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Expression profile of some important genes related to carbohydrates metabolism under drought stress in bean (*Phaseolus vulgaris* L.)

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Abstract

This study was carried out to investigate the influence of drought stress during the flowering stage, on the content of water soluble carbohydrates and the expression of genes related to carbohydrate metabolic enzymes (*FBPA*, *FBPase*, *PGM*, *PRK*, *RBCL*, *RBCS*, *SBPase*, *TK*, *TPI*, and *GAPDH*) in bean (*Phaseolus vulgaris* L.) leaves. A factorial experiment was conducted based on a randomized complete block design with four replications. Factors included different bean cultivars, Taylor and COS16 (as tolerant cultivars), Khomein and Akhtar (as sensitive cultivars), drought stress included normal irrigation (100% FC) (available water), moderate stress conditions (60% FC), and high stress conditions (30% FC) and stress duration. Significant differences were observed among cultivars and levels of drought stress in carbohydrates content and gene expression. Drought stress caused a decrease in sucrose and an increase in water soluble carbohydrate concentration, glucose, and fructose. These analyses revealed that expression levels of most genes encoding chloroplast enzymes involved in carbon fixation (Calvin cycle) were reduced in the leaves during prolonged drought stress. The expression levels of most genes were higher in tolerant cultivars compared to susceptible ones under drought conditions. Calvin cycle related genes expression showed significant negative correlations with water soluble carbohydrates concentration, glucose, and fructose. In deficient water condition, tolerant cultivars (Taylor and COS16) accumulated more soluble sugars, and Khomein and Akhtar as susceptible cultivars had the lowest soluble sugar in both conditions. The soluble sugar content of different bean cultivars was typically increased, showing that sugar metabolism was influenced greatly by soil water stress.

Key words: Bean, Calvin cycle, Carbohydrate metabolism, Drought stress, Gene expression.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important crop from the Fabaceae family that is cultivated worldwide for human consumption. Drought is one of the most important environmental factors influencing growth and physiological characteristics of common bean especially during the flowering and grain-filling stages (Zlatev and Stoyanov, 2005). Plants have developed different mechanisms (morphological, physiological, biochemical and molecular) to adapt in drought conditions (Bartels and Salamini, 2001; Faical *et al*., 2009). The response of plants to drought

stress depend on several factors including plant developmental stage, genotype, the duration and severity of the stress applied (Bray, 2002; Torres *et al*., 2006). Photosynthesis is among the primary processes to be affected by drought (Chaves, 1991). The effects can be, as the decreased CO2 availability for fixation or the alterations of photosynthetic metabolism (Lawlor and Cornic, 2002). Despite the reduced photosynthetic assimilation of CO2 in drought-stressed leaves, plants accumulate a large content of water soluble carbohydrates such as glucose, fructose, sucrose, fructans, polyols, together with other (Pinheiro *et al*., 2001; Bartels and Salamini, 2001; Villadsen *et al*., 2005). Indeed, osmolyte accumulation in plant cells results in a decrease in the cell osmotic potential and thus in the maintenance of water absorption and cell turgor pressure (Blum *et al*., 1983). Also, carbohydrates serve as a source of energy and signaling molecules in regulation of metabolic pathways. They are also involved in the response to abiotic stresses and the expression of a large number of stress responsive genes corresponding to the enzymes of carbohydrate metabolism are regulated by the sugar status of the cell (Ho *et al*., 2001; Price *et al*., 2004). The determination of expression pattern of the genes involved in the primary carbohydrate metabolism (Figure 1) are helpful to improve the efficiency of breeding for increased drought tolerance (Price *et al.*, 2004). The identification and expression analysis of some genes involved in carbohydrate metabolism during drought stress have been reported in many different studies (Ramanjulu and Bartels, 2002; Chaves *et al*., 2002; Bray, 2002; Zhu, 2002; Bartels and Sunkar, 2005; Yamaguchi-Shinozaki and Shinozaki, 2006). The Calvin cycle is one of the basic pathways of carbohydrate metabolism. Therefore, the study of expression profile of genes related to the Calvin cycle under drought stress, and elucidating how they are regulated would be helpful to understanding their importance in drought tolerance.

The aim of this study was to evaluate the transcription level of important genes related to carbohydrates metabolism under drought stress in bean (*Phaseolus vulgaris* L.) using quantitative RT-PCR.

MATERIALS AND METHODS

This study was carried out to investigate the influence of drought stress during the flowering stage on some physiological characteristics and expression of carbohydrate metabolic genes in bean (*Phaseolus vulgaris* L.) leaves. This factorial experiment was based on a completely randomized design and was conducted in 4 replications (2 plants in each pot and 4 pots per replication). Factors included different bean cultivars, Taylor (tolerant cultivar), COS16 (tolerant cultivar), Khomein (sensitive cultivar) and Akhtar (sensitive cultivar), drought stress included normal

