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Evaluation of Drought Stress-responsive Genes Expression of Durum Wheat Using Comparative Genomics

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ABSTRACT

As the water resources become more limited, the production levels of crops are declining, and following the rapid increase in population, the use of mankind from staple food products has increased. So, the development of drought-tolerant crops aimed at cultivation in the arid regions will be of great worth to provide the country's nutritional necessity. The following research aimed to elucidate the drought tolerance of durum wheat (*Triticum turgidum* ssp. durum) through comparative expressed sequence tags/EST analysis of unstressed library with 2534 EST and drought-stressed library with 4485 EST. Preliminary data were gathered from the Harvard university database. All unigenes were assembled using EGassembler software to detect similarities between the two libraries and were then analyzed through X-blast by CLCbio software against a non-redundant protein database. To identify statistically differentially expressed genes, the IDEG6 web tool was used. Over 150 differentially ESTs were detected by Audic-Claverie statistics of IDEG6 software, in which over 85% ESTs were upregulated in response to drought. The GoMapMan comparative classification tool was used to categorize gene functional annotations. All significant differential unigenes were divided into seven functional categories *i.e.,* photosynthesis and energy (19%), regulatory pathways (25%), transport (5%), hormones, plant defense, response to drought stress (11%), cell metabolism (19%), cell organization and development (4%) and miscellaneous as well as unclassified processes (17%). Comparative analysis revealed that some of the promising traits in *T. turgidum* are specifically regulated in drought stress including genes related to response to stress and hormones pathways, development and growth (helicases and *CPL* phosphatase), maintenance of cell water content (transporters and osmolites), membrane stability (*HSP*) and preventing the accumulation of toxins. This study prominently demonstrates the helicase role under durum wheat's drought tolerance as well as provides indices for assessing the drought tolerance of *T. turgidum* aims to the use of tolerant varieties in breeding programs in arid and semi-arid regions in Iran.

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Introduction

Today, owing to the ever-increasing need for food products following the rapid increase in population, the use of crops has increased. On the other hand, due to drought, crop production is declining. Drought is a significant limiting factor in crop production particularly in Iran where approximately 80% of the country is located in arid and semi-arid regions. To deal with these crises, crop researchers recommend the cultivation of plants that show tolerance to drought stress. *Triticum turgidum* ssp. durum is one of the most substantial cereals whose seed product is the raw material of universal foods. By achieving high yielding, adaptable, and

drought-tolerant *T. turgidum* cultivars, their cultivation can be developed in Iran, especially in arid areas, and thus increasing crop production took positive steps. Various physiological traits of plants such as relative leaf water content and photosynthesis-related parameters are impaired during drought stress conditions leading to significantly repressed plant growth and yield (Ortiz *et al.,* 2015). Plant species and drought intensity are the determinative factors affecting the responses of plants to drought. The drought tolerance mechanism, also, differs between species of plant. Some varieties of plants have developed sophisticated responses and adaptations to maintain optimal growth under stress conditions. Assessing the ability of plants to tolerate drought stress and realizing the drought tolerance mechanisms is of remarkable value and should be considered, particularly in semi-arid and arid regions (Heidari *et al.,* 2020). Over the last decade, tremendous efforts have been devoted based on genetic and biochemical studies to identify physiological response-related genes of drought stress in the plant (Kaur and Asthir, 2017). Numerous genomic and molecular approaches have been developed to study of drought tolerance of crop plants. Recent genomics tools will identify candidate genes and physiological pathways underlying drought stress tolerance in plants. However, sequencing of the entire genome is impracticable and high-cost when large genome sizes of organisms are present. Elimination of long, costly, and time-consuming steps of the whole genome sequencing has made the EST analysis approach a widely used tool. Besides, in plants, the presence of retrotransposon repeats that lead to genome expansion makes sequencing of the entire genome less desirable. Hence, the expressed sequence tags method has been used as an alternative to performing the sequencing of the genomes of many organisms (Bennetzen, 2002). EST method provides the most valuable resources for transcriptome recognition and is an approach that has been widely published in the scientific literature over the past decade. ESTs are short about 200-800 bp in length, unedited, incidental selected single-pass sequence derived from any cDNA library providing a direct document for all of the transcription samples. The analysis of EST plays a vital role in gene discovery, and genome annotation complementarity, facilitating proteomic recognition and attribution of genes to specific physiological pathways (Jongeneel, 2000). Some gene expression studies have documented several plant responses to drought stress by EST analysis. Sudhakar *et al*. (2020) using EST libraries of safflower, Ct-D-EST (drought tolerant safflower cultivar), and Ct-N-EST (sensitive) studied drought stress on safflower. They considered S-adenosylmethionine synthetase and myo-inositol 1-phosphate synthetase as promising candidate genes for developing drought-tolerant cultivar by which many physiological processes such as plant growth, hormone, and secondary metabolites production proceed. Heidari *et al*. (2012) using analysis of EST library in crop plants such as bread wheat and rice through IDEG6 and mapman classification tools reported the most important genes involved in the physiology of drought stress including lipid transferase, LEA, metallothionein, glutathione s-transferase, dehydrin, and phosphatase. In the present study, to identify important genes regulated under drought stress affecting the main physiological processes in *T. turgidum*, a comparative analysis of two *T. turgidum* EST libraries under stressed and unstressed conditions was performed to use tolerant cultivars in breeding programs in semi-arid and arid regions in Iran.

