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Expression Analysis of Candidate Genes in Common Vetch (*Vicia sativa* L.) Under Drought Stress

A. R. Abbasi[1*], B. Mohammadi[1], R. Sarvestani[1], and F. Miratei[1]

ABSTRACT

***Vicia sativa L.*, an annual winter growing leguminous plant, is a valuable source of protein and minerals for cattle. Drought is one of the key stress factors that influence plant growth and development. In order to investigate common vetch physiological and molecular responses under Normal irrigation (N) and two levels of drought stress [S_1= 30% and S_2= 10% Field Capacity (FC)], a greenhouse experiment was carried out on two genotypes, namely, Mahalimaraghe and 41, and some physiological traits [e.g. Relative Water Content (RWC), Electro Leakage (EL), total protein, chlorophyll (a, b), and carotenoid content] were measured and expression patterns of three genes (sod, aq1 and bzip) were evaluated by real-time quantitative RT-PCR analysis. Results showed that expression pattern of all three genes and physiological responses had significantly changed in response to the stress. The highest increase in the expression of each of the three genes was observed in Mahalimaraghe genotype in S_1 condition compared to N. In contrast, under S_2 condition compared to N, the highest increase in expression of the three genes was observed in genotype 41. In comparison of S_2 with S_1, the highest changes in expression of all the three genes was observed in Mahalimaraghe genotype. All together, the obtained results may facilitate the understanding of molecular mechanism of *V. sativa* in response to drought stress, and also provide the basis of effective genetic engineering strategies for improving stress tolerance of *V. sativa*.**

Keywords: Dought tolerance, Expression pattern, Gene Expression, Molecular response, Osmotic Adjustment.

INTRODUCTION

Common vetch (*Vicia sativa L.*) is one of the most widely distributed annual leguminous crops throughout the Mediterranean basin, western Asia, and in countries of the former Soviet Union (Dhima *et al*., 2007). It can be used for pasture or as grain legume, showing high attractiveness at all its growth stages. (Abbasi *et al.*, 2014). Drought is one of the most important abiotic stresses affecting plant growth and crop productivity. Drought limits plant growth and development mainly by photosynthetic decline, osmotic stress-imposed constraints on plant processes, and interference with nutrient availability (Chinnusamy *et al*., 2004). Therefore, osmotic stress and associated oxidative stress appear to be common consequences of exposure to drought. The regulatory factors [such as transcription factors, protein kinases, and enzymes involved in the Abscisic Acid (ABA) biosynthesis] play important roles in improving plant tolerance to drought and other abiotic stresses (Chen *et al*., 2010). Although several abiotic stress-induced genes have been characterized from model species such as *Arabidopsis thaliana, Nicotiana tabaccum and Oryza sativa*, little is known in economically important species such as forage crop, so far. Identification to

[1] Department of Agronomy and Plant Breeding, Faculty of Agricultural Science and Engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Islamic Republic of Iran.
[*] Corresponding author; email: rezabbasi@ut.ac.ir

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novel genes, analysis of their expression patterns in response to these stresses, and evaluation of their potential functions in stress adaptation will provide the basis of effective engineering strategies to improve crop stress tolerance (Chen *et al*., 2010).