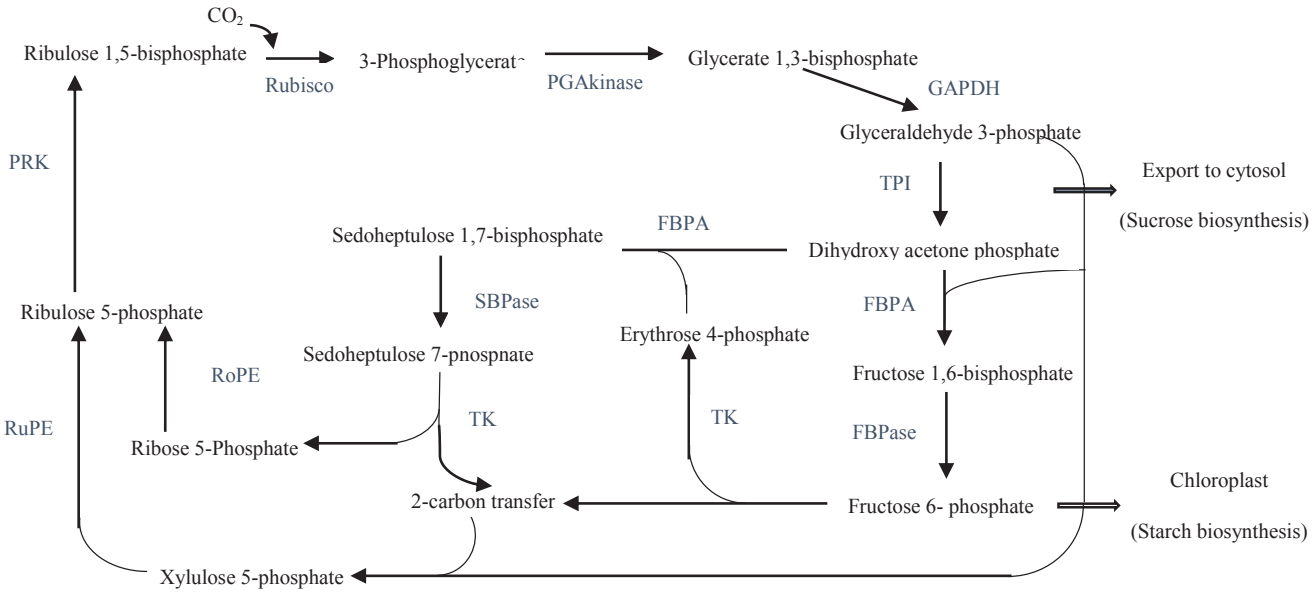

Figure 1. The pathway of Calvin cycle in *phaseolus vulgaris*. Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; PGA, phosphoglycerate kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TPI, triose-phosphate isomerase; FBPA, fructose-1,6-bisphosphate aldolase; FBPase , fructose-1,6-bisphosphatase; TK, transketolase; SBPase, sedoheptulose-1,7-bisphosphatase; RuPE, ribulose-phosphate epimerase; RoPE, ribose-5-phosphate isomerase; PRK, phosphoribulokinase (Xue *et al.,* 2008).

irrigation (100% FC) (available water), moderate stress conditions (60% FC), and high stress conditions (30% FC) and stress duration (3 and 9 days). Plants were kept in optimum conditions of soil moisture before drought stress was implemented in the beginning of the flowering stage. To apply water, the weight method was used. Once every day all pots were weighed using a precision portable balance device with an accuracy of ±5 g. Then, the moisture of the pots based on the soil moisture curve was set. Relative water content (RWC) was determined by the method of Schonfeld *et al*. (1988) and electrolyte leakage index (ELI) was determined by recording the electrolyte leakage (EL) as described by Valentovic *et al*. (2006) with a few modifications. Total soluble sugars content was measured by spectrophotometer (Dubois, 1956). A chromatographic system consisting of a Knauer HPLC (USA) equipped with a 50 µL sample loop, degasser, quaternary pump, column oven, and RI detector was used for carbohydrate analyses. Gene expression was performed using a reverse transcriptase polymerase chain reaction (RT-PCR). Sampling was carried out at 3 and 9 days after stress treatment. Leaf samples were frozen in liquid nitrogen and stored at -80 °C until RNA extraction. The samples were ground into a powder with liquid nitrogen. Total RNA was extracted using Gene JET Plant RNA Purification Mini Kit (Thermo Scientific, Fermentas, Vilnius), according to the manufacturer's protocol. The quantity and purity of the purified RNA were assessed with a spectrophotometer (Nano drop) at wavelengths of 230, 260, and 280 nm. The Quality and integrity of isolated RNA was verified by electrophoresing RNA on 1.2% agarose gel and staining with ethidium bromide. Then, RNA samples were treated with DNase I (Fermentas, Vilnius, Lithuania) to degrade any residual genomic DNA. The synthesis of cDNA was performed with Reverse Transcription Kit (Vivantis, Malasia) according to the manufacturer's protocol. All cDNAs were diluted with nuclease free water to 50 ng/µL to be used as templates in qRT-PCR. The qRT-PCR primers for carbohydrate metabolism genes fructose-1,6-bisphosphate aldolase (*FBPA*), fructose bisphosphatase (*FBPase*), phosphoglucomutase (*PGM*), phosphoribulokinase (*PRK*) ribulose-1,5-bisphosphate carboxylase large subunit (*RBCL*), ribulose-1,5-bisphosphate carboxylase small subunit (*RBCS*), sedoheptulose bisphosphatase (*SBPase*), transketolase (*TK*), triose-phosphate isomerase (*TPI*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and *actin* (as housekeeping) were designed using Primer 3 Plus software (http://frodo.wi.mit.edu/primer3/input.htm). The primers details are shown in Table 1. The specificity of primers was checked by a BLAST search of NCBI databases and standard PCR and electrophoresis on the agarose gel. To analyze the expression profiles of genes after different treatments, quantitative real-time RT–PCR was performed in an optical 96-well plate with a BIO-RAD iQ5 real-time PCR system (Bio-Rad, USA) using the SYBR Green method (Li *et al.*, 2010). The total reaction volume was 20 µL, containing 2 µL of template cDNA (40 ng) , 4 µL of SYBR Green Master Mix (Applied Biosystem) according to the manufacturer's instructions, 1µL of primer mix (0.5 µL each), and 13 µL of water. The PCR thermal cycle conditions were as follows: Predenaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 34 s and extension at 72 °C for 20 s. Relative fold changes in gene expression were calculated based on the $2^{-\Delta\Delta CT}$ comparative method by Livak and Schmittgen (2001). The data were statistically analyzed for variance using the SAS Software and the means were compared using Duncan multiple range test at the 0.01 and 0.05 P levels.

RESULTS

Relative water content

Under drought stress condition, leaf water content decreased gradually in all bean varieties with the increase in the severity of the stress compared to control. Both tolerant cultivars (Taylor and COS16) showed higher RWC than the sensitive cultivars (Akhtar and Khomein) on all the treatments (stress condition and control). The lowest RWC% was observed in Akhtar (49.42) under high stress conditions, and the highest RWC% was measured in Taylor (89.78) under normal condition (Figure 2A).

In both stress conditions (60% and 30% FC), there was a significant reduction in RWC with an increase in the duration of water stress from the 3rd to 9th day. Exposing the plants to drought stress of 60% field capacity in the duration of the 3rd to 9th day resulted in an decrease of 10% - 30% RWC, respectively. While, 30% FC treatment at the 3rd to 9th day of drought showed a larger decrease (14 to 33.4%) in RWC as compared to control (Figure 2B).

Electrolyte leakage index

In 4 cultivars, stress conditions significantly increased ELI compared to control condition and the sensitive cultivars (Akhtar and Khomein) showed higher ELI than tolerant cultivars (Taylor and COS16). The lowest and highest ELI were observed in Taylor (14.8%) under severe drought conditions (30% FC) and in

Table 1. Specific primers used in qRT-PCR.

