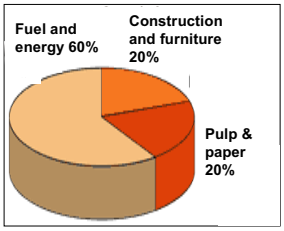
Engineering lignin biosynthesis for biofuels

Wout Boerjan
EPSO meeting
London
May 28-29, 2008

Wood consumption



World wood consumption

Forests and Wood

Need for genetic improvement

Plantation forests occupy 5% of all forests and deliver 35% of industrial roundwood

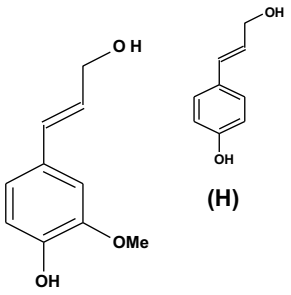
FAO: ⟹ higher wood yield / ha
⟹ improved wood quality

Outline of presentation

1. Introduction to lignin
2. Engineering low-lignin trees
3. Deep phenotyping
4. Transgenic trees for bio-ethanol
5. Science and society

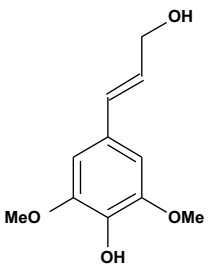
1. Introduction to lignin

Predominant monolignols



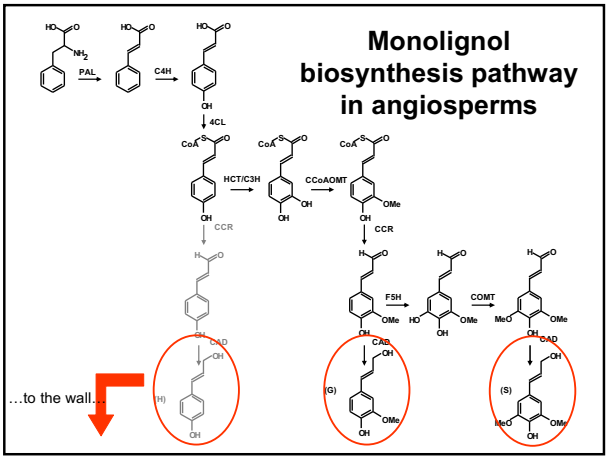
Coniferyl alcohol (**G**)

(H)

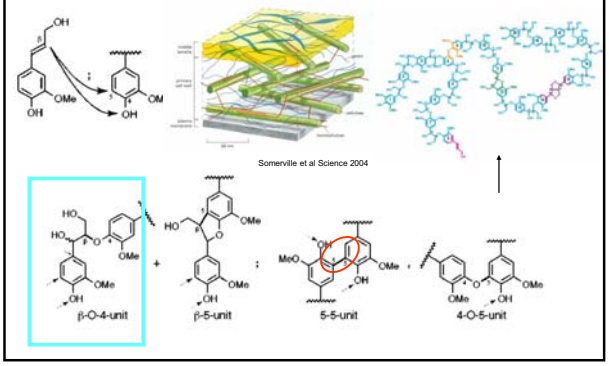


Sinapyl alcohol (**S**)

