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Original article

Study on expression of transcription factors AP2-Domain, HD-ZIP, WRKY and MYB in oily sunflower (*Helianthus annuus* L.) under drought stress

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Extended abstract

Introduction

Sunflower (*Helianthuse annuus* L.) is an annual plant from Composite with a chromosome number of 2n = 2x = 34 which is widely cultivated for supplying edible oil. Drought is one of the most important environmental stresses that limit the growth and distribution of plants more than other factors. This plant is classified as semi-tolerant to drought stress; however, its performance is negatively affected by drought. Transcription factors are molecules that play an important role in the understanding and transmission of stress messages as well as many physiological processes. One of the most effective ways to deal with stress is to produce resistant hybrids. Investigation and study of the expression of genes post-stress application and identification of genes involved in resistance and especially regulatory genes such as transcription factors are vital and necessary for molecular breeding programs.

Materials and methods

In order to investigate the effect of drought stress on the expression of transcription factors: AP2-Domain, HD-ZIP, WRKY and MYB in oilseed sunflower, two lines with different susceptibility to drought stress were selected and cultivated in a completely randomized design with three replications in the greenhouse. The seeds were planted in 3 cm depth of 30 × 25 cm pots containing farm soil and sand mixture in the ratio of 2:1. The plants were grown in controlled conditions at 25 ± 3 °C, 65% relative humidity and 12 h dark-light photoperiod and were irrigated regularly at 100% of field capacity up to 8-leaf stage. After this stage, a number of pots were kept at the same field capacity however, some others were exposed to 80, 60 and 40% of field capacity. Samplings were done in two times, one and three weeks after drought stress application. The study of the expression of genes was performed using real-time PCR by SYBR Green method. RNA extraction kit RNX-plusTM (Sinoclon Co., Iran) and complementary DNA (cDNA) synthesis Kit (Fermentas LIFE SCIENCE # K1621) were used according to the manufacturer's protocols. Quantitative reverse transcription-PCR (qRT-PCR) was performed in

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triplet using 6.25 µl of Maxima SYBR Green/ Fluorescein qPCR Master Mix (2X) (Thermo Fisher Scientific, Germany), 5 pM of forward and reverse primers and 50 ng of cDNA for each reaction in a final volume of 12.5µl. Relative gene expression was analyzed by comparative Ct method, $2^{-\Delta\Delta C}$. The target gene was normalized by the reference gene, ACTIN and calibrated for each sample against the control.

Results

The results of statistical analyzes showed that the expression of the genes in the susceptible and resistant lines of sunflower is different. Mean comparisons of expression of AP2-Domain, WRKY and MYB transcription factors in the two genotypes ENSAT254 (tolerant) and LC1064C (susceptible) showed that the expression level was not tangible in the first week after drought stress application, but the expression of genes was increased in 40% of field capacity in the third week post drought stress application especially in ENSAT254 genotype. In relation to HD-ZIP transcription factor, the expression was much higher in ENSAT254 genotype than LC1064C genotype in the first week of sampling at 40% stress intensity. In the third week of sampling, the expression level of both genotypes increased in 40% of field capacity, although the expression was slightly higher in LC1064C genotype.

Conclusions

Early expression of HD-ZIP transcription factor appears to be involved in increasing genotype resistance to drought stress. The results of the present study can be useful in sunflower improvement programs for producing and developing drought tolerant cultivars.

***Keywords*:** Gene expression, Real-time PCR, Transcription factors, Water-deficit stress

	Tolerant line		
	Replication		
Smpling time	1	2	3
Frist	100 80	100 80	100 80
	100 60	100 60	100 60
	100 40	100 40	100 40
Second	100 80	100 80	100 80
	100 60	100 60	100 60
	100 40	100 40	100 40
	Susceptible line		
	Replication		
Smpling time	1	**2**	**3**
First	100 80	100 80	100 80
	100 60	100 60	100 60
	100 40	100 40	100 40
Second	100 80	100 80	100 80
	100 60	100 60	100 60
	100 40	100 40	100 40

Fig. 1. General scheme of experiment to investigate the effect of drought stress on the expression level of transcription factors in 2 oilseed sunflower lines; 7 and 21 days after stress application. It shows, for example, an experimental unit in which the plant grows under 40% field capacity near to control plant under 100 field capacity conditions.

