# ROLE OF SIGNAL TRANSDUCTION IN REGULATION OF GENE EXPRESSION OF DROUGHT TOLERANCE IN WHEAT

By

## SARAH ADEL ABDULLAH ABD EL-BEIN

B.Sc. Agric. Sc. (Biotechnology, English section), Cairo University, 2007

A Thesis Submitted in Partial Fulfillment Of

the Requirements for The Degree of

## **MASTER OF SCIENCES**

in

Agricultural Sciences (Genetics)

Department of Genetics Faculty of Agriculture Ain Shams University

# ROLE OF SIGNAL TRANSDUCTION IN REGULATION OF GENE EXPRESSION OF DROUGHT TOLERANCE IN WHEAT

By

### SARAH ADEL ABDULLAH ABD EL-BEIN

B.Sc. Agric. Sc. (Biotechnology, English section), Cairo University, 2007

#### **Under the supervision of:**

#### Dr. Eman Mahmoud Fahmy

Prof. Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University (Principal Supervisor).

#### Dr. Fatthy Mohamed Abdel-Tawab

Prof. Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University.

## Dr. Lamyaa Mostafaa Kamal

Lecturer of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University.

#### **ABSTRACT**

Sarah Adel Abdullah Abd El-Bein: Role of signal transduction in regulation of gene expression of drought tolerance in wheat, Unpublished Master Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2017.

A Differential Display Polymerase Chain Reaction (**DD-PCR**) technique was applied for gene expression profiling to detect the up and down regulation of gene expression in shoots and roots of the two cultivars Sahel 1(tolerant) and Gammeiza 7 (sensitive) in response to drought in bread wheat (*Triticum aestivum* L.). Twenty differentially expressed ESTs fragments were obtained from shoots and roots. The sequences of ESTs were identified by using bioinformatics analysis. Seven of which were from shoots and 13 from roots. The results of fragments showed a significant homology to some predicted proteins such as: TaAP2 which has important roles in directing changes in gene expression during stress, glycosyltransferases (GTs) which catalyzes the transfer of sugar moieties to a wide range of acceptor molecules, nelumbo nucifera protein NEN-4 which plays a role in cellular protection, restoration of plant functions under stress and exhibits protein-repair activity after heating to 100°C, fructan 1-exohydrolase which is important for regrowth of leaf tissue after defoliation, ageratina adenophora ICE1 protein that activates many downstream genes that confer chilling and freezing tolerance to plants. RNA polymerase II gene which regulates transcription, the synthesis of RNA from a DNA template, is one of the most important steps in control of cell growth and differentiation. Serine/threonine-protein kinase maintains unperturbed photosynthesis inducing an oxidative stress response and plays a role in salt stress signaling, NRC-1 which is highly responsive to its environment and provided insights into some of the specific responses at the level of gene expression and prolamine genes stable proteins which is accumulated at

massive levels due to the high expression level from extensively duplicated genes in endoreduplicated cells. Elucidation of the functions of these genes can understand the signal transduction of response mechanism for drought in the two cultivars which can be used to improve the quality and productivity of Egyptian wheat in future investigations.

## **Key words:**

*Triticum aestivum*, ESTs, Differential display, drought and signal transduction.

#### ACKNOWLEDGMENT

Firstly, I'd like to thank my God Allah, the greatest and almighty for give me the force and support during the master work.

I'd like to thank **Prof. Dr. Fatthy Mohamed Abdel-Tawab,** Professor Emeritus of Genetics, Genetics Dept., Fac. of Agric., Ain Shams University for designing a fantastic master plan which it's important for solving the recent and future wheat plant problem.

I'd like to thank **Prof. Dr. Eman Mahmoud Fahmy**, Professor Emeritus of Genetics, Genetics Dept., Fac. Of Agric., Ain Shams University for her kind supervision and helping me either materially or morally.

