

# Xylan biosynthesis and modification in poplar

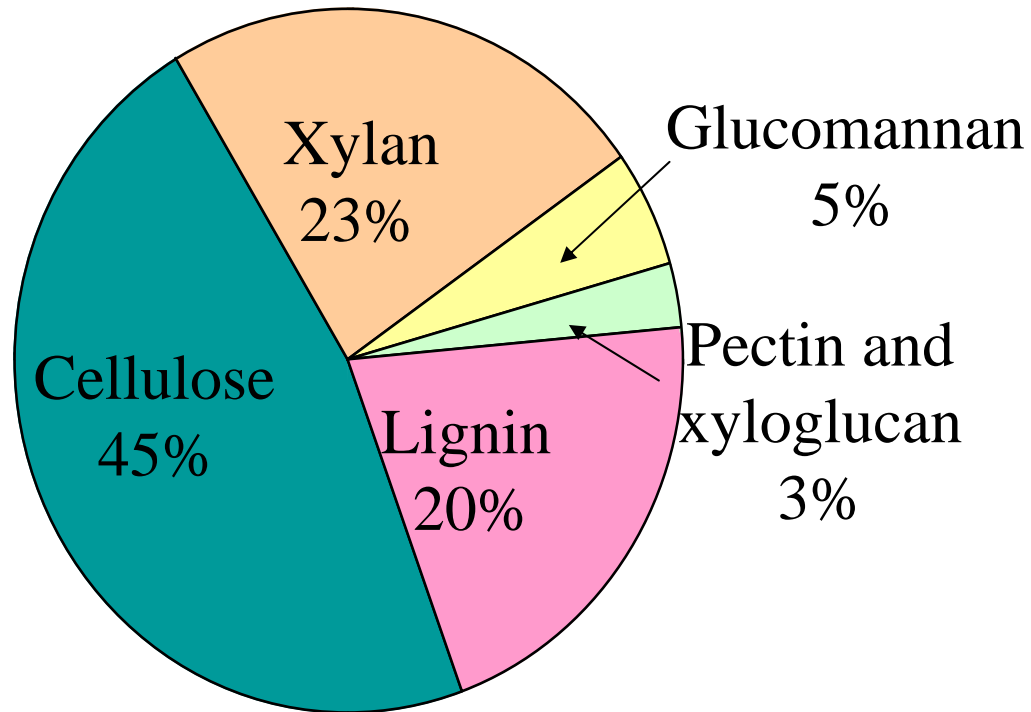
Ewa Mellerowicz

Umea Plant Science Center, Sweden



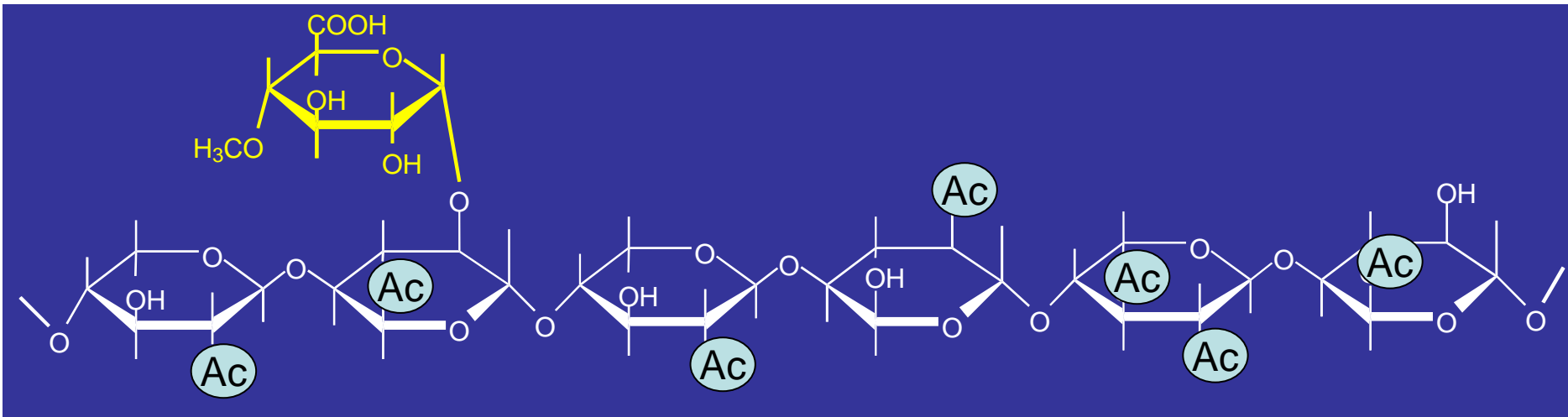
# Xylan is the 3d most abundant biopolymer on Earth