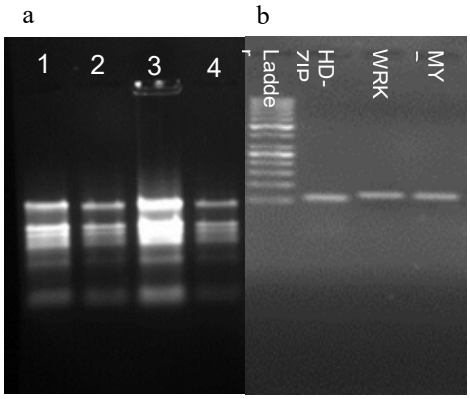

Fig. 2. Electrophoresis results on 1% agarose gel for the extracted RNA (Figure a) and PCR product of HD-ZIP, WRKY and MYB transcription factors on the synthesized cDNA (Figure b). The first column in Figure b is a molecular ruler (ladder) of 50 bp containing bands of 50-1000 bp (Fermentase Company)

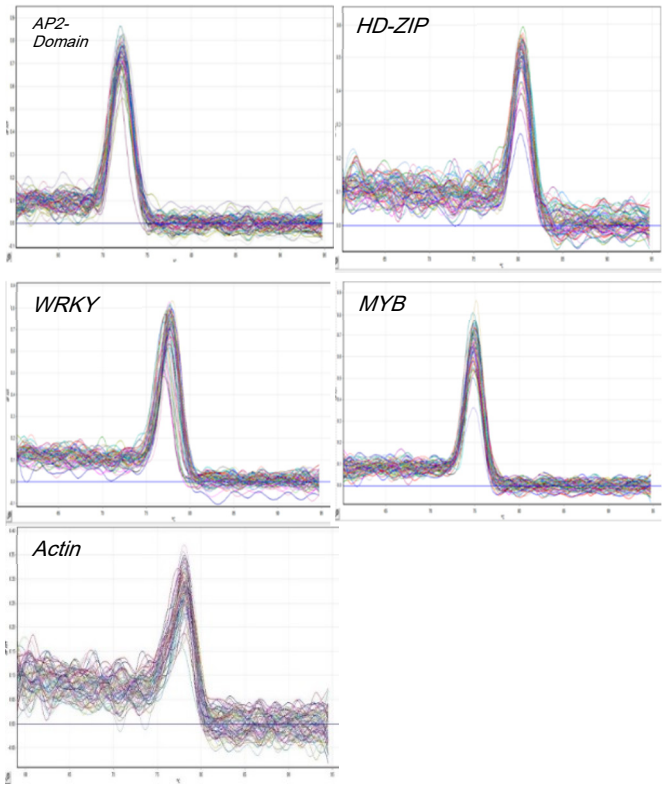

Fig. 3. Melting curves of studied transcription factors (*AP2-Domain*, *HD-ZIP*, *WRKY*, *MYB* and *Actin*) in real time polymerase chain reaction of sunflower plants under drought stress. In all curves, X axis represent temperature (℃) and Y axis represent the dF/dT ratio. dF/dT ratio is the derivative of the function 'fluorescence vs. Temperature melting' that represents the rate of the fluorescence variation in the reaction

Table 1. Oligonucleotide primers used in real-time RT-PCR of sunflower plants under drought stress

