

ANALYSIS OF LEAF TRANSCRIPTOME IN TWO CITRUS VARIETIES UNDER PEG-INDUCED DROUGHT STRESS

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Desertification and overexploitation of water pose significant challenges to crop yield and food security for the growing world population. Especially in citrus cultivation, water scarcity negatively affects tree growth and fruit quality. Understanding the molecular mechanisms of drought stress tolerance in citrus trees is essential for enhancing their resilience in arid conditions. In this study, we investigated the transcriptional responses of Citrus rootstocks under drought stress to gain insights into the plant's adaptive response to water stress. To achieve this objective, one-year-old plants of two distinct citrus rootstock genotypes (Carrizo citrange, Bitters C22) were grown in aerated half-strength Hoagland's nutrient solution in a hydroponic station prototype with a 16 h photoperiod (250 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and 25/21 °C day/night temperature for a period of ten days. Subsequently, the plants were exposed to a nutrient solution containing either 0% (control) or 15% PEG-8000 (PEG) for a duration of ten days. To assess the extent of oxidative stress induced by the imposed water stress condition, the malonyl dialdehyde (MDA) content of the leaves was quantified. Then, RNA was extracted from the leaves and subjected to sequencing on the Illumina platform and *de novo* assembly. Our results indicate that Carrizo citrange and Bitters C22 exhibited different sensitivities to drought stress. Carrizo displayed pronounced susceptibility to the PEG treatment, as evidenced by the assessment of MDA levels and leaf phenotype alterations. The analysis of differentially expressed genes (DEGs) reveals that both genotypes exhibited transcriptomic rearrangements in response to water stress. Notably, Bitters plants displayed a more pronounced and extensive reprogramming of gene expression than Carrizo plants (5022 and 2084 DEGs, respectively). Compared

to Carrizo, the observed higher abundance of DEGs in Bitters suggests that a more robust stress response occurred which would explain the enhanced tolerance to drought conditions. Furthermore, the GO enrichment analysis emphasizes the crucial role played by the “oxidoreductase activity” category in the response to water scarcity in both genotypes. This elevated number of DEGs highlights the predominant response of plants to the secondary oxidative stress induced by water deprivation. Nevertheless, Bitters exhibits a significant enrichment in GO categories involved in maintaining overall plant stability and coping with water stress, whereas Carrizo shows enrichment in GO categories which regulate the utilization of carbohydrates for energy and osmotic balance during water stress. Our results, within the PON “Ricerca e Innovazione” Water4AgriFood project, led to the identification of numerous unigenes exhibiting promising potential in the development of citrus varieties with enhanced resilience to drought stress.