Materials and Methods

Leaf's EST preliminary data from drought tolerant cultivar OS-GA of *T. turgidum* include unstressed (control) and stressed condition (at fully developed seedlings, stress was induced by the applying 15% PEG6000 (-1.65 MPa)) were collected from the Harvard university database. The web pages of DFCI gene index (Http://compbio.dfci.harvard.edu/tgi/, Antonescu *et al.,* 2010) allow access to lot of resources for EST and unigenes upon 114 species. In the preprocessing step, using VecScreen database (Https://www.ncbi.nlm.nih.gov/tools/vecscreen/, Schäffer *et al.,* 2018), sequences with less than 100 bp, vector sequences and poor-quality sequences were trimmed from the raw single-pass sequences with percentage of N≥95 for cutoff matching. In the processing step, using EGassembler software (http://egassembler.hgc.

jp/, Masoudi Nejad *et al.,* 2006), the cDNA sequences were clustered for constructing contigs with 95% identity over 40 bp for the parameters set. Contigs have two or more ESTs and singletons have only one EST indicating unit expression of gene. Bioinformatics pipeline for comparative genomics through EST analysis has been shown in figure 1. Through Egassembler software, unisequences were assembled to detect similarities between the two libraries and were then analyzed using X-blast by CLCbio workbench software (installed on a linux system using the installer script which allows special control of the software) against non-redundant protein database with E-value$\leq 10^{-5}$. Audic-Claverie statistics and the IDEG6 web tool (http://telethon.bio.unipd.it/ideg6, Romualdi *et al.,* 2003) were used to identify genes with differential expression among libraries. Generally, cDNA libraries encompass a lot of different expressed genes and the existence of a certain cDNA is known as a scarce event which estimate through Poisson distribution. For an EST indicating a slight part of a lot of n clones in library, the probability of existence x tags of the similar gene will be measured up through the Poisson distribution parametrized by $\lambda \geq 0$ of the $P(X = x \mid \lambda) = e^{-\lambda} \frac{\lambda^{x}}{x!}$ (Tiňo, 2009).

The parameter λ implies the EST number of the certain type (tag) per n clones in the cDNA library. Under the null hypothesis of not differentially expressed genes, it is presumed that the tag count x in one library comes from the similar underlying Poisson distribution $P(\cdot|\lambda)$ as the tag count y in the other library, when comparing two libraries. Under the null hypothesis that the counts of tag are made from the similar but unknown poisson distribution, the pragmatic gadget of the Audic-Claverie method is a distribution P(y|x) upon counts of tag y in a library informed by the count of tag x in the one another library ($P(Y \mid X) = \frac{1}{2^{x+y+1}} \frac{(x+y)!}{x!y!}$). P(y|x) is generated through Bayesian averaging in infinite mixture of whole feasible Poisson distributions (Tiňo, 2009).