Target gene	GenBank accession number	Primer sequences (5′ → 3′)	Amplicon length (bp)
FBPA	JX869951.1	F: GTCTCTCTCCCGATCCGC R:CCTGTAGGCCTGGCGATT	173
PGM	XM006577370.1	F:CATGCCAACCTCTGCTGC R:GGCCAGAACTGCCCAGAT	189
SBPase	XM003538398.2	F:TGAGGCGTGCCTCTATTACAA R:GAGCCGTCAAGGGGATCAAA	105
TPI	KF569617.1	F:TCGTCGGTGGAAACTGGA R:CACCACCTTTGCGAACCC	203
GAPDH	KF569621.1	F:TGGCACGGTCGTAAAGAGTC R:CAGGCTTGACAACAGCATCG	127
PRK	XM003516804.2	F:AAGAGGGGCAGCAACAGT R:GGTGGTTCTGCTGCTCCT	154
FBPase	XM003552168.2	F:GCAGCAACAGCATCCTCC R:TTCTGTGGTTGCTTGCCC	176
RBCS	EC997022	F:TTGGAGCATGGTTTCGTGTA R:ATGCACTGCACTTGACGAAC	165
RBCL	EC997037	F:TTGGGGTTATCCGCTAAGAA R:ATGCCCTTTGATTTCACCTG	172
TK	XM00355603.2	F:TCTGTCTCAGGCCCTTTTGG R:TCTGCGTGAACCTGCAGAAA	142
Actin	CV537379	F:TGGCCGTACAACTGGTATTG R:GCTCTGCAGATGTGGTGAAA	163

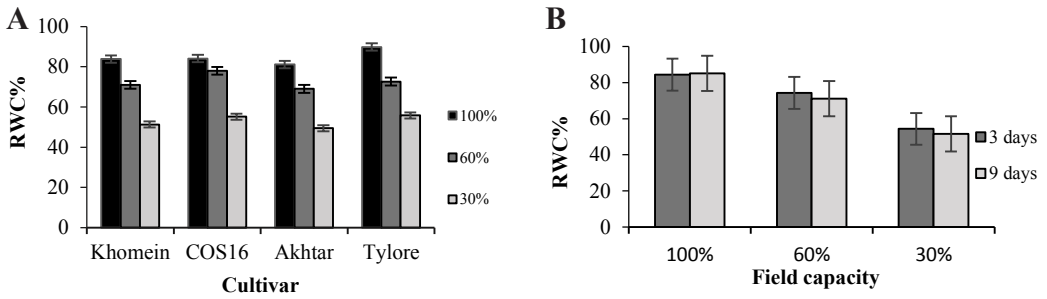

Figure 2. Effect of **A:** water stress and cultivar, **B:** water stress and duration of water stress on the relative water content in leaves.

Khomein (44.8%) under normal condition (100% FC), respectively (Figure 3A). At 9th day of drought stress, the level of ELI% was higher than 3rd day of drought stress in all treatments (Figure 3B).

Carbohydrates

The glucose, fructose and water soluble carbohydrate (WSC) contents increased significantly in drought-stressed bean leaves compared to the control plants. However, the degree of increase in soluble sugars content was dependent on species tolerance potential, severity and duration of water deficit.

This result showed that tolerant cultivars (Taylor and COS16) accumulated more soluble sugars in response to drought stress than susceptible cultivars (Akhtar and Khomein). The highest amount of glucose (121.18 µmol g^{-1} dw) and fructose (118.92 µmol g^{-1} dw) were observed under severe drought stress (30% FC) and the lowest content of glucose (75.38 µmol g^{-1} dw) and fructose (67.67 µmol g^{-1} dw) were related to the optimal condition or 100% FC (Figure 4). Analysis of variance

showed that the effect of drought stress duration was significant for contents of glucose, fructose and WSC. Total soluble sugars, glucose and fructose contents were increased significantly with the increase in stress duration (3th to 9th day) in all cultivars (Figure 5).

Sucrose content declined significantly with increasing severity of stress in all cultivars. Optimal condition (100% FC) and severe stress (30% FC) showed the highest and lowest sucrose contents (333.5 and 302.8 µmol g^{-1} dw), respectively (Figure 4C). Among the cultivars, Taylor had the highest sucrose content (323.1 µmol g^{-1} dw) and Akhtar showed the lowest sucrose levels (309.86 µmol g^{-1} dw). It was also observed that the increase in drought stress duration decreased sucrose level (Figure 4C).

Gene expression of *GAPDH*

In four cultivars, the level of *GAPDH* gene expression decreased under drought stress compared to the normal conditions. However, the amount of decrease was significantly higher under high stress condition (30% FC) than moderate stress (60% FC).

Under drought stress, the expression of *GAPDH* in tolerant cultivars (Taylor and COS16) was higher than those of sensitive one (Akhtar and Khomein). Among cultivars, the highest and lowest *GAPDH* relative expression ratios were observed in Taylor and Khomein, respectively (Figure 6A). The expression level of *GAPDH* was higher at the 3rd day of drought stress compared to 9th day in all drought treatments (Figure 6B). However, the relative expression of

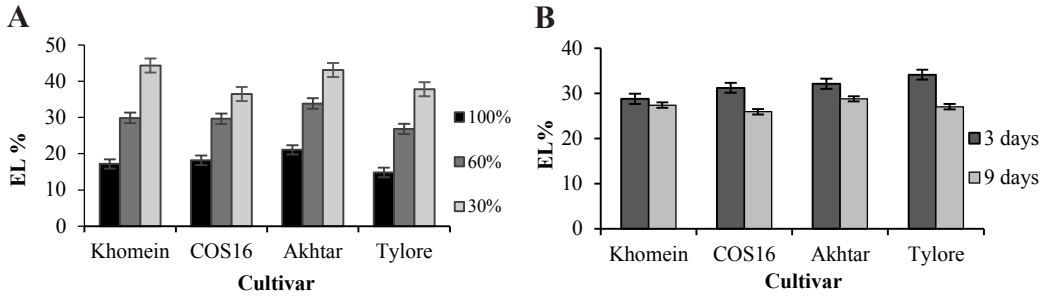

Figure 3. Effect of **A:** the water stress and cultivar, **B:** water stress and duration of water stress on the electrolyte leakage index in leaves.