Plant cells have evolved several enzymatic and non-enzymatic antioxidant systems to keep low concentrations of Reactive Oxygen Species (ROS). One of the enzymatic antioxidant defenses in plants is Superoxide Dismutase (SOD). It is well known that SOD is important in plant stress tolerance (Gill and Tuteja, 2010). In several plant species, it has been observed that Cu/ZnSOD expression can be induced by paraquat herbicide, ozone, NaCl, hormones, high or low temperature and high irradiance (Kayihan *et al*., 2012; Abercrombie *et al*., 2008). Another important component involved in adaptation to many conditions such drought is Aquaporins. Aquaporins are proteinaceous pores that facilitate the passive diffusion of water across membranes of living cells. Rice (*O. sativa)* and Tobacco (*N. tobacco)* plants overexpressing a PIP1 protein increased their drought tolerance (Yu *et al*., 2005). Water stress may also cause changes in the post transcriptional regulation of aquaporin abundance, since aquaporin activity has been shown to be regulated by phosphorylation; divalent cations and pH (Luu and Maurel, 2005). Drought, ABA application and methotrexate caused an increase in the expression of *pvpip2;1* gene expression and PIP1 protein abundance in bean leaves. In contrast, in the roots, only the drought treatment raised the expression of the three examined *pip* genes (Aroca *et al*., 2006). Transcription level of genes involved in signal transduction such as *bZIP* transcription factors are usually altered by drought stress (Bray, 2002). The expression of root-specific *bZIP* transcription factor in water stressed *Phaseolus acutifolius* and *Phaseolus vulgaris* plants suggested that *bZIP* may regulate the expression of other water stress-responsive genes (Rodriguez-Uribe and O'Connell, 2006). In our study, the aim was to evaluate expression of 3 genes (sod, aq1, bZIP) related to drought stress in *V. sativa* by real-time quantitative RT-PCR analysis and to determine their relationship with physiological traits.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Common vetch seeds of two local varieties (*V. sativa. cv mahalimaraghe and V. sativa. cv maraghe*) and a drought-tolerant (V. sativa. cv 39) and a drought-sensitive (V. sativa. cv 41) genotypes were used to study the response of common vetch to drought stress. Seeds were surface sterilized with 1% hypochlorite and planted into pots (15 cm in diameter) containing 1.5 kg sandy clay loam soil with FC= 16.2% and PWP= 7.8% in greenhouses using a factorial experiment and a completely randomized design with three replications. The number of seeds planted in each pot was 6–8. All of the cultivars were grown in similar pots so that they were exposed to the same soil moisture content. Plants were subjected to three irrigation regimes including 10%, 30%, and FC. The Available Water Capacity (AWC) was calculated as: AWC= FC–PWP, where, AWC is the Available Water Capacity; FC is Field Capacity and, PWP is the Permanent Wilting Point (Arin and Kiyak, 2003). After sowing, the pots were irrigated with water to reach FC level. After reaching to FC, by means of the mentioned methods above, pots were weighed until determining PWP. AWC was maintained by weighing each pot every day, and adding the required amount of water based on the irrigation regime of each treatment. The irrigation regime began when the plants reached the four-leaf stage. Plants were harvested 10 days after begin stressed, when they showed drought symptoms, and were frozen for more analysis. Also, Relative Water Contents (RWC), Electrolyte Leakage (EL) (Lutts et al., 1996), chlorophyll a, b and carotenoids content of the leaves were

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measured at the corresponding times following standard method (Arnon, 1967).

Statistical Analysis

Statistical analysis was carried out using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and the *t*-test was used to evaluate differences between mean values**.**

RNA Extraction and RNA Quantitation

Total RNA was extracted from 50 mg of plant material (leaves) by Biozol reagent, following the manufacturer's protocol for RT-PCR. RNA samples were quantified by absorbance at 260 nm using NanoDrop (Thermo scientific,USA) and the structural integrity of the RNAs of each sample was checked with agarose gel. To remove contaminating DNA, RNAs (1 µg) were treated with DNase I, RNase-free (Thermo Fisher Scientific, Fermentas).

Reverse Transcription (RT-PCR)

DNase-treated RNA samples (0.5 µg) were reverse-transcribed using Revert Aid First Strand cDNA Synthesis Kit (Fermetase,Thermo Scientific, USA) For First-strand cDNA synthesis and the oligo (dT)16 as primer**.** Gene-specific RT-PCR primers were designed according to the genes derived sequences from NCBI. All the primers used for SYBR real-time RT-PCR are listed in Table 1. As internal control, 18S transcripts were analyzed. The PCR program was denatured at 94°C for 4 minutes, followed by PCR cycles of 94°C for 30 seconds, 56°C for 45 seconds, and 72°C for 1 minute, and then a final extension at 72°C for 7 minutes. The PCR products (10 µL) were analyzed by electrophoresis in 1.2% agarose gels. The cDNAs were then stored at -20°C until used in real-time PCR amplification.

Table 1. Gene specific primer pairs used in the real-time RT-PCR experiments.

