

FLOODING RESPONSES ON GRAPEVINE: A PHYSIOLOGICAL, TRANSCRIPTIONAL AND METABOLIC PERSPECTIVE

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Abstract:

Context and purpose of the study - Studies on model plants have shown that temporary soil flooding exposes roots to a significant hypoxic stress resulting in metabolic re-programming, accumulation of toxic metabolites and hormonal imbalance. To date, physiological and transcriptional responses to flooding in grapevine are poorly characterized. To fill this gap, we aimed to gain insights into the transcriptional and metabolic changes induced by flooding on grapevine roots (K5BB rootstocks), on which cv Sauvignon blanc (*Vitis vinifera* L.) plants were grafted.

Material and methods - A preliminary experiment under hydroponic conditions enabled the identification of transiently and steadily regulated hypoxia-responsive marker genes and drafting a model for response to oxygen deprivation in grapevine roots. Afterwards, over two consecutive vegetative seasons, flooding was imposed to potted vines during the late dormancy period, to mimic the most frequent waterlogging events occurring in the field. Untargeted transcriptomic and metabolic profiling approaches were applied to investigate early responses of grapevine roots during exposure to hypoxia and subsequent recovery after stress removal.

Results - The initial hypoxic response was marked by a significant increase of the hypoxia-inducible metabolites ethanol, GABA, succinic acid and alanine which remained high also one week after recovery from flooding with the exception of ethanol that levelled off. Transcriptomic data supported the metabolic changes by indicating a substantial rearrangement of primary metabolic pathways through enhancement of the glycolytic and fermentative enzymes and of a subset of enzymes involved in the TCA cycle. GO and KEGG pathway analyses of differentially expressed genes showed a general down-regulation of brassinosteroid, auxin and gibberellin biosynthesis in waterlogged plants, suggesting a general inhibition of root growth and lateral expansion. During recovery, transcriptional activation of gibberellin biosynthetic genes and down-regulation of the metabolic ones may support a role for gibberellins in signaling grapevine rootstocks waterlogging metabolic and hormonal changes to the above ground plant. The significant internode elongation measured upon budbreak during recovery in plants that had experienced flooding supported this hypothesis. Overall integration of these data enabled us to draft a first comprehensive view of the molecular and metabolic pathways involved in grapevine's root responses and in the coordination of scion-rootstock signaling during and after exposure to waterlogging.

Keywords: Waterlogging, Hypoxia, Root, Transcriptome, Gene Expression, *Vitis*.

1. Introduction

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1. Context and purpose of the study

Studies on model plants have shown that temporary soil flooding exposes roots to a significant hypoxic stress resulting in metabolic re-programming, accumulation of toxic metabolites and hormonal imbalance. To date, physiological and transcriptional responses to flooding in grapevine are poorly characterized. To fill this gap, we aimed to gain insights into the transcriptional and metabolic changes induced by flooding on grapevine roots (K5BB rootstocks), on which cv Sauvignon blanc (*Vitis vinifera* L.) plants were grafted.

2. Material and methods

A preliminary experiment under hydroponic conditions enabled the identification of transiently and steadily regulated hypoxia-responsive marker genes and drafting a model for response to oxygen deprivation in grapevine roots (Figure 1). Afterwards, over two consecutive vegetative seasons, flooding was imposed to potted vines during the late dormancy period, to mimic the most frequent waterlogging events occurring in the field. Untargeted transcriptomic and metabolic profiling approaches were applied to investigate early responses of grapevine roots during exposure to hypoxia and subsequent recovery after stress removal.

3. Results

The initial hypoxic response was marked by a significant increase of the hypoxia-inducible metabolites ethanol, GABA, succinic acid and alanine which remained high also one week after recovery from flooding with the exception of ethanol that levelled off (Figure 2). Transcriptomic data supported the metabolic changes by indicating a substantial rearrangement of primary metabolic pathways through enhancement of the glycolytic and fermentative enzymes and of a subset of enzymes involved in the TCA cycle. Gene Ontology and KEGG pathway analyses of differentially expressed genes showed a general down-regulation of brassinosteroid, auxin and gibberellin biosynthesis in waterlogged plants, suggesting a general inhibition of root growth and lateral expansion. During recovery, transcriptional activation of gibberellin biosynthetic genes and down-regulation of the metabolic ones may support a role for gibberellins in signaling grapevine rootstocks waterlogging metabolic and hormonal changes to the above ground plant (Figure 3). The significant internode elongation measured upon budbreak during recovery in plants that had experienced flooding supported this hypothesis (Figure 4).