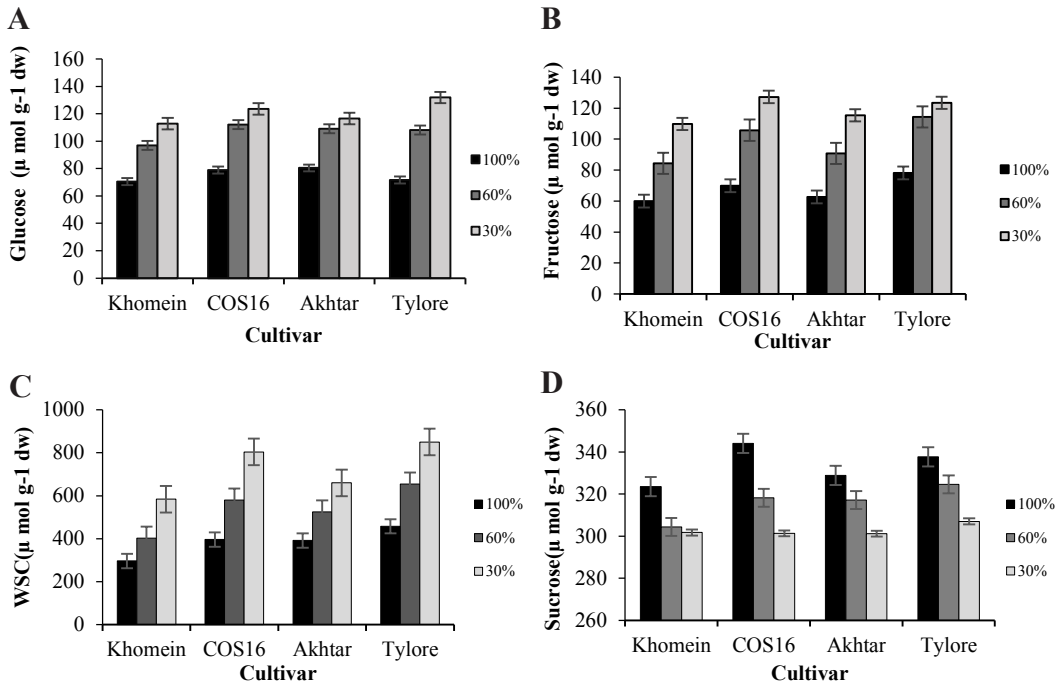

Figure 4. Effect of water stress on the content of **A:** glucose, **B:** fructose, **C:** sucrose and **D:** water soluble carbohydrate in four cultivars.

GAPDH under moderate stress condition (60% FC) was significantly higher than sever stress condition (30% FC).

Gene expression of *Rubisco*

The expression level of small subunit and large subunit of ribulose 1, 5-bisphosphate carboxylase/oxygenase (RBCS/RBCL) significantly decreased during drought stress condition as compared to the control condition. The transcript level of *RBCL* gene was higher than *RBCS* gene under drought stress condition (60% and 30% FC). However, it was revealed that the decrease in the level of *RBCL* and *RBCS* genes expression in response to drought stress in 30% FC was higher than that in 60% FC. In between cultivars, the highest relative expression ratio of *RBCL* and *RBCS* genes were observed in Taylor and the lowest relative expression ratio of *RBSC* and *RBCL* genes were observed in Khomein (Figure 7A, 8A). Also, the relative expression ratio of *RBSL* and *RBCS* (Figure 7B, 8B) in treatment of 9th day of drought stress (0.55 and 0.45) decreased significantly compared to the treatment of 3rd day (0.79 and 0.65).

Gene expression of *PGM*

Under drought stress, the highest relative expression of *PGM* was observed in Taylor cultivar (1.09) and the lowest was observed in Khomein cultivar (0.74), however, in relative expression of *PGM* Khomein, Akhtar and COS16 cultivars were not significantly different. The interaction effect of cultivar and duration of drought stress was significant on relative expression of *PGM* (Figure 9A). In Taylor cultivar, relative expression of *PGM* was not significantly different

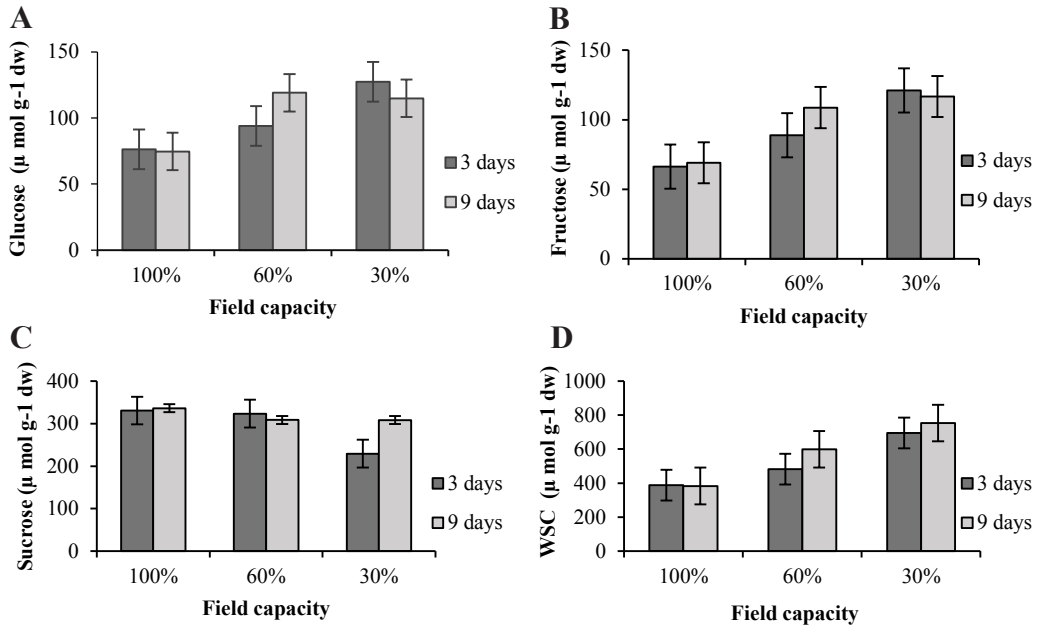

Figure 5. Interaction of level and duration of stress on **A:** glucose, **B:** fructose, **C:** sucrose and **D:** water soluble carbohydrate in bean cultivars.