Monolignol biosynthesis pathway in angiosperms

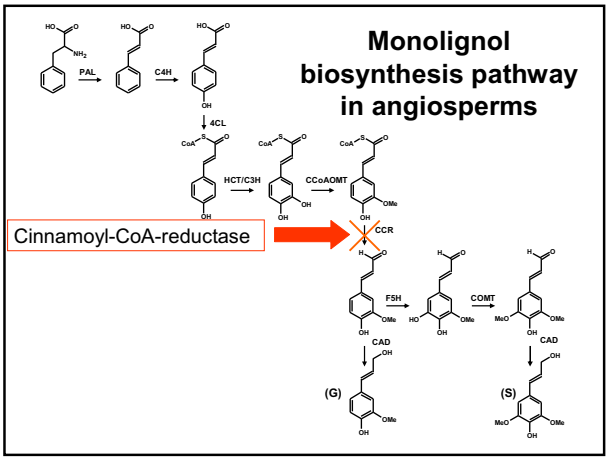


Coupling of monolignols in the wall



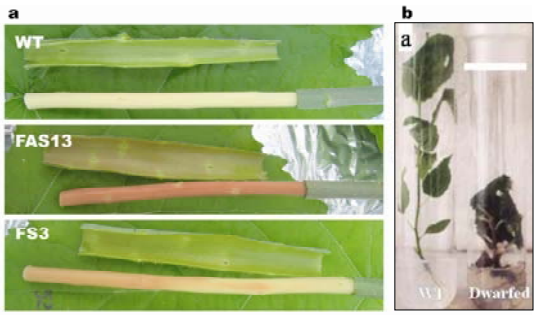
2. Engineering low-lignin trees

Monolignol biosynthesis pathway in angiosperms



CCR down-regulated poplar

Phenotype



Twigs from field-grown trees

WT, 2 months; CCR, 7 months

CCR down-regulated poplar

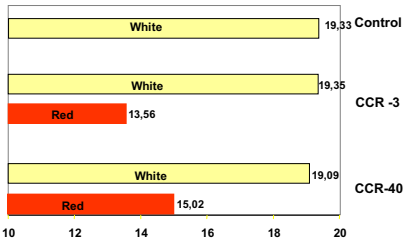
Differential suppression

Debarked stem

Phloroglucinol staining

CCR down-regulated poplar

Lignin amount

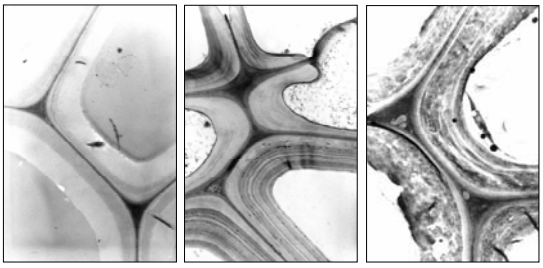


Debarked stem

Klason lignin

CCR down-regulated poplar

Cell wall phenotype



WT CCR ↓ CCR↓

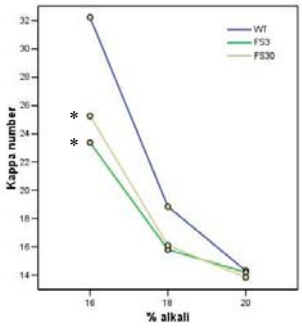
Field trial CCR down-regulated poplar

Orléans, France (4-year-old)

4 lines + WT; 5 blocks; 2 plants/line/block = 10 plants per line

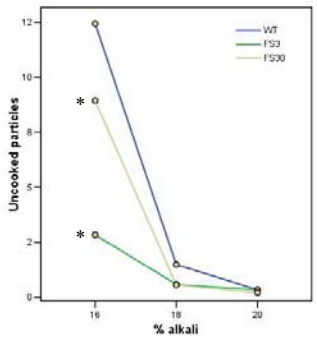
Pulping CCR down-regulated poplar

Kappa number versus % alkali



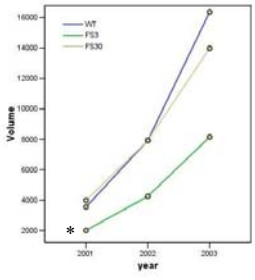
Pulping CCR down-regulated poplar

Uncooked particles versus % alkali



Growth CCR down-regulated poplar

Volume



Leplé et al. The Plant Cell, 2007

Conclusions

-> CCR down-regulation translates to improved pulping (lower Kappa, higher pulp yield, less uncooked particles)

-> But, ….growth is affected in strongly down-regulated lines

-> molecular breeder needs to exploit allelic diversity

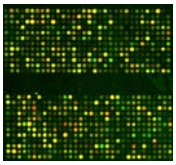
-> what are the causes of pleiotropic effects?