## Poplar wood

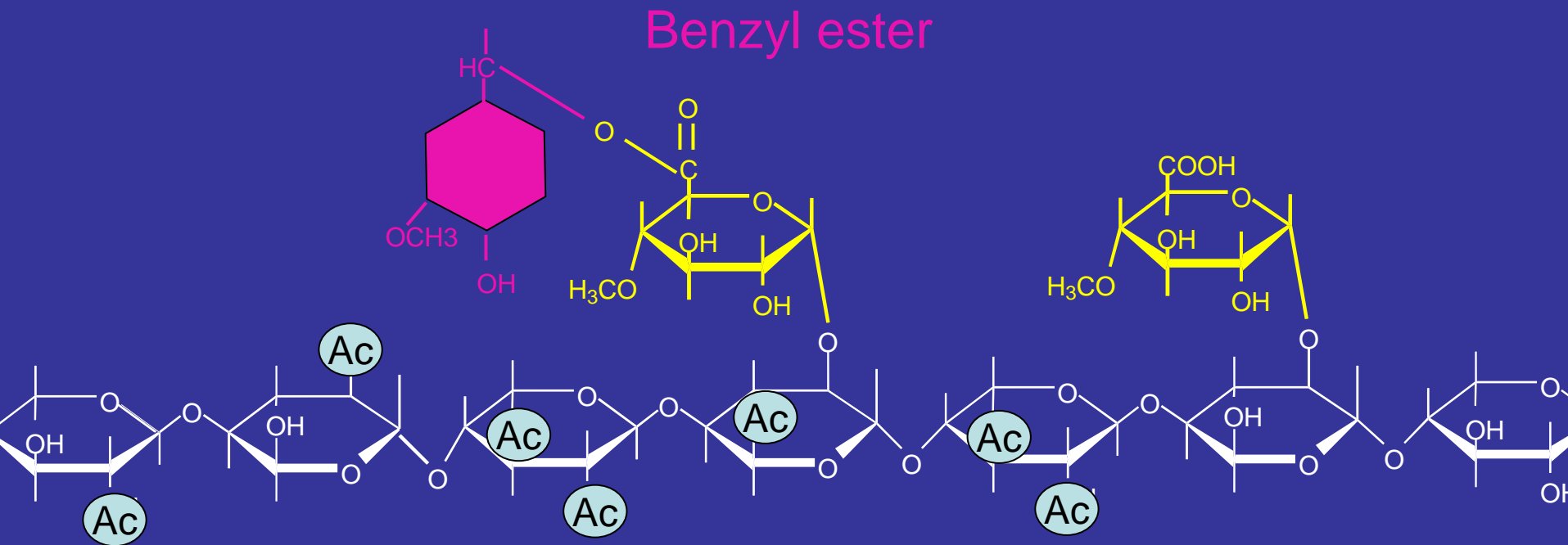


Simson and Timell, 1978

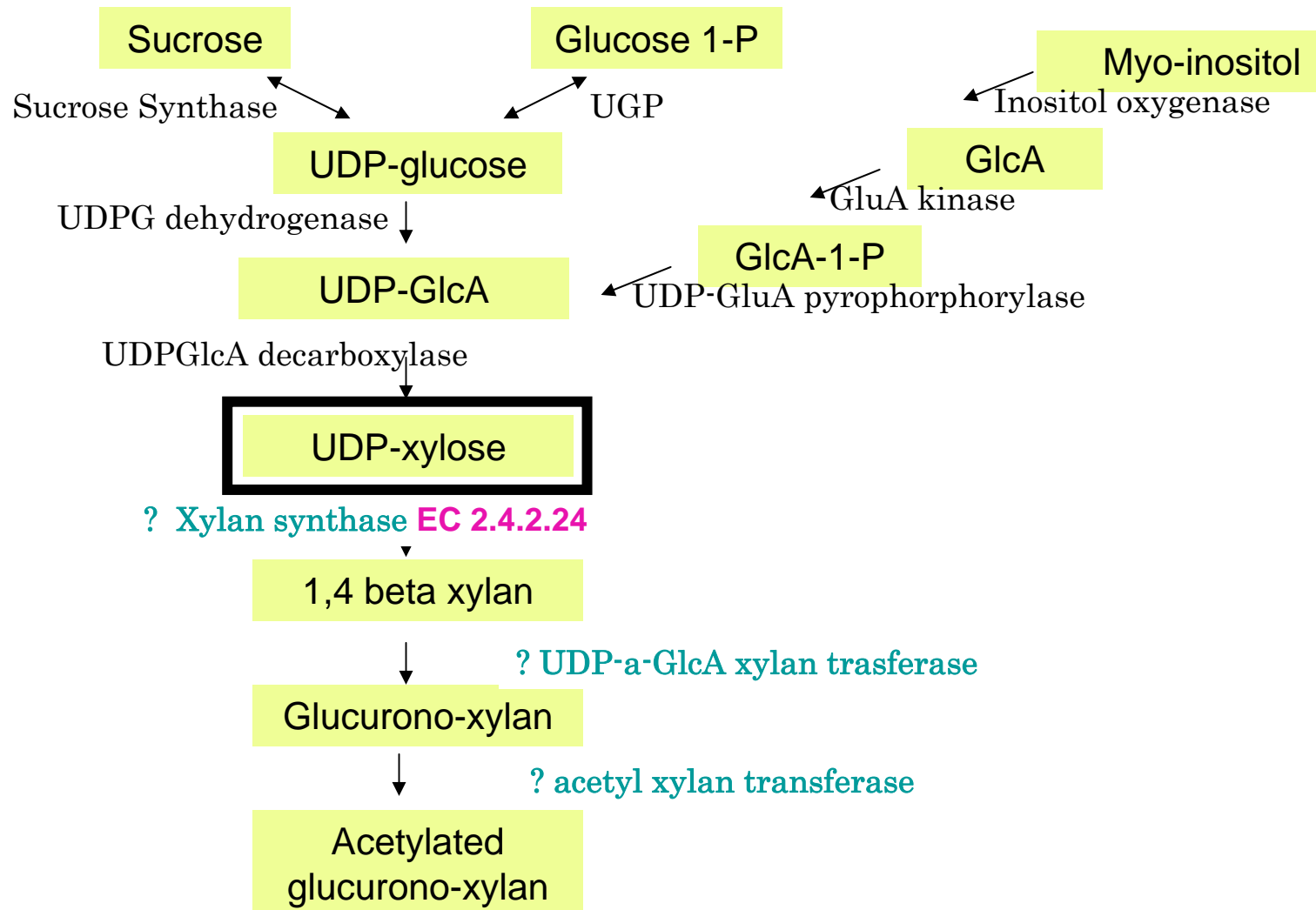
# (4-O-Methylglucurono)-xylan



# Xylan-Lignin connections

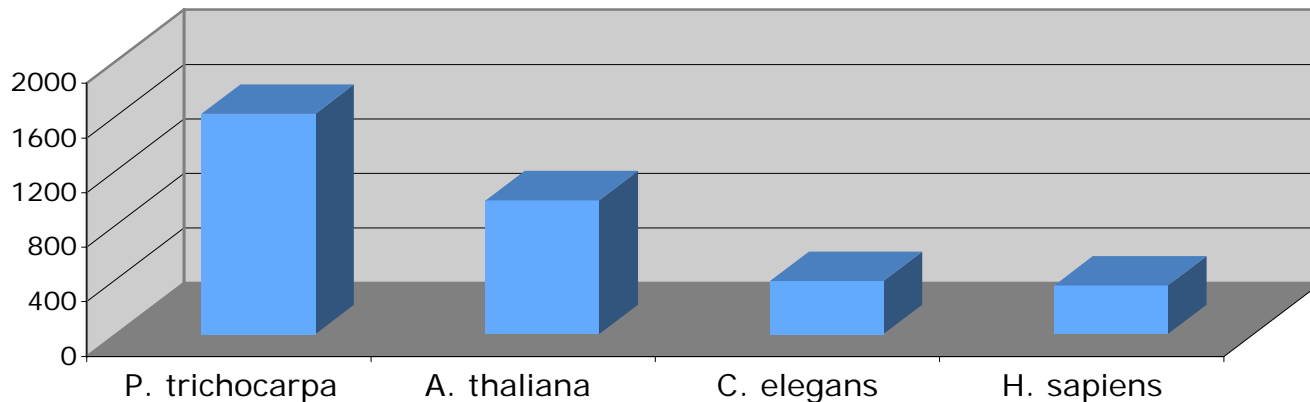


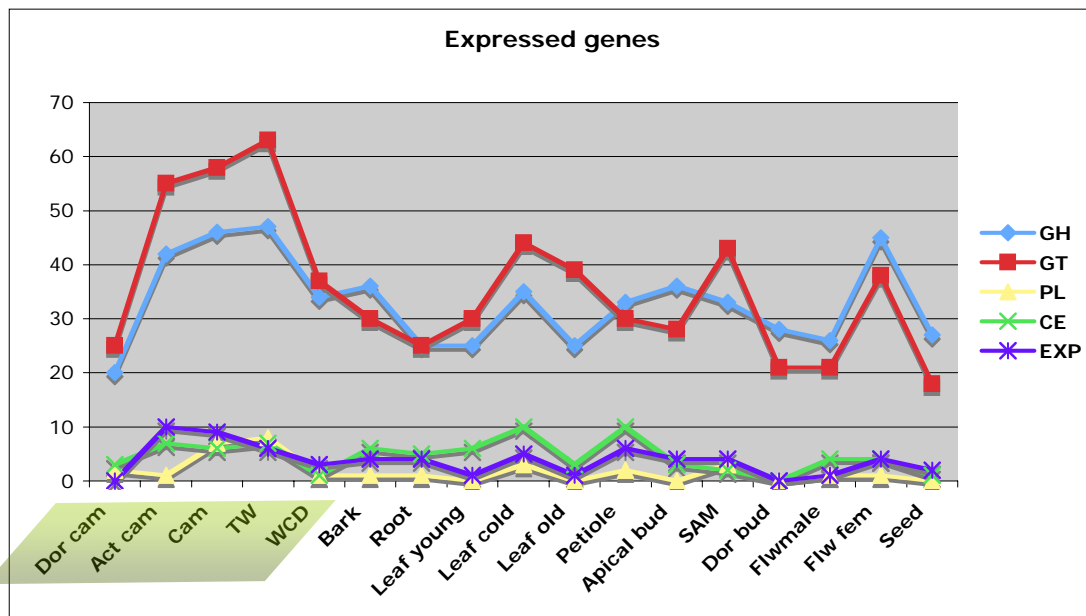
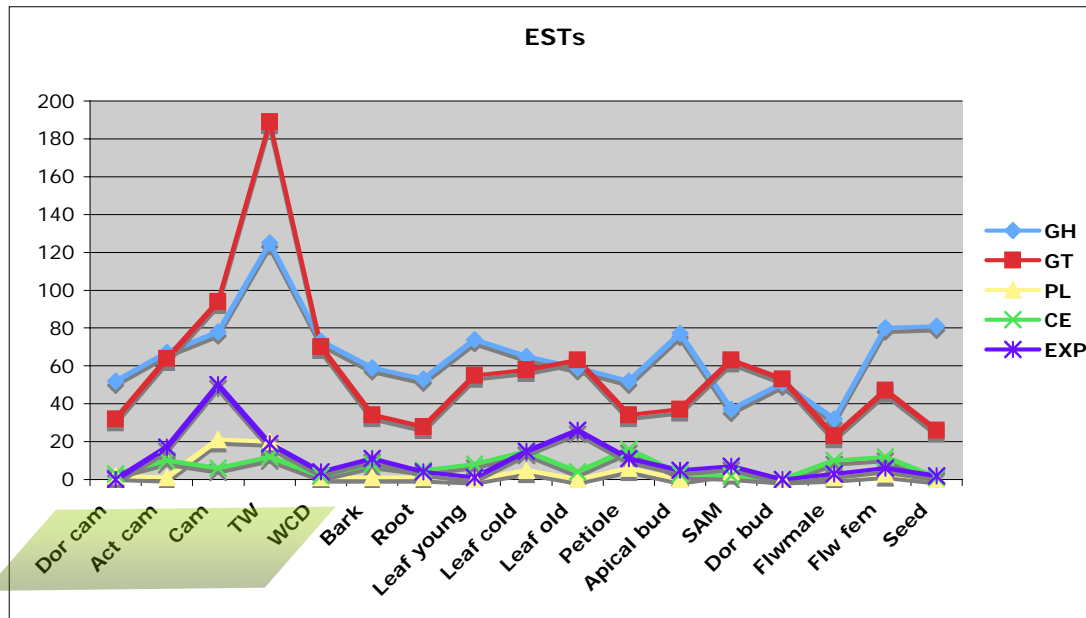
# Xylan biosynthesis in dicots