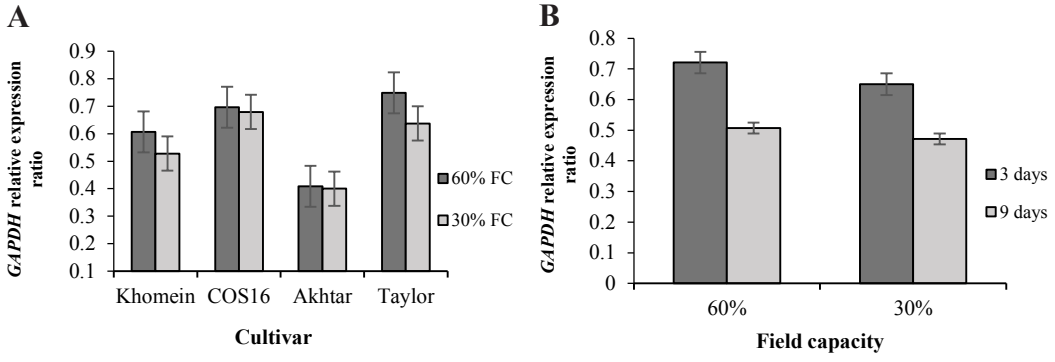

Figure 6. Interaction of **A:** stress level×cultivar and **B:** stress level×stress duration on *GAPDH* relative gene expression ratio.

at the 3rd day of drought stress compared to control treatment but with an increase in stress duration to the 9th day of drought its expression increased by about 14%. In other cultivars decreased relative expression of *PGM* at the 3rd to 9th day of drought stress occurred compared to control treatment and the lowest relative expression of *PGM* was observed in cultivar Khomein at the 9th day of drought stress (0.69).

Gene expression of *SBPase*

The relative expression level of *SBPase* gene significantly decreased by about 29% and 41% under drought stress condition of 60% and 30% FC, respectively. At the 3rd day of drought stress, the relative expression of *SBPase* decreased by about 25% compared to control treatment, while it decreased by 43% at the 9th day of drought stress. At the 3rd day of drought stress, the highest transcript of *SBPase* was related to COS16 cultivar (0.81) and the lowest transcript of SBPase was related to Khomein (0.67) compared to their control plants. At the 9th day of

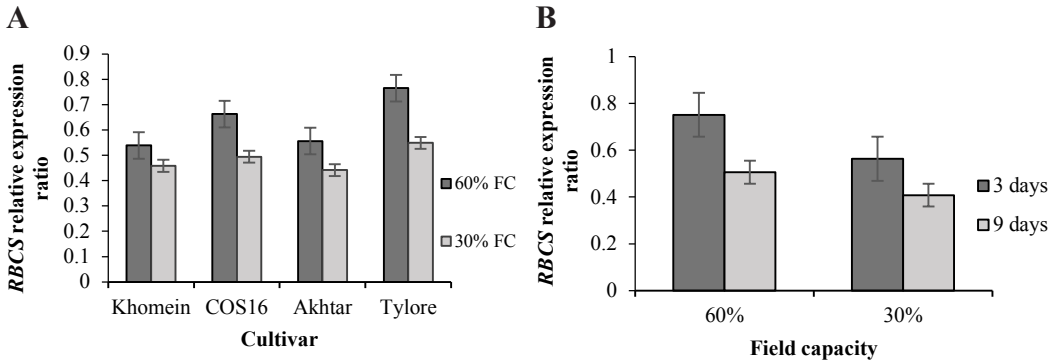

Figure 7. Interaction of **A:** stress level×cultivar and **B:** stress level×stress duration on *RBCS* relative gene expression ratio.

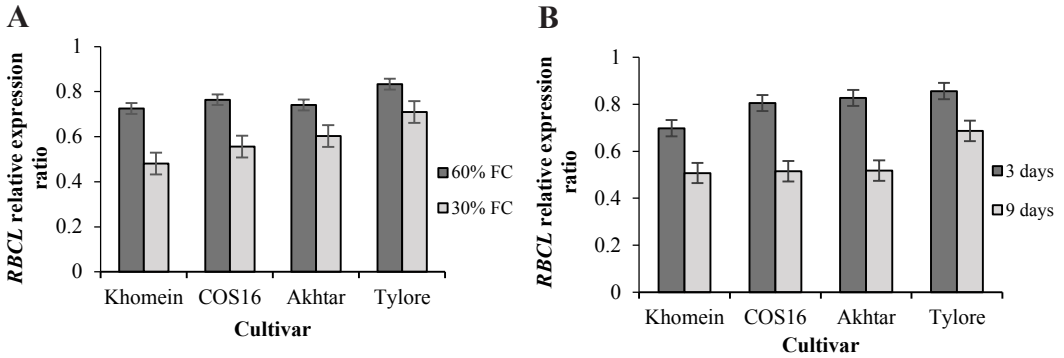

Figure 8. Interaction of **A:** stress level×cultivar and **B:** cultivar×stress duration on *RBCL* relative gene expression ratio.

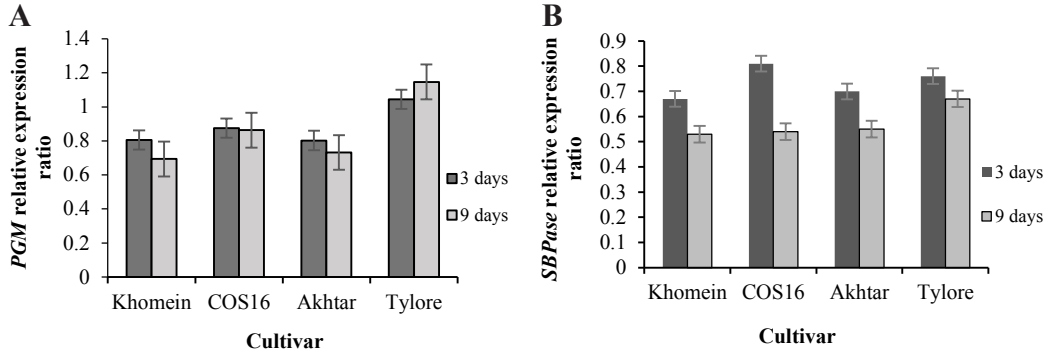

Figure 9. Interaction of **A:** cultivar×stress duration on *PMG* and **B:** *SBPase* relative gene expression ratio.

drought stress, the highest and lowest transcript of *SBPase* were observed in Taylor (0.67) and Khomein (0.53), respectively (Figure 9B).

Gene expression of *FBPA*

The highest and lowest relative expression of *FBPA* were observed in tolerant cultivars Taylor (1) and COS16 (0.98), and in sensitive cultivars Khomein (0.66) and Akhtar (0.67), respectively. In sensitive cultivars (Figure 10A), relative expression of *FBPA* decreased by 35% under drought stress (30% and 60% FC). However , the tolerant cultivars showed a lower decrease (5%) in relative expression of *FBPA* under 60% FC drought stress but there were no significant differences compared to control treatment under high stress condition (30% FC). The relative expression of *FBPA* (Figure 10C) decreased with increase in stress duration from the 3rd day (0.9) to 9th day (0.73) under moderate stress (60% FC). In contrast, the relative expression of *FBPA* was higher at the 9th day (0.89) than the 3rd day (0.79) under high stress condition (30% FC). In tolerant cultivars, the relative expression of *FBPA* showed a slight decrease at the 3rd day and then slightly increased at the 9th day of stress in comparison to control. In sensitive cultivars, the relative expression of *FBPA* decreased at the 3rd to the 9th day of drought stress compared to control (Figure 10B).

