

# Transcriptome dynamics during salt stress in olive (*Olea europaea* L.) cultivar Koroneiki

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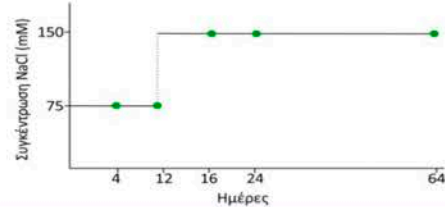
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**ABSTRACT:** Olive (*Olea europaea* L.), one of the most economically important fruit tree in the Mediterranean basin is under threat due rapid changes in environmental conditions. Acute heat-stress (drought) are followed with heavy rains could change rapidly the global picture of olive cultivation. In combination with low quality saline water often used for irrigation could result in detrimental production in the next years to come. There are certain molecular studies about the salt tolerance in olive, though, the perplexed salt tolerance of a tree that is already adapted to drought, needs further investigation. Herein, a transcriptomics approach was used to unravel gene regulatory networks underlying salinity responses by introducing acute stress conditions of salinity. A 64-day long salinity experiment using one-year old trees exposed to irrigation scheme containing two NaCl concentrations: at day 0 plants were irrigated with 75 mM NaCl for 12 days and then the concentration was raised to 150 mM NaCl for 52 days. Leaves were detached from plants at day 4 (at 75 mM NaCl) and at day 16 (at 150 mM NaCl), and RNA was isolated from Koroneiki cultivar. In total, 93,794 transcripts were identified and from those 1382 were characterized as DEGs. These DEGs were divided into 9 clusters depending on the expression profile. Q-PCR analysis confirmed the DEGs derived from in depth RNA sequencing. Based on comparative amino acid analysis a number of genes involved in hormonal synthesis/response were differentially expressed. This approach will provide key factors for crop breeding and engineering, facilitating the tolerance of the olive tree under drought environmental conditions.



**Figure 1.** Experimental design and samplings at 4 and 16 days after treatment. RNA was isolated and sequenced.

4d: 46864 transcripts identified

16d: 46930 transcripts identified

↓  $\log_2FC (NaCl/Control) \geq 1$  or  $\leq -1$   
Padj  $\leq 0.05$

4d: 627 DEGs (393 Up- / 234 Down- regulated)  
16d: 885 DEGs (398 Up- / 487 Down- regulated) **1382 unique DEGs**

↓ Perseus Clustering (z-score of expression values)  
pick early UP, late UP, early DOWN, late DOWN, both UP, both DOWN

**1061 DEGs**

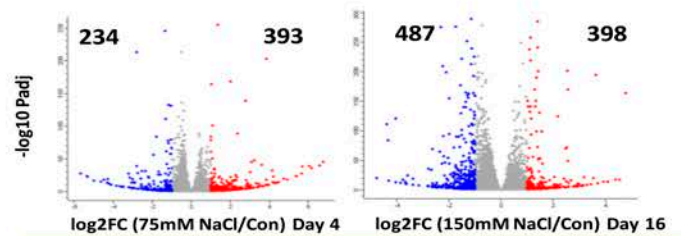
↓ FPKM filtering of the Clusters  
(FPKM  $\geq 5$ )

**187 DEGs FPKM  $\geq 5$  qRT-PCR verification**

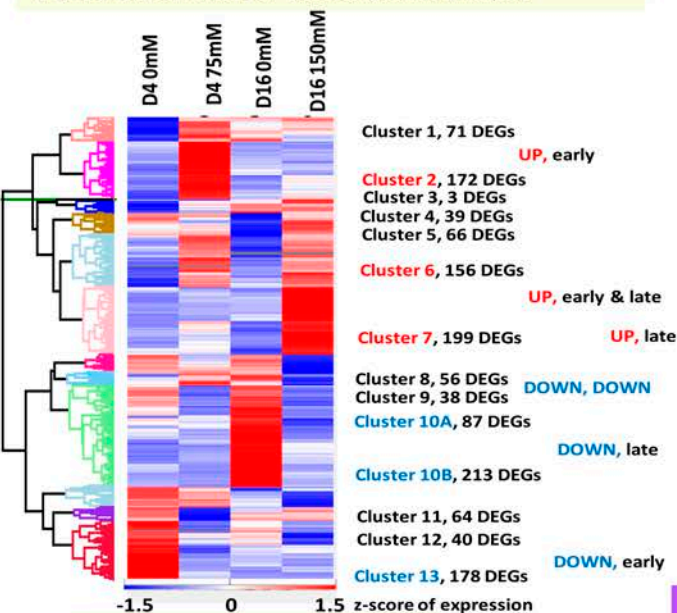
**Figure 3.** Flow chart of transcriptome analysis



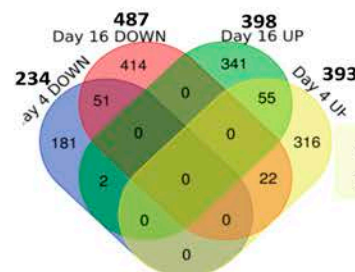
**Figure 2.** Phenotypes of cv. Koroneiki plants. A. Control plants at 4 days after treatment (dat). B. 4 days-old plants treated with 75 mM NaCl. C. 64 days-old control plants. D. 64 days-old plants treated with 150mM NaCl.



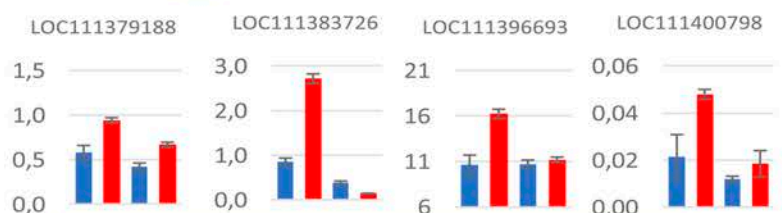
**Figure 4.** Differentially expressed genes (DEGs) of cv. Koroneiki treated with NaCl at  $\log_2FC \geq 2$  or  $\leq -2$  Padj  $\leq 0.05$ .



**Figure 6.** Clustering of DEGs.



**Figure 5.** Venn diagram of DEGs of cv. Koroneiki treated with NaCl.



**Figure 7.** Q-PCR analysis verified the RNA seq results.

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