Multiple Approaches Provide Novel Information on Flax Cell Wall Biology

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The cell wall biology of **bast fibers**
- present in outer stem tissues of certain species (e.g. hemp, flax)
- mechanically extracted and used for textiles and composites

extremely long, single cells with a small diameter and a **thick cell wall**

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Research on bast fiber cell walls

1) Construction
   Regulation of the biosynthesis and deposition of different cell wall polymers

2) Modifications
   Effects of stress (abiotic) on cell wall metabolism and structure

3) Break-down
   Composition and dynamics of microbial communities during retting (natural enzymatic degradation of cell walls)

Models used: Flax, arabidopsis, hemp

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1) Construction: Regulation of the biosynthesis and deposition of different cell wall polymers

- the secondary cell wall in most plant species contains **HIGH** amounts of lignin

- the secondary cell wall of bast fibers contain **LOW** amounts of lignin

**Fibre Quality Workshop, Lille 18/01/2016**
1) Construction: Regulation of the biosynthesis and deposition of different cell wall polymers

i) whole genome transcriptomics\textsuperscript{1,2}

Separation stem outer tissues (OT) from inner tissues (IT), extraction RNA
- 775 unigenes differentially expressed (Log2 ratio > 1.5) between IT and OT

\textsuperscript{1}\text{Fénart et al., (2010), BMC Genomics;}
\textsuperscript{2}\text{Huis et al. (2012) Plant Physiol.}
**Whole genome transcriptomics**

ALL ‘lignin genes’ (B/S and polymerisation) are more highly expressed in IT.

Lignin : cellulose/NCP ratio in the flax stem is regulated at the transcriptional level.
ii) EMS mutant population (5,000 families) for reverse genetics (TILLinG)\(^1\)

Other tilled cell wall-related flax mutants

- CAD, C3H (monolignol biosynthesis)
- MYB4, KNAT7, XIP, (transcription factors, stem structure)
- UGTs, (monolignol glycosylation)
- pectin-related genes

\(^1\)Chantreau et al. (2013) BMC Plant Biol.  *Fibre Quality Workshop, Lille 18/01/2016*
ii) EMS mutant population (5,000 families) for forward genetics

- identification of flax lignified bast fiber (lbf) mutants

3,391 M2 families

Screen stem cylinders (UV autofluorescence) → 540 families

Screen stem cross sections (Phloroglucinol/HCl) → 319 families
  - 150 families class 3
  - 176 families class 2
  - 93 families class 1

‘core collection’ of lignified bast fiber (lbf) mutants

1Chantreau et al. (2014) Plant Cell. Fibre Quality Workshop, Lille 18/01/2016
Characterization lignified bast fiber1 (lbf1) mutant

<table>
<thead>
<tr>
<th>Mutants</th>
<th>All fiber wall</th>
<th>ML and P1</th>
<th>Bundle periphery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>44 families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>10 families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3 families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>13 families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>1 family</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>16 families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>4 families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>2 families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0 family</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>Wild-type bast fibers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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- lignin monomer (monolignol) biosynthesis

- up-regulation *CCR*, *COMT* and *CAD* genes (outer tissues only)

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- lignin monomer (monolignol) polymerisation
- NO laccase genes differentially regulated (*lbf1* vs wt)

- **14 peroxidase** genes up-regulated in *lbf1*
- **11 peroxidase** genes specifically up-regulated in OT

9 are orthologs of Arabidopsis peroxidases involved in lignification

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The flax *lbf* mutant core collection – a useful biological resource for:

- Investigating lignification regulatory mechanisms
- Investigating cross-talk between lignification and other cell wall polymers
- Evaluating impact of increased lignification on fiber chemical/physical properties
- Improving flax and fiber plant quality

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iii) Targeted (organ-specific) proteomics

- If you have transcriptomics, why bother with proteomics??
The Flax Proteome Atlas!

- stem inner-tissues (1), stem outer-tissues (2), leaves (3), roots (4)
- fractions: soluble proteins, cell wall proteins, membrane proteins
The Flax Proteome Atlas!

- ID 2,686 proteins (1,242 non-redundant)

- inner stems: 712 proteins
- outer stems: 773 proteins
- leaves: 418 proteins
- roots: 452 proteins
- seeds\(^1\): 1,516 proteins
- total stem\(^2\): 1,135 proteins

Total of 2,665 non-redundant proteins (6.7% predicted proteins)

\(^2\)Day et al. (2013) Proteomics

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Flax cell wall-related proteins

How do we define what is/isn’t a ‘cell wall protein’?

- physically located in the cell wall and involved in cell wall metabolism (14) ?
- physically located in the cell wall and NOT involved in cell wall metabolism (14) ?
- NOT physically located in the cell wall but involved in cell wall biosynthesis (15, 16) ?
Flax cell wall-related proteins

How do we define what is/isn’t a ‘cell wall protein’?