The sequences of contigs and singletons of each library were then analyzed by the X-blast against the resource of *Arabidopsis* data downloaded from the TAIR database (ftp://ftp.arabidopsis.org, Bassel *et al.,* 2011). The comparative classification tool of GoMapMan (http://www.gomapman.org, Ramšak *et al.,* 2014) was used to categorize functional catalogs. GoMapMan is a web tool for gene functionality annotations in the sciences of plants. It was expanded to simplify betterment, visualization and stabilization of annotations of gene amidst several plant species. Outputs of Mapman are used to characterize disparate catalogs in libraries that can detect effective catalogs in multiple sample experiments. IDEG6 software and Audic-Claverie test were used to find functional differential catalogs in libraries. The identified sequences were classified into three categories of GO gene ontologies.

Results

Assembly of contigs and EST annotation

2534 and 4485 ESTs were obtained from unstressed (control) (CTT) and drought-stressed (STT) *T. turgidum* leaves library respectively after eliminating unwanted and vector sequences. The constructed contigs and singletons number are given in Table 1.

Table 1. The number of ESTs, contigs and Singletons in each library and determining the hit associated with sequences.

Library name	CTT	STT
Unisequences	2534	4485
Contigs	341(789 EST)	560 (1877 EST)
Singletons	1745(69%)	2608(58%)
Contigs with distinct hit	280(82%)	485(87%)
Singletons with distinct hit	1543(88%)	2113(81%)

*Unstressed (Control) *T. turgidum* (CTT), Drought-stressed *T. turgidum* (STT).

The result of X-blast searches using CLC Workbench software revealed that in the (CTT) library, 15% ESTs and in (STT), (16%) ESTs had a very weak homology (E-value $>10^{-5}$) against the non-redundant protein database of NCBI or there was no similar sequence with

them while other EST showed high or moderate homology (E-value<10^{-5}). The results of the present study showed, sequences that failed to show significant homology to the public database protein are good candidates as new genes under drought stress. Many contigs and singletons were matched to unknown or hypothetical functions of proteins (results not shown).

Functional classification and GO ontology

In present study, 150 unisequences including contigs and singletons showed statistically significant differences (E-value≤10^{-5}) and most of these (over 85%) were upregulated in response to drought. The "only unisequences" that had very significant expression differences have been shown in table 2 (E-value≤10^{-150}). Annotated ESTs matched with other organisms (out of Plantae kingdom' spectrum) are not given in the table 2. Well-annotated significant differential unigenes based on IDEG6 software results were divided into seven functional categories (Fig. 2).

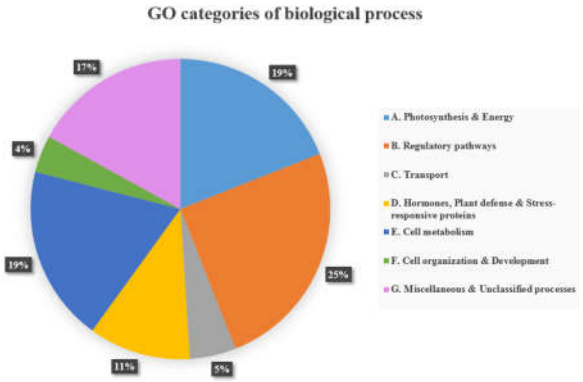

Fig. 2. Distribution of GO categories in biological process assigned to all assembled unigenes.

Moreover, based on subcellular localization, the identified proteins were categorized into eleven groups (Fig. 3).