# Analysis of poplar CAZyme genome with Bernard Henrissat et al.

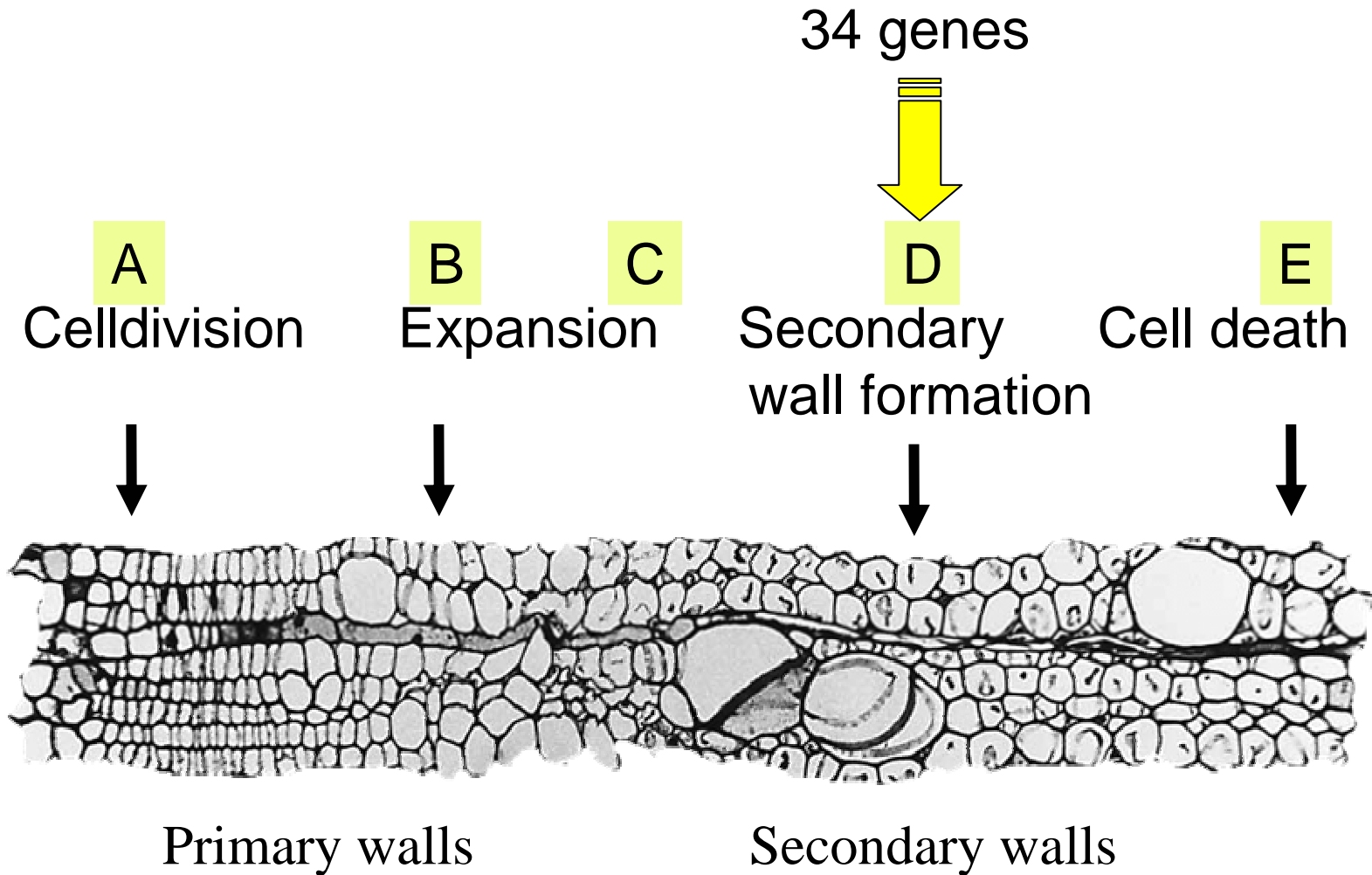
- Poplar genome contains over 1600 carbohydrate active enzyme (CAZyme) genes



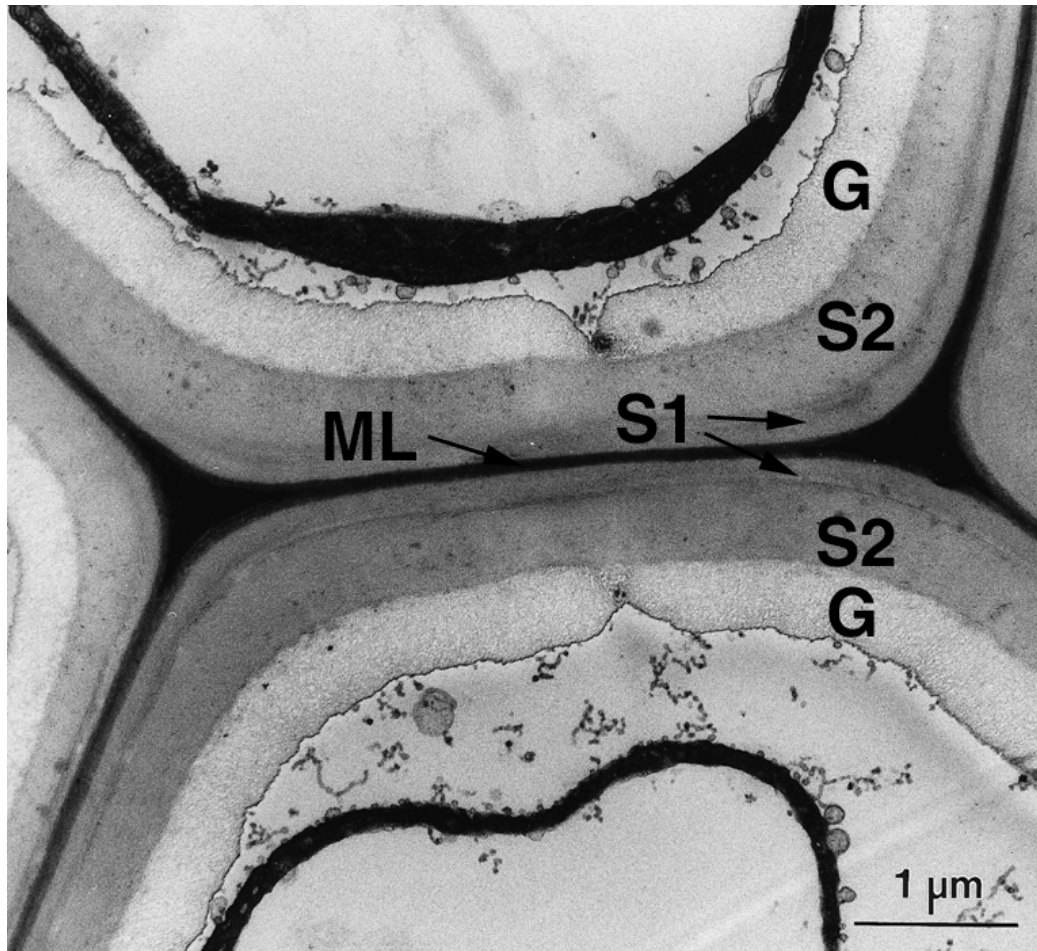


# D-zone upregulated CAZyme genes in poplar

Aspeborg et al., Plant Physiol. (2005) 137: 983



# Using tension wood transcriptomics data to identify xylan biosynthetic genes



27 highly expressed CAZyme genes were downregulated in TW

Andersson-Gunnerås et al., Plant J (2006)

Micrograph by K. Pickering and J. R. Barnett

# Bioinformatics approach to find xylan-biosynthetic genes in Arabidopsis

Brown et al., Plant Cell (2005) 17: 2281.

Genes co-regulated with irx3

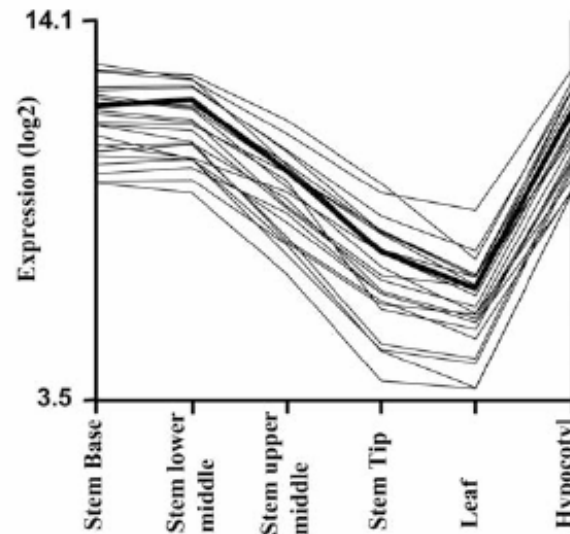


Figure 2. Interpretation and Analysis of Microarray Data.

# Novel *irx* mutants with reduced xylan content identified

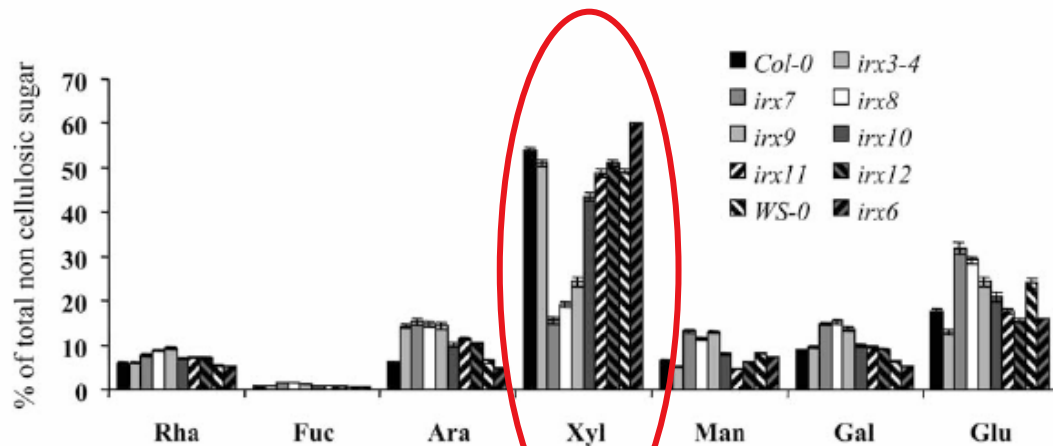
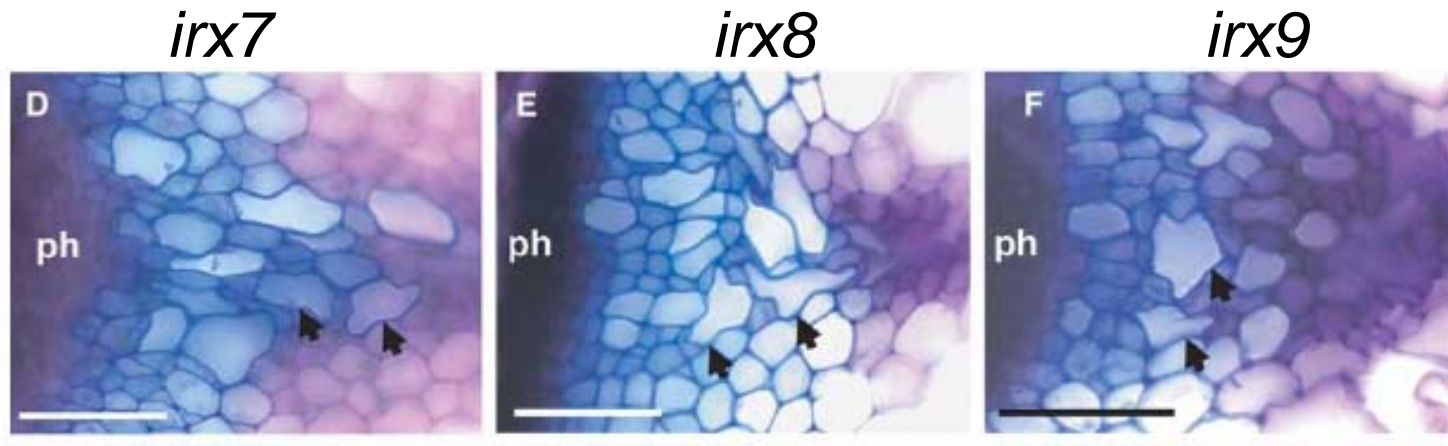


