Understanding scion/rootstock interactions at the graft interface of grapevine

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• In Europe, grapevines are grafted because of the Phylloxera outbreak of the end of the 19th century.
• Phylloxera is a soil dwelling aphid pest that is native to America & was introduced accidentally to Europe.
• American grapevine species have tolerance to Phylloxera & used as rootstocks.
• Successful graft union formation is key to viticulture today.

Transversal section of a graft interface many years after grafting (Photo: JP Tandonnet)
• In Europe, grapevines are grafted because of the Phylloxera outbreak of the end of the 19th century.
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• American grapevine species have tolerance to Phylloxera & used as rootstocks
• Successful graft union formation is key to viticulture today

Although essential, producing grafted plants is not so easy.

215.3 millions grafts produced in France in 2015, 120-130 million grafts sold = 58 % sold¹

This could be improved!
1. How does the graft union form?
2. Is hetero-grafting different from homo-grafting?
3. What are the causes of graft incompatibility?
Graft union formation – wound responses & healing processes

- Formation of a necrotic layer
- Adhesion
- Callus cell proliferation
- Fragmentation of the necrotic layer
- Contact between the cells of the two partners
- Formation of plasmodesmata & differentiation of xylem & phloem
- Development of functional connections between the scion & rootstock

Introduction

Cellular connections

Xylem connections

Transcripts & metabolites

Ongoing & Future work

Grapevine graft interface 1 month after grafting

Scion

Rootstock
Grapevine graft interface 1 month after grafting

Outline

1. Physical connections between the scion & rootstock
   - Cellular connections
   - Xylem connections

2. Transcripts & metabolites involved

3. Ongoing & future work
Plasmodesmata – tiny channels connecting almost every cell

- Key elements for cell to cell communication
- Transport proteins, RNAs...
- Permeability which is regulated

Plasmodesmata allowing communication (arrows) between different cells; n, nucleus.
Plasmodesmata – tiny channels connecting almost every cell

- Flattened endoplasmic reticulum (ER) runs through them
- How plasmodesmata form across existing cell wall is a mystery
- Where does the ER in the middle come from?

Cross sectional image of a plasmodesmata¹

¹Maule et al., 2012 Frontiers in Plant Science
Plasmodesmata have been shown to form at the graft interface.

- Are they functional?
- Are they important for grafting success or graft incompatibility?

Electron micrographs of interface of *Vicia faba*/*Helianthus annuus* grafts

1Kollmann & Glockmann, 1984, Protoplasma
Plasmodesmata have been shown to form at the graft interface. Are they functional? Are they important for grafting success or graft incompatibility?

Electron micrographs of interface of *Vicia faba*/*Helianthus annuus* grafts

See poster 79: Clément CHAMBAUD

Understanding the establishment of scion/rootstock interactions in grapevine

- Are they important for grafting success or graft incompatibility?

Electron micrographs of interface of *Vicia faba*/*Helianthus annuus* grafts

¹Kollmann & Glockmann, 1984, Protoplasma
Xylem formation

- Have studied xylem formation at the graft interface
  1. Imaging xylem vessels
  2. Measuring hydraulic conductivity

Transport of blue stain from the rootstock to the scion 3 months after grafting
Imaging xylem formation – X-ray tomography

X-ray computed tomography (CT) with a relatively low resolution; scion, Sc; rootstock, Rc

High resolution CT, but deadly & image size difficult to handle – 3D reconstruction to come...

Hydraulic conductivity gives an indication of flux in the xylem for a given driving force

Across the graft interface gives an indication of formation of xylem vessels

We have used two techniques:

1. High Pressure Flow Meter – not suitable for young grafts
2. Low pressure flow meter (gravity) – can be used from 8 weeks after grafting
Hydraulic conductivity of different genotypes

Where measurements were made

Hydraulic conductivity of an internode cutting during early stages of growth

GN, Vitis vinifera cv. Grenache; RGM, V. riparia cv Gloire de Montpellier; 110 R, V. berlandieri x V. rupestris cv. 110 Richter.
Hydraulic conductivity of different genotypes

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Hydraulic conductivity of an internode cutting during early stages of growth

No difference in hydraulic conductivity in cuttings of different genotypes studied
Hydraulic conductivity of the graft interface

Where measurements were made

Hydraulic conductivity of the graft interface 8 weeks after grafting

Hydraulic conductivity of the graft interface

Hydraulic conductivity reduced >100 times 8 weeks after grafting

Hydraulic conductivity is different between the scion/rootstock combinations
Future questions

- Can the measurements of hydraulic conductivity be linked to xylem formed at the graft interface?
- Are there differences in xylem connections between different scion/rootstock combinations?
- Is xylem connection involved in graft incompatibility &/or dieback?
Transcripts involved in graft union formation & heterografting?

