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# Exploring the Impact of Water Stress on Grapevine Gene Expression and Polyphenol Production: Insights for Developing a Systems Biology Model †

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**Abstract:** This scientific paper delves into the effects of water stress on grapevines, specifically focusing on gene expression and polyphenol production. We conducted a controlled greenhouse experiment with three hydric conditions and analyzed the expression of genes related to polyphenol biosynthesis. Our results revealed significant differences in the expression of ABCC1, a gene linked to anthocyanin metabolism, under different irrigation treatments. These findings highlight the importance of anthocyanins in grapevine responses to abiotic stresses. By integrating genomics, metabolomics, and systems biology, this study contributes to our understanding of grapevine physiology under water stress conditions and offers insights into developing sensor technologies for real-world applications in viticulture.

**Keywords:** *Vitis vinifera*; precision viticulture; metabolism



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## 1. Introduction

Deficit irrigation strategies are increasingly adopted in viticulture to enhance wine production by influencing grape quality [1–3]. Mild-to-moderate drought stress can lead to increased sugar and phenolic compound accumulation in grapes. However, severe droughts may reduce sugar content and affect phenolic compounds and grape aromas, which is commercially undesirable [4].

Water stress can also alter gene expression, affecting various pathways such as phenylpropanoid biosynthesis, flavonoid synthesis, ABA (Abscisic acid) signaling, carbohydrate metabolism, amino acid metabolism, ROS (Reactive Oxygen Species) production, photosynthesis, and signal transduction [5,6]. Figure 1 illustrates the multidisciplinary approach used in this study, incorporating genomics, metabolomics, and systems biology to bridge the gap between field observations and laboratory analyses.

Research in grapevine studies encompasses proteomics, metabolomics, transcriptomics, and genomics, generating extensive data on temporal and spatial dynamics and responses to external factors [7]. In addition to traditional chromatography and mass spectrometry data, metabolomics, transcriptomics, and proteomics provide crucial insights into grapevine responses [7]. Precision Agriculture (PA) employs sensors for mechanistic plant physiological diagnosis under various environmental conditions [6]. This study emphasizes the importance of omics data, connecting phenotypes, metabolites, and genes

to enhance precision viticulture solutions. Water stress, as observed in other studies, can significantly impact gene expression, metabolites, enzymes, and phenolic compounds. Our goal is to evaluate water stress levels in grapevines and identify genes associated with polyphenol production in response to abiotic stresses.

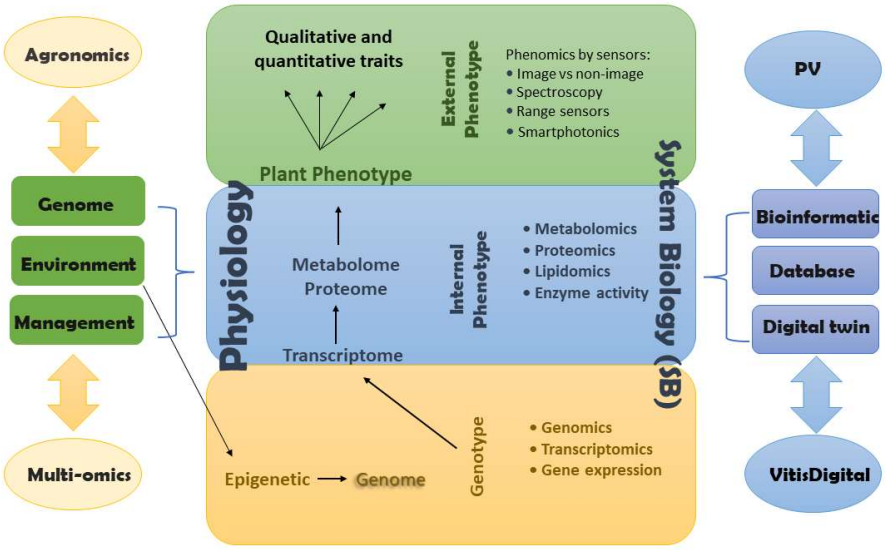


**Figure 1.** A holistic approach combining genomics, metabolomics, and systems biology to understand grapevine responses to water stress and connect laboratory and field data. This study also explores sensor integration for molecular component detection and plant physiological state monitoring (VitisDigital).