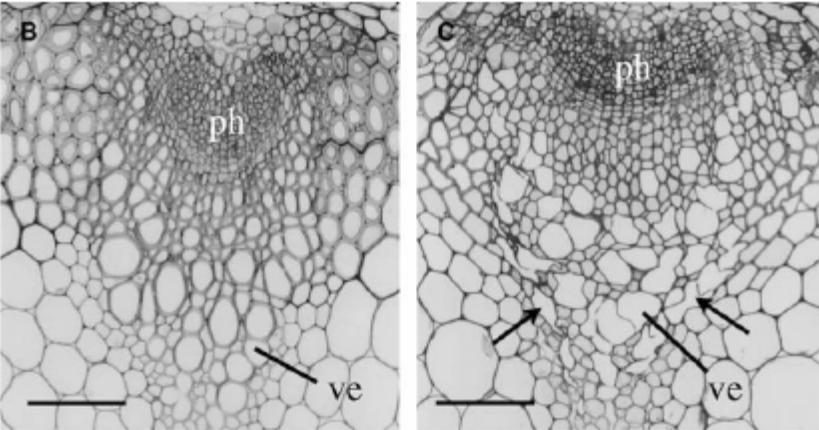
Figure 10. The Noncellulosic Carbohydrate Composition of Cell Wall Material from Stems of Wild-Type and Insertion Mutant Lines.

Individual sugars are expressed as a percentage of the total cell wall sugar. Material used was from the late stage of development. Standard error bars are shown ( $n = 5$ ).

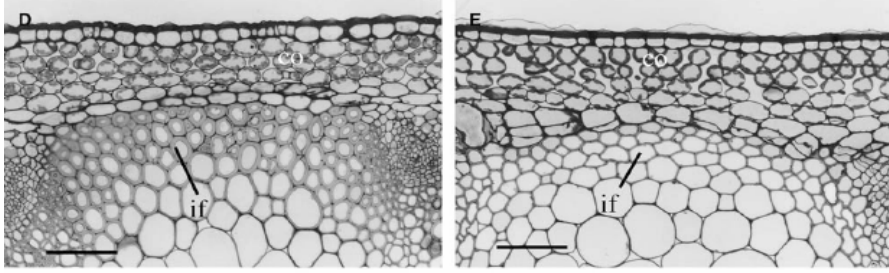
# *Arabidopsis Fragile Fiber8*, Which Encodes a Putative Glucuronyltransferase, Is Essential for Normal Secondary Wall Synthesis

Ruiqin Zhong,<sup>a,1</sup> Maria J. Peña,<sup>b,1</sup> Gong-Ke Zhou,<sup>a</sup> C. Joseph Nairn,<sup>c</sup> Alicia Wood-Jones,<sup>c</sup> Elizabeth A. Richardson,<sup>a</sup> W. Herbert Morrison III,<sup>d</sup> Alan G. Darvill,<sup>b</sup> William S. York,<sup>b,2</sup> and Zheng-Hua Ye<sup>a</sup>

## Irregular Xylem Phenotype



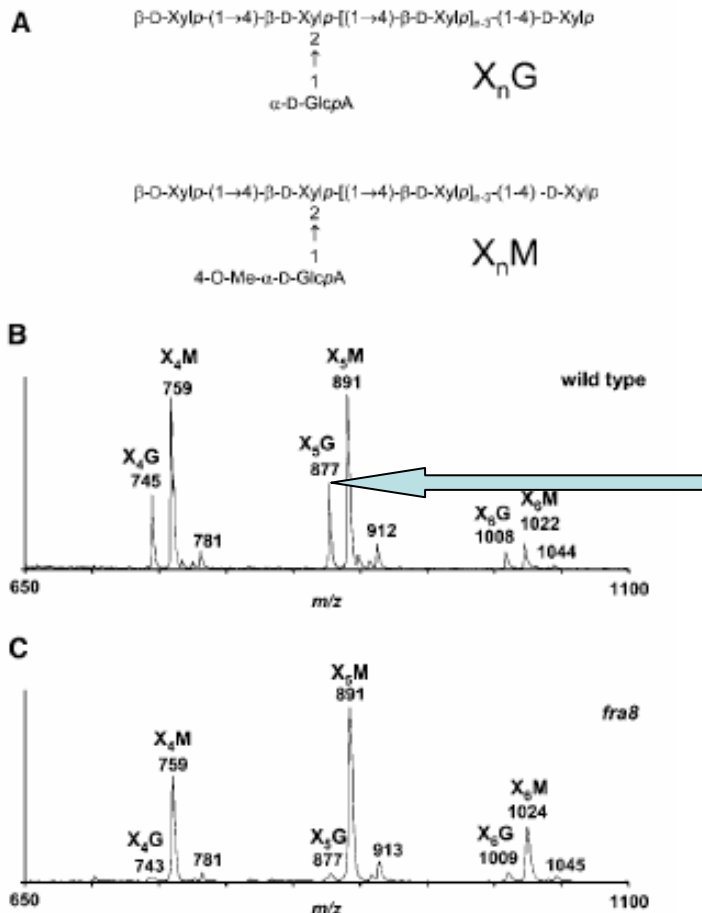
## Fiber cell wall defect



# *fra8* is deficient in xylan and cellulose

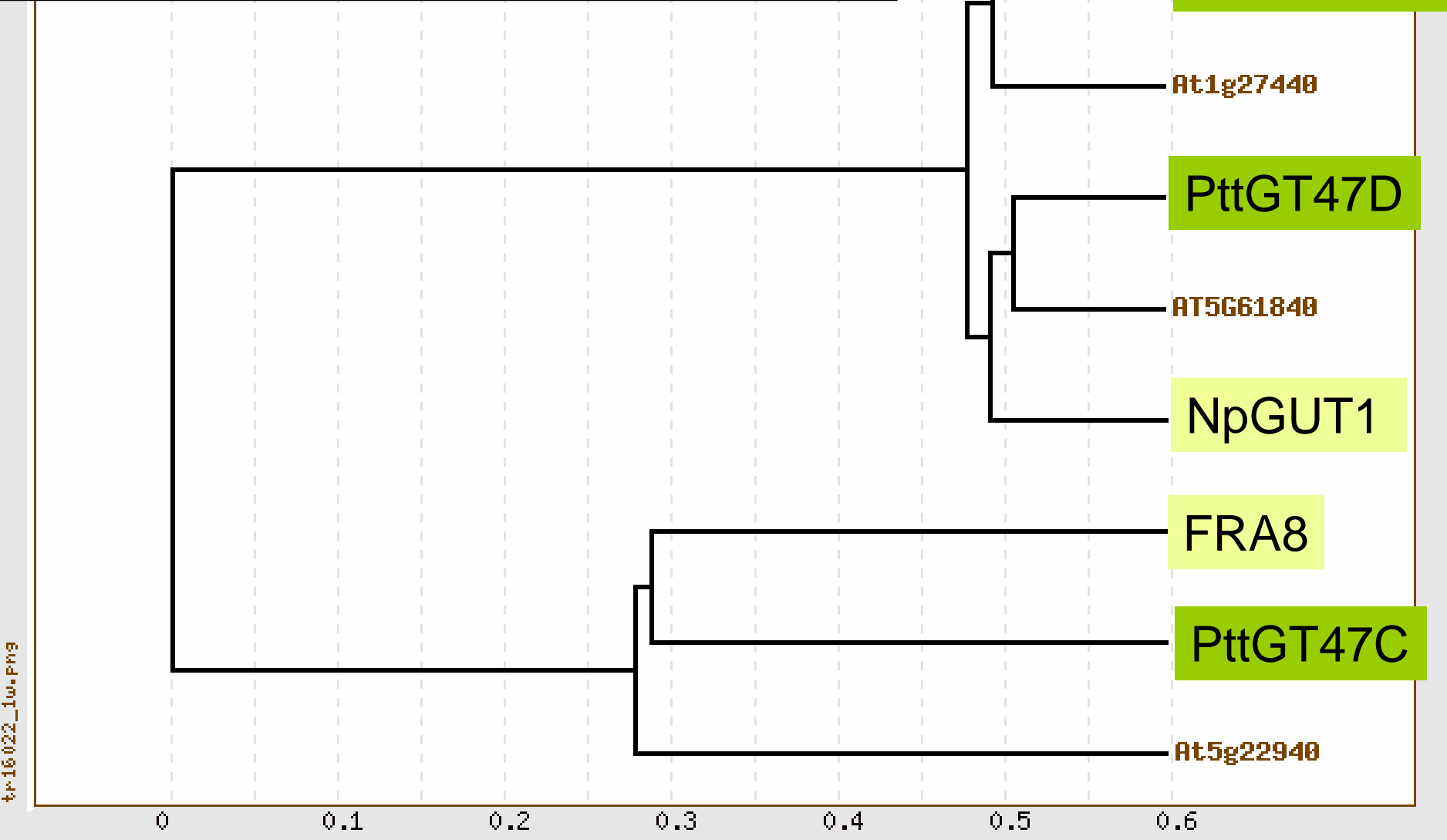
**Table 4.** Glycosyl Compositions of Fractions from Wild-Type and *fra8* Cell Walls

