BIOTECHNOLOGICAL STUDIES ON SUGAR BEET (*BETA VULGARIS*)

By

ABO BAKR AL SEDIK AHMED ALY YOUSSEF
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Azhar Univ., 2007

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
Cairo University
EGYPT

2018

Format Reviewer **Vice Dean of Graduated studies**

APPROVAL SHEET

Biotechnological studies on sugar beet (*Beta vulgaris*)

M.Sc. Thesis
In
Agric. Sci. (Genetics)

By

Abo Bakr Al Sedik Ahmed Aly Youssef
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Azhar Univ., 2007

APPROVAL COMMITEE

Dr. GHADA AHMED ABO EL- HEBA ……..
Associate Professor of Molecular Biology , AGERI, ARC

Dr. ABDEL HADI ABDALLAH ……..……
Professor of Genetics, Fac. Agric., Cairo University

Dr. SALAH EL-DIN SAYED EL-ASSAL.....
Professor of Genetics, Fac. Agric., Cairo University

Dr. NAGLAA ABDALMONEIM ABDALLAH.....
Professor of Genetics, Fac. Agric., Cairo University

Date: / / 2018

SUPERVISION SHEET

BIOTECHNOLOGICAL STUDIES ON SUGAR BEET (*BETA VULGARIS*)

M.Sc. Thesis
In
Agric. Sci. (Genetics)

By

ABO BAKR AL SEDIK AHMED ALY YOUSSEF
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Azhar Univ., 2007

SUPERVISION COMMITTEE

Dr. NAGLAA ABDALMONEIM ABDALLAH
Professor of Genetics, Fac. Agric., Cairo University

Dr. SALAH EL-DIN SAYED EL-ASSAL
Professor of Genetics, Fac. Agric., Cairo University

Dr. ROBA MEDHAT ISMAIL
Associate Professor of Genetics transfer , AGERI, ARC

Name of Candidate: Abo Bker Al Sedik Ahmed Aly **Degree:** M.Sc.
Title of Thesis: Biotechnological studies on sugar beet (*Beta vulgaris*)
Supervisors: Dr. Naglaa A. Abdallah
Dr. Salah El-Din Sayed El-Assal
Dr. Roba Medhat Ismail

Department: Genetics **Approval: / / 2018**

ABSTRACT

Sugar beet (*Beta vulgaris L.*) is an important industrial crop to produce sugar. The sugar beet production is often limited by environmental conditions that cause reduced photosynthesis rates, root development and sucrose accumulation. In order to improve development of sugar beet crop with increased drought tolerance, is the understand plant response to drought stress at the genomic level needed. Recent research efforts have focused on studying gene expression under environmental stresses to identify water deficit inducible genes. This work focused on using qRT- PCR for studying gene expression in sugar beet under in vitro drought stress conditions. Plants were grown for 30 days on micropropagation media with 0%, 3%, 5% and 7% PEG. The glutamine synthetase is housekeeping gene, was used as endogenous control, while the target genes were Heat stress transcription factor HSF, alpha amylase and osmotin-like protein. This study showed that there is a significantly up regulation expression of the three studied genes, Heat stress transcription factor HSF, alpha amylase and osmotin-like protein genes under drought stress. The obtained results indicated that qRT-PCR protocol was efficient and accurate in studying the potential of expression analysis for the candidate genes under water stress in sugar beet.. The Heat stress transcription factor HSF have been chossen for bioinformatics characterization, cloning and transfered into *Arabidopsis thaliana* in order to study expression of the isolated heat shock factor under drought stress. Therefore this research could help in deep understanding of plant response to stress.

Key words

Drought – qPCR – Heat shock factor - Amylase – osmotin –Sugar beet

DEDICATION

I dedicate this work to whom my heartfelt thanks: to my mother, father, brothers, sisters and my friends for their endless support along the period of my post-graduation.

ACKNOWLEDGEMENT

Special thanks and sincere appreciation to ***Dr. Naglaa A. Abdallah,*** *Professor of Genetics, Fac. of Agri. Cairo Uni., for her assistance, guidance and support throughout my postgraduate studies, writing of the thesis and research paper publication.*

Sincere appreciation to ***Dr. Salah El-Din Sayed El-Assal*** *Professor of Genetics, Fac. of Agri. Cairo Uni., for his supervision, and his great efforts in data analysis, writing of the thesis and research publication.*

Also, I would like to thank to ***Dr. Roba Medhat Ismail****, Associate Professor at the gene transfer lab, AGERI, ARC for her supervision, encouragement and support along the period of this work.*

Additionally, I would like to express my gratitude to ***Dr Samy el Aladwy*** *and his lab members for their support to carry out my Master thesis .They providing me many materials, supplies and facilities for this work to be done.*

Sincere appreciation to ***Dr. Abdelhadi A. Abdallah,*** *Professor of Genetics, Fac. of Agri., Cairo Uni., for his great efforts and support during our research article publication process.*