## 2. Materials and Methods

### *2.1. Experimental Conditions and Sample Preparation*

Genomic data, including genetic information on genes, proteins, and metabolites, were collected from databases such as http://www.grapegenomics.com/ (access on 20 February 2023) and https://www.ncbi.nlm.nih.gov/ (access on 20 February 2023). These data supported genomics and metabolomics analyses and the development of grapevine models in systems biology. Grapevines were grown in a controlled greenhouse, and three hydric conditions were analyzed: no-irrigation (C0), 100% of crop evapotranspiration, hydric comfort (Etc, C100) and moderate stress, around 50%Etc (C50). These conditions were controlled for the induction of water stress. The leaves were collected and stored at −80 °C. For the analysis, the leaves were placed in liquid nitrogen, and maceration was carried out until a powder of leaves was obtained.

### *2.2. Gene Expression by RT-qPCR*

For gene expression analysis, the first step was RNA extraction. The samples from leaves were stored at −80 °C, and for the analysis, were kept in liquid nitrogen (C0, C50 and C100). Around 100 mg of leaf tissue was weighed and then homogenized in microtubes (bead beater tubes) containing beads. The samples were agitated in the bead beater for 20 s at 3.5 v. After centrifugation at 11,000× *g* for 10 min, RNA extraction was performed using the RNA Purification Systems kit (GeneMatrix, EURx, Gdańsk, Poland) following the provided protocol. The RNA was quantified using spectrophotometry. From RNA, cDNAs were synthesized to perform qPCR using the NZY First-Strand cDNA Synthesis Kit (NZYtech). RT-qPCR was performed to analyze gene expression using the NZYSupreme qPCR Green Master Mix (2x) with ROX plus on a CFX-Bio-Rad instrument. The genes analyzed in this study were ABCC1, CHS1, DFR, MATE1, and UFGT1. Actin (ACT), Elongation Factor (EF), and GAPDH were used for endogenous control. The results were analyzed using ANOVA and Duncan tests, and significance was determined with a $p$-value $\leq 0.05$. The Graph Pad Prism 8.0® program was used.

## 3. Results

Gene expression analysis revealed that among the evaluated genes (ABCC1, CHS1, DFR, MATE1, and UFGT1), only ABCC1 exhibited significant differences in response to different irrigation treatments. Other genes showed variations, but these differences were not statistically significant. The absence of significance may be attributed to the single sampling date. In some conditions, the expression values remained at more controlled levels despite presenting, for some genes, also higher values [8].

ABCC1, related to anthocyanin transport and metabolism, displayed higher expression levels in the C0 condition, indicating its sensitivity to water stress. CHS1, associated with flavonoid biosynthesis, showed higher expression levels in conditions with reduced irrigation, consistent with the known impact of water stress on flavonoid levels [9–11]. Figure 2 shows the gene expression level of the anthocyanin pathway (ABCC1, MATE1, DFR).

Figure 3 shows the genes CHS1 and UFGT1 related to the flavonoid routes [10]. It can be observed that in CHS1, the conditions with less irrigation present a higher level of gene expression. On the other hand, in UFGT1, C50 and C100 showed a higher expression than compared with C0. These results did not show a significant difference, but some punctual differences can be essential in genetics and metabolic modulation related to the hydric stress response in grapevine.

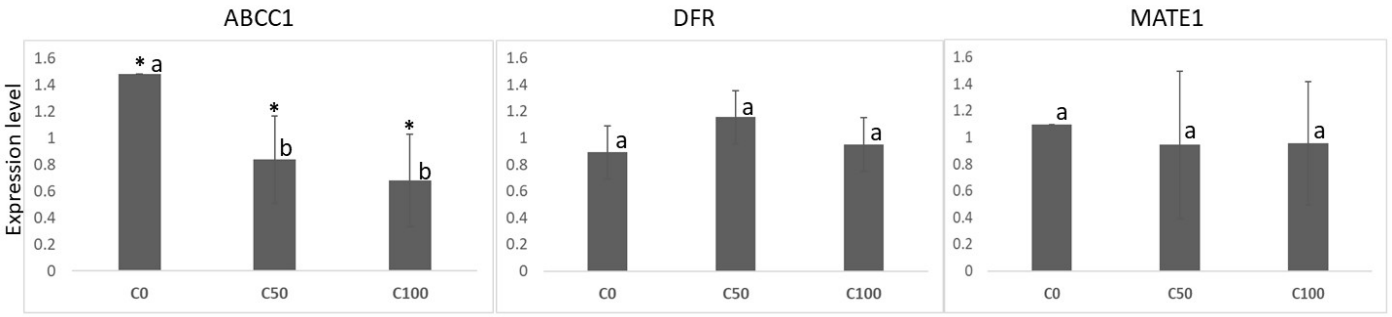


**Figure 2.** Gene expression analysis of the ABCC1, DFR and MATE1 genes of grapevine related with polyphenols, specifically with the anthocyanin's metabolism. The RT-qPCR (CFX-BioRad®, Herculies, CA, USA) method was analyzed with the conditions of hydric stress (C0, C50, and C100), and the figures showed a gene expression level. Only ABCC1 showed statistically significant differences between the treatments. * Statistically significant. The letters a and b represent the statistical differences between the samples, the "a" samples are statistically different from the "b" samples. Normalization: quotient transformation (x/mean).