Gene expression of *TPI*

Results showed that there was a significant difference in relative expression of *TPI* gene among cultivars, drought stress in the duration of stress. The relative expression of *TPI* gene decreased by 33% and 55% under drought stresses of 60% and 30% of field capacity, respectively. In different cultivars, the relative expression of *TPI* decreased between 18 to 46% under drought stress of 60% FC, although this decrease was between 41 to 62% under 30% FC stress (Figure 11A). The highest relative expression of *TPI* gene was observed in Taylor (0.82) under moderate stress condition (60% FC) and the lowest relative expression of TPI (0.38) was observed in Khomein under severe stress condition (30% FC). The expression of *TPI* gene showed a larger decrease at the 9th day of drought stress compared to the 3rd day. At the 3rd and the 9th day of drought stress, the highest and lowest expression of *TPI* were related to cultivars Taylor and Khomein, respectively (Figure 11B).

Gene expression of *TK*

The expression of *TK* gene was significantly affected by drought stress, cultivar and duration of stress. Under drought stress of 60% and 30% FC, expression level of *TK* gene decreased approximately by 45% and 50% relative to the control conditions. The highest and lowest expression levels of *TK* were detected in cultivars Taylor (0.70) and Khomein (0.47), respectively (Figure 12A). At the 3rd and 9th days of drought stress, gene expression of *TK* decreased 36%

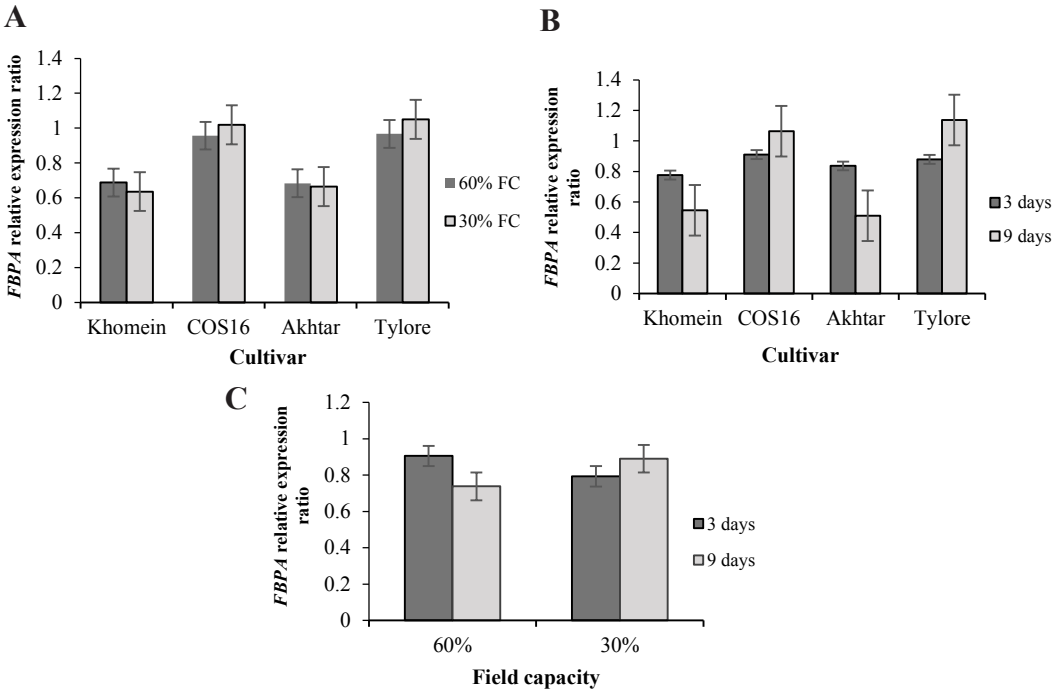

Figure 10. Interaction of **A:** stress level×cultivar, **B:** stress duration×cultivar and **C:** stress level×stress duration on *FBPA* relative gene expression ratio.

and 49% compared to control, respectively. The effects of interaction between cultivar and stress, cultivar and duration of stress on expression of *TK* gene were significant. The highest relative expression of *TK* was observed in Taylor (0.81) under normal conditions, and the lowest *TK* relative expression was observed in Khomein (0.39) under high stress conditions. At the 3rd and 9th days of drought stress, the lowest relative expression levels of *TK* were observed in cultivars Akhtar (0.56) and (0.34), respectively (Figure 12B). In contrast, Taylor cultivar had the highest relative expression (0.75 and 0.64) in both conditions.

Gene expression of *PRK*

Relative expression level of *PRK* decreased by about 37% and 54% under drought stresses of 60% and 30% FC, respectively. Under 60% FC treatment, expression of *PRK* decreased by 26% in tolerant cultivars (Taylor and COS16), while in sensitive cultivars, it decreased by 47% (Akhtar) and 51% (Khomein). Expression of *PRK* gene showed a larger decrease under drought stress of 30% FC in all cultivars, the highest and lowest expressions of *PRK* were observed in Taylor and Khomein by 52% and 38% relative to the control (Figure 13A). Under drought stress of 60 and 30% FC, expression of *PRK* decreased with the increase of stress duration. At 3th and 9th day of drought stress, expression of *PRK* decreased by 39 and 53%, respectively (Figure 13B).

Gene expression of *FBPase*

The sensitive cultivars (Akhtar and Khomein) showed more decrease in expression of *FBPase* under drought stress in comparison to tolerant cultivars (Taylor and COS16). Under mild drought condition (60% FC), expression of *FBPase* decreased by 7 and 20% in Taylor and COS16 cultivars, respectively. In the sensitive cultivars, the reduction in expression of *FBPase* was by about 26%. Under sever water stress (30% FC), relative expression of *FBPase* reduced by about 44-47% in sensitive cultivars, while it did not decrease in tolerant cultivars.

Expression of *FBPase* decreased at 3th day of drought in all cultivars except Taylor. At 9th day of drought stress, expression of *FBPase* drastically decreased in sensitive cultivars by about 45% (Figure 14A). The expression of *FBPase* decreased by 12% and 29% at 3rd and 9th days of moderate stress, respectively (Figure 14B). At the 3rd day of stress, relative expression of *FBPase* showed a higher decrease under severe drought stress (0.8) in comparison to moderate stress (0.88). At the 9th day of stress, a slight increase in the relative expression level of *FBPase* was observed under severe drought stress (0.81) relative to the moderate stress (0.71).

