

**BIOTECHNOLOGICAL STUDIES ON SUGAR  
BEET ( *BETA VULGARIS* )**

**By**

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**B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Azhar Univ., 2007**

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### **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 chosen for bioinformatics characterization, cloning and transferred 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.*

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# CONTENTS

	page
<b>INTRODUCTION</b> .....	1
<b>REVIEW OF LITERATURE</b> .....	5
1. Effect of Abiotic stresses on agriculture production.....	5
2. Sugar beet and abiotic stress.....	6
3. Regulatory mechanisms involved in drought stress response and tolerance.....	8
4. Osmotins , Alpha amylase and Heat shock factor Genes expression under drought defect.....	31
a. In vitro drought stress by using polyethylene glycol .....	13
b. Osmotins (osmotin-like proteins or OLPs).....	13
c. Alpha amylase .....	15
d. Heat shock transcription factors and other transcription factors response to drought stress .....	15
5. Real-time PCR analyses for study gene expression.....	31
6. Arabidopsis thaliana as a model plant and Floral-dip Transformatio .....	19
<b>MATERIALS AND METHODS</b> .....	13
<b>MATERIALS</b>	
1. Sugar beet and Arabidopsis seeds.....	21
2. Primers design.....	21
3. Vectors .....	11
4. Bacterial strains .....	24
5. Programs and online tools .....	24
<b>METHODS</b>	
1. In vitro drought stress treatment by using (PEG).....	25
2. Growth parameters and morphological changes .....	26
3. Real time PCR analysis.....	26
a. RNA isolation and cDNA synthesi .....	26

b. Real time PCR reaction and conduction.....	27
c. Relative quantification .....	28
<b>4. Isolation of heat shock factor from sugar beet.....</b>	<b>28</b>
<b>5. The manipulation and transformation of E.col .....</b>	<b>29</b>
a. Preparation of competent E. coli DH10 $\beta$ .....	30
b. Transformation of chemically competent E. coli DH10 $\beta$ cells.....	13
c. Screening of recombinant plasmids .....	31
<b>6. Subcloning of the hsf gene into plant binary vector .....</b>	<b>31</b>
<b>7. Manipulation and transformation of competent Agrobacterium</b>	
<b>8. tumefaciens .....</b>	<b>32</b>
a. Preparation of electro-competent A. tumefaciens GV310.....	32
b. Transformation of HSF gene into competent A. tumefaciens GV3101	32
c. Transformation of Heat stress transcription factor HSF into <i>Arabidopsis</i> .....	33
<b>8. Screening of transgenic seed .....</b>	<b>11</b>
<b>9. Confirmation of transgen.....</b>	<b>13</b>
a. DNA extraction from plant leaves .....	13
b. Polymerase chain reaction .....	13
<b>10. Bioinformatics analysis .....</b>	<b>15</b>
a. Multiple sequence alignment.....	15
b. Protein prediction features.....	15
<b>RESULTS AND DISCUSSION .....</b>	<b>16</b>
<b>1. Morphological changes.....</b>	<b>16</b>
<b>2. RNA isolation and cDNA synthesis .....</b>	<b>19</b>
<b>3. Quantitative PCR (qPCR) .....</b>	<b>41</b>
<b>4. Relative expression ratio.....</b>	<b>42</b>
<b>5. Isolation of Heat stress transcription factor (hsf) cDNA .....</b>	<b>45</b>
a. RT-PCR for Heat stress transcription factor HSF gene.....	45
b. Cloning of the sugar beet hsf gene .....	46
<b>6. Bioinformatics analysis .....</b>	<b>47</b>
a. Sequencing and Basic Local Alignment Search for (hsf).....	48
b. Motif-based sequence analysis .....	49
<b>7. Subcloning of the hsf into plant binary vector.....</b>	<b>50</b>
<b>8. Transformation of hsf construct to Arabidopsis .....</b>	<b>53</b>
<b>9. Screening and Molecular analysis of transgenic plants .....</b>	<b>57</b>
a. Screening of transgenic Arabidopsis plants.....	58
b. PCR analysis for the transformed plants .....	58
<b>10. PEG treatment for transformed Arabidopsis to induce</b>	

<b>drought stress .....</b>	<b>59</b>
<b>SUMMARY .....</b>	<b>61</b>
<b>REFERENCES .....</b>	<b>69</b>
<b>ARABIC SUMMARY</b>	
<b>ARABIC SUMMARY</b>	

## LIST OF FIGURES

No.	Title	Page
1.	Functions of drought stress-inducible genes in stress tolerance and response.....	9
2.	Physical map of the pGEM-Teasy vector.....	23
3.	pRI201-AN expression vector.....	24
4.	Physical maps of the binary vectors pRI201-AN-HSF used for Floral-dip-transformation .....	32
5.	Effect of different concentrations of PEG (A: 0% (control) B: 3% C: 5% and D: 7%) treatments on morphological characterization of sugar beet plants.....	37
6.	Effect of 0% (control), 3%, 5% and 7% (w/v) PEG added to the MS media on the growth parameters of sugar beet plants.....	37
7.	Effect of drought stress on growth parameters; (A) Shoot dray weight, (B) Root length, (C) Shoot numbers and (D) Shoot fresh weight.....	39
8.	Isolated RNA from sugar beet plants treated with PEG ....	40
9.	Amplified cDNA using (A) heat shock factor (qHSF 227bp),(B) osmotin-like protein (Osmo65bp) and (C) alpha-amylase (Alpha 73bp) primers giving the expected size fragments, used as preliminary test before starting real-time PCR analysis.....	42

10. Effect of drought stress by using different concentration of PEG on the expression of heat shock factor, Alpha amylase and osmatin like protein genes.....	43
11. Gel electrophoresis for the amplified hsf full length cDNA <i>hsf</i> (1030 bp) M : 1Kb plus.....	46
12. The nucleotide sequence of the hspcDNA.....	47
13. BLASTn results between the isolated hsf and the <i>B. vulgaris</i> subsp. <i>vulgaris</i> hsf A-7a (XM_010698225.2)	51
14. BLASTp results between the amino acids sequence of the isolated HSF and the <i>B.vulgaris</i> HSF (xm010698225.2).	52
15. Phylogenetic analysis of heat shock factor, produced by ETE Toolkit website.....	53
16. The conserved motif analysis of HSF using MEME Motif-based sequence alignment result .....	55
17. Agarose gel electrophoresis showing (A) 1.digested recombinant pGEM-Teasy with NdeI to release the hsf cDNA and 2. linearized pRI201-AN with the same enzyme, (B) 1. linearized pRI201-AN 2. Purified hsf cDNA.M: 1kb plus.	56
18. Confirmation of recombinant plasmid contains HSF; 1- NdeI digestion, 2- PCR by using the core HSP primers with isolated plasmid as a template, 3- positive control using pGEM-Teasy recombinant plasmid with core HSP primers, and 4- PCR by using full length HSF primers.	56
19. Steps of the dipping protocol. (A) Arabidopsis plants growing conditions, (B) beginning of flowering stage and (C) shoot dipping step.	57
20. Figure 22. Screening of transgenic plants on MS media contains kanamycin.	58

21. PCR analysis of the transformed plants using core primers for hsf gene.	59
22. Transgenic drought treatment (A) Control, (B) Transgenic <i>Arabidopsis</i> grown on MS media with 5% PEG	60
23. $\Delta$ ct values for HSF in <i>Arabidopsis</i> 1-4 control 5-6 plants treated with 5% PEG	60

# 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