UNDERSTANDING GRAFT UNION FORMATION BY USING METABOLOMIC AND TRANSCRIPTOMIC APPROACHES DURING THE FIRST DAYS AFTER GRAFTING IN GRAPEVINE

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What is going on here ?

INTRODUCTION

In viticulture most grapevines are grown grafted onto Phylloxera tolerant rootstocks, so **successful graft union formation is essential** to most wine production today. The mechanisms underlying **graft union formation are complex**, involving the reprogramming of transcription, metabolic pathways, cell division and differentiation, and cell-to-cell communication. We have previously identified some metabolite markers (particularly **stilbenes**) associated with poor grafting success (Loupit et al., 2022).

→ The objective of this work was to 1.) **identify genes** involved in graft union formation, and 2.) characterize the **spatial and temporal changes in secondary metabolites** occurring during graft union formation.

MATERIAL AND METHODS

We studied graft union formation in *Vitis vinifera* cv. Pinot noir homo-grafts **(PN/PN)**, in comparison to wounded cuttings **(PNw)** and intact control cuttings **(PNc)**.

- **Gene expression analysis** above and below the graft interface at 0 and 14 **days after grafting (DAG)** using RNA sequencing.
- **HPLC-QqQ analysis** of 41 phenolic compounds (20 stilbenes) in scion, rootstock and at the graft interface for 2 weeks.
- **MALDI-MS analysis** on longitudinal sections in the middle of the graft interface at 0, 16 and 30 DAG.

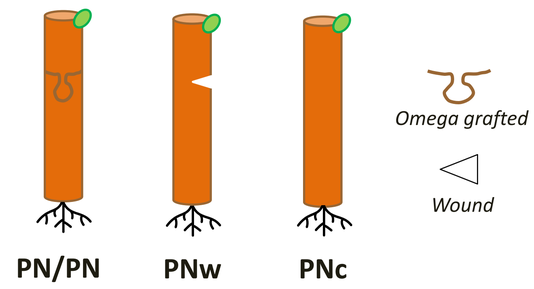
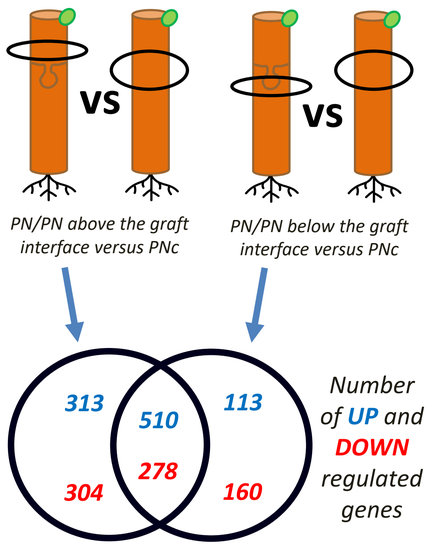


Figure 1: Experimental design for *Vitis vinifera* cv. Pinot noir genotype (realized in 2020 for metabolic analysis, in 2021 for transcriptomic analysis, and in 2022 for MALDI-MS analysis)

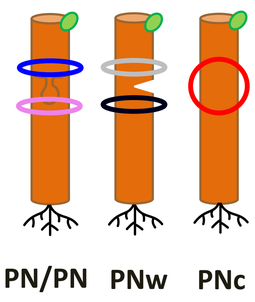
RESULTS

Which genes are differentially expressed at the graft interface 14 DAG ?



- **More DEGs above than below** the graft interface (Figure 2).
- **Stilbene synthases** are expressed almost **3 times more in homo-grafts and wounded cuttings** compared to intact controls (Figure 3A).
- The biosynthetic pathway of **polyphenols is oriented towards the accumulation of stilbenes** (Figure 3).

Figure 2: Venn diagram of the number of DEG in PN/PN grafts between above and below the graft interface compared to intact controls (PNc); (LogFC > 1.5 and < -1.5, LogCPM > 3, adjusted p-value < 0.05).



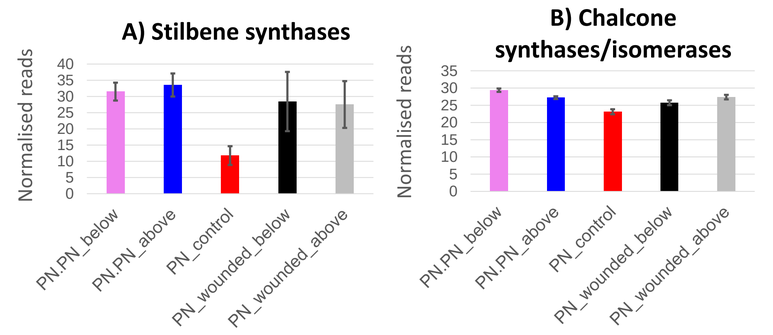
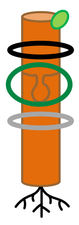


Figure 3: Expression of highly expressed (A) stilbene synthases (11 genes) and (B) chalcone synthases/isomerases (5 genes) above and below the graft/wound interface and in intact controls at 14 DAG.