Name of sequence	Putative function	Accession number	Forward primer 5'→3'	Reverse primer 5'→3'	Annealing temp. (°C)	Product size (bp)
DH0AQA11ZA10RM1	AP2-domain	CX946549	CAAGAACTCGGCCAATTCGT	AGGAGTAGCAAGGCACCATCA	56	59
DH0AQA17ZH04RM1	ZIP-HD	CX946945	GCAGCACATCGAGGACATCA	GGATCGCACCTCGTGGTTT	57	56
AJ412452	WRKY	AJ412452	TTGGATTGAAGATGGTCATCTGTGA	CCCTATTCAATTCTTCCACCAAA	54	67
DH0AC002ZF08F08FM1	MYB-related transcription factor	CD848175	CCGCCACACGCATTCTCT	CGAGCGCAGCAGCATCTA	60	66
AF282624	Actin		TCAATGTTCCCGCCATGTAT	GACCACTGGCATAGAGGGAAAG	57	60

Table 2. Analysis of variance for transcriptome variations of studied transcription factors (AP2-Domain, HD-ZIP, WRKY, and MYB) in two oilseed sunflower lines (ENSAT254 and LC1064C) under drought stress at different sampling times

			Wald type statistics / Wald			
S.O.V	**df_N**	**df_D**	**AP2-Domain**	**HD-ZIP**	**WRKY**	**MYB**
Drought (D)	2	24	6.07^{*}	22.52^{**}	1.37^{**}	67.43^{**}
Genotype (G)	1	24	4.54^{*}	0.07^{ns}	2.87^{ns}	3.19^{**}
Time (T)	1	24	1.27^{ns}	7.25^{**}	12.84^{**}	32.29^{**}
D × G	2	24	3.22^{ns}	1.80^{ns}	1.15^{**}	56.45^{ns}
D × T	2	24	10.38^{**}	2.16^{ns}	0.65^{**}	27.43^{ns}
G × T	1	24	0.00^{ns}	2.90^{ns}	0.29^{ns}	2.91^{**}
T × D × G	2	24	2.29^{ns}	7.02^{*}	9.56^{**}	32.04^{**}

S.O.V: Source of variation

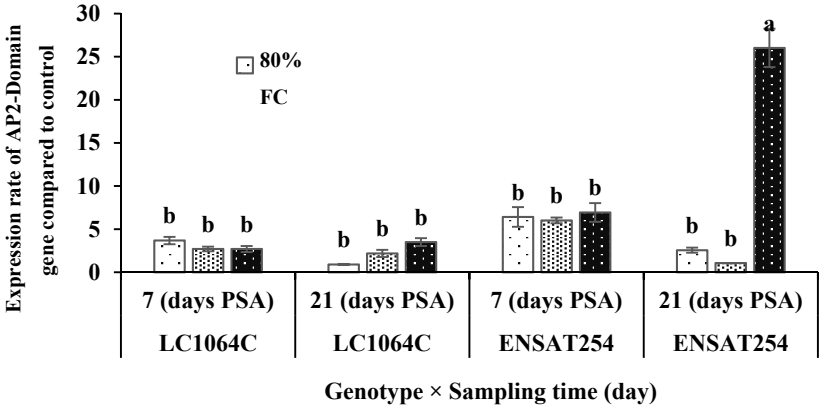

Fig. 4. Pattern of AP2-Domain transcription factor expression in ENSAT254 (tolerant) and LC1064C (sensitive) oilseed sunflower lines at 80, 60 and 40% drought stress levels, one and three weeks post stress application. The vertical lines on the columns show the standard error (SE). FC: Field capacity (The concept of field capacity is used to express the water holding capacity of soil). PSA: Post stress application