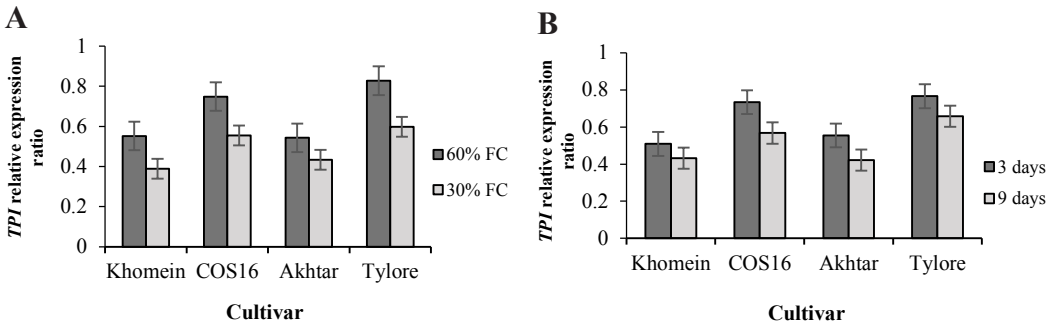

Figure 11. Interaction of **A:** stress level×cultivar and **B:** stress duration×cultivar on *TPI* relative gene expression ratio.

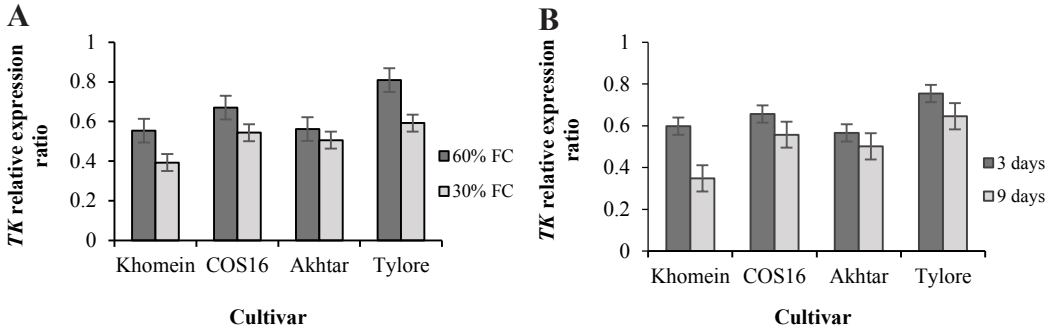

Figure 12. Interaction of **A:** stress level×cultivar and **B:** stress duration×cultivar on *TK* relative gene expression ratio.

Correlation analysis between traits under drought stress

Correlation analysis revealed that there were significant positive correlations in expression levels of Calvin cycle genes (*RBCS*, *RBCL*, *TPI*, *TK*, *SBPase*, *PRK*, and *GAPDH*) (r values ranging from 0.47 to 0.98). Significant negative correlations were observed between concentration of glucose, fructose, WSC and ELI with expression levels of genes. The levels of fructose, glucose and WSC were highly correlated with RWC% (r=-0.79, r=-0.80, r=-0.74 P<0.01, respectively). The levels of fructose, glucose and WSC were inversely associated with sucrose concentrations (r=-0.79, r=-0.69 and r=-0.55, P<0.01). The expression levels of genes were correlated with sucrose content. There were significant positive correlations between the levels of expression genes with RWC% (Table 2).

DISCUSSION

Drought stress is an important factor limiting photosynthesis of plants and leading to major alternations in carbohydrate metabolism (Hare *et al*., 1998) and decreasing the activities of Calvin cycle enzymes (Monakhova and Chernyadev, 2002). The current study showed that drought stress increased the content of fructose, glucose and WSC in leaves of bean with changes in the expression of the genes related to carbohydrates metabolism. These results indicated that the tolerance mechanism to drought stress may be associated with the storage of osmoprotectants such as soluble carbohydrate within the cells. As a result of water soluble carbohydrates accumulation, the cell osmotic potential reduces, and water is absorbed into the cell for turgor maintenance (Farooq *et al*., 2009).

Under drought stress, the levels of glucose, fructose and WSC in the leaves of the tolerant cultivars (Taylor and COS16) were also higher than the sensitive cultivars (Akhtar and Khomein), probably to achieve better osmotic adjustment. The content of sucrose was decreased with increasing of drought stress. The reduction of sucrose in leaves might indicate that sucrose synthesis had become reduced and also sucrose hydrolyzed into fructose and glucose by invertase (Keller and Ludlow, 1993). Su *et al*. (2004) expressed that increased soluble sugars have a direct relationship with tolerance and stability of performance under stress conditions.

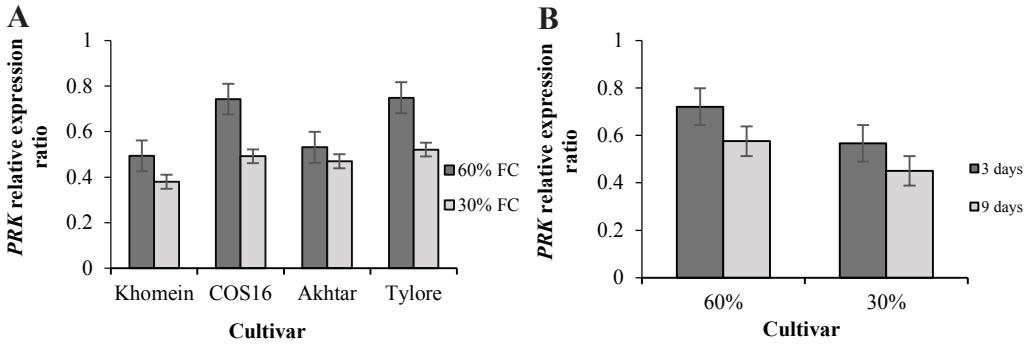

Figure 13. Interaction of **A:** stress level×cultivar and **B:** stress duration×stress level on *PRK* relative gene expression ratio.