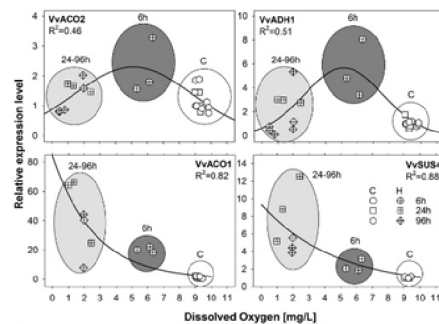


Figure 1 - Scatter plot of the expression of the candidate hypoxia-responsive markers VvACO1 and VvACO2 (1-aminocyclopropane-1-carboxylic acid oxidases), VvADH1 (alcohol dehydrogenase) and VvSuS4 (sucrose synthase) plotted against levels of dissolved oxygen in hydroponic solution under well oxygenated (empty symbols) and progressive hypoxic conditions (crossed symbols). Gray-shaded ellipses/circles indicate the expression levels measured at 6 h (dark gray) and 24–96 h (light gray); white circles group the expression levels measured in roots of control plants at all time-points. Solid lines indicate three-parameter Gaussian and hyperbolic decay best fit curves for (VvACO2, VvADH1) and (VvACO1, VvSuS4), respectively.

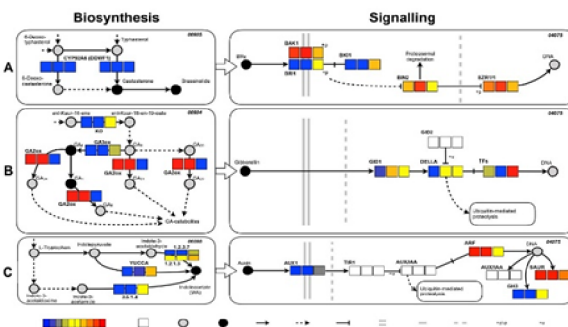


Figure 3 - Expression patterns of the most relevant genes related to hormone biosynthesis/homeostasis, perception, and signal transduction plotted within the correspondent KEGG maps (the pathway code is also indicated at the top-right of each map). Only the most relevant part of the pathways of brassinosteroids (A), gibberellins (B), and auxin (C) were extracted. The differential expression level was calculated as log₂-ratio (Flooded versus Control), scale-centered for each time point, and color-coded as displayed in the legend (blue: <-1; red: >+1). Each box, representing a specific enzymatic or regulatory step, is divided in three sub-boxes representing the different time points: from left to right: T1, T2, and T6.

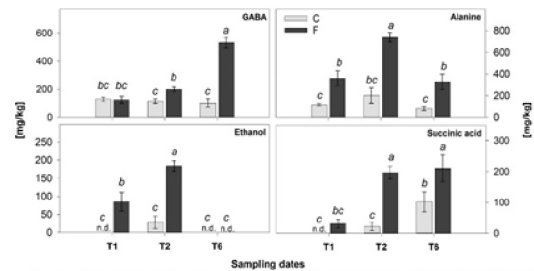


Figure 2 - Quantification of the main metabolites identified by 1H-NMR in flooded (F) and control (C) samples at T1 (24h), T2 (48h), and T6 (21 Days). Values are mean ± SE (n = 5). Significant differences (Duncan's test, p < 0.05) are marked by different letters, n.d., not detectable.

4. Conclusions

Overall integration of these data enabled us to draft a first comprehensive view of the molecular and metabolic pathways involved in grapevine's root responses and in the coordination of scion-rootstock signaling during and after exposure to waterlogging.

For more details see: Ruperti B., Botton A., Populin F., Eccher G., Brilli M., Quaggiotti S., Trevisan S., Cainelli N., Guarracino P., Schievano E. and Meggio F., (2019). Flooding Responses on Grapevine: A Physiological, Transcriptional, and Metabolic Perspective. *Front. Plant Sci.* 10:339.

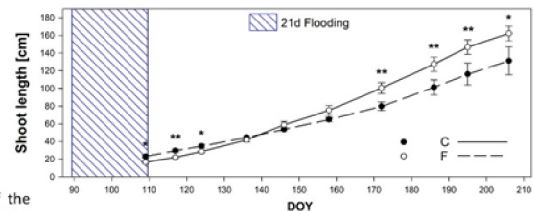


Figure 4 - Shoot length growth dynamics among (C, black circles) and flooded (F, white circles) treatments throughout the 2017 season. Asterisks denote mean values that are statistically different at *p < 0.05, **p < 0.01. DOY, day of the year.

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