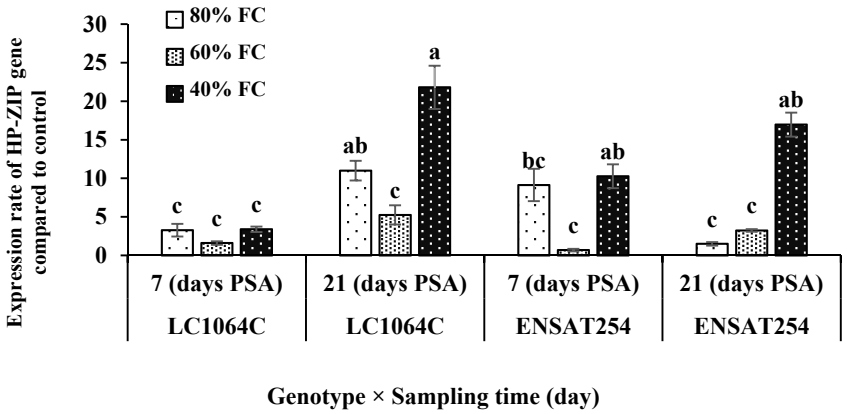

Fig. 5. Pattern of *HD-ZIP* transcription factor expression in ENSAT254 (tolerant) and LC1064C (sensitive) oilseed sunflower lines at 80, 60 and 40% drought stress levels, one and three weeks post stress application. The vertical lines on the columns show the standard error (SE). FC: Field capacity. PSA: Post stress application

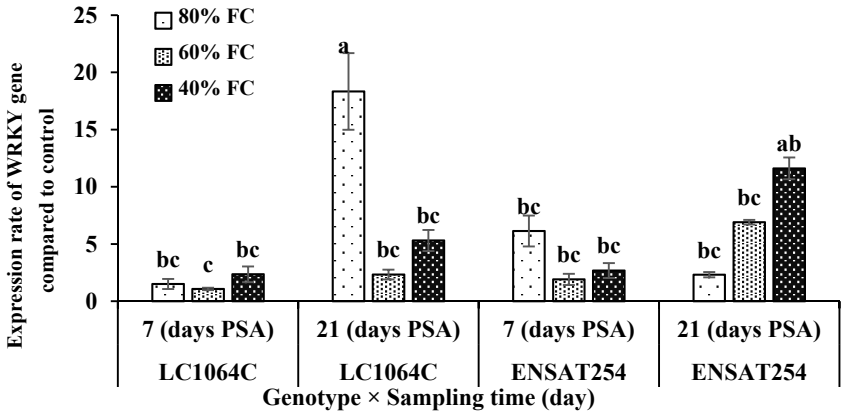

Fig. 6. Pattern of WRKY transcription factor expression in ENSAT254 (tolerant) and LC1064C (sensitive) oilseed sunflower lines at 80, 60 and 40% drought stress levels, one and three weeks post stress application. The vertical lines on the columns show the standard error (SE). FC: Field capacity. PSA: Post stress application.

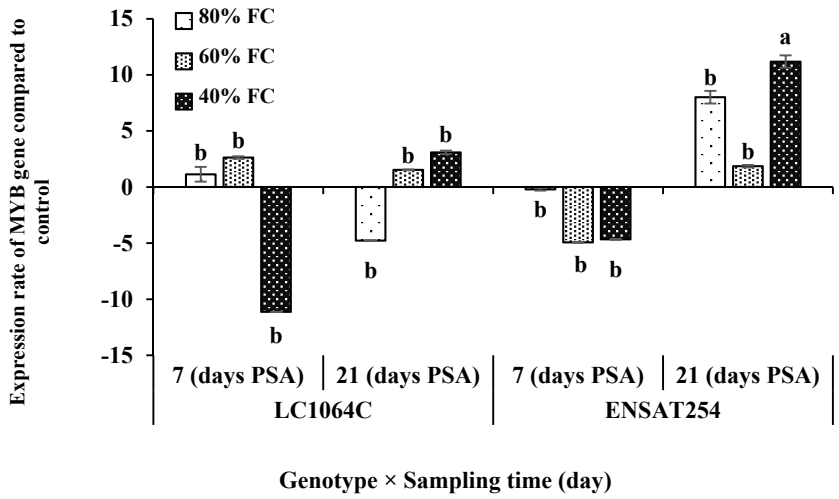

Fig. 7. Pattern of MYB transcription factor expression in ENSAT254 (tolerant) and LC1064C (sensitive) oilseed sunflower lines at 80, 60 and 40% drought stress levels, one and three weeks post stress application. The vertical lines on the columns show the standard error (SE). FC: Field capacity. PSA: Post stress application. The relative gene expression was reported as Log2 fold change.