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Differential Gene Expression in Roots of Wild Emmer Wheat Genotypes Contrasting in Drought Resistance

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Introduction

Drought is the most important environmental factor limiting plant development and crop productivity worldwide. Roots are the primary sensors of drought in the soil, transmitting signals influencing stomatal behavior, leaf initiation, expansion, and other developmental processes. The understanding of mechanisms involved in plant root adaptation to drought is required to ensure further progress in drought resistance improvement of crop plants.

Wild emmer wheat (*Triticum dicoccoides*), the progenitor of domesticated wheat, is a promising source for improvement of drought resistance (Peleg et al., 2005).

The main objective of the current research is to discover adaptive mechanisms and potential candidate genes associated with drought resistance, by comparative transcriptome analysis. Transcripome analysis is considered to be an efficient approach to reveal the components responsible for abiotic stress response and tolerance. We describe here a comparison of global gene expression in roots of drought resistant genotype (Y12-3) *vs.* drought susceptible genotype (A24-39) of wild emmer wheat, under water stress *vs.* well-watered conditions using microarray analysis (Affymetrix GeneChip®).

Results

A. Gene expression patterns

- A total of 4,969 transcripts were differentially regulated between drought stress and control conditions and/or genotypes. Of them, 3,570 differentially expressed transcripts (DETs) showed significant effect of treatment (E effect).
- Common regulation patterns, in response to drought stress, were observed for 1,323 DETs in the R and S genotypes (FC > 1.4 between drought and control) (Figure 1 A).
- Unique pattern of regulation in each of the genotypes, in response to drought stress, was observed for 995 DETs in the R genotype and 310 DETs in the S genotype. Example is are presented in Figure 1B.
- A visual characterization of differential expression patterns of transcripts is presented by Volcano plots (Figure 2 A-C) and two-way hierarchical clustering (Figure 3).
- **Candidate genes involved in drought resistance** were identified by filtering the data of DETs using a set of selection criteria (see M & M) that enabled to identify transcripts with the largest difference in expression level between the R and S genotypes in response to drought. A Venn diagram in Figure 4.A describes the numbers of up- and down DETs with common and differential gene expression between treatment/genotypes before filtering and Figure 4B describes the selected transcripts (DETRs and DETSs) after filtering .
- The subset of the selected transcripts were subjected to annotation analysis (Figure 4.B & Figure 5)

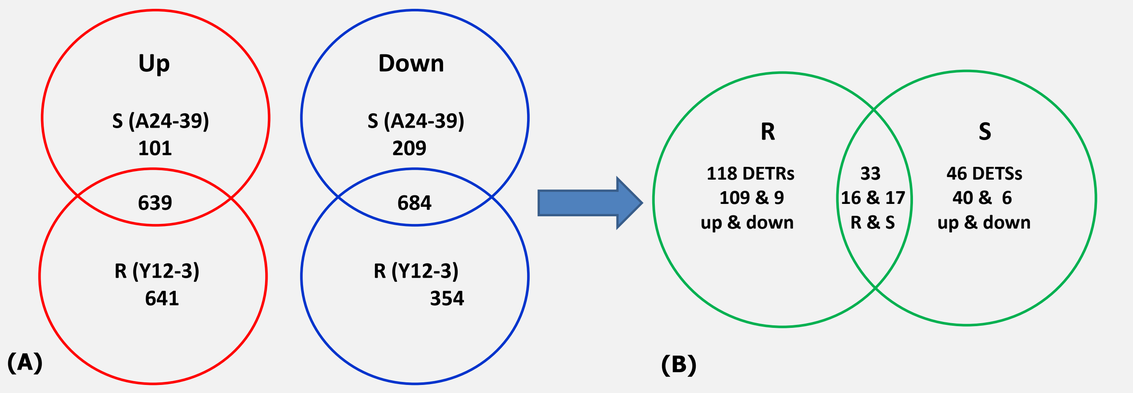

Figure 4. Venn diagrams of differentially expressed transcripts (DETs) in roots of R and S wild emmer wheat genotypes in response to drought: A. numbers of up- and down differentially and commonly expressed transcripts in response to drought; all transcripts show FC > 1.4 between treatments or between genotypes; B. the f selected DETSs and DETRs showing significant G, E and G × E effects & FC>1.4 in each comparison; p<0.05.