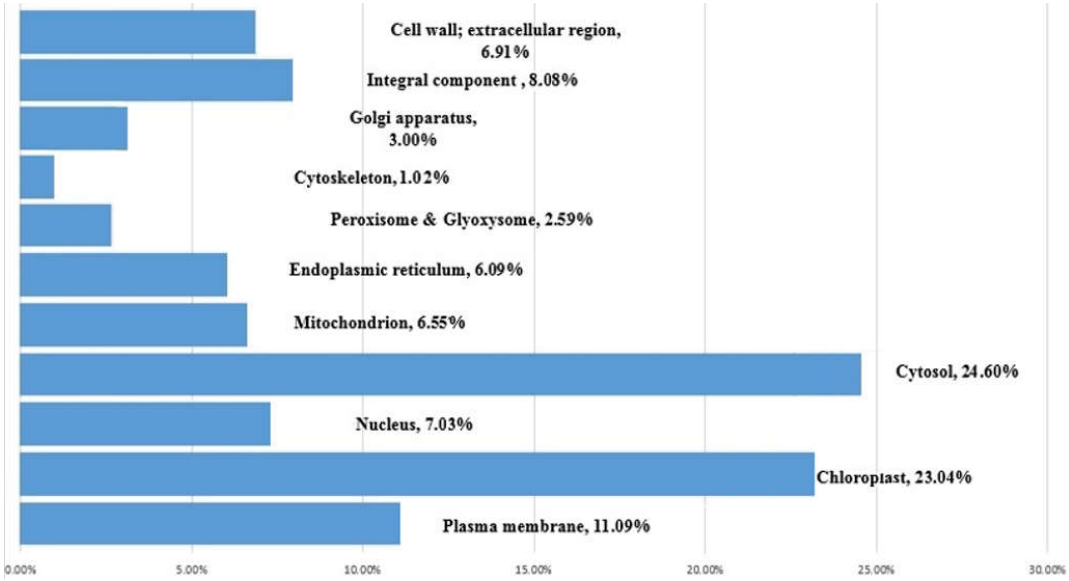

Fig. 3. Subcellular localization of categorized proteins in *T. turgidum* under drought stress.

Since the protein function is generally associated with its subcellular localization, the subcellular localization prediction will be effective for discovering functions of the protein. According to the results of this study, most of the subcellular localization happened in cytosol and chloroplast. This prediction includes the proteins's subcellular localization of transport in cytosol and chloroplast, the subcellular localization of proteins related to stress and defence in the cytosol, chloroplast and endoplasmic reticulum, photosynthetic proteins in chloroplast, proteins of glycolysis in the cytosol, detoxification proteins in the chloroplast, extracellular, and cytoplasm as well as proteins of transcription regulatory pathways in nucleus.

Table 2. The annotation and expression levels of the unisequences which are statistically different between libraries.

