EXPRESSION DIVERGENCE OF THE DREB1 TRANSCRIPTION FACTOR AMONG CONTRASTING WHEAT GENOTYPES UNDER DROUGHT STRESS

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Abstract

DREB, the Dehydration Responsive Element (DRE)-binding proteins belonging to the superfamily of AP2/ERF plant transcription factors known to regulate diverse processes of plant development and stress responses. The expression level of the DREB1 transcription factor gene was examined under drought in wheat genotypes of Azerbaijan origin differing in drought resistance: two tetraploid wheats (*Triticum durum* Desf.), Barakatli 95 (tolerant), Garagylchyg 2 (sensitive) and two hexaploid wheats (*Triticum aestivum* L.), Azamatli 95 (less sensitive), Qiymatli 2/17 (sensitive), as well as German hexaploid winter wheat cultivar Batis and the synthetic hexaploid wheat accession Syn022L. The seeds were sown in pots in control and drought blocks inside the growth chamber and 12-day-old seedlings were exposed to drought stress. These genotypes were thoroughly phenotype for variability of vegetation indices like NDVI, SR, OSAVI and ARI1 using spectro-radiometer PolyPen RP400 & RP410 under control and drought stress conditions. Statistically significant differences between genotypes were observed for all index under drought. In parallel, the expression of DREB1 gene was analyzed at 7 days after stress, when the visible stress-related traits were observed. The transcript level was determined by qRT-PCR using the elongation factor 1 alpha (EF-1α) gene as an internal control. The fold change in expression was determined according to the 2−ΔΔCt method. There was no difference in the DREB1 expression levels in various genotypes of the control plants. The transcript level in all drought-exposed genotypes increased and was significantly variable among genotypes. In general, under drought, the expression level of the DREB1 transcription factor gene in tolerant genotypes increased more than in drought sensitive ones. The German wheat genotype Batis revealed the highest up-regulation under drought stress condition. Under severe drought, lower NDVI score was observed in the German genotypes having higher expression levels of DREB1. These data will help to screen drought stress tolerance at large scale among the diverse wheat gene-pool non-invasively using digital phenotyping.

***Keywords**: Bread wheat, Durum wheat, Drought stress, DREB1 transcription factor, Vegetation indices*

Wheat Gene Pool of the Research Institute of Crop Husbandry of Azerbaijan

MATERIALS AND METHODS

Plant materials and growth conditions

Experiments were undertaken on the four Azerbaijan and two German winter wheat cultivars. Plants were grown in the growth chamber at 16:8 h light/dark period 24°C and 19°C respectively, relative humidity 50%. The 3-leaf stage (12-day-old) plants were subjected to water deficiency by withholding irrigation. At 7 days after stress plants were harvested for gene expression analysis. The remaining plants were re-watered to evaluate recovery processes.

Analysis of vegetation indices

A portable handheld spectro-radiometer PolyPen RP400 & RP410 was used for a measurement of spectral reflectance of leaves. Spectral reflectance measurements were made at 3, 5, 7 days after stress in ten replicates and 1, 2, 3 days after rewatering in six replicates on each well-watered and exposed to drought genotypes.

Multispectral vegetation indices employed in the study*

ABBREVIATION	NAME	EQUATION
NDVI	Normalized Difference Vegetation Index	NDVI = (RNIR - RRED) / (RNIR + RRED)
SR	Simple Ratio Index	SR = RNIR / RRED
OSAVI	Optimized Soil-Adjusted Vegetation Index	OSAVI = (1 + 0.16) * (R790- R670) / (R790- R670 + 0.16)
ARI1	Anthocyanin Reflectance Index	1/R550-1/R700

**R indicates reflectance and the subindex indicates the wavelength (in nm)*

RNA extraction and cDNA synthesis

Total RNA was extracted from leaf material using the Monarch Total RNA Miniprep Kit (New England Biolabs, Inc.). The single-stranded cDNA synthesis was carried out from total RNA using LunaScript RT SuperMix Kit (New England Biolabs, Inc.).

Quantitative real-time PCR

Real-time PCR was carried out using a 7500 fast real-time PCR system in a volume of 20 µl containing 10 µl Luna Universal qPCR Mix (New England Biolabs, Inc.). Elongation factor 1 alpha (EF-1α) was used as an internal control gene. Primers were designed by complete cds of *Triticum aestivum* AP2-containing protein (Dreb1) mRNA (Accession Nr. AF303376) from NCBI using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus. cgi). The fold change in expression (stressed compared to control) was determined according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analysis

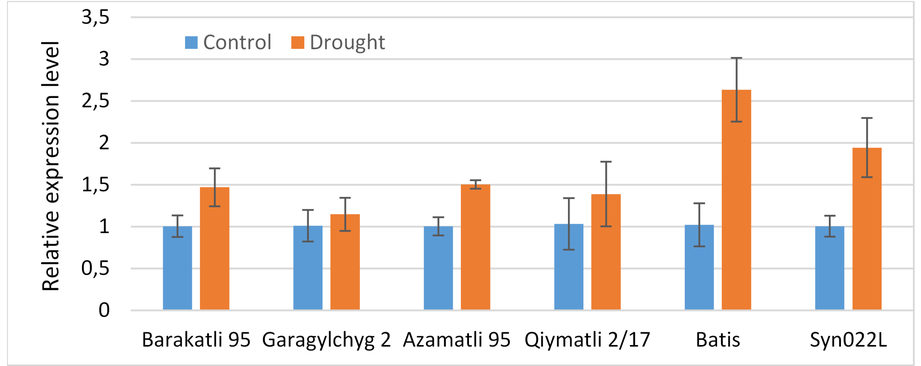
The statistical analysis was carried out using SAS software ver9.1 (SAS Institute, Cary, NC, USA). Standard deviation (SD) values are from at least three biological replicates, and the significance of differences was evaluated at the $p<0.05$ level.

RESULTS

Phenotypic evaluation of vegetation indices

Variation of vegetation indices (VI) in wheat genotypes under drought condition and rewatering. DAS-days after stress, DAR-days after rewatering. Each value means ± standard deviation of ten biological replicates for DAS, and six biological replicates for DAR.

Quantitative expression analysis of DREB1



Transcript levels of DREB1 in leaves of wheat plants under drought stress. Level of mRNA determined by qRT-PCR using elongation factor (EF-1α) gene as an internal control. The fold change in expression was determined according to the $2^{-\Delta\Delta Ct}$ method. Each value means ± standard deviation of three biological replicates.

CONCLUSION

The expression of the Dreb1 gene in wheat was induced by drought stress in most genotypes. Obtained results also showed correlation between established tolerant wheat genotypes and the expression level of Dreb1 gene. Transcripts of the Dreb1 were more accumulated in resistant genotypes as compared to sensitive ones. Depending on the origin of the wheat, a significant difference was observed in the expression of the Dreb1 gene. Thus, as a whole, it can be concluded that DREB proteins are the central regulator of abiotic stress response in wheat. Further, the genotypes which showed more yield losses under drought were showing lower vegetation indices as compared to the tolerant genotypes. Therefore, screening wheat diversity using vegetation indices as well as mining new Dreb alleles will help to identify novel variants which can directly be utilized to make drought adaptive cultivar in wheat.

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