Cell Wall Fraction		Rha	Fuc	Ara	Xyl	Man	4-O-Me-GlcA	Gal	Glc	GalA	GlcA	Total
Cellulose	WT								331.65			
	<i>fra8</i>								243.45			
Total	WT	11.62	3.28	13.42	118.75	8.64	12.18	23.29	358.09	22.46	8.57	580.29
	<i>fra8</i>	15.22	4.89	19.76	53.50	14.24	9.24	31.50	276.83	31.96	3.10	460.23



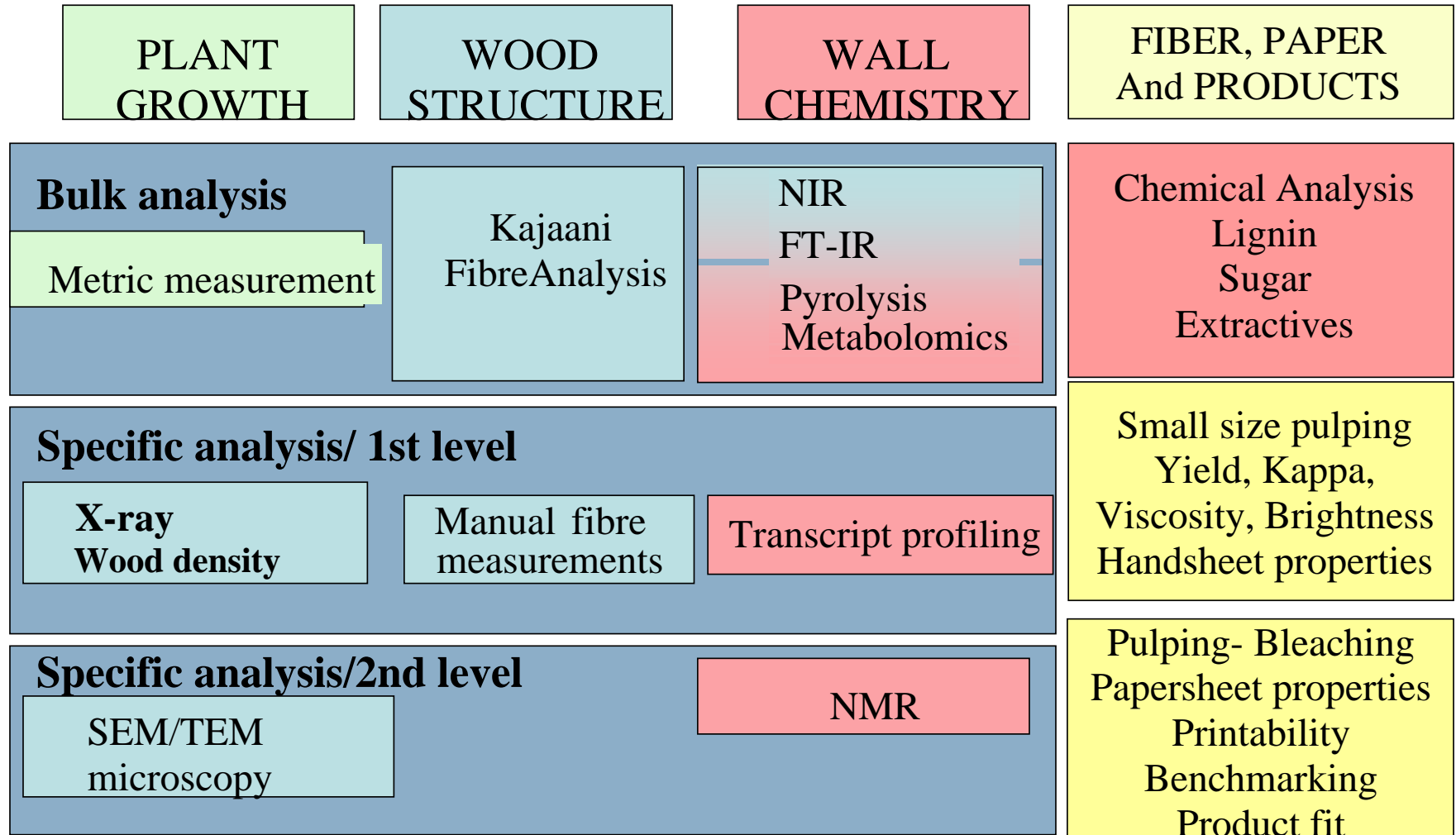
...and specifically affected in non-methylesterified GlcA

# Sub-branch I of sub-family A of GT47



tr:16.022\_1w.png

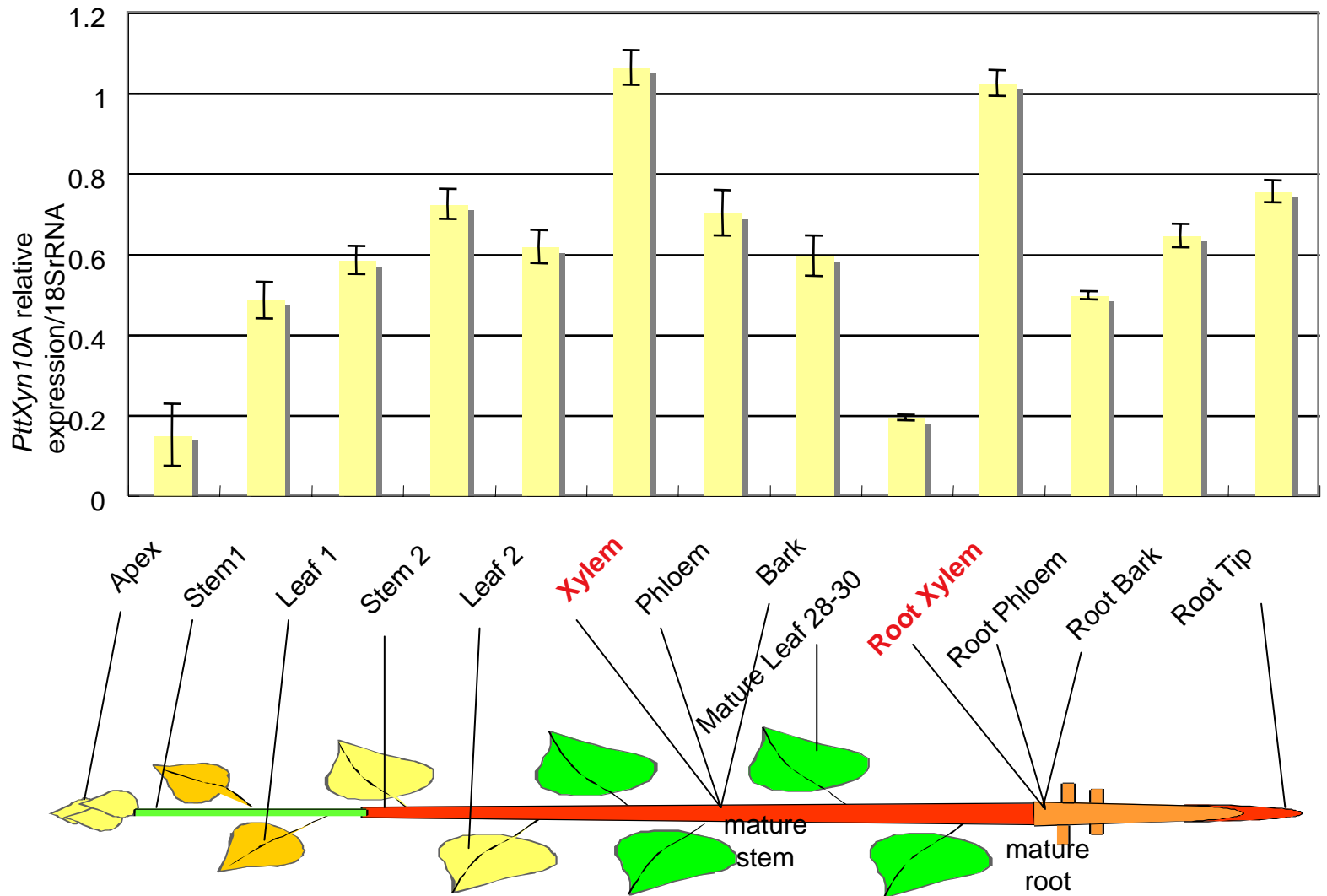
# 13 CAZyme genes included in SweTreeTechnologies functional screening program