Unisequence	AC ID	Putative identity	Cellular location	Biological process	CTT	STT	p-value
Contig 106	Q9LDY8	Putative NAC transcription factor	Nucleus	Transcription regulation	1	8	$1E^{-161}$
Contig 30	A0A446MCK1	RNA polymerase II C-terminal domain phosphatase-like	Nucleus	Signal transduction	3	16	$3E^{-194}$
Singleton	ACI16353	Trehalose phosphate synthase	Cytoplasm	Carbohydrate metabolism	0	1	$5E^{-187}$
Contig 207	S4Z9C8	50S ribosomal protein	Chloroplast	Translation- protein synthesis	1	6	$6E^{-184}$
Contig 5	CAC24843	Chlorophyll a-b binding protein 1	Chloroplast	Photosynthesis	4	11	$2E^{-192}$
Contig 101	C6K7G4	Lipoxygenase	Cytoplasm, plastid	JA biosynthesis, fatty acid metabolism	2	7	$8E^{-196}$
Contig 33	BAH20800.1	Protein disulfide isomerase	Endoplasmicreticulum	protein folding	0	5	$1E^{-158}$
Contig 44	Q6YLX9	Two pore calcium channel protein 1	Membrane	Transport	1	7	$7E^{-182}$
Contig 95	ACO90195	Superoxide dismutase	Mitochondrion	Detoxification	4	13	$3E^{-174}$
Contig 8	O24578	Adenylosuccinate synthetase	Chloroplast	Nucleotide metabolism	15	2	$3E^{-170}$
Contig 17	Q9LHA8	Heat shock 70 kDa protein 4	nucleus; cytosol	Stress response, protein folding	2	17	$1E^{-151}$
Contig 78	Q0JHF8	Fructose-1,6-bisphosphatase 1	Cytoplasm	Carbohydrate metabolism	5	0	$6E^{-163}$
Contig 25	Q84TB6	Actin-depolymerizing factor	Cytoskeleton	Actin filament depolymerization	0	6	$1E^{-199}$
Singleton	O22850	Putative glutathione peroxidase 3	Mitochondrion	detoxification	0	1	$3E^{-172}$
Contig 179	P25858	Glyceraldehyde-3-phosphate dehydrogenase	Cytosol	Glycolysis	1	3	$3E^{-169}$
Contig 90	F4J9M5	ATP-dependent DNA helicase	Nucleus	DNA replication	2	16	$1E^{-154}$
Contig 72	P41343	Ferredoxin-NADP reductase	Chloroplast	Photosynthesis	0	5	$3E^{-162}$
Contig 132	A0A251SGR0	Cytidine triphosphatesynthetase	Cytosol	Nucleotide metabolism	1	9	$2E^{-187}$

*The columns CTT and STT show the ESTs number in contigs in unstressed (control) and drought-stressed *T. turgidum* libraries, respectively. The p-value refers to the Audic-Claverie statistics for very significant expression differences (E-value≤10^{-150}); AC ID: Accession ID (Uniprot).

Discussion

Photosynthesis and energy-related genes

Nearly 19% of differential ESTs were annotated to photosynthesis and energy processes. In drought-stressed library increased chlorophyll a-b binding protein 1 (*CAB1R*) expression was observed which was consistent with the findings of Zhou *et al.* (2015) that discerned upregulation of CAB1 in a variety of apples with tolerance to drought stress. It has been shown that upregulation of any member of the CAB family increase sensitivity of stomatal movement to ABA and so results in an increased *A. thaliana* tolerance to drought stress (Xu *et al*., 2012). In our study, Ferredoxin-NADP reductase (*FNR*) was upregulated under stress. Lehtimäki *et al.* (2010) indicated that the overall expression of the *FNR* protein increased in drought stress. A relation between tolerance to oxidative stress and *FNR* content is well characterized although the detailed mechanism remains unknown. It has been shown that *FNR* has a crucial function in the appropriation of the high energy electrons in the chloroplast and contributing distributing electrons to CO2 fixation (Kozuleva *et al.,* 2016). Therefore, drought tolerance of *T. turgidum* could be closely related to the involvement of the role of *FNR* protein during the carbon fixation process. Drought stress also upregulated the expression of ESTs annotated to energy processes such as Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). This is a central enzyme in glycolysis and was found that significantly upregulated under drought stress. The enhancement of respiration at less relative water content would relate to an incrementing metabolism as the plant triggers adaptation mechanisms to tolerate drought stress which would increase the retention of the entirely rate of respiration and production of energy to keep their structures and respond to their ambient. It has been shown that upregulation of *GAPDH* provide an increased potato tolerance to drought and *GAPDH* has a remarkably positive relation to drought tolerance (Kappachery *et al.,* 2015). Hence, this protein may also be associated with *T. turgidum* tolerance to drought but the physiological significance requires further *in vivo* investigation.