I would like to thank all the members of GTL lab members, specially Thanks for ***DR. GIHAN HOSNY*** *Head of Plant Genetic Transformation, AGERI, ARC*

Special thanks to ***Dr. Ghada Abu el-Heba*** *Head of Department of Nucleic Acid and Protein Structure, Agricultural Genetic Engineering Research Institute (AGERI) Many thanks must go to* ***Dr. Mohamed Slaeh*** *Associate Professor at AGERI, ARC for for his great support and provided Plasmid and seeds. Special thanks to* ***Dr. Mohamed Abd El Star*** *Researcher at AGERI, ARC for his great efforts and supplies of facilities for this Real-time PCR during this work.*

CONTENTS

LIST OF FIGURES

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is an economic crop for sugar production. It is a second crop that is used for sugar production worldwide after the sugarcane (Sen and Alikamanoglu 2012). Sugar beet is a species of Beta genus belong to Chenopodiaceae family and sub-family Betoideae, chromosome number notation, 2n = 18 (UniProt database). It produces about 25% of worldwide sugar production (http://www.fao.org). Egyptian sugar production from beet increases yearly. According to USDA'S Global Agriculture Information Network Annual report 2018, Cairo expects area planted to increase and, subsequently, area harvested to rise by 9.5 percent or 20,000 ha, to reach 230,000 ha. with increased area comes an associated increase in production, expected to reach 9.75 million metric tons. This is an increase of 9.6 percent from post's MY 2017/18 estimate of 8.9 million metric tons. The sugar-beet root crop is moderately tolerant to drought (Brown *et al.,* 1987). The production of sugar beet is often limited by environmental conditions that cause reduced rates of photosynthesis, canopy expansion, root development and sucrose level (Ober and Rajabi 2010). Water stress consider as one of the most serious problems for plant production in general. It reduces nutrient uptake and decreases metabolism during growth and development, which is reflected in reduced productivity and quality. (Sen and Alikamanoglu 2012).

Although it is primarily grown in countries with temperate climate, there are many production areas where irrigation is not usually applied and summer rainfalls are unpredictable and insufficient to fully meet the crop's water requirements. Since the summer, drought can

severely limits root yield and quality, as well as sugar content in sugar beet (Sadeghian and Yavari 2004), it becomes clear that the most economically viable solution for overcoming this problem is the development of cultivars with increased drought tolerance. Drought is one of the most abiotic stress that adversely affect plant development, growth and crop productivity (Toker *et al*., 2007). Tolerance to drought is a complex phenomenon, because it changes according to drought intensity and duration, plant's developmental stage during which drought occurs and ability of genotype to tolerate situations of stress (Micheletto *et al.,* 2007). Understanding of the tolerance process is one of the main aim of plant breeders in order to manipulate the genetic variability for produce of more tolerant cultivars (Santiago *et al.,* 2009).

There are several genetic variations in genotypic reaction to drought stress within a sugar beet germplasm (Ober and Luterbacher 2002). Although many papers devoted to this topic (Hoffmann 2010, Ober and Rajabi 2010), breeding for drought tolerance is very difficult and complex because of many participating traits. *In vitro* culture techniques can be useful in the study of stress tolerance mechanisms, as they minimize environmental variations and at the same time enable studying large number of samples in a limited space and short period of time.

Since an understanding of how plants respond to water stress at the gene level is essential for crop breeding and improvement of production, recent research efforts have focused on the molecular responses of the plant, in order to identify water deficit inducible genes (Bray 2004, Stolf-Moreira *et al.,* 2010). Common experimental techniques used to quantify relative levels of gene expression are

microarrays and real-time reverse transcription PCR (qRT-PCR). Microarray analysis are the preferred method for large-scale (e.g., whole-genome) expression profiling, while the qRT-PCR has become the method of choice for measuring gene expression levels in multiple samples, involving a limited number of genes (VanGuilder *et al.*, 2008). It provides accurate and sensitive quantification of gene transcript levels, even for those genes with fairly low transcript levels (Bustin *et al.*, 2006).

In sugar beet, drought causes yield reductions between 10 and 30 % (Romano *et al.*, 2013) There are many molecular mechanisms play a role when plant face Drought stress. Such as, signal transduction pathway (MAP kinase and ROS pathways), producing of regulatory protein, gene expression, synthesis of osmolytes (proline, glycine betaine, fructans) and other event that takes place under drought stress, heat stress and cold stress. (Dinesh *et al.*, 2016)

Heat shock factors (HSFs) are modular transcription factors encoded in plants by a large gene family. They bind to the consensus sequence 'nGAAnnTCCn' found in the promoters of many defence genes, and are thought to function as a highly redundant and flexible gene network that controls the response of plants to different environmental stress conditions, including biotic and abiotic stresses (Miller and Mittler 2006). Expression of HSPs is mainly regulated by heat shock transcription factors (hsfs) on a transcriptional level, and they play a critical role in high-temperature stress responses (Liao *et al.*, 2016) In sugar beet, NPR1 is a gene of central importance in enabling plants to resist pathogenic attack found in cluster with a heat shock