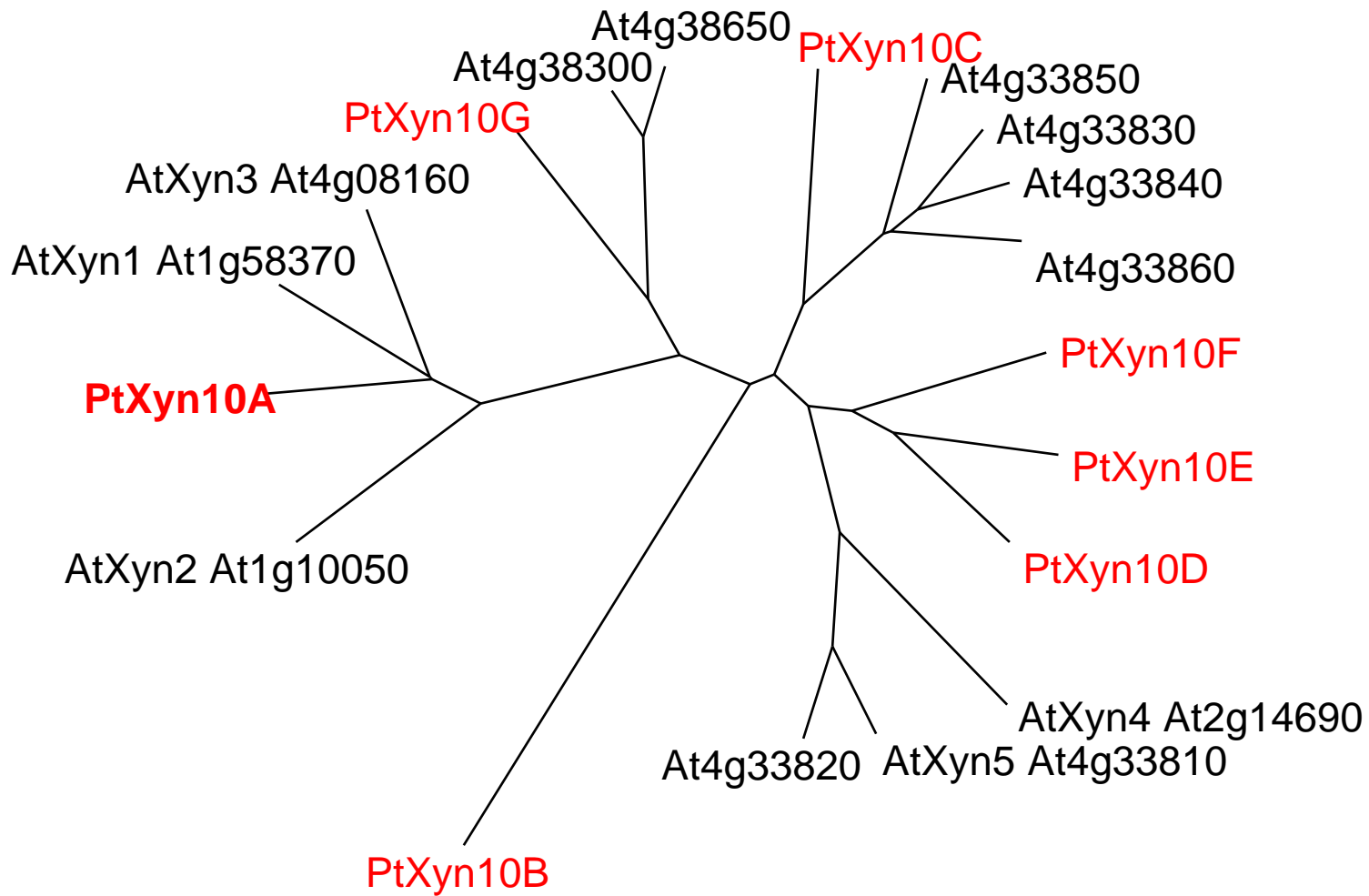
# Functional studies of putative endoxylanase PttXyn10A in poplar

Junko Takahashi, Tatsuya Awano, Åsa Kallas, Joanna Lesniewska, Anne Gouget, Fredrik Berthold, Tuula T. Teeri, Björn Sundberg, and Ewa J. Mellerowicz. In prep.

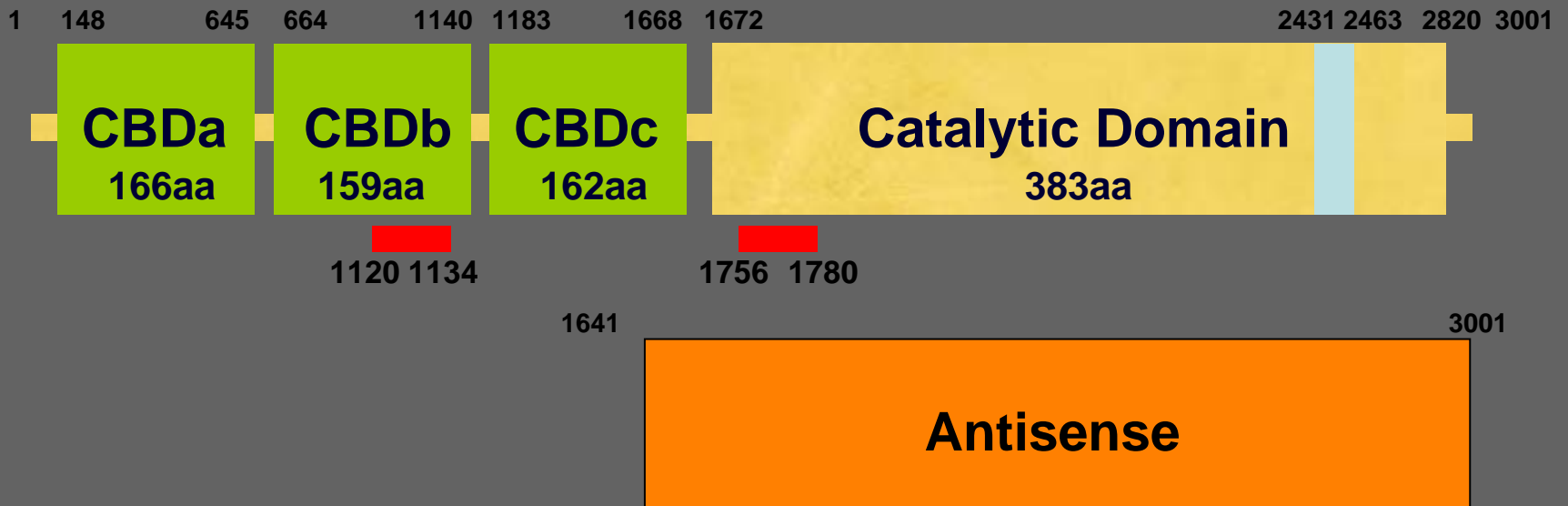
# PttXyn10 is highly expressed in developing xylem