B. Functional classification of differentially regulated transcripts

- Annotation analysis was focused on the selected 118 DETRs and 46 DETSs. Transcripts were annotated using HarvEST Affymetrix Wheat1 Chip (http://harvest.ucr.edu/) (ver. 1.55). Homology with known proteins, gene ontology (GO) and a putative biological function was assigned for 69 DETRs and 24 DETSs (54%). Classification of the annotated DETRs into functional groups is described in **Figure 5**.

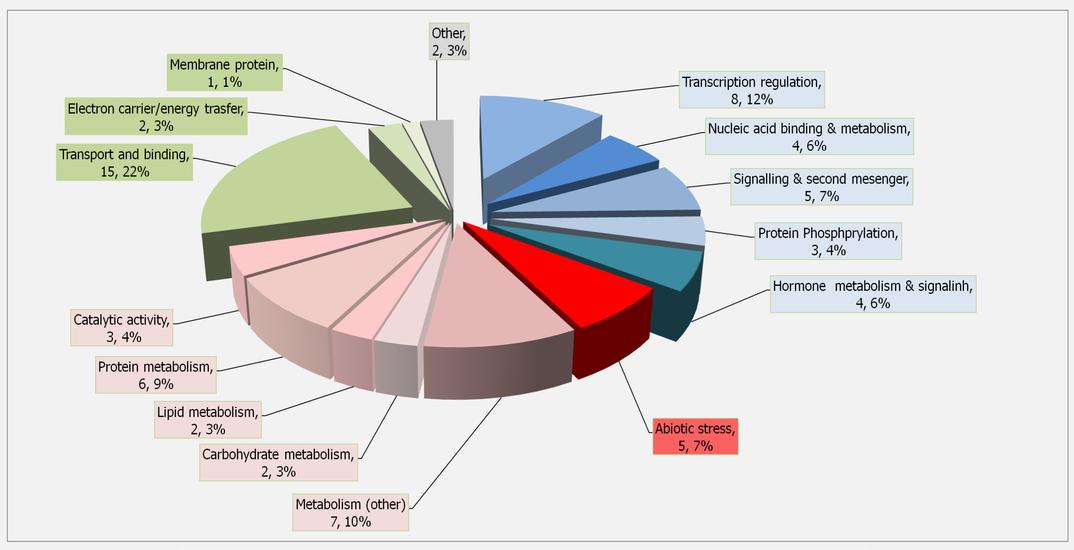

Figure 5. Functional classification of the 69 annotated DETRs

- Many of the annotated DETRs (35%) are involved in ***multilevel regulation***, some are indicated as involved in adaptation to abiotic stress in other plant species. Following are few examples for such proteins

1) Transcription regulation: MCB1 a MYB protein; and MICKc type box26 - a MADS-box protein.
2) Post transcription regulation: CBP80/ABH1 involved in ABA signal transduction; and DEAD box RNA helicase.
3) Protein phosphorylation activity: phosphatidylinositol 4-phosphate 5-kinase (PIPK).
4) Plant hormone signaling and metabolism – Gibberellin receptor (GID1L2); and ABA signaling and biosynthesis (HAB1, a protein phosphatase 2C; aldehyde oxidase AAO1).
5) Signaling and second messenger: WD-40 proteins; and calcium binding proteins (CBP1 and calmodulin).

- The group of ***membrane transporters*** included: transport of potassium (KUP6), lipids (LPT), oxysterol-binding, sodium/calcium exchanger protein (CAX3) and others.

* Gene names are based on Arabidopsis (TAIR) database.

