Transcriptomic Analysis of 'Garnem' Rootstock by RNAseq Reveals Genes and Gene Ontologies Involved in Drought Response

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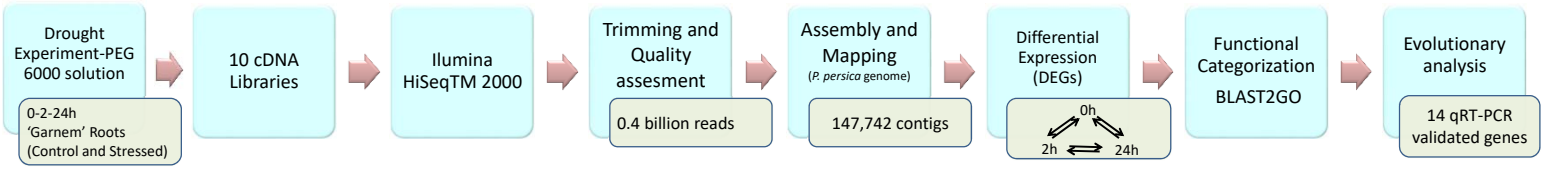
In light of changing environmental conditions, accentuated by climate change, the impact of drought on agriculture has become critical. There is now a pressing need to select drought tolerant rootstocks that can adapt to limited water availability. We performed a time course RNAseq-based transcriptome analysis of roots in an almond x peach hybrid [*P. amygdalus* Batsch, *syn P. dulcis (*Mill.) *x P. persica* (L.) Batsch] using an Illumina platform. The analysis revealed gene ontology and signaling pathway information about the significantly up and down-regulated genes during drought response. An orthologous study of 14 qRT-PCR validated genes revealed different evolutionary patterns in a number of plant species.



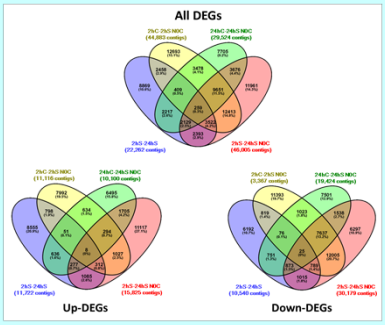


Figure 1. Venn Diagrams. Number of DEGs (Differentially Expressed Gene) for the four pairwise comparisons between control and stressed samples collected at different time points: 2h stressed vs. 24h stressed (2hS-24hS), in which drought responsive genes are revealed; 2h control vs. 2h stressed normalized to 0h control (2hC-2hS N0C); 24h control vs. 24h stressed normalized to 0h control (24hC-24hS N0C); and 2h Stressed vs. 24h stressed normalized to 0h control (2hS-24hS N0C). These three last comparisons helped to identify additional drought tolerant genes, as well as those that were differentially expressed because of PEG solution.

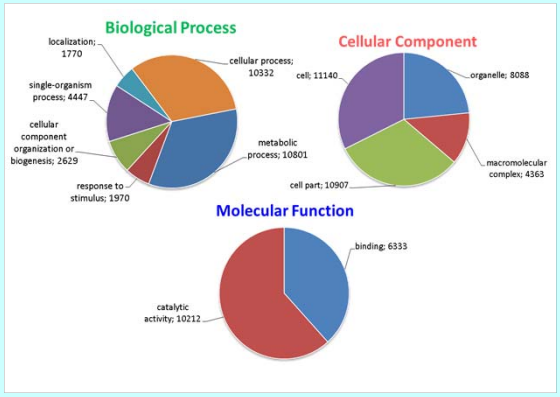


Figure 2. Annotated Gene Ontology (GO) term distribution at 2-level for the three GO categories after GO-slim analysis. In total, 26,700 DEGs were annotated and categorized by biological process (BP) (15,870 DEGs), molecular function (MF) (22,595 DEGs) and cellular component (CC) (13,883 DEGs) sets.

