TRANSCRIPTIONAL RESPONSE TO HYDROGEN PEROXIDE IN GRAPEVINE BERRY SKIN AT THE BEGINNING OF RIPENING

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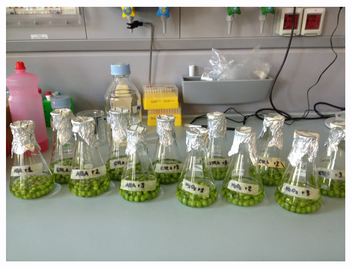
Introduction

Fleshy fruit **ripening** represents the final stage of fruit development, when pulp and skin undergo many metabolic and morphological transformations to become attractive for seed dispersing animals. In grapevine, the beginning of ripening can be identified by the **softening** and coloring of the berries (**véraison**). The transition from mature green to ripening berries is controlled by many internal **signals**, such as hormones, transcription factors and metabolites, tuned to external stimuli, mainly light, temperature and water availability. Ripening onset is characterized by the gradual loss of photosynthetic activity and a transient shift to an aerobic fermentative metabolism (grapevine is a non climacteric plant) which are likely to favor an **oxidative stress**. We reported the **transient accumulation of hydrogen peroxide (H_2O_2)** and singlet oxygen ($^1O_2$) respectively in the **cytosol** and plastids of **Pinot noir berry skin** at the beginning of ripening (Pilati *et al*, BMC Plant Biology 2014). In order to ascertain the signaling function of H_2O_2, we collected pre-véraison berries and treated them *ex-vivo* for 20 hours either with **1 mM H_2O_2, 0.2 mM abscisic acid (ABA)**, known to be an important signal for ripening transition, a cocktail of **ROS scavengers** or a combination of them. RNA extracted from berry skin has been anayzed to study transcriptional response at the genome-wide level by **RNA-seq** for the H_2O_2 and ABA treatment and at the gene level by **Real-time PCR** considering all the conditions. Main results are described here.

Experimental setting

E-L 33
mature hard green berries
(approx. two weeks before véraison)

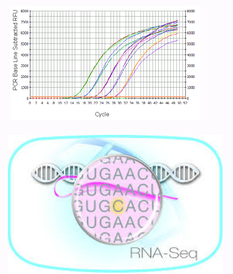


20 hours in mild agitation in the dark

Treatments (in biological triplicates):

- control
- H_2O_2 1mM
- ABA 0,2 mM
- ABA 0,2 mM + anti-oxidants (pyridoxal-phosphate 1mM, Na/Ascorbate 1mM, tocopherol 0,25 mM)
- ABA 0,2 mM + H_2O_2 1 mM
- anti-oxidants (pyridoxal-phosphate 1mM, Na/Ascorbate 1mM, tocopherol 0,25 mM)

Gene expression analysis
(gene and genome-wide level)



RNA-seq analysis results

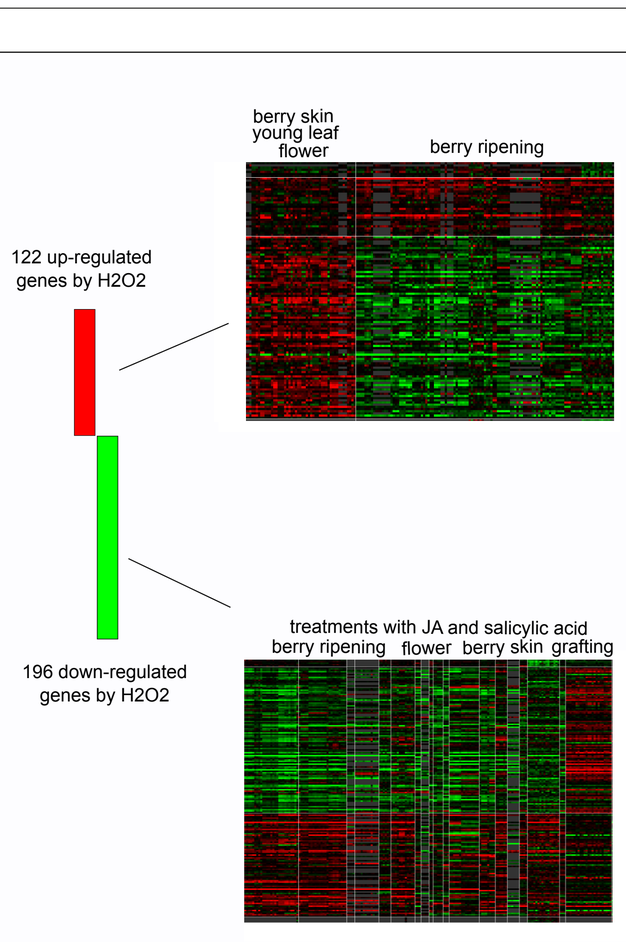
After reads quality check, mapping and normalization, differentially expressed genes have been selected by t-test analysis imposing thresholds on p-value (<0.05) and on fold change (|FC| > 1.5).
318 differentially expressed genes were obtained for berries treated with H_2O_2 and 871 for berries treated with ABA.