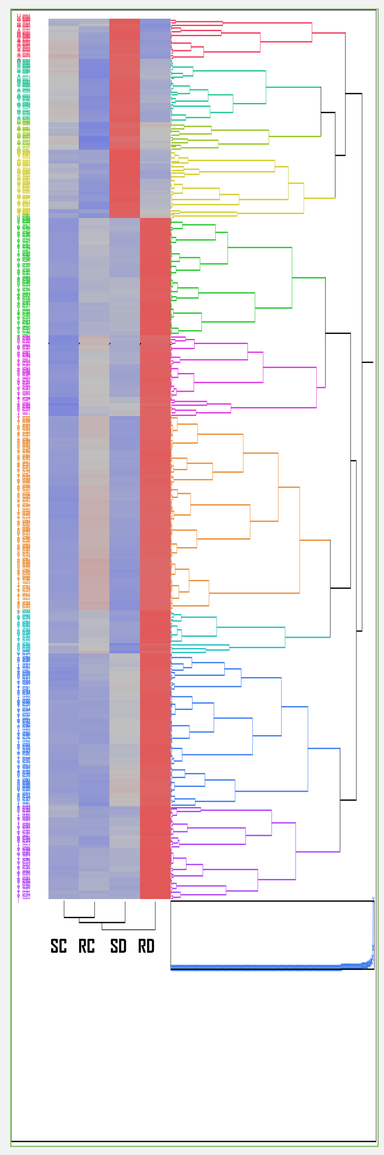

Figure 3. Two way hierarchical clustering of the selected differentially expressed transcripts in the resistant (R) genotype (DETRs) and in the susceptible (S) genotype (DETSs) of wild emmer wheat grown under drought stress or control conditions. Light red to deep red indicates increasing levels of expression and light blue to deep blue represent decreasing levels of expression .