Regulatory pathways-related genes

The largest number (25%) of differential EST influenced by the drought stress were annotated

to regulatory pathways. ESTs annotated to DNA synthesis and repair significantly altered in drought-stressed *T. turgidum* compared to unstressed controls. Proteins identified as ATP-dependent DNA helicase was upregulated under drought stress. Helicases nearly participate in all sorts of DNA metabolisms specially in eukaryotic DNA replication during initiation and elongation steps in the cell cycle's S phase and hence have pivotal function over development and growth of plants (Bell and Dutta, 2002). The function of helicases in plant's stress tolerance is not completely known. Sanan-Mishra *et al*. (2005) showed that the upregulation of plant helicase confers salinity stress tolerance. Dang *et al.* (2011) described expression of helicases in *Pisum sativum* response to cold and high salinity stress. Increased expression of these helicases in drought stress response also confirms their function during establishing tolerance to drought which is probably related to the control of physiological processes of development and growth under stress. EST analysis showed that 8% of the proteins involved in the drought-responsive are attributed to the functions such as protein synthesis, processing and degradation which are the fundamental processes to tolerate drought stress. In present study, expression of some ribosomal protein increased which are associated with protein synthesis under drought stress, such as 50S ribosomal protein. Xu *et al.* (2013) reported that ribosomal protein genes are adjusted in abiotic stresses response. A similar conclusion has been drawn that expression of ribosomal proteins was induced in high and low temperature in Rice. Although, their tolerance mechanism to abiotic stresses has not been characterized. Furthermore, the proteins involved in processing and folding of proteins displayed increased change between libraries. In present study expression of the protein disulfide isomerases (*PDIs*) were increased. This protein plays role in catalyzing of protein disulfide bond preventing aggregation of misfolded proteins, and in endoplasmic reticulum it has function in isomerization of protein folding contributing tolerance to abiotic stresses. Kayum *et al*. (2017) reported that 24 *PDI* were overexpressed under drought and salt stress in Canola. These results propose that retaining the protein folding in correct manner is a mechanism that *T. turgidum* adopts to maintain the normal physiological function of plants to tolerate drought stress. In this study, the upregulation of putative NAC transcription factor (*NAC*) was observed in *T. turgidum* under drought stress. Extensive studies have identified these stress-responsive transcription factors (TFs) that regulate response of plants to abiotic stress. The function of *NAC* in plants have been extensively reviewed under different stress but there is little information over these TFs in *T. turgidum*. Tweneboah and Oh (2017) demonstrated the function of *NAC* TF in abiotic stress responses in which *NAC* TF activate the genes expression related to defense via the ABA and JA signaling pathways. *NAC* TF has pivotal functions in responses of plants to the drought and salt stress by modulating of expression of the target genes. Yuan *et al*. (2019) through functional analysis method in Rice demonstrated that *NAC* is a positive regulator of drought and oxidative stress tolerance. This result suggests that modulation of stress-responsive genes expression in term of transcriptional regulation is an essential step to regulate the mechanisms underlying *T. turgidum* tolerance responses to drought stress. So, these TFs may be important indices for identifying of crop plants with enhanced drought stress tolerance. 4% of differential EST affected by the stress were annotated to signal transduction. Signaling components which highly upregulated under drought stress was a phosphatase i.e. RNA polymerase II C-terminal domain phosphatase-like (*CPL*). This phosphatase is versatile regulator that regulates phytohormones, stress responses and plant growth and development. Many reports indicated that *CPL* is associated to the regulation of signaling pathways by modulating the kinase activity or mitogen-activated protein kinase (*MAPK*) to respond to abiotic stresses. In *Arabidopsis, CPL1* have been functioning in the development, growth and stress response (Koiwa *et al.,* 2004). Xiong *et al*. (2002) have displayed that the *CPL* contributes to the plant's response including salinity, low temperature and drought. The overexpression of a *CPL1* increases the tolerance of *Arabidopsis* to heat by controlling of the *NAC019* phosphorylation status and activity of HSPs (Guan *et al.,* 2014). Besides, a few interaction partners of *CPL1* related to the abiotic stress

