurs in all plants, but their tolerance levels and rates of growth reduction at lethal concentrations of salt vary widely among different plant species.

One of the most common stress responses in plants is overproduction of different types of compatible organic solutes **(Serraj and Sinclair, 2002**). Proline accumulation is one of the most frequently reported modifications induced by water and salt stresses in plants and is often considered to be involved in stress resistance mechanisms, although its precise role still remains a controversial subject. Cytoplasmic accumulation of this amino acid is thought to be involved in osmotic adjustment of stressed tissues **(Delauney and Verma 1993, Kavi Kishor *et al.,* 1995)**.

Ornithine δ-aminotransferase (OAT) is an important enzyme in proline biosynthetic pathway and is implicated in salt tolerance in higher plants. OAT transaminates ornithine to pyrroline-5-carboxylate, which is further catalyzed to proline by pyrroline-5-carboxylate reductase **(Sekhar *et al.,* 2007)**.

It was reported that the activity of δ-OAT as well as the proline contents in plants increased when treated with cold **(Charest and Phan, 1990)**, salt **(Roosens *et al.,* 1998)**, dehydration **(Hervieu *et al.,* 1994)** and ABA (Abscisic acid) **(Yang *et al.,* 2000)**.

There are two research topics which elucidate the significance of studying OAT: (i) production of genetically modified plants for increased production of the osmoprotectant proline, (ii) considering human OAT as a potential target for development of new therapeutic drugs **(Stránská *et al.,* 2008)**.

The present study aims to isolate and characterize ornithine δ-aminotransferase gene using ABA as inducer for gene expression and to give a primary estimates of the encoded protein function.