Here, we focus on results concerning H_2O_2 response and the comparison with ABA treatment.

H_2O_2 response is slightly unbalanced towards gene **repression** (62%).

24% of the genes are unknown and 65% of the annotated genes have been assigned to a metabolic pathway (https://www.sdstate.edu/ps/research/vitis/pathways.cfm).
The more represented metabolisms are:

- **lipid metabolism**
- **response to stress**
- **photosynthesis**
- **secondary metabolism**
- **plant-pathogen interaction**
- **cell wall metabolism**
- **transport**
- **regulation of transcription**



MarcoPaolo: a gene expression compendium for Vitis vinifera

MarcoPaolo is an atlas based on COLOMBOS technology which incorporates 1883 samples coming from 64 microarray and RNA-seq experiments publicly available at GEO, ArrayExpress, PlexDB and SDRA.

Here, MarcoPaolo has been used to select and visualize conditions in which genes responding to H_2O_2 in pre-véraison berry skin are highly modulated and then to cluster genes according to their co-expression behaviour.

It becomes evident that the genes we find up- and down-regulated by H_2O_2 are co-expressed not only in other experiments on berries (focused on development or tissues) but even in other conditions, like flower and leaf development, grafting and response to jasmonic and salicylic acid.
Moreover, we can distinguish blocks of co-expressed genes, suggesting a modular organization of gene expression.

This analysis supports the role of H_2O_2 in signaling in Vitis physiology and provides candidates for gene expression regulation.

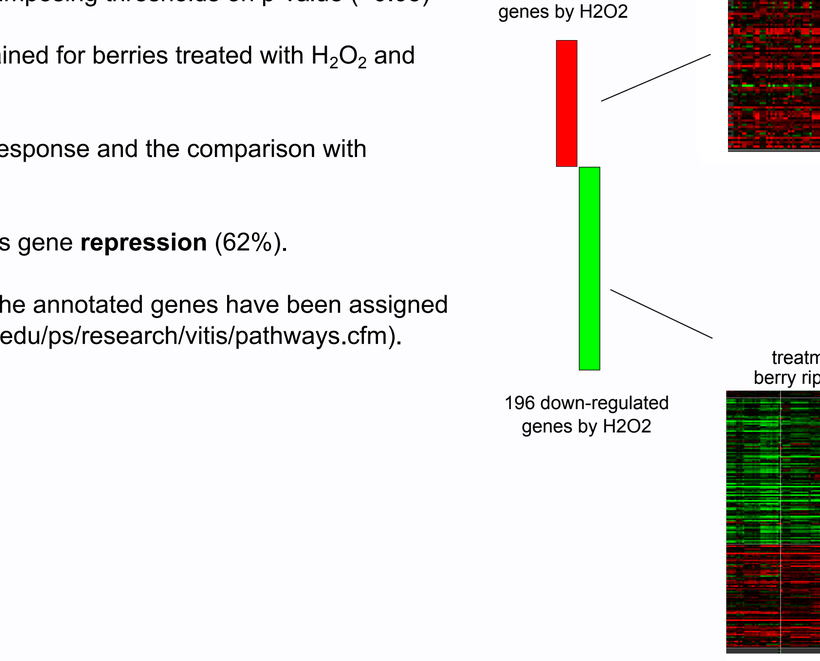
Comparison between ABA e H_2O_2 responses in pre-véraison berry skin

18% of the genes modulated by H_2O_2 treatment are modulated also by ABA, 50% of them with a coherent trend.

This overlap suggests that H_2O_2 could act as secondary signal down-stream of ABA at ripening onset.

Lipoxygenase (PnLOXA), a gene modulated by ABA and H_2O_2, and 9-cis-epoxycarotenoid dioxygenase (NCED), modulated only by ABA, were analyzed by RT-PCR in all the conditions tested. This analysis confirms that PnLOXA requires not only ABA but also H_2O_2 for its full induction, and suggests that H_2O_2 accumulation is a consequence of the presence of ABA.

This hypothesis will be further investigated.



Future work: H_2O_2 visualization with hyper

Hyper, a recently developed cell probe for H_2O_2-specific and quantitative measurements in living cells, has been stably introduced in a dwarf mutant of Vitis vinifera. Two constructs have been employed, for cytosolic and plastidial probe localization.
So far, we can see the probe in the leaves of acclimated plants. This probe will allow to monitor oxidative stress in berries at ripening onset. Then, the same treatments describe above (i.e. with ABA, H_2O_2 and anti-oxidants) will be repeated in order to test the hypothesis of a cause-effect relationship between ABA increase and H_2O_2 accumulation.