Gene	Accession number	Protein	Primer pairs	Primers sequences (5'–3')	Tm (°C) /Length (bp)
aq1	AJ289701.1	plasma membrane aquaporin	VIP1-F / VIP1-R	*ACTGCACCGCTGGAATCTCT/CCTTAACCACACCAGCACCA*	56 / 147
SOD	JQ043347.1	Cu-Zn Superoxide dismutase	SOD-F / SOD-R	*ACTCACTGGCCCCAATTCAG/TGGAGTCAAGCCAACCACAC*	56 / 145
bZIP1	X97903.1	bZIP transcription factor	bZIP1-F / bZIP1-R	*TCATTCCAAAAGTGGCGATAGTGCA/CGCTGAACTTCCATTTTGAGACGG*	56 / 117
18S	-	18S ribosomal RNA	18s rRNA-F/ 18s rRNA-R	*GCAACAAACCCCGACTTCTG/TGCGATCCGTCGAGTTATCA*	56 / 110

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Real-time PCR

Real-time quantitative RT-PCR was carried out using a Biorad iQ5 Real time PCR. The *18S* gene, used as reference gene, was amplified in parallel with the target gene allowing gene expression normalization and providing quantification. Detection of Real-time RT-PCR products was done by using the SYBRR Green BioPars kit (Agricultural Sciences and Natural Resources University of Gorgan, Iran) following the manufacturer's recommendations. Two microliters of cDNA were used as template for PCR. PCR cycling conditions comprised one cycle at 95°C for 2 minutes, followed by 35 cycles at 95°C for 10 seconds , at 56°C for 10 seconds, and 72°C for 10 seconds and then a final extension at 72°C for 5 minutes. For each sample, reactions were set up in triplicate to ensure the reproducibility of the results. cDNAs to be amplified (target and reference) were made with the same PCR master mix and within a single Biorad iQ5 run. For the accurate amplification of each specific target gene, we carefully designed the primer sets for each gene based on the sequences corresponding to 3′-untranslated region. To achieve optimal amplification, PCR conditions for each primer combination were optimized for annealing temperature, and PCR products were verified by melting curve analysis and confirmed on an agarose gel. Mean values and standard errors (bar) were calculated from three replicates of leaves materials. The Ct (Cycle threshold), defined as the PCR cycle at which a statistically significant reporter fluorescence is first detected, is used as a measure for the starting copy numbers of the target genes. Relative quantity of the target gene expression level was normalized using 18S as a reference gene and was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

RESULTS

Physiological Traits

The effect of drought stress on Relative Water Content (RWC), Electro Leakage (EL), chlorophyll (include a and b) content and total of carotenoids were significant ($\alpha \leq 0.05$) (data not shown). The *RWC* of leaves declined under water stress, but the reduction varied in different genotypes and at different water stress levels. Here, the highest decrease in *RWC* under drought vs. normal condition was found for genotype 41 (which showed 36% decrease in RWC relative to normal condition) (Figure 1-a). In the case of *EL*, the Maraghe cultivar showed highest ion electro leakage from leaves and genotype number 39 showed the lowest impact (Figure 1-b).

Water deficit significantly decreased pigment content in plants. Photosynthetic pigments including chlorophyll *a*, *b* decreased along with increase in drought stress levels. Our results showed also remarkabe decrease in total carotenoids. The same decreasing trends were observed in all samples for photosynthetic pigments and total carotenoids (Figures 1-c, -d and -e).

Gene Expression

Superoxide Dismutase (sod)

In S_1 condition (30% FC), expression of *sod* gene in all of genotypes, except Mahalimaraghe genotype, increased in response to drought stress (Figure 2-a). But, in S_2 condition (10% FC), a significant increase was observed in expression of *sod* gene in all studied genotypes, except Mahalimaraghe genotype. Increase in expression of *sod* gene in S_1 and S_2 conditions was greater in genotype 41 as a sensitive genotype than genotype 39 as a tolerant genotype. The highest and lowest gene expression variations were observed in Mahalimaraghe and genotype 39, respectively (Figure 2-a).