3. Deep phenotyping

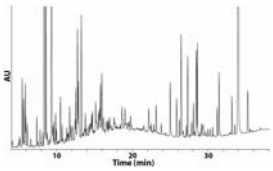
Transcriptome/metabolome

Aim: Relation between lignin biosynthesis and other metabolic pathways

- Transcriptome
- Metabolome

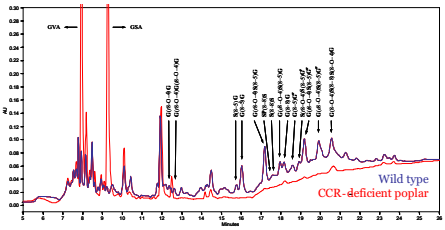


≈ 25K poplar array

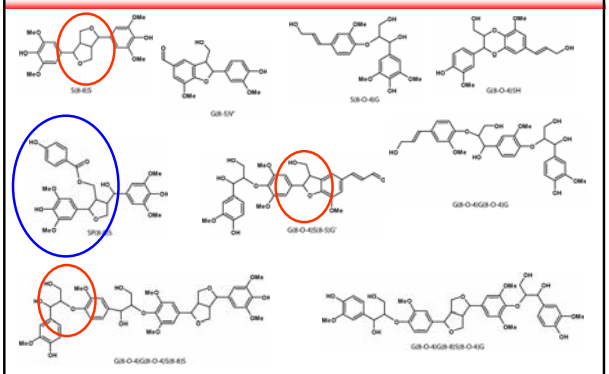


LC and GC-MS profiling

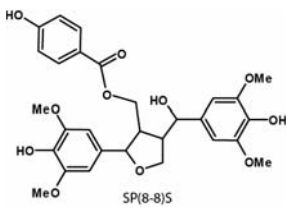
LC-MS analysis of phenolics

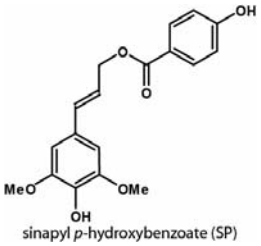


Monolignol coupling products



Sinapyl-*p*-hydroxybenzoate is a monomer



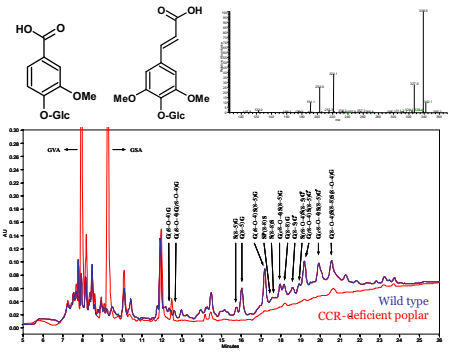


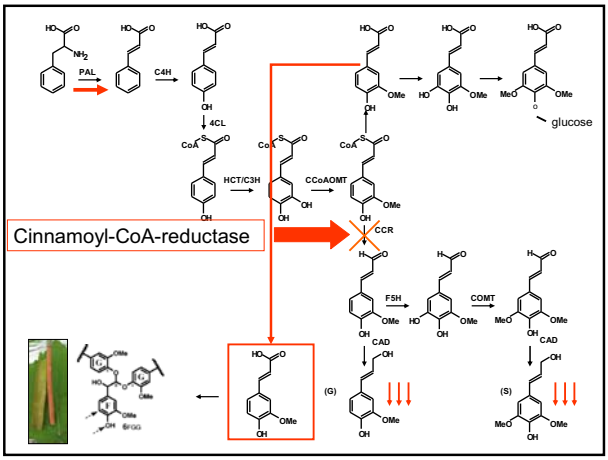
sinapyl *p*-hydroxybenzoate (SP)

Lignomics: LC-MS analysis of phenolics

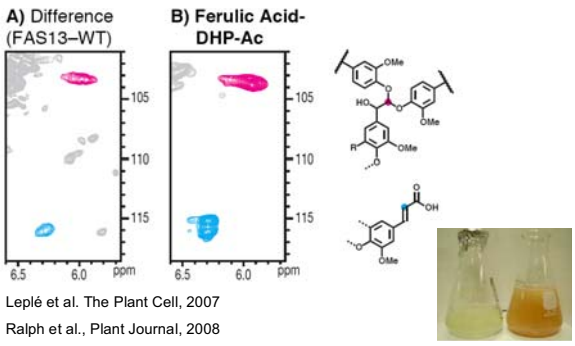
Morreel et al., Plant Physiol 2004 a; 2004 b