response have been lately recognized. The results of this study show the positive role of signaling pathways affecting the physiological processes of *T. turgidum* in creating drought tolerance. Under drought stress conditions, a group of antioxidant detoxification and redox genes including superoxide dismutase (*SOD2*) as well as putative glutathione peroxidase 3 (*Gpx3*) were upregulated and accounted for 6% differential expressed ESTs. When plants subject to abiotic stresses, oxidative stress occurs that result in the enhancement of reactive oxygen species (ROS). ROS can attack plant molecules, metabolites and organelles leading to interrupting metabolic pathways and it eventually causes cell death. This study displayed that tolerant *T. turgidum* have evolved mechanisms through the antioxidants generation to detoxify the ROS and to defend the plant against oxidative damage. In agreement with present results, Heidari *et al.* (2018) was shown that there is an increase in the accumulation of antioxidants in Durum wheat to combat drought and reported that more antioxidant detoxification enzymes generate in drought tolerant cultivars. These results are important indices for evaluating *T. turgidum* as a drought tolerant plant for use in crop breeding programs in arid regions.

Transport-related genes

A large number of ESTs were annotated to transport (5%). Under drought stress, two pore calcium channel 1 (*TPC1*) was upregulated. Plants use the calcium ion (Ca^{2+}) in the regulation of responses to environmental stresses as a second messenger (Berridge, 2005). Different signals of abiotic and biotic stress are sensed by plant cells and induce rapid spatiotemporal changes in Cytoplasmic Ca^{2+} $[Ca^{2+}]_{cyt}$ resulting in the generation of calcium signals in order to establish specific cellular responses, such as altered phosphorylation and target gene expression (Webb *et al.,* 1996; Dodd *et al.,* 2010; Kudla *et al.,* 2010). In plants, induction of $[Ca^{2+}]_{cyt}$ in response to a stimulus usually arises via different channel such as two pore calcium channel protein 1 (TPC1) indicating that these transporters have remarkable features to confer drought stress tolerance in the plant (Peiter *et al.,* 2005; Choi *et al.,* 2014). It was reported that calcium-permeable ion channels such as *TaTPC1* functioning in Ca^{2+} import in wheat cytosol can regulate plant water use efficiency (WUE) by involving the midway process of ABA-induced stomatal closure and the change of plant WUE. Song *et al.* (2008) also showed a relationship between TPC1 and plant drought resistance as well as other abiotic stresses. In *Arabidopsis*, increased sensitivity to drought stress was observed in the loss-of-function mutant of transporter whereas tolerance to drought stress happened in overexpressed mutant (Kapilan *et al.,* 2018). More details concerning its biological mechanism of TPC1 need to be further studied and clarified.

Hormones, plant defense and stress responsive proteins-related genes

In this study, 11% of the differential EST were annotated in the Hormones, plant defence and stress-responsive proteins groups. The genes encoding Lipoxygenase (*LOX*), which are involved in the JA biosynthetic and metabolism pathway, were expressed more highly during drought stress. Lipoxygenases catalyzes the addition of an oxygen molecule to polyunsaturated fatty acids including linolenic acid and linoleic acid. Rai *et al*. (2021) identified LOX expression as the considerable transcription (increase 15.76-fold) in leaves of *Brassica rapa* under drought. Drought stress also upregulates the genes expression involved in other functional catalogs which are specifically expressed in response to abiotic and biotic stress. This included ESTs annotated to heat shock proteins (*HSPs*). *HSPs* detoxifies ROS as well as enhance membrane stability through upregulation of the antioxidant enzymes conferring tolerance to drought stress (SU Haq *et al.,* 2019).