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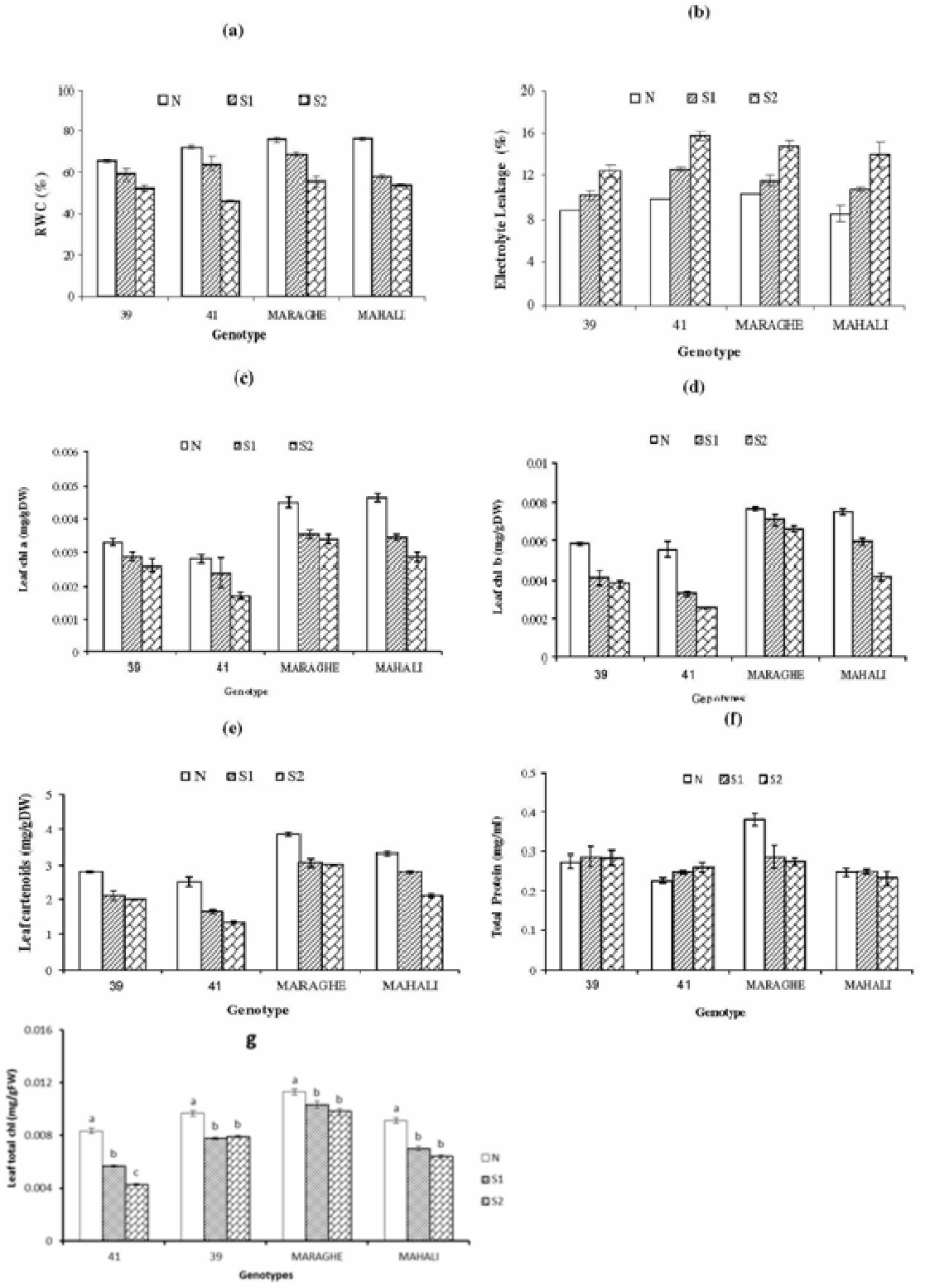

Figure 1. Physiological parameters of common vetch plants under drought stress: (a) Relative Water Content (RWC); (b) Electrolyte Leakage (El), (c) Leaf chlorophyll ***a*** content; (d) Leaf chlorophyll ***b*** content; (e) Leaf carotenoid content, (g) Total chlorophyll. The values are mean±SE (n= 3). N= Control; S_1= 30% FC drought stress, S_2= 10% FC drought stress. Vertical bars represent the standard deviation.

