IMPLEMENTATION OF BIOTECHNOLOGY FOR IMPROVING SALT STRESS TOLERANCE IN PLANT USING PRROTEOMIC STUDY

Submitted By

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B.Sc. of Agricultural Sciences (Biochemistry), Faculty of Agriculture,
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A thesis submitted in Partial Fulfillment
Of
The Requirement for the Doctor of Philosophy Degree
In
Environmental Sciences

Department of Environmental Agricultural Sciences
Institute of Environmental Studies and Research
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APPROVAL SHEET

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ABSTRACT

A proteomic approach was used to identify proteins affected by salt in the halophyte plant *Atriplex nummularia L*. A total of twenty seven protein spots were reproducibly detected and analyzed on 2 DE gel. These proteins showed changes under salinity condition in *Atriplex* leaves and were up-regulated. The identified protein spots were involved in biosynthesis, ATP generation, metabolism and photosynthesis. In an attempt to explore the data of the proteomic experiment, we have studied the effect of some compounds on wheat plant under salinity condition. The relevant of such compounds have been used as exogenous application under salinity. The selected compounds were proline, ascorbic acid, arginine, glutathione and glutamine. The results obtained from this experiment revealed that growth parameters (shoot length, root weight and grain weight), biochemical compounds (chlorophyll, carbohydrate, starch, protein, fiber, ash and fat content) and some antioxidant enzymes such as ascorbate peroxidase, peroxidase and catalase activity were affected by both salt and treatments. The selected compounds mitigate the negative effects of salt stress and improved growth parameters and biochemical compounds compared with control plants under different salinity level.

Key words: Salt stress, salt tolerance, proteomic approach, exogenous application, *Atriplex* plant and wheat plant.

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LIST OF ABBREVIATIONS

AAT	Aspartate aminotransferase
ABA	Abscisic acid
ABF_3	Abscisic acid responsive elements-Binding Factor 3
ABF_4	Abscisic acid responsive elements-Binding Factor 4
ALDHs	Aldehyde dehydrogenase
APX	ascorbate peroxidase
ASA	Ascorbic acid
ATP	Adenine triphosphate
Ca	Calcium
CBB	Coomassie brilliant blue
CHCA	a-cyano-4-hydroxycinnamic acid
CID	Collision induced dissociation
Cl	Chloride
CMO	Choline mono-oxygenase
CO_2	Carbon dioxide
DHAR	Dehydroascorbate reductase
DHB	Dihydroxybenzoic acid
DNA	Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
ECD	Electron capture dissociation
ESI	Electrospray ionization
ESTs	Expressed sequence tags
ETD	Electron transfer dissociation
EUI	elongated uppermost internode
FAD	Flavin adenine dinucleotide
FBP aldolase	Fructose-1,6-bisphosphatase aldolase
GAs	Gibberellins
GB	Glycinebetaine
GPX	Glutathione peroxidase

GR	Glutathione reductase
GS	Glutamine synthetase
GSR	Glutathione-disulfide reductase
H	Hydrogen
H_2O_2	Hydrogen peroxide
ha	Hectare
HSP	Heat-shock proteins
IAA	indole-3-acetic acid
IEF	Isoelectric focusing
IPG	Immobilized pH gradient
IPG	immobilized pH gradient
JA	Jasmonic acid
K	Potassium
KD	Kilo Dalton
LC	Liquid chromatography
Li	Lithium
MALDI	Matrix-assisted laser desorption/ionization
mg	Milligram
Mg	Magnesium
min	Minute
ml	Milliliter
mRNA	Messenger RNA
MS	Mass spectrometry
MW	Molecular weight
Na	Sodium
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
OEEs	Oxygen-evolving enhancer proteins
P5CS	Pyrroline-5-carboxylate synthase
PA	Picolinic acid
pI	Isoelectric point
PMSF	Phenylmethyl sulfonyl fluoride
PPDK	Orthophosphate dikinase
PSII	Photosystem II
PTMs	Post-translational modifications
RNA	Ribonucleic acid

ROS	Reactive oxygen species
SA	salicylic acid
SAGE	serial analysis of gene expression
SAM	S-adenosyl-L-methionine
SAMS	S-adenosyl-L-methionine synthase
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SID	Surface induced dissociation
SOD	Superoxide dismutase
SOS	Salt Overly Sensitive
SOS	Salt overly sensitive
TK	Transketolase
TPI	Triosphosphate isomeraseas
DMSP	Dimethyl sulfonium propironate
ng	Nanogram
PMF	Peptide mass fingerprinting
TOF	Time-of-flight
2 DE gel	Two-dimensional gel electrophoresis

I. INTRODUCTION

Crop plants are often exposed to various biotic and abiotic stresses that greatly reducing the productivity of the crop worldwide. Salinity is a major abiotic stress limiting crop productivity worldwide and factor seriously affecting crop production in different regions, particularly in arid and semi–arid regions and affects more than 800 million hectares of land, equivalent to more than 6% of the total global area of the Earth (**Munns and Tester, 2008; Taffouo *et al.*, 2009; Deinlein *et al.*, 2014; Carmelina and Stefania 2016**).

Salt stress causes detrimental effects on crop yield by altering plant metabolism, including reduced water potential, ion imbalances and toxicity (**Krishnamurthy *et al.*, 2016**). Understanding the molecular mechanisms underlying the plant's response to salinity stress will facilitate efforts to develop crop plants with enhanced resistance to high salinity (**Peng *et al.*, 2014**). Hence, improved salt tolerance of crops has also become an urgent task to reduce the spread of salinity and sustain increases in food production worldwide (**Munns, 2005**).

Actually, plants have evolved sophisticated mechanisms to cope with salinity stress. The salt signal is primarily perceived through roots, which rapidly respond and transmit signals to the shoot for appropriate changes in function, regulating the transcription and translation of intracellular genes associated with stress response, ultimately generating a series of physiological and biochemical responses in plants (**Lv *et al.*, 2016**).

Plants have been divided into salt-tolerant 'halophytes' and salt-sensitive 'glycophytes', depending on growth performance of these Plants in saline habitats. Glycophytes display severely subdued growth and even death in