I'd like to thank the group of Biochemical Genetic Laboratory specially; Dr. lamyaa mostafaa kamal, lecturer of Genetics, Genetics Dept., Ain Shams University, Shereen khaled, Heba Hassen and Marwa Mohmoud for guiding and helping me.

I'd like to thank cytogenetics lab. "**Prof. Dr. El sayed Husseien**", **Professor Emeritus of** Genetics, Genetics Dept., Fac. of Agric., Ain Shams Univ. and special thanks to **Mona Moghazy.** 

All thanks and greetings for Plant Pathogen Interaction Lab.(PPI) In Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC) with leading **Dr. Sameh El-Sayed**, Research associated Professor of Molecular Genetics for his supervisions, and his staff **Mohamed El Mahdy and Rasha Abdel Kader** for their learning and facilitating the master work.

I'd like to thank the staff of Environmental Stress Lab. (ESL), AGERI, ARC specially; **Dr. Mohamed Abdel-Sattar.** 

I'd like to thank the Field Crops Research Institute, Agricultural Research Center for supplied me wheat grains.

I'd like to thank the Molecular Genetics Lab "Pro. Dr. Mohamed Abd El-Salam", Professor Emeritus of Genetics, Genetics Dept., Fac. of Agric., Ain Shams Uni. and his group; Prof. Dr. Mahmoud Abd-El Hafiez, Dr. Nouh Eid, Dr. Mahmoud Mossallamy, Hala Zoghly and Hager Tarek.

Thinner salute for all **Professors of Genetics**, Department of Genetics, Faculty of Agriculture at Ain Shams University.

I'd like to thank the Central laboratory in the Faculty of Agriculture, Ain Shams University, specially; **Prof. Dr. Sayed Shaban, Dr. Sayed Abdel El-Moneam, Dr. Manal Moubarek and Dr. Ashraf.** 

Special thanks to the Academy of Scientific Research and its contribution to the support of this thesis.

I cann't express my feels to thank my parents and my brother to enhance and encourage me.

Special thanks for new freinds which I acquired them, specially the soul of **Asmaa Hamed**.

Finally, I cann't find nothing wrong to thank Myself which posses patience, calm self and faith in **God** for illogic reasons, thanks **Sarah Adel.** 

## **CONTENTS**

		Page
I. I	Introduction	1
II.	Review of Literature	4
	1. Wheat genome	4
	2. abiotic stress.	7
	3. Physiological effects	11
	4. Signal transduction.	13
	5. Gene profiling technique	15
	6. Differential display technique	18
III	. Materials and Methods	21
	1. Materials	21
	2. Methods.	21
	3. Physiological evaluation	21
	4. Osmotic potential measurement	21
	5. Determination of calcium and potassium	22
	6. Isolation of RNA from wheat	27
	7. Differential display PCR reaction	30
	8. Silver staining of the sequencing gel	33
	9. Isolation of band (expressed sequence tags) from gel	34
	10. Reamplification of bands by PCR	35
	11. Sequencing and bioinformatics tools	36
IV	. Results and Discussion	37
	1. Drought experiment	37

VI. References	86
V. Summary	83
9. Appendix 1	74
8. DD fragments showing homology with predicted proteins	63
7. The purification of selection bands	59
6. Molecular genetic analysis	47
5. Calcium level	44
4. Potassium level	41
3. Osmotic potential measurement	39
2. Physiological evaluation	38

# LIST OF TABLES

Table	]	Page
<b>Table (1):</b>	The readings of potassium concentration on flame	
	photometry	26
<b>Table (2):</b>	The sequence of primer combinations was used in	
	(DD-PCR)	31
<b>Table (3):</b>	The osmotic potential of the shoots of Sahel 1 cultivar	39
<b>Table (4):</b>	The percentage of potassium in shoots of Sahel 1	
	(tolerant) and Gemmeiza 7 (Sensitive) wheat cultivars	
	under control and drought stresses	
		43
<b>Table (5):</b>	The percentage of Ca2+ in shoots and roots of Sahel	
	1(tolerant) and Gammeiza7 (sensitive) under drought	
	treatments	44
<b>Table (6):</b>	The up and down ESTs for shoots and roots of the two	
	cultivars; Sahel 1 and Gemmeiza 7	56
<b>Table (7):</b>	BLASTn analysis of ESTs fragments from shoots and	
	roots compared with wheat database	60

