MOLECULAR GENETIC STUDIES ON SOME DROUGHT AND SALINITY TOLERANCE GENES IN BREAD WHEAT

By

HAYAM FOUAD IBRAHIM SAYED

B.Sc. Agric. Sci. (Biotechnology), Fac. Agri., Cairo Univ., 2001
M.Sc. Agric. Sci. (Agric. Biochemistry), Fac. Agri., Cairo Univ., 2008

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agriculture Sciences
(Agricultural Biochemistry)

Department of Biochemistry
Faculty of Agriculture
Cairo University
Egypt

2017

APPROVAL SHEET

MOLECULAR GENETIC STUDIES ON SOME DROUGHT AND SALINITY TOLERANCE GENES IN BREAD WHEAT

Ph.D. Thesis
In
Agric. Sci. (Agricultural Biochemistry)

By

HAYAM FOUAD IBRAHIM SAYED

B.Sc. Agric. Sci. (Biotechnology), Fac. Agri., Cairo Univ., 2001
M.Sc. Agric. Sci. (Agric. Biochemistry), Fac. Agri., Cairo Univ., 2008

APPROVAL COMMITTEE

Dr. SALAH MOUSTAFA MAHMOUD SAAD.....
Professor of Biochemistry, Fac. Agric., Benha university.

Dr. FOUAD ABDEL-RAHEM AHMED.....
Professor of Biochemistry, Fac. Agric., Cairo University.

Dr. ABDEL- KADER MORSY ABDEL-SAMAD.....
Professor of Biochemistry, Fac. Agric., Cairo University.

Dr. OSAMA KONSOWA AHMED.....
Professor of Biochemistry, Fac. Agric., Cairo University.

Date: 2 / 7 / 2017

SUPERVISION SHEET

MOLECULAR GENETIC STUDIES ON SOME DROUGHT AND SALINITY TOLERANCE GENES IN BREAD WHEAT

Ph.D. Thesis
In
Agricultural Sci. (Agricultural Biochemistry)

By

HAYAM FOUAD IBRAHIM SAYED

B.Sc. Agric. Sci. (Biotechnology), Fac. Agri., Cairo Univ., 2001
M.Sc. Agric. Sci. (Agric. Biochemistry), Fac. Agri., Cairo Univ., 2008

SUPERVISION COMMITTEE

Dr. ABDEL-KADER MORSY ABDEL-SAMAD

Professor of Biochemistry, Fac. Agric., Cairo University

Dr. OSAMA KONSOWA AHMED

Professor of Biochemistry, Fac. Agric., Cairo University

Dr. SABRY ABD-ALLA MOHAMMED KHATAB

Researcher Professor of Genetics, National Research Center, Egypt

Name of Candidate: Hayam Fouad Ibrahim Sayed **Degree:** Ph.D.
Title of Thesis: Molecular genetic studies on some drought and salinity tolerance genes in bread wheat
Supervisors: Dr. Abdel-kader Morsy Abdel-samad
Dr. Osama Konsowa Ahmed
Dr. Sabry Abd-allaha Mohammed Khatab
Department: Agricultural Biochemistry **Branch:**
Approval: 2 / 7 /2017

ABSTRACT

The aims of this study were identifying of some wheat varieties and double haploid response to drought and salinity stress to determine and isolation of tolerant genes and select a gene for cloning it in tobacco plant. The behavior of seven wheat (*tritium aestivum*) genotypes under salinity and drought stress was studied. The genotypes used were double haploids (DH1, DH2, DH3 and DH4) and local verities (Sakha93, Sids1 and Gemmiza9) and relative water content (RWC) was estimated for the varieties and DH under drought stress 30% polyethylene glycol 6000, after 72 hours of treatment, The genotypes DH4 had the higher RWC (91.93%) while Sids1 had the lowest RWC of 68.23%. under water stress 30% PEG-6000. While, at salinity level 2.5% NaCl, DH3 displayed the highest relative water content (92.71%) and Gimmeza9 had the lowest RWC 75.88%.

Using primers and polymerase chain reaction (PCR) technique, the genes *WZY2, WUB3, ZFP22, TaOF1b, STRP* and *Di19a* were detected in all genotypes and gave fragments with MW. 194,234,182,204,228 and 747bp respectively, with variance expression between different genotypes. Three genes were selected for study the expression of whole sequence genes. DH4 the highest RWC genotype under drought stress and DH3 was had the highest RWC genotype under salinity stress at different times 0, 3, 6,12,24,48 and 72h, in leaves and roots, The wzy2 gene appeared 474bp which varied in expression between leaves and roots, while *STRP* with 879bp appeared in DH4 in root only at 48h, however in DH3 under salinity stress no gene expression was appeared. On the other hand, *Di19a* with 747bp appeared in DH3 at 24 and 48h under salinity stress. The three genes were isolated and the sequences of the genes were compared with genbank, *wzy2* gene was selected and cloned by carried on pBi121 vector, propagated in *E. coli* and transformed in tobacco leaf disc mediated Agrobacterium by using tissue culture technique the callus, leaves and roots were obtained, the whole plant was transferred to adaptation. The transformed *wzy2* gene was detected using PCR reaction technique by using primers compared with control.

Key words: wheat, *tritium aestivum*, drought and salinity stress, relative water content, cloning, agrobacterium, transformation.

Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisor ***Dr. Abdel-Kader Morsy Abdel -Samad*** *Professor of Biochemistry, Dept. of Biochemistry, Faculty of Agriculture Cairo University for the continuous support of my Ph. D study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis, also* ***Dr. Osama Konsowa Ahmed*** *prof. of Biochemistry, Department Biochemistry, Faculty of Agriculture Cairo University for his continuous support of my Ph.D. study.*

I am extremely grateful to ***Dr. Sabry Abd-Allaha Mohammed Khatab and Dr. Abdel-Hamid Mohammed Abdel-Hamid*** *Genetics and Cytology Department, National Research Center for their insightful comments and encouragement. I would like to express my sincere thanks to* ***Dr. Tarek Hewezi*** *for providing the seeds of double haploid genotypes and brought them from France to work in this study and* ***Dr. Haggag Salah Zien*** *assistant prof. of Genetics, Genetics Department, Faculty of Agriculture, Cairo University for his guidance in writing of this thesis.*

LIST OF ABBREVIATIONS

6BA	6-Benzylaminopurine
ABA	Absisic acid
bZIP	basic region/leucine zipper protein
CaMV35s	Cauliflower Mosaic Virus promoter
CIMMYT	International Wheat and Maize Improvement Center
DMSO	Dimethyl sulfoxid
DH	Doubled Haploid
DHNS	Dehydrin (DHN) is a multi-family of proteins present in plants
GTC	Guanidine thiocyanate
GUS	β-glucuronidase
HR	Hypersensitive response
IAA	Indole-3- acetic acid
LEA	Lata embryogenesis abuandant
MS	Murashige and Skoog media
NOS	Nopaline synthase terminator which induce termination process rate
NPTII	Neomycin phosphotransferase
PCD	Programmed cell death
PEG	Polyethylene glycol
ROS	Reactive Oxygen species
RWC	Relative water content
SA	Salicylic acid
SOS	Salt overlay sensitive genes
STRP	Salt Tolerance Related Protein
SUMO	Small Ub-related modifier
TFS	Transcription factors
Ub	Ubiquitin proteins
UPS	The ubiquitin 26S proteasome system
USDA	United States Department of Agriculture
ZFP	Zinc finger protein
DH	Double Haploid

CONTENTS

LIST OF TABLES

LIST OF FIGURES