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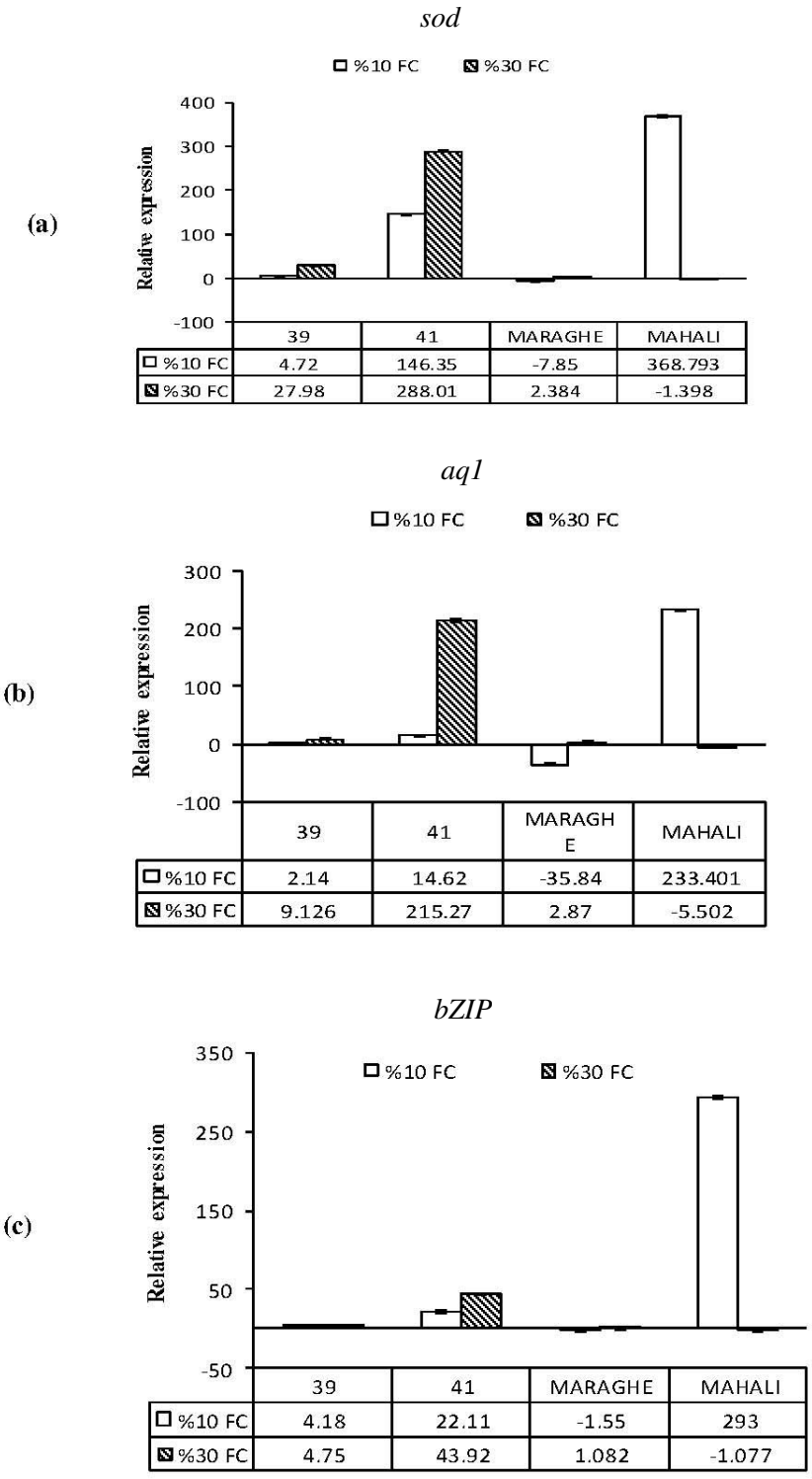

Figure 2. Relative expression profile of the three candidate genes: (a) *sod*; (b) *aq1* and, (c) *bZIP* in common vetch under drought stress. Mean values and standard errors (bar) were shown from three independent experiments.

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These results indicate plants with increase in expression of *sod* gene prevent cell dehydration damage caused by drought stress.

Aquaporins (*aq1*)

In S_1 condition (30% FC), expression of *aq1* gene increased in all genotypes, except Maraghe genotype, in response to drought stress. But, in S_2 condition (10% FC), an increase in expression of *aq1* gene was observed in all genotypes, except Mahalimaraghe genotype. Increase in expression of *aq1* gene in S_1 and S_2 conditions was greater in genotype 41, as a sensitive genotype, than genotype 39, as a tolerant genotype. In S_1 condition, the highest increase in expression *aq1* was observed in Mahalimaraghe genotype, while in S_2 condition, this genotype had an opposite change. The highest and lowest gene expression variations were observed in Mahalimaraghe and genotype 39, respectively. (Figure 2-b)

bZIP1 Transcription Factor

An increase in expression of *bZIP1* gene was observed in all studied genotypes, except Maraghe genotype in S1 treatment (30% FC). But, in S2 treatment (10% FC), expression of *bZIP1* gene in all of the genotypes, except Mahalimaraghe genotype, increased. The highest and lowest gene expression variations were observed in Mahalimaraghe and genotype 39, respectively. In S_1 condition, the highest increase in expression of *bZIP1* was observed in Mahalimaraghe genotype, while in S_2 condition, the maximum increase in expression of *bZIP1* was observed in genotype 41 (Figure 2-c).