# LIST OF FIGURES

Figure		Dogo
No.		Page
1	The standard curve for the reading of potassium	
	concentration on flame photometry	26
2	Seedlings of Sahel 1 as tolerant cultivar at three weeks	
	stage with two-three leaves	38
3	Seedlings of Gemmeiza 7 as sensitive cultivar at three	
	weeks stage with two-three leaves	38
4	Diagram for the osmotic potential of Sahel 1 and	
	Gemmeiza 7 shoots (MPa)	40
5	Diagram for the percentage of potassium in shoots of	
	Sahel1 (tolerant cultivar) and Gemmeiza7 (sensitive)	
	under drought stress treatments	43
6	Digram for the percentage of Ca <sup>2+</sup> in shoots of Sahel	
	1(tolerant cultivar) and Gammeiza7 (sensitive cultivar)	
	under drought treatments	45
7	Differential display PCR reaction to confirm	
	polymerization results	48
8	DD polyacrylamide gel for ESTs of shoots and roots of	
	Sahel 1 cultivar from combination of anchor A and	
	arbitrary 6 primers at control, 6h, 9h and 12h drought	
	treatments	49
9	DD polyacrylamide gel for ESTs of shoots and roots of	
	Gammeiza 7 cultivar from combination of anchor A	
	and arbitrary 6 primers at control, 6h, 9h and 12h	
	drought treatments	50
10	DD polyacrylamide gel for ESTs of shoots and roots of	
	Sahel 1 cultivar from combination of anchor A and	
	arbitrary 7 primers at control, 6h, 9h and 12h drought	
	treatments	51
11	DD polyacrylamide gel for ESTs of shoots and roots of	
	Gemmeiza 7 cultivar from combination of anchor A	
	and arbitrary 7 primers at control, 6h, 9h and 12h	
	drought treatments	52
12	DD polyacrylamide gel for ESTs of shoots and roots of	53

Figure No.		Page
	Sahel 1 cultivar from combination of anchor A and	
	arbitrary 3 primers at control, 6h, 9h and 12h drought	
	treatments	
13	DD polyacrylamide gel for ESTs of shoots and roots of	
	Gemmeiza 7 cultivar from combination of anchor A	
	and arbitrary 3 primers at control, 6h, 9h and 12h	
	drought treatment	54

#### LIST OF ABBREVIATIONS

Abscisic acid ABA AP Arbitrary primers

APS Ammonium persulfate ATI Abjotic tolerance index AWC Available water content

Bacterial artificial chromosome BAC Basic local alignment search tool **Blast** 

Blastn Basic local alignment search tool nucleotide Basic local alignment search tool protein **Blastp** 

Basic local alignment search tool nucleotide to protein Blastx

**bZIP** Basic Leucine Zipper Domain

Complementary DNA **cDNA** 

Complementary DNA amplified fragment length

**CDNA-AFLP** polymorphism

**COR** Cold-stress-responsive

**CVG** Germination and coefficient of velocity

DD Differential display

DI Drought resistance index **DREB** DRE binding proteins DRI Drought response index

**EDTA** Ethylenediaminetetraacetic acid

**EST** Expressed sequence taq EtBr Ethedium bromide Expected value E-value

**FEH** Fructan 1- exohydrolase

**G7** Gemmeiza 7

**GAD** Glutamic Acid Decarboxylase The glutenin-to-gliadin ratio Glu/Gli Geometric mean productivity **GMP GST** Glutathione-s-transferase

GT Glycosyltransferase

High K utilization efficiency cotton genotype **HEG** 

**HSPs** Heat shock proteins

ICE1 Inducer of CBF expression 1 **KUE** Potassium utilization efficiency