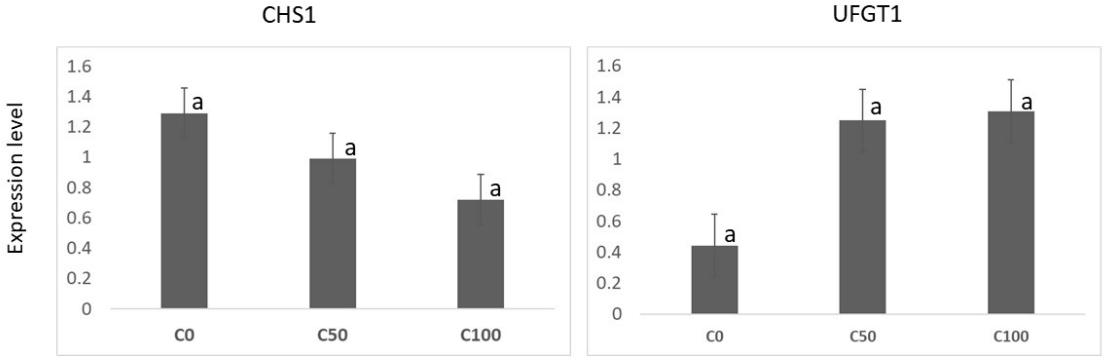


**Figure 3.** Gene expression analysis of the CHS1 and UFGT1, genes of grapevine related with polyphenols, specifically with the flavonoids routes. The RT-qPCR (CFX-BioRad®, Herculies, CA, USA) method was analyzed with the conditions of hydric stress (C0, C50 and C100), and the figures showed a gene expression level. There were no statistically significant differences. The letters a represent the statistical differences between the samples, that no shown differences. Normalization: quotient transformation (x/mean).

## 4. Discussion

In this study, the differences in the ABCC1 gene, which significantly differed between water stress conditions (C0, C50, and C100), showed the importance of anthocyanins in responding to abiotic stresses. Pioneering research used an Affymetrix Gene Chip *Vitis vinifera* oligonucleotide microarray to explain mRNA expression in berry skin, flesh, and seeds in well-watered and water-deficit plants at fruit maturity. This study showed many genes involved in drought stress [8].

Another critical study identified a hundred grape polyphenols by UHPLC/QTOF, classifying several grape flavanols, anthocyanins and stilbenes with different functions. In recent years, metabolomics has been coupled to transcriptomics, providing information about pathways, metabolites, mechanisms, and genes of grape development and the response of biotic and abiotic factors [7]. In a study that performed the transcriptomic and genomic analysis of the *Vitis vinifera* (cultivars *Autumn royal* and *Italia*) in a water deficit, the study identified 29 genes involved in the water stress and the ABA/hormone signal transduction in *Autumn royal* [12].

On the other hand, the *Italia* cultivar identified 1037 genes differentially expressed, related to osmotic and hormone stress, carbohydrate metabolism, ROS response and Cell wall modification [12]. In an analysis in grapevine at abiotic stress, with *Vitis Vinifera* and *Pinot noir*, PCR-based expression analyses were performed, and the whole transcriptome from mRNA-seq, the primarily investigated genes are related to polyphenols, being VvSTS and VvCHS. All the treatments showed a significant difference in the biotic and abiotic stress [13]. Another group that tested the hydric stress in grapevine used combined stresses, such as drought and high temperature and observed anthocyanin levels were down-regulated; however, when there was only drought, there was an increase in anthocyanin genes [5]. Another study used two types of vine grafts and a *Cabernet Sauvignon* cultivar, and tested two levels of water deficit, 20 and 50%, verifying that genes involved in primary and secondary metabolism were affected, as well as responses to stimuli [6]. The differences between these components, in our evaluation, can indicate the alterations suffered by the vines in a situation of water stress, in addition to providing us with the necessary data for the application of systems biology and the assembly of the biological model in genomic scale coupled to Digital- twin for sensor development). These results are good indicators for our study, considering that we need to link genes and plant compounds with sensors and incorporate data with the construction of a model based on systems biology.

## 5. Conclusions

The results of the study showed differences between the presented conditions, as well as differences between the target genes. It suggests that water stress affects gene expression, as well as the general metabolism of the grapevine. Also, it can then be a strong point for an in-depth analysis, using systems biology to connect these laboratory results with real field conditions, enabling the creation of tools and technologies.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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