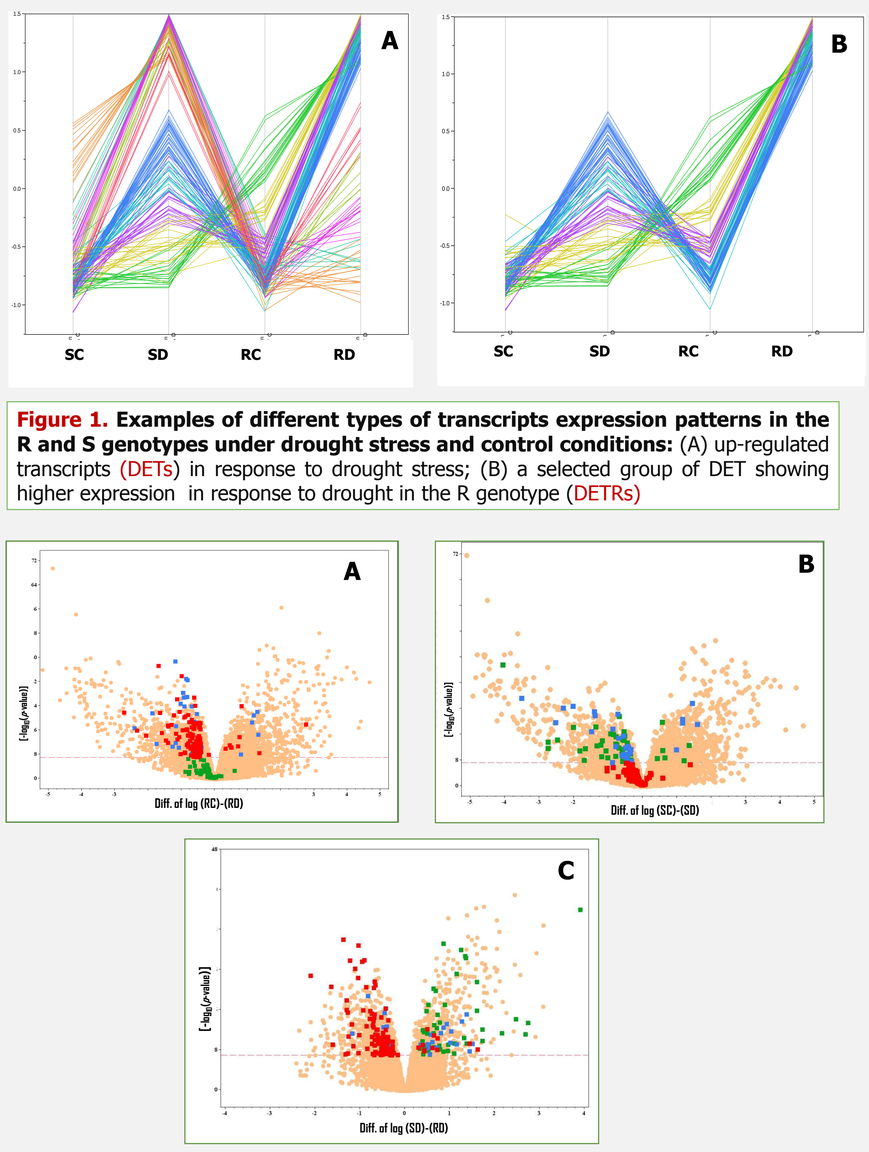

Figure 2. Volcano plots providing a visual characterization of the differential expressed transcripts. The Y-axis describes increasing significance levels [-$\log_{10}$(p-value)]; the X-axis ($\log_2$FC) describes differences in expression level between two conditions/genotype (fold change). Transcripts with statistically significant differential expression lie above a horizontal threshold line, whereas genes with large fold change values lie outside a pair of virtual vertical fold change (FC) thresholds. The selected DETs in the S genotype (DETSs) are marked in green; DETs in the R genotype (DETRs) are marked in red, DETs that answered the selection criteria in both genotypes are marked in blue; all other DETs are marked in orange colour. The Volcano plots present transcripts that show differential expression between: A. control (C) and drought (D) in the R genotype (RC-RD); B. C and D in the S genotype (SC-SD); and C. genotypes under drought conditions (SD-RD).

Conclusions and Prospects

- The current study relies primarily on the hypothesis that the DETRs may be associated with drought resistance mechanisms, and thus, they may be regarded as potential source for candidate genes for drought resistance. Annotation analysis of the DETRs show that some of the up-regulated DETRs can be considered as potential candidate genes that may contribute to drought resistance in wheat.
- Further studies are designed to explore the contribution of the candidate genes to drought resistance by molecular genomic approaches and by testing their co-localization with drought related QTLs.
- These results further demonstrate that wild emmer wheat gene pool is a promising source for potential candidate genes for improvement of drought resistance in cultivated wheat.

Materials & Methods

Wild emmer wheat genotypes: Drought resistant and drought susceptible wild wheat genotypes were selected for wide genome expression analysis based on previous results described by Peleg et al., (2005):

1. Y12-3, a resistant (R) genotype is characterized by combining high productivity under water-limited conditions with high yield stability, collected in Yehudiyya, Israel.
2. A24-39, a susceptible (S) genotype characterized by low productivity under water-limited conditions with low yield stability, collected in Amirim, Israel.

Drought stress: Plants were grown in a controlled environment greenhouse (23/18 ºC; 12 h day/12 night), with three biological replicates. Drought stress was applied at 5-6 leaf stage by withholding water for 7 days. Roots were frozen in liquid nitrogen and stored at -80ºC for RNA extraction.

Microarray hybridizations: Total RNA was used to synthesize biotin-labelled cRNAs that were hybridized with the Affymetrix GeneChip® Wheat Genome Arrays. Hybridization, staining and washing steps were performed following the manufacturer's instructions (www.affymetrix.com/support/technical/manual/expression_manual.affx).

Data analysis: JMP Genomics® (ver. 3.2) was used for statistical analyses of expression data. Normalization was performed by the standard deviation (STD) procedure. Analysis of variance (ANOVA) was employed to test the effects of: genotype (G) (A24-39 vs. Y12-3), environment (E) (drought stress vs. control), and their interactions (G×E). In the ANOVA model E, G and 'probe-sets' were considered as fixed effects and 'array' as random effect. A threshold of significance was based on the Bonferroni correction of p = 0.05 (Hochberg 1988). Volcano plots **(Figure 2 A-C)** and two-way hierarchical clustering **(Figure 3)** were used to provide a visual characterization of differential expression patterns of transcripts. The hierarchical clustering was based on the Stdlsmean (least square mean standardized using STD method as described by SAS) of the transcript expression levels using Ward's method (Ward 1963).

Selection of candidate transcripts: The differentially expressed transcripts (DETs) that showed the following significant effects were selected for further analysis: G × E interaction effect; E effect for the resistant *or* susceptible genotypes; and G effects under drought condition. A Fold change (FC) was calculated as the -$\log_2$ of the differences of 'least square means' (Lsmeans) between: *(i)* genotypes under drought; *and (ii)* between control and treatment in each of the genotypes. A FC > 1.4 was set as threshold for each of the two comparisons.

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