DISCUSSION

Understanding plant responses to the external environment is of great importance, and it also is a fundamental part of making stress tolerant crops (Farahani *et al*, 2011). We exposed some common vetch genotypes to two drought and normal treatment (30, 10% FC and normal condition) and some physiological traits were measured. Decrease of Relative Water Content (RWC) is one of the early symptoms of water deficiency in plant tissues (Valentovic *et al.*, 2006) and many of researchers have reported that under drought stress *RWC* decreases (Valentovic *et al.*, 2006; Ghaderi *et al.*, 2011). Drought stress results in increased electro leakage from plant leaves. Cells membranes from all genotypes were affected by drought stress. Valentovic *et al*. (2006) reported that the electrolyte leakage of the sensitive maize cultivar increased from 11 to 54%, more than the tolerant cultivar. Sreenivasulu *et al.*, (2000) also showed that there was a positive correlation between stress sensitivity and membrane injury. It is generally accepted that the maintenance of cell membrane integrity and stability under water stress conditions is a major component of drought tolerance in plants (Bajji *et al.*, 2001). Often, plant membranes are subject to changes associated with the increases in permeability and loss of integrity under environmental stresses (Blokhina *et al.*, 2003). Therefore, the ability of cell membranes to control the rate of ion movement, in and out of cells, has been recommended as a valuable criterion for identification of stress resistant cultivars in several crop species.

Stress-induced transcription factors have been considered as powerful tools, as they can regulate expression of several genes. *bZIP* is one of the key transcription factors that play important roles in response to drought stress. *bZIP* transcription factor alarms stress condition to plant and also causes activation of genes involved in response to drought stress. For example, transgenic plants overexpressing *OsbZIP16* showed significantly improved drought resistance at both the seedling and tillering stages when compared with wild type plants (Chen *et al*., 2012). Although, *bZIP* gene

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expression in genotypes 39 and 41 (both S_1 and S_2 conditions) increased (Figure 2-c), the overall gene expression variation in S_2 condition compared to S_1 condition was not significant. Previous studies showed that several expression of *bZIP* genes to be up-regulated in rice under drought and high salinity stress (Nijhawan *et al*., 2008 and Chen *et al*., 2012). As mentioned above, *bZIP* transcription factor causes activation of multiple genes which is followed by production of relevant proteins in response to drought stress. Therefore, the results of gene expression are comparable with the results of measurements of total protein (Figure1-f). Total protein variations in genotypes 39 and 41 were not significant (Figure 1-f), which are similar to the results of gene expression variations. In addition, another role of *bZIP* is to mediate dehydration responses which is carried out by ABA (Rodriguez-Uribe and O'Connell., 2006 and Chen *et al*., 2012). Probably, *bZIP* causes activation of genes related to ABA, which, through aquaporin channel (aq1), leads to closing of stomata and prevents cell water loss. Thus, we can indirectly say that *bZIP* expression is associated with expression of *aq1*. Figure 2 (2-c and -b) shows that expression levels of *bZIP* and *aq1* genes have the same pattern. Also, another study has shown that overexpression of this transcription factor increased the plant's ability to remove reactive oxygen species by scavenging systems (e.g. SOD) (Sun *et al*., 2010). Thus, the gene expression of this transcription factor may be associated with the expression of *SOD* gene. Figure 2 (2-a and -c) shows that *bZIP* and *SOD* genes expression are consistent with each other.