Homograft: *V. vinifera* cv. Cabernet Sauvignon (CS)/CS

Homo- & heterografs: CS/CS vs. CS/RGM

Cookson et al., 2013; 2014. J. Exp. Bot
Transcriptome of the rootstock & graft interface

Number of transcripts differentially expressed between the rootstock & graft interface

3 days
76 0
28 days
13 0
220 13

Number of UP and DOWN regulated genes

Cookson et al., 2013. J. Exp. Bot
Transcriptome of the rootstock & graft interface

Graft interface is associated with the **UP-REGULATION** of gene expression.

Genes up-regulated at the graft interface associated with cell wall formation, secondary metabolism (stilbenes), auxin, the regulation of transcription (e.g. MYB102), oxidative stress, jasmonic acid...

Cookson et al., 2013. J. Exp. Bot
Transcriptome of homo- & heterografts

Homo- & hetero-graft: CS/CS vs. CS/RGM

Time course: 3, 7, 14 & 28 days after grafting

>4000 genes were differentially expressed between the scion/rootstock combinations

~1100 genes showed a rootstock genotype x time after grafting interaction

Cookson et al., 2014. J. Exp. Bot
Introduction

Cellular connections

Xylem connections

Transcripts & metabolites

Ongoing & Future work

- Polyamine oxidase
- Secondary metabolism
- Cell organisation

- Jasmonic acid
- Pathogenesis-related (PR) proteins
- Oxidative stress

- Secondary metabolism
- Cell wall
- Receptor kinases

- Senscence associated genes
- Stress
- Jasmonic acid
- Transcription factors

Cookson et al., 2014. J. Exp. Bot
Hetero-grafting induces the expression of genes involved in oxidative stress, jasmonic acid, secondary metabolism... → **STRESS & DEFENSE** responsive transcripts

28 days after grafting many transcripts associated with secondary metabolism accumulated – what about metabolites?

Cookson et al., 2014. J. Exp. Bot
Metabolomics of interface & wood of homo- & heterografts

Measured:

- Activity of phenylalanine ammonia lyase (PAL)
- Stilbenes
- Flavanols
- Amino acids
- Sugars
- Starch
- Protein

Homo- & hetero-grafts:
CS/CS vs. CS/RGM & CS/1103P
PCA of metabolite profiles of CS/CS, CS/RGM & CS/1103P
PCA of metabolite profiles of CS/CS, CS/RGM & CS/1103P

Separation of metabolomes of wood & interface
PCA of metabolite profiles of CS/CS, CS/RGM & CS/1103P

**Rootstocks**
- RGM & 1103P + flavanols

**Scion**
- Arg
  - + cis-Astringin
  - + Starch...

**Interface**
- + Gln, Ileu, Val...
  - + PAL activity
  - + Piceid, Astringin...

**Variables - PCA**
- Dim1 (31.3%)
- Dim2 (17.7%)
PCA of metabolite profiles of CS/CS, CS/RGM & CS/1103P

See poster 113: Duyen Prodhomme

Metabolite profiling at the graft interface of grapevine
**Cellular connections**

- Are plasmodesmata functional across the graft interface?
- Is plasmodesmata formation or function linked to grapevine incompatibility &/or dieback?

**Xylem connections**

- Are these differences in the formation of xylem in different scion/rootstock combinations?
- Are xylem connections involved in graft incompatibility &/or dieback?
Limitations to previous studies in perennial crops

- Lack of appropriate controls (homo-grafts, & un-grafted scions & rootstocks)
- Insufficient sampling density in time course
- Graft interface sample a mixture of wood, callus & cells from both partners
- Microarrays rather than RNAseq
- No previous studies have measured stilbenes

Future transcriptomics project – understand how each grafting partner functions using RNAseq in perfectly controlled experiment ($$$$$$

Future metabolomics project - study a wider range of scion/rootstock combinations of different levels of compatibility with the objective of identifying metabolite markers of incompatibility
• Understand the mechanisms of graft union formation in woody plants

• Identify the origins of incompatibility responses in grapevine

• Use this knowledge to improve grafting success – applying chemicals? Antioxidants?

• One day study the genetic architecture of graft compatibility
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