## *A. thaliana* and *Populus trichocarpa* GH10genes



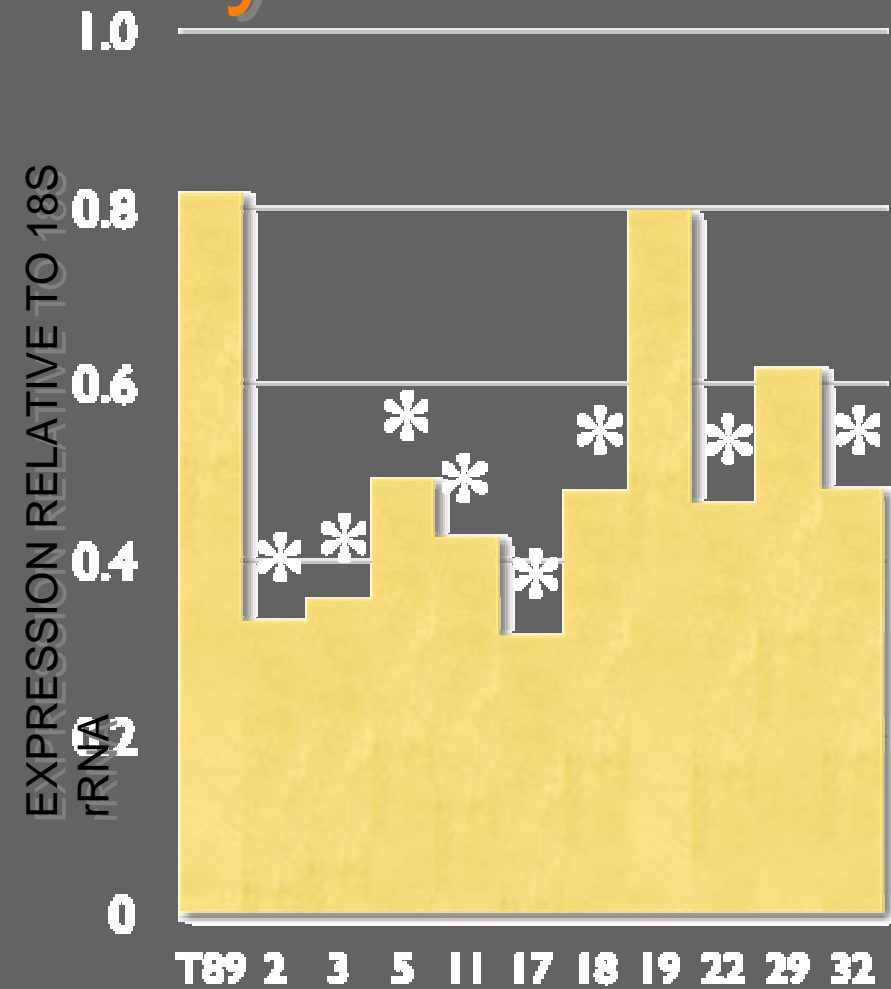
# Structure of PttXyn10



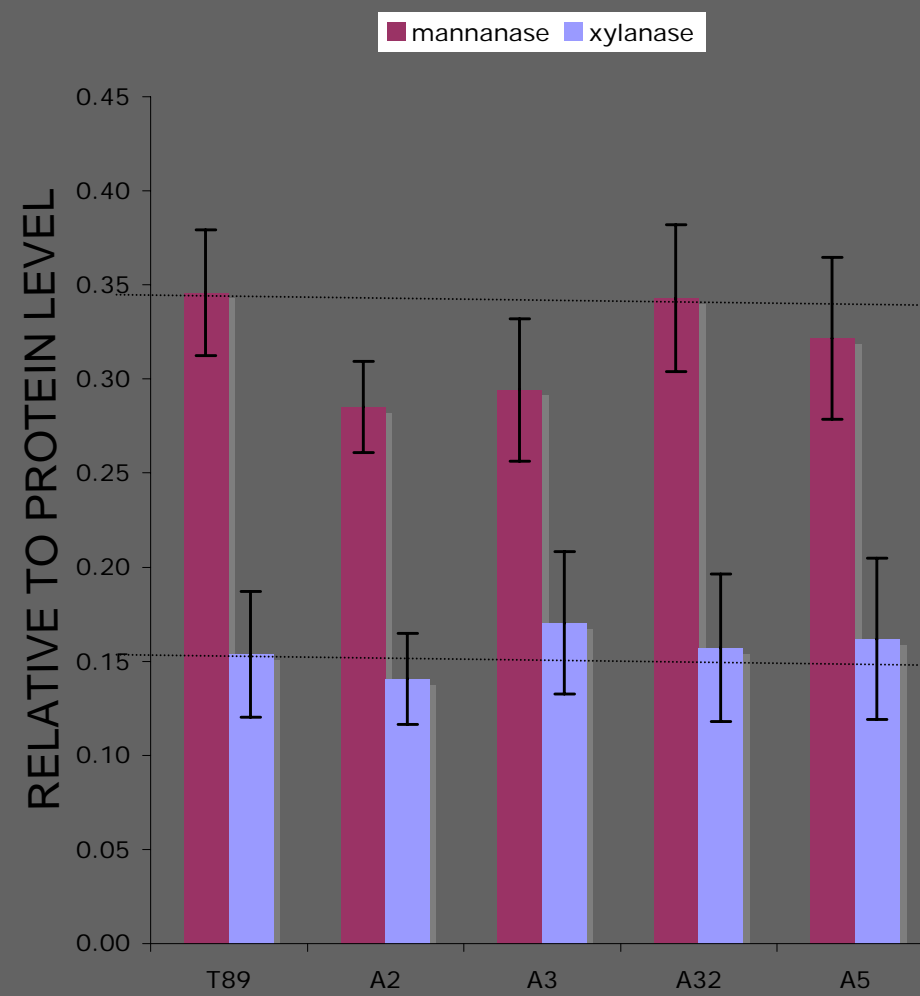
■ Glycosyl hydrolase family 10  
Active site

■ Primer for RT-PCR

# mRNA Expression by RT-PCR

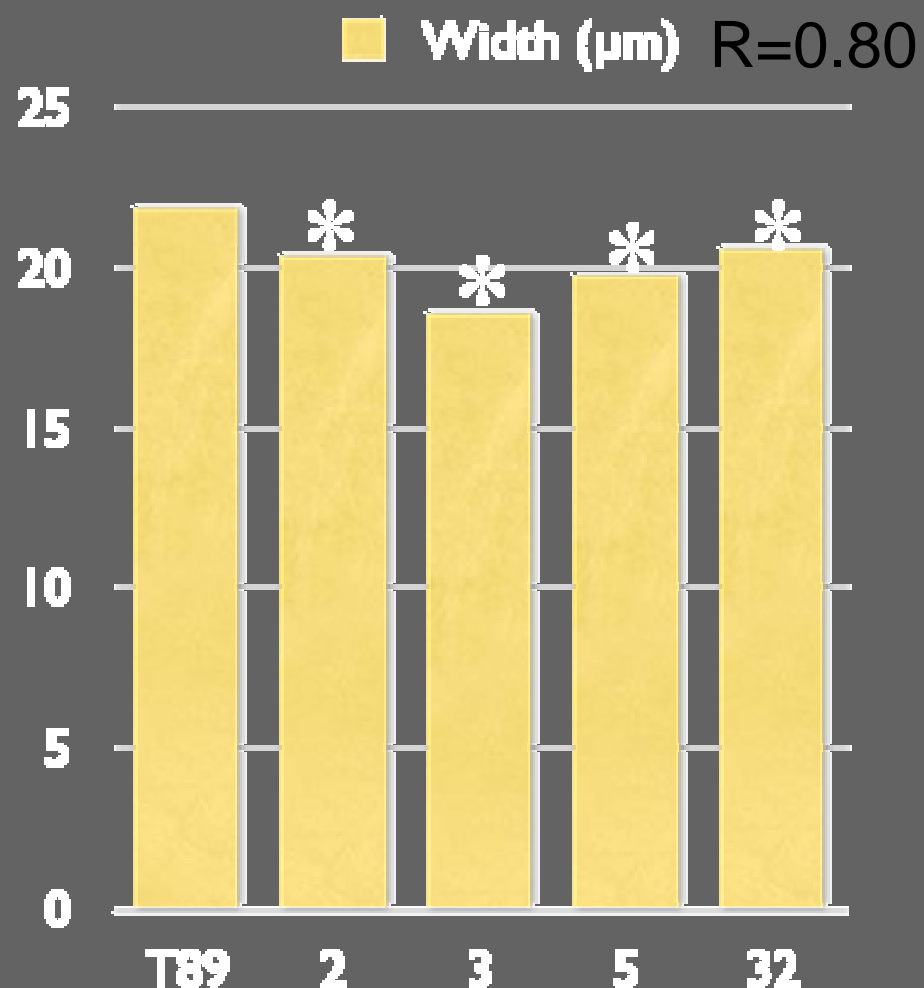
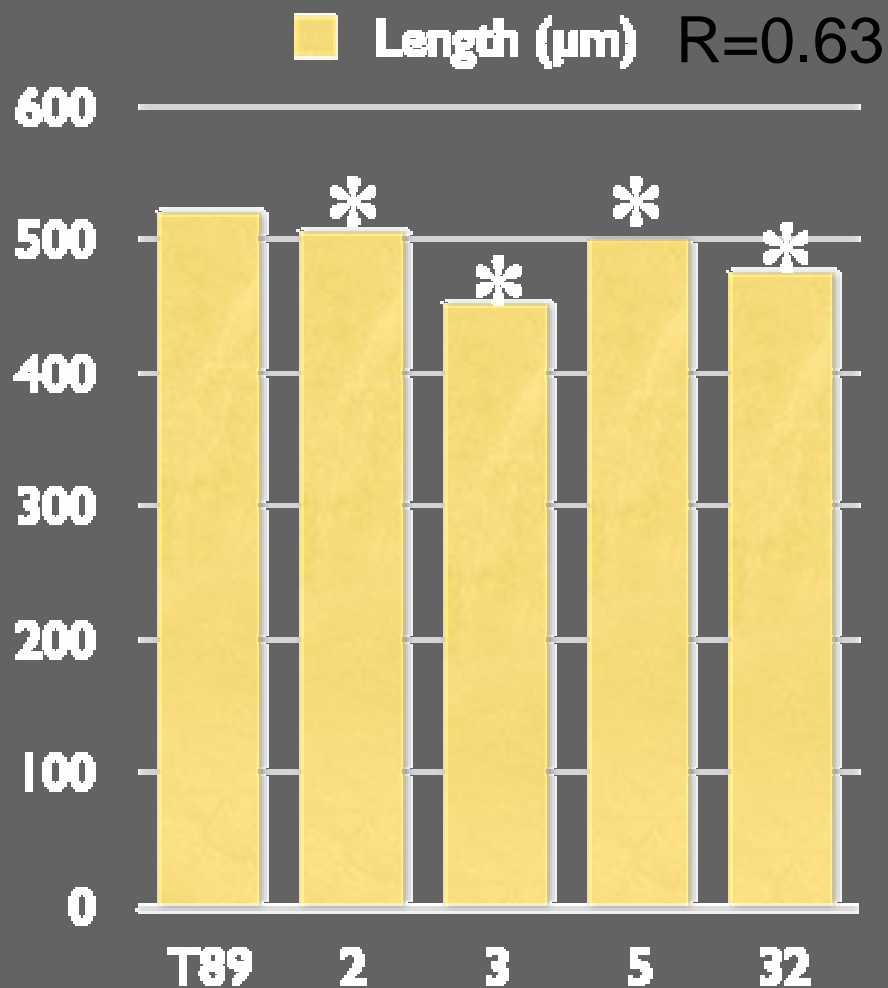