The discovery of aquaporins was a major breakthrough in the understanding of water flow through living plant cells. The role of this protein channel (AQ1) is quite prominent in response to drought stress (Maurel and Chrispeel, 2001) in the sense that increasing level of *aq1* gene expression leads to closing of stomata, which in turn prevents cell water loss in response to drought stress. Expression of several genes that encode for aquaporins were shown to be upregulated in *Arabidopsis* (Yamaguchi-Shinozaki *et al*., 1992) and in *Phaseolus vulgaris* (Cui *et al*., 2008) in response to drought or ABA. Furthermore, when transcription levels of *aq1* gene are compared to *RWC* rate (Figure 1-a), a relation was found between *aq1* expression (Figure 2-b) and *RWC*. For example, in genotype 39, the *RWC* rate was little decreased in both stress condition compared to normal irrigation, and expression levels of *aq1* gene were increased in both stress condition *vs.* normal. So, these results indicate that increase in *aq1* transcription may enable genotype 39 to prevent water loss under stress condition, which in turn leads to an efficient response to drought stress. In contrast, *aq1* gene expression changes in genotype 41, as a sensitive genotype, has wider variations than genotype 39. Although expression level of *aq1* gene in S_2 increased significantly compared to the control condition, this increase of gene expression mentioned could not prevent water loss due to inherent sensitivity of genotype 41. In addition, the greatest *RWC* changes among all of the studied genotypes was observed in genotype 41, which reflects excessive cell water loss in this genotype under stress conditions. But, it is quite different in the two other genotypes (Mahali and Mahalimaraghe genotypes). Expression of *aq1* gene in Maragheh genotype in S_1 condition due to shock caused by drought stress was severely decreased. In line with these observations, Mesembryanthemum PIPs decline upon osmotic stress and then recover during adaptation (Yamada *et al*. 1995). In any case, there must be other concomitant measures by the cells suffering from water deficit that allow them to attract water from regions of increasingly lower water potential (Bray, 1997). Conversely, in S_2 condition Maraghe genotype was able to increase *aq1* gene expression. However, the results of *RWC* measurement (Figure1-a) indicate that this genotype could not prevent excessive cell water loss. Unlike Maraghe genotype,

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expression of *aq1* gene in Mahalimaraghe genotype under S_1 condition was severely increased. But, this increased expression could not prevent excessive cell water loss. In contrast, this genotype was able to adapt to S_2 conditions and prevent excessive cell water loss. Several previous studies reported that gene expression for this protein family increased by water and osmotic stresses (Kawasaki *et al*., 2001). During drought stress، CO_2 fixation is limited due to stomatal closure which، in turn, leads to reduced $NADP^+$ regeneration through the Calvin cycle. Due to lack of electron acceptor, over reduction of the photosynthetic Electron Transport Chain (ETC) occurs which leads to a higher leakage of electrons to O_2 by the Mehler reaction. This, in turn, leads to generation of ROS which are potentially dangerous under drought stress conditions (Sharma *et al*., 2012). Superoxide dismutase enzyme as one of the most important defense antioxidant mechanisms plays a key role in the removal of reactive oxygen species (ROS) (Sharma *et al*., 2012). Among *SOD* gene expression and physiological traits such as electrolyte leakage, chlorophyll (a, b) and carotenoid contents, there is a significant relationship. Increase in *SOD* activity is expected to decrease *ROS* accumulation, which in turn leads to lower damage to cell membranes, and also less degradation of chlorophyll (a and b) and carotenoid contents. Overexpression of superoxide dismutase has been implicated in free radical detoxification and suggested to have a major role in defending the alfalfa and mangrove species against severe abiotic stresses (Yan and Guizhu, 2007). When the results of *sod* gene expression (Figure 2-a) were compared with physiological traits such as electrolyte leakage (Figure 1-b), chlorophyll *a*, *b* (Figures 1-c and -d)) and carotenoid content (Figure 1-d), the findings were interesting. For instance, *sod* gene expression in genotype 41 as a sensitive genotype was increased approximately 2-fold under S_2 compared to S1 condition. While expression level of this gene in tolerant genotype (39) was approximately 6-fold increase. Comparative study of the antioxidant responses in drought tolerant and drought sensitive genotypes revealed higher antioxidant capacity in tolerant genotypes (Sairam *et al*., 1998). These results show that genotype 39 as tolerant genotypes was able to increase the sod gene expression, in turn preventing accumulation of reactive oxygen species effectively in response to drought stress. A previous study showed that the expression level of genes in Superoxide Dismutase (SOD) family was higher in tolerant lines. Hence, reducing superoxide (O^-_2) could be higher in tolerant lines than susceptible lines in rice (Moumeni *et al*., 2011). According to the results of physiological traits, rate of changes in electrolyte leakage, chlorophyll (a, b) and carotenoid contents in genotype 39, as a tolerant genotype, is lower than genotype 41 as a sensitive genotype. These results are validated by those of *sod* gene expression. In fact, by increase in sod gene expression, accumulation of reactive oxygen species declined significantly, leading to less damage to cell membranes (low Electro Leakage changes) and also prevention of chlorophyll (a, b) degradation. Increased production of ROS leads to oxidative stress in growing plants. Rice seedlings subjected to drought showed increased concentration of O^-_2, increased level of lipid peroxidation, chlorophyll bleaching, and loss of some antioxidants (AsA, GSH, α-tocopherol, and carotenoids), total soluble protein, and thiols (Boo and Jung, 1999; Sharma and Dubey, 2005). Expression of *sod* gene in Maragheh genotype was associated with minimum gene expression changes. Expression of *sod* gene was reduced in S_1 condition but in the severe stress conditions (S_2) this genotype was able to adapt to environmental conditions and, on the other hand, expression of *sod* gene was increased, leading to reduced accumulation of ROS. In addition, these results corresponded to the results of physiological traits such as chlorophyll (*a*, *b*) and carotenoids contents.