Which defense metabolites accumulate at the graft interface ?

- Strong **accumulation of stilbenes** in PN/PN graft interface from **1 DAG**.
- **Oligomerization of stilbenes** over time (Figure 4).



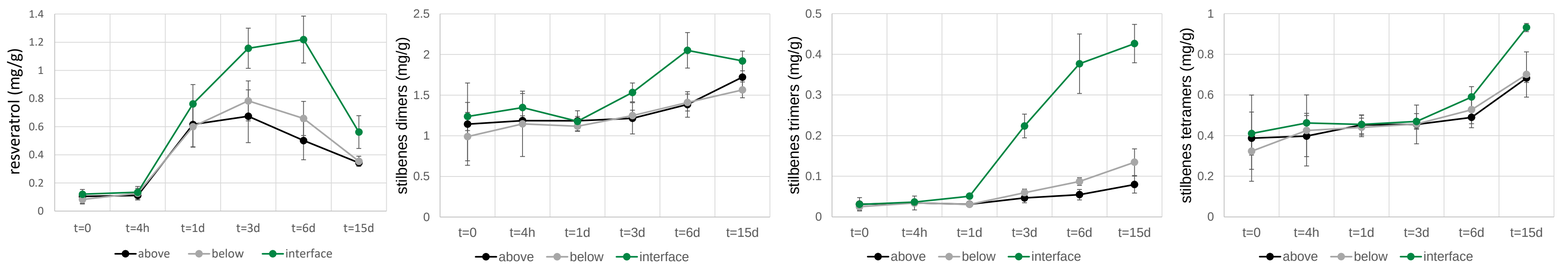


Figure 4: Concentrations of resveratrol, total stilbene dimers, total stilbene trimers and total stilbene tetramers quantified above, below and at the graft interface of PN/PN for 2 weeks (mg/g FW, error bars correspond to standard deviations).

In which tissues do the stilbenes accumulate ?

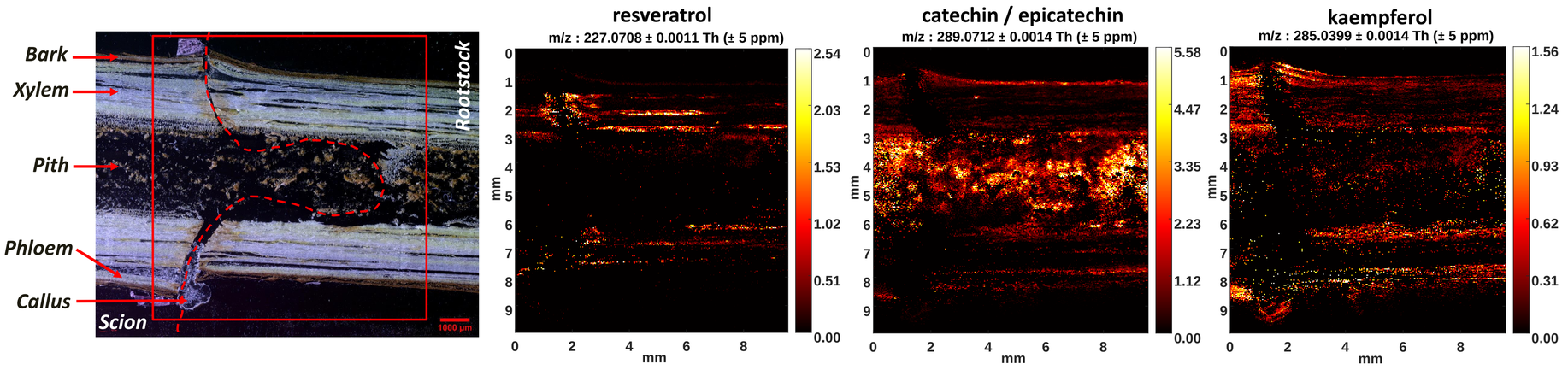


Figure 5: MALDI-MS of PN/PN grafts at 16 DAG. Graft section (20 µm thick). Analysis were done in full scan negative mode (between 100 and 1000 m/z, TIC normalization).

- **Resveratrol accumulates in necrotic tissues** especially in xylem zone (Figure 5).
- Flavanols monomers are mostly in the pith and between the bark and the phloem (Figure 5).
- Kaempferol is mostly in the xylem and phloem zone as also a little bit in the new callus tissue (Figure 5).
- This is the **first MALDI-MS analysis** at the graft interface level.

CONCLUSION / WAY FORWARD

- **Stilbenes** seem to be **specific in response to grafting/wounding** compared to flavanols, flavonols or phenolic acids.
- Many genes differentially expressed in response to grafting, **the response of the scion is more pronounced than the rootstock.**
- **MALDI-imaging provides insights into the tissue specific metabolite profile** in woody samples of grapevine and can identify the accumulation of metabolites at the graft interface (in the callus and along the cut surface of the stem).
- The **integration of these transcriptomic and metabolomic datasets** will allow us to further our **understanding of graft union formation** in grapevine and to **identify candidate genes and pathways regulating**.

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