# Enzyme activity



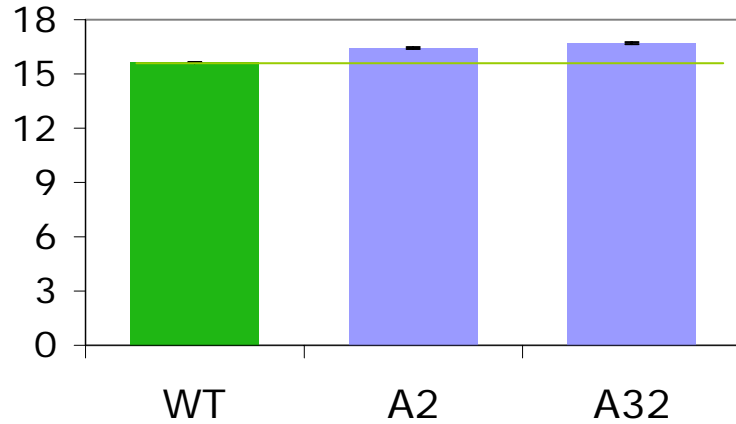
# Antisense Xyn10 lines have shorter and thinner fibers

But no effect on vessel elements

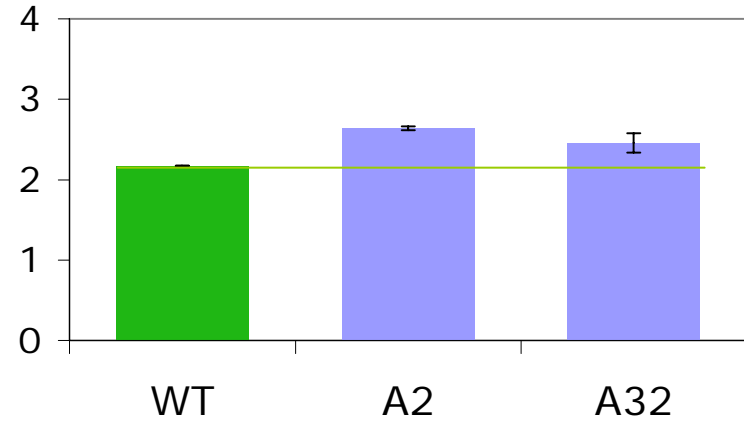


# Wood carbohydrates, g/100g dw

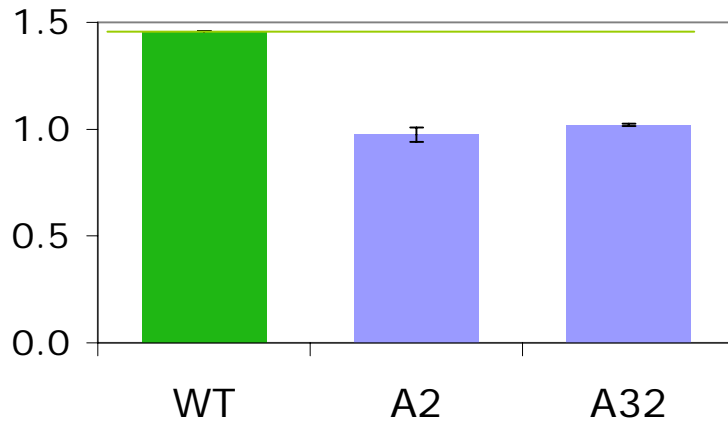
xylan



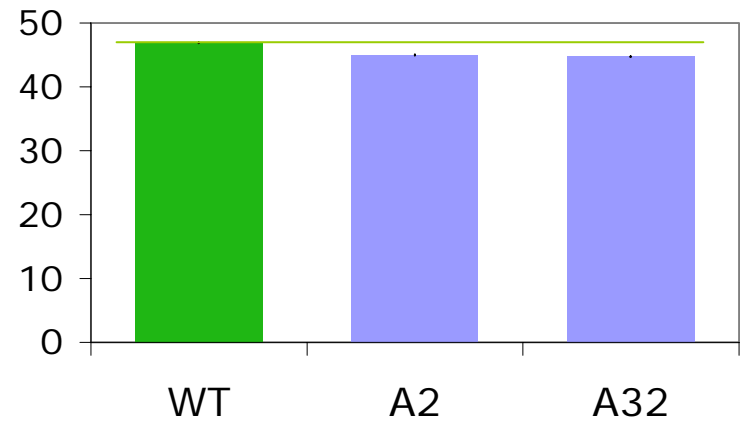
mannan



galactan

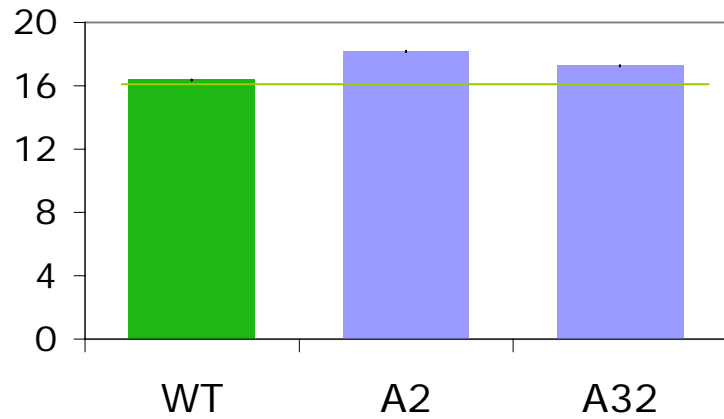


glucan

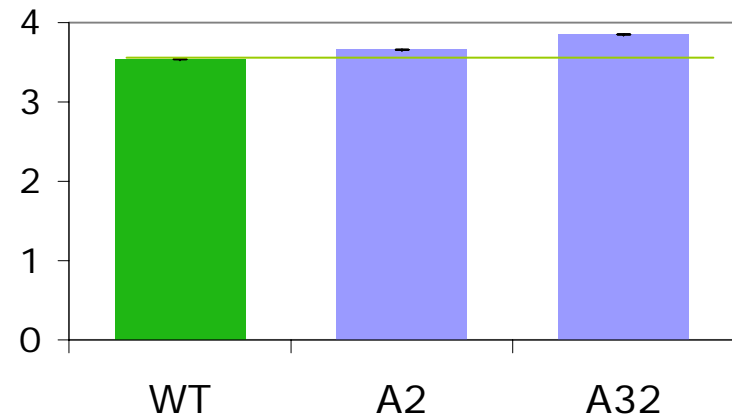


# Wood lignin, g/100g dw

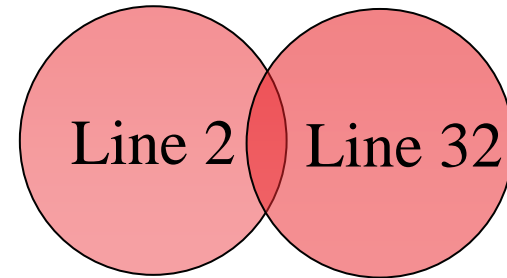
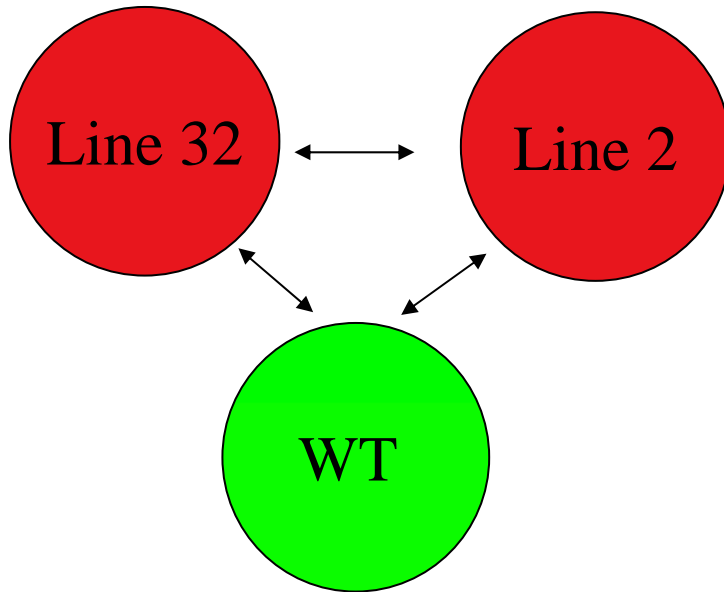
Klason lignin



Acid soluble lignin



# Transcriptome of antisense plants by 25K poplar EST array



- Downregulation of genes associated with cellulose biosynthesis:
  - AGP FLA12,
  - COBRA-like
  - fructokinase
- No effect on monolignol biosynthetic genes

# Antisense Xyn10A - Conclusions:

- Xyn10A, putative xylanase, is involved in the regulation of xylan content
- Xylan content regulates fiber growth but does not affect vessel growth
- Xyn10A regulates carbohydrate wall composition perhaps by regulating xylan recycling: downregulation of Xyn10A inhibits cellulose biosynthesis
- Xylan amount correlates with lignin content

# Future plans:

- Overexpression of Xyn10A in poplar
- Functional studies of candidate xylan biosynthetic/modifying genes in poplar to affect xylan content and xylan composition

# Other ongoing projects

- Cellulases in wood development (Junko Takahashi **PhD student**)
- XTH genes expressed in wood (Dr. Alicja Banasiak, Dr. Satoshi Endo)
- Screening for genes involved in the onset of secondary wall formation (Dr. Satoshi Endo)
- Role of PME in wood development (David Öhman **MSc student**, Dr. Joanna Lesniewska)

## Collaborations

Emma Master  
Luc Richard  
Fabienne Micheli  
Bernard Henrissat  
Herman Höfte  
Henrik Scheller  
Björn Sundberg  
Tuula Teeri  
Paul Gatenholm  
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STT

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