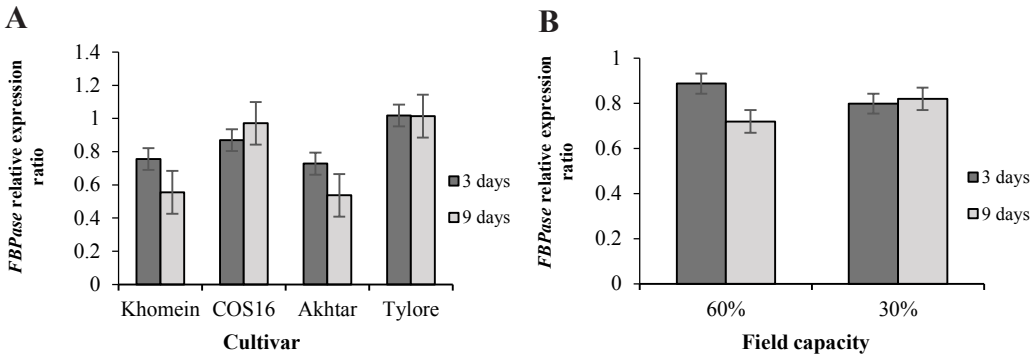

Figure 14. Interaction of **A:** stress duration×cultivar and **B:** stress duration×stress level on *FBPase* relative gene expression ratio.

Table 2. Correlation between RWC, EL, glucose, fructose and water soluble carbohydrate concentration with Calvin cycle genes expression under drought stress.

Traits	RWC	EL	Sucrose	Glucose	Fructose	WSC	*GAPDH*	*RBCS*	*PGM*	*SBPase*	*FBPA*	*TPI*	*TK*	*PRK*	*RBCL*
EL	0.95**														
Sucrose	0.82**	-0.78**													
Glucose	-0.80**	0.79**	-0.79**												
Fructose	-0.79**	0.77**	-0.69**	0.92**											
WSC	-0.74**	0.69*	-0.55**	0.86**	0.93**										
GAPDH	0.78**	-0.86**	0.72**	-0.73**	-0.66*	-0.57*									
RBCS	0.86**	-0.91**	0.84**	-0.83**	-0.78*	0.69*	0.93**								
PGM	0.49*	-0.60**	0.52*	-0.23	0.19	-0.005	0.62*	0.60*							
SBPase	0.87**	-0.92**	0.80**	-0.80**	-0.79**	-0.70**	0.94**	0.98**	0.95**						
FBPA	0.40	-0.49*	0.45	0.27	-0.19	0.05	0.56*	0.49*	0.75**	0.49*					
TPI	0.90**	-0.95**	0.82**	-0.75**	0.71*	-0.60*	0.94**	0.96**	0.68*	0.96**	0.57*				
TK	0.87**	-0.94**	0.82**	-0.79*	-0.73*	-0.63*	0.91**	0.97**	0.67*	0.96**	0.55*	0.97**			
PRK	0.89**	-0.92**	0.86**	-0.82**	-0.77*	-0.65*	0.91**	0.98**	0.60*	0.96**	0.55*	0.98**	0.98**		
RBCL	0.83*	-0.87*	0.74**	-0.72**	-0.74*	-0.67*	0.84**	0.93**	0.60*	0.95**	0.47*	0.90**	0.89**	0.89**	
FBPase	0.46*	-0.58*	0.50*	-0.32	-0.26	-0.01	0.65*	0.58*	0.83**	0.56*	0.89**	0.63*	0.62*	0.58*	0.55*

ns, * and ** : non-significant , significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

EL: Electrolyte leakage index, WSC: Relative water content, *GAPDH*: Glyceraldehyde-3-phosphate dehydrogenase, *RBCS*: Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit, *PGM*: Phosphoglucomutase, *SBPase*: Sedoheptulose-1,7-bisphosphatase, *FBPA*: Fructose-1,6-bisphosphate aldolase, *TPI*: Triose-phosphate isomerase, *TK*: Transketolase, *FBPase*: Fructose-1,6-bisphosphatase, *PRK*: Phosphoribulokinase, *RBCL*: Ribulose bisphosphate carboxylase large subunit.

Also, relative water content (RWC) as an integrative indicator of internal plant water status under drought conditions has successfully been used to identify drought-resistant cultivars of common bean (Parsons and Howe, 1984). In this study, the relative water content decreased under water stress in all the cultivars as compared to control. COS16 and Taylor cultivars had the lowest reduction in RWC while, sensitive cultivars such as Khomein and Akhtar had a higher one. These results indicated that sensitive cultivars respond to drought with a faster decrease in RWC than tolerant cultivars. Indeed, under water stress conditions, tolerant cultivars are able to hold a greater amount of bound water in the cell wall (Bajji *et al*., 2001). Similar observations have been reported in common bean (Korir *et al*., 2006).Varietals differences in RWC may be a result of their varied genetic ability to absorb sufficient water through their root systems and the ability to control water decline through stomata closure in response to soil drying. It may also be due to differences in the ability of tolerant and sensitive cultivars to accumulation of osmoprotectants such as soluble sugar under drought stress and to maintain tissue turgor and hence physiological activities. These results are in agreement with those reported by Sinclair and Ludlow (1985).

We also found that transcription of many genes for the Calvin cycle were down regulated in drought-stressed leaves compared with control. In tolerant cultivars, the relative expression of genes were higher under stress condition than the sensitive cultivars. Decreases in the expression levels of these genes matched with a marked increase in the glucose, fructose and WSC levels in the drought-stressed leaves. Under drought stress, the expression level of genes encoding enzymes of the Calvin cycle were downregulated thus reduced photosynthetic assimilation of CO_2 in drought-stressed leaves. In contrast, genes encoding cytoplasmic enzymes in the pathways leading to glucose and fructose production were up-regulated under drought stress (Xue *et al*., 2008) and content of water soluble carbohydrates in the drought – stressed bean leaves increased markedly. It seems that increasing water soluble sugars to reduce osmotic potential is a resistance mechanism of plants against drought stress. Reduced osmotic potential upon the occurrence of stress can be one way to induce tolerance against drought stress and prevent cell dehydration. Also, decrease photosynthetic activity under drought is due to reductions in stomatal conductance and Rubisco activities leading to lower carbon fixation at the step of Calvin cycle (Xue *et al*., 2008). The significant positive correlations were observed between transcript levels of genes encoding enzymes of Calvin cycle. Therefore, the reduction of Rubisco would reduce the demand for the cellular levels of enzymes involved in subsequent reactions within the Calvin cycle.

CONCLUSION

Drought stress is known to reduce photosynthesis rates and to induce the accumulation of water soluble carbohydrates. These changes were accompanied by alteration in expression levels of a large number of carbohydrate metabolic genes, as shown in this study. These genes might be key components of the drought tolerance of Taylor and COS16 cultivars.

The results showed that under drought stress, the relative expression of genes in susceptible and tolerant cultivars had a similar trend and with increasing stress intensity reduced the relative expression of genes. The expression levels of most genes were higher in tolerant cultivars compared to susceptible ones under drought conditions. Also, the level of transcription of genes in different genotypes also decreased with increasing the duration of stress.

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