- 1) Carbohydrate Active enZymes (CAZy proteins)
- 2) Apoplastic proteins (with signal peptide and 1 transmembrane domaine)
- 3) Lignin (monomer) biosynthesis (cytosolplasm)
- 4) Other proteins potentially related with cell wall metabolism

Identification of 465 different flax ‘cell wall proteins’
Flax cell wall-related proteins
- identification 209 proteins in different CAZy families

<table>
<thead>
<tr>
<th>CAZy family protein</th>
<th>Targeted cell wall polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH1 : β-glucosidase</td>
<td>glucan, cellulose, hemicellulose (xyloglucan)</td>
</tr>
<tr>
<td>GH3 : β-xylosidase</td>
<td>hemicellulose (xylan)</td>
</tr>
<tr>
<td>GH16 : xyloglucan endotransglycosylase/hydrolase (XTH)</td>
<td>hemicellulose (xyloglucan)</td>
</tr>
<tr>
<td>GT2 : cellulose/glucan synthase</td>
<td>cellulose, hemicellulose (xyloglucan, mannan, MLG)</td>
</tr>
<tr>
<td>GT47 : xylan synthase</td>
<td>hemicellulose (xylose, xyloglucan), pectin (RGI)</td>
</tr>
<tr>
<td>AA1/2: laccase/peroxidase</td>
<td>lignin</td>
</tr>
<tr>
<td>CE8: pectin methylesterase (PME)</td>
<td>pectin</td>
</tr>
<tr>
<td>PL4 : pectate lyase</td>
<td>pectin</td>
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</table>
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2) Modifications: Effects of stress (abiotic) on cell wall metabolism and structure

Effect of drought stress on flax cell wall metabolism

- Drought is a major abiotic stress (33% cultivated lands)

How does this stress modify cell wall metabolism?

- Consequences on the quantity/quality of plant products (e.g. fibers)?
- Consequences on the growth/survival of plants in sub-optimal conditions?
2) Modifications: Effects of stress (abiotic) on cell wall metabolism and structure

Approaches

- Transcriptomics (2,828 gènes DE)
- Proteomiques (402 prots DE)
- Metabolomiques (underway)

Functional classification, data integration and focus on cell wall genes
4) Transversal projects and new approaches...

- development of different imaging techniques

- light/electron microscopy

- micro FT-IR spectroscopy and FPA cartography

- **In situ** hybridization

- click chemistry (marked coniferyl alcohol)

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**Improved capacity to analyze fiber cell wall structure and metabolism**

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Flax cell wall-related proteins - identification 209 proteins in different CAZy families

**CAZy family protein** | **Targeted cell wall polymer**
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GH1 : β-glucosidase | glucan, cellulose, hemicellulose (xyloglucan)
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AA1/2: laccase/peroxidase | lignin
CE8: pectin methylesterase (PME) | pectin
PL4 : pectate lyase | pectin

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1) Construction

The xyloglucan endotransglycosylase/hydrolase family (XTH; GH16)

i) high number of flax XTH genes
   - arabidopsis (33 genes)
   - poplar (44 genes)
   - flax (67 genes)

ii) important paralogy in the
    flax IIIA XTH class
The xyloglucan endotransglycosylase/hydrolase family (XTH; GH16)
XTH activity is intimately involved with cell wall expansion during growth
The xyloglucan endotransglycosylase/hydrolase family (XTH; GH16)

XTH activity probably plays an important role in aspen tension wood G-fiber expansion/structure*

- wall loosening (cell expansion) via XEH activity
- wall rigidification (reticulation) via XET activity
- wall structural integrity (S-G boundary) via XET activity


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The xyloglucan endotransglycosylase/hydrolase family (XTH; GH16)

XET activity necessary for maintaining (repairing) xyloglucan-dependant structural integrity between fiber G-/S-layers

G – S layer junction is structurally weak

*Fig. from Gorshkova et al. (2015) Plant Physiol.*
The xyloglucan endotransglycosylase/hydrolase family (XTH; GH16)

Do XTHs play similar roles in flax fiber formation/structural integrity?

- flax fibers can also be considered to be “G-fibers”

- cell wall structured in P, S1 and cellulose-rich S2
Do XTHs play similar roles in flax fiber formation/structural integrity?

- flax fibers can also be considered to be “G-fibers”

- or alternatively, cell wall structured in P, S1/S2 and cellulose-rich G layers
Do XTHs play similar roles in flax fiber formation/structural integrity?

1) flax fibers are extremely long cells ("logical" role for XEH activity in cell expansion)

2) certain XTH genes are more highly expressed in flax stem outer tissues compared to inner tissues*

3) certain XTH genes are more highly expressed in mature outer stem tissues*

- *lbf1* shows changes in cellulose and NCP content.

**Increasing lignin content**

**Increasing lignin content**

- Lignin associated with total sugars: glucose, other sugars
- Lignin associated with proteins

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