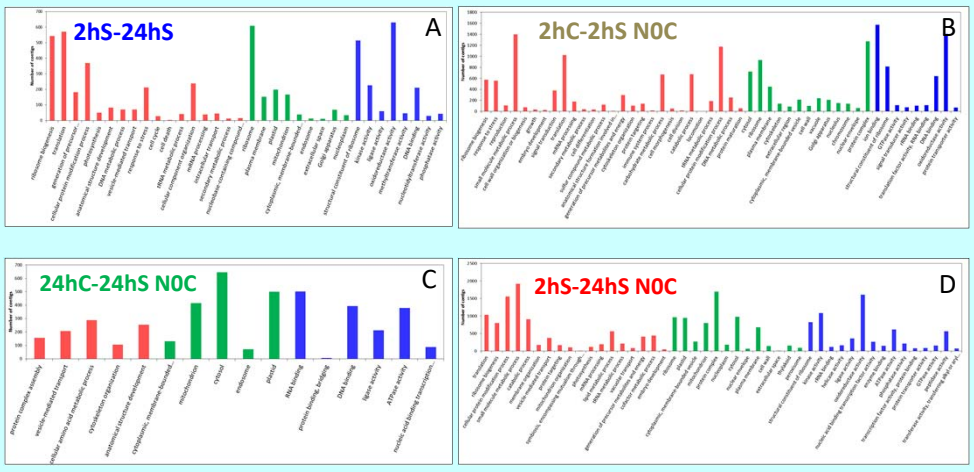


Figure 3. Number of contigs within each enriched GO term related to BP-biological process (red bars), CC-cellular component (green bars) and MF-molecular function (blue bars) for the four pairwise comparison categories: (A) 2hS-24hS; (B) 2hC-2hS N0C; (C) 24hC-24hS N0C; and (D) 2hS-24hS N0C. GO enrichment analysis was performed using the two-tailed Fisher's exact test (FDR < 0.05) in order to reveal the over- and underrepresented functions during drought stress. From these enriched functions, 14 drought-related differentially expressed genes were arbitrarily selected for qRT-PCR validation and perform an evolutionary study with the homologs of these genes identified from other tree species.

Table 1. List of the drought-related, differentially expressed 14 genes chosen from the transcriptome analysis and validated by qRT-PCR.

Gene Name	Garnem' gene and contig name	*P. persica* gene name
9-cis-epoxycarotenoid dioxygenase chloroplastic-like	*GN_NCED_32686*	*ppa014647m*
late embryogenesis abundant protein d-34-like	*GN_LEA34_122663*	*ppa019572m*
transcription factor myb44-like	*GN_Myb44_122842*	*ppa017892m*
r2r3 myb transcription factor	*GN_R2R3MYBTF_48673*	*ppa023768m*
transcription factor myb108	*GN_Myb108_50472*	*ppa025672m*
nac domain-containing protein 2	*GN_NAC2_130671*	*ppa009380m*
probable wrky transcription factor 53	*GN_WRKY53_785*	*ppa007986m*
transcription factor bhlh36-like	*GN_BHLH36-like_73622*	*ppa015702m*
calcium-transporting atpase plasma membrane-type-like	*GN_CaATPase_108825*	*ppa018972m*
bzip transcription factor bzip79	*GN_bZIPTF79_82605*	*ppa001262m*
zinc finger family protein (C2H2 ZnF)	*GN_ZnF_C2H2_67026*	*ppa014890m*
zeaxanthin chloroplastic-like	*GN_Zeoxanthin_48875*	*ppa016605m*
calcium-independent phospholipase a2-gamma	*GN_Ca_Phosp2- γ _47372*	*ppa000303m*
calcium-transporting atpase plasma membrane-type	*GN_CaATPase_26608*	*ppa000672m*

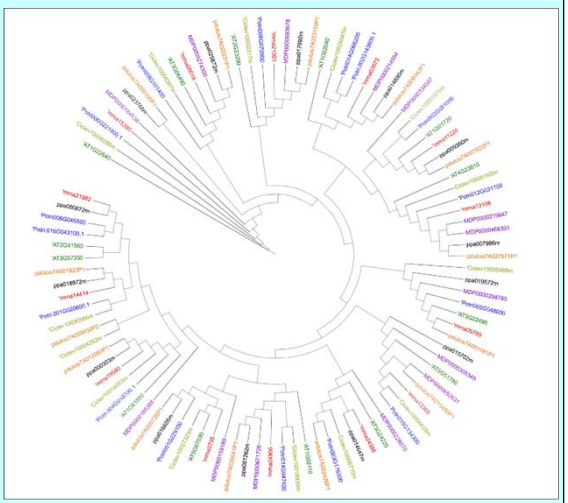


Figure 4. Dendrogram showing the phylogenetic relationship between the selected 14 genes and corresponding homologs from seven tree species: *P. dulcis* (orange), *P. persica* (black), *M. domestica* (purple), *F. vesca* (red), *C. sinensis* (light green), *P. trichocarpa* (blue), *A. thaliana* (green). The sequence of the homologs was derived from genome assemblies of the seven tree and used for phylogenetic analysis using the maximum-likelihood approach (RAxML). Our results indicate that these drought-related genes may have enhanced diversification across closely related genera.

CONCLUSION

This study revealed drought-related temporal variation in the transcriptomic profile in roots of the almond x peach hybrid 'Garnem' rootstock. The knowledge regarding genes which change in expression due to drought stress will facilitate discovery of targets for genetic improvement of 'Garnem'. Furthermore, phylogenetic studies of genes which regulate drought responses will allow this knowledge to be extended to other agronomically important tree fruit rootstock varieties.

Although further investigation is required to understand the molecular mechanisms associated with drought tolerance in 'Garnem' rootstock, this research has laid the foundation for target gene identification and, ultimately, genetic improvement of crops' ability to respond to the changing environmental conditions.

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