Cell metabolism-related genes

Most ESTs annotated to metabolism group were mainly involved in carbohydrates, amino acids, lipids, nucleotide and secondary metabolism and account for 19% of differential ESTs. In carbohydrates metabolism, Trehalose phosphate synthase (*TPS*) was upregulated under drought stress and Fructose-1,6-bisphosphatase (CFBP1) was downregulated. When are subject to stressful conditions, plants accumulate specified

compatible solutes known as osmolytes particularly sugars and amino acids, to safeguard the cellular machinery and prevent cellular damage caused by oxidative stress (Farooq *et al.,* 2009). Sugars are considered as pivotal regulators retaining the water of the cells and facilitating a lot of physiological processes encompassing photosynthesis under abiotic stresses (Rosa *et al.,* 2009). Due to the increased gene expression related to some sugars and the decrease of others, it is possible that the genes expressing these osmolytes act specifically under stress conditions. However, there are no reports on the specificity of expression of osmolytes under stress and more research is needed in this area. ESTs annotated to nucleotide metabolism also altered with downregulation in purine and ATP synthetase, and upregulation for pyrimidine and cytidine triphosphate synthetase (*CTPS*). Adenylosuccinate synthetase (*PURA*) acts as an enzyme that has a key function in purine biosynthesis and is downregulated in drought stress. *CTPS* catalyzes the last step in pyrimidine nucleotide biosynthesis and is upregulated in drought stress. The change in the genes expression related to nucleotide metabolism probably resulting from their role in various cellular mechanisms under drought stress.

Cell organization and development genes

The 4% of ESTs were mainly involved in cell organization and development processes. In present study, it was found that abundance of the cytoskeleton-related protein such as actin-depolymerizing factor (*ADF*) was induced by drought stress. Actin regulates different cellular and physiological functions through function of numerous actin-binding proteins that are vital for grow and response to environmental changes in plants (Henty-Ridilla *et al.,* 2013; Higaki *et al.,* 2007). Many studies have explained roles of *ADFs* in plant development, growth and various abiotic stress responses. Yang *et al.* (2003) reported that drought stress upregulates expression of *OsADF2* in the rice cultivar Azucena which has function at filamentous actin (F-actin) depolymerization as a dynamics F-actin regulator and contribute to the development and growth of organism. *OsADF3* accumulates in the cultivars of Nipponbare and Zhonghua 8 during drought and osmotic stresses (Ali and Komatsu, 2006; Huang *et al.,* 2012). Proteomic analysis revealed that two *ADF-1* and *ADF-2* of rice (Taichung native 1) accumulate in response to ABA application (Chen *et al.,* 2006). The induction of *TaADF3* expression was also observed during drought (Tang *et al.,* 2016). Therefore, *ADF* may play an important role in regulating physiology processes such as cell organization and development in drought tolerance of *T. turgidum*.

Miscellaneous and unclassified processes

It is noteworthy that 17% of the significant differential ESTs remained miscellaneous enzyme and unclassified functions, which were regulated under drought stress. A catalog of ESTs, attributed to miscellaneous enzyme are associated to different metabolic processes having moderately great alteration in expression. But the unclassified genes are interesting, as they harboring the potential to provide drought adaptation and therefore serve as novel drought tolerance genes.

Conclusion

Evaluation of drought stress tolerance is a very challenging task for crop researchers. Following the understanding of drought stress in the plant, various metabolic processes begin. The effective can be considered as a promising horizon in the evaluation of plant species (Heidari *et al.,* 2019). In addition to the adoption of directly mechanisms involved in response to stress *T. turgidum* recruits mechanisms which indicate the necessity of using that in semi-arid and arid regions in Iran. So that in the process of photosynthesis, it adjusts genes that are involved in carbon fixation process. Effective carbon fixation can allow for a wide range of development and growth conditions under drought stress. *T. turgidum* has a pivotal function in enhanced membrane stability by regulating the expression of genes associated with *HSP* stress-responsive proteins. In regulatory pathways, it regulates genes involved in DNA synthesis (helicases) and CPL phosphatase, which are somehow related to cell cycles and plant growth indicating its importance for providing enough growth to achieve maximum yield efficiency. In addition, it contributes maintain the cell water content by regulating the

genes expression involved in transport and cellular metabolism (osmolites). It prevents the accumulation of toxins in cells and cell death under drought stress by modulating redox processes. These results provide indices for assessing the drought tolerance of *T. turgidum* in order to the use of tolerant varieties in breeding programs in semi-arid and arid regions in Iran.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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