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Although, a severe increase in *sod* gene expression in Mahalimaraghe genotype under S_1 condition was observed, but in S_2 condition it was reduced. These results suggest that Mahalimaraghe genotype, similar to genotype 41, was severely affected by drought stress as evidenced by physiological traits results. Although expression level of *sod* gene in this genotype under S_1 was severely increased, it was not able to prevent inflicted damages on the membrane (high electrolyte leakage), or reduction of chlorophyll (a, b) and carotenoid contents. Plant adaptive responses to drought are coordinated by adjusting growth and developmental processes as well as molecular and cellular activities. Our results indicate that the genes *sod*, *aq1* and *bzip* are likely to play important roles in *V. sativa* that are potentially involved in regulating cellular activities during the changes that occur under drought stress.

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بررسی بیان برخی ژنهای کاندید در ماشک گل خوشه ای تحت تنش خشکی

ع. ر. عباسی، ب. محمدی، ر. سروستانی، و ف. میر عطایی

چکیده

ماشک گل خوشه ای (*Vicia sativa*) یک گیاه علوفه ای یکساله است که منبع با ارزش پروتئین و مواد غذایی برای احشام به شمار می رود. خشکی یکی از فاکتور های کلیدی تنش است که رشد و عملکرد گیاهان را تحت تاثیر قرار می دهد. به منظور بررسی واکنش های فیزیولوژیکی و مولکولی ماشک گل خوشه ای به تنش خشکی، آزمایشی با دو تیمار تنش خشکی و یک تیمار آبیاری نرمال (۱۰ ٪ و ۳۰ درصد ظرفیت زراعی به ترتیب به عنوان تیمارهای S1، S2 و N) در گلخانه انجام شده و برخی صفات فیزیولوژیکی مانند محتوای نسبی آب، نشت یونی، پروتئین کل، محتوای کاراتنوئید و کلروفیل a و b اندازه گیری شده و الگوی بیان سه ژن در سطح رونویسی نیز با RT-PCR زمان واقعی بررسی شد. نتایج نشان داد که الگوی بیان هر سه ژن و پاسخ های فیزیولوژیکی به طور معنی داری تحت تنش خشکی قرار گرفتند. بیشترین افزایش در بیان هر سه ژن در تیمار S1 نسبت به N در ژنوتیپ Mahalimaraghe مشاهده شد. بر عکس، در شرایط S2 نسبت به N، بیشترین افزایش در بیان ژن های اندازه گیری شده در ژنوتیپ ۴۱ دیده شد. همچنین، بیشترین افزایش بیان در شرایط S2 نسبت به S1 در ژنوتیپ Mahalimaraghe مشاهده شد. در مجموع، نتایج بدست آمده ممکن است درک مکانیسم مولکولی پاسخ *V.sativa* به تنش خشکی را تسهیل کرده و پایه ای را برای ارائه راهکار های مهندسی ژنتیک برای رسیدن به گیاه مقاوم به خشکی فراهم آورد.

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