LC-MS analysis of phenolics





Ferulate cross-couples with lignin

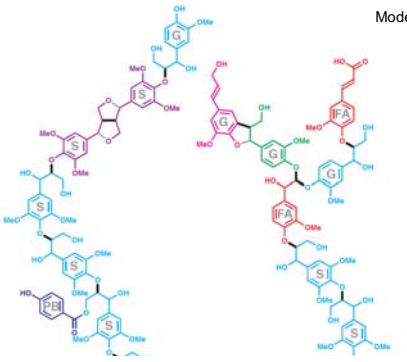


Leplé et al. The Plant Cell, 2007

Ralph et al., Plant Journal, 2008

Ferulate cross-couples with lignin

Model by J. Ralph



Vanholme, Morreel, Ralph, Boerjan *Curr. Op. Plant Biol.*, 2008, in press

Conclusions

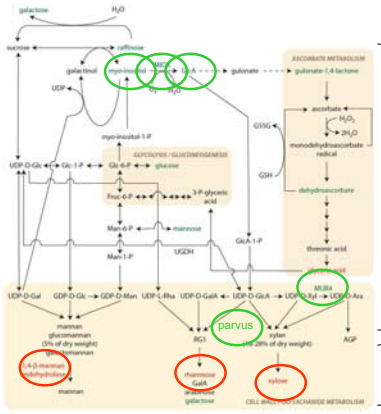
- Ferulate is a new monomer
- Incorporation of ferulate might cause the red-brown xylem coloration
- New bonds are made: acetal bonds that are cleavable in mild acid
- This opens perspectives for modifying lignin structure

25K microarray -> 49 differential genes

- Cell cycle & processing (1)
  - ↑ Ras-rel. GTP-bind.
- Transcription (3)
  - ↑ PHD finger fam.
  - ↓ Zinc finger fam.
  - ↓ bHLH fam.
- Protein synthesis & fate (3)
  - ↑ Peptidyl-prolyl cis-trans isomerase
  - ↑ Ser carboxypeptidase
- Signal transduction (4)
  - ↑ Inositol 1,3,4-triP 5/6 kinase
  - ↓ Prot. Kinase fam.
  - ↓↑ LRR fam.
- Cell rescue, defense (6)
  - ↑ Metallothionein
  - ↑ Glutathion S-transferase
- Control of cell organization (2)
  - ↓ **Laccase**
- Cell/Environment regulation (2)
  - ↓ **AGP**
  - ↑ Vac. Proton ATPase
- Metabolism & energy (18)
  - ↑↑ PAL (2)
  - ↓ CCR
  - ↑ GS
  - ↑ NDP kinase II
  - ↓↓ **Glyc. Transf. (GT8) (2)**
  - ↑ **Mannan endohydrolase**
  - ↓ **UDP-xylose-4-epimerase**
  - ↓ **MIOX**
  - ↑ G6PDH
  - ↑ PSII reaction center fam
- Unclassified (13)

GC-MS profiling

- Organic acids (6)
  - Maleic acid
  - Fumaric acid
  - Malic acid
  - Succinic acid
  - Cis-Aconitic acid
  - Glyceric acid
- Hemicellulose (4)
  - ↑ Xylose
  - ↑ Rhamnose
  - ↓ Inositol
  - ↓ Glucuronic acid
- Sugar (6)
  - Melezitose
  - Raffinose
  - Glucose
  - Mannose
  - Galactose
  - Gulono-1,4-lactone
- Other (4)
  - Norvaline
  - Allantoin
  - Dehydroascorbic acid dimer
  - Benzoic acid



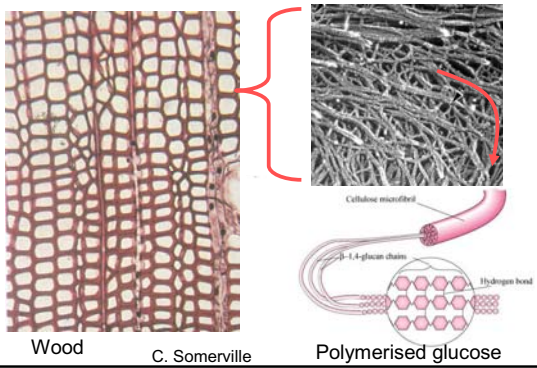
Wet chemistry indicates less lignin, less hemicellulose and more cellulose

Line	*n*	*Klason Lignin*	*Acid-insoluble Lignin*	*Acid-soluble Lignin*	*α- Cellulose*	*Hemi-cellulose*
WT	6	20.65 ± 0.22	17.70 ± 0.21	2.95 ± 0.05	48.22 ± 0.69	30.72 ± 0.69
FS3	5	***16.75 ± 0.16***	***14.54 ± 0.15***	***2.21 ± 0.05***	***56.55 ± 0.49***	***23.19 ± 0.70***
FS40	5	***16.64 ± 0.18***	***14.24 ± 0.18***	***2.40 ± 0.04***	***57.07 ± 0.81***	***24.10 ± 0.44***
		-20%	-20%	-22%	+ 17%	-23%

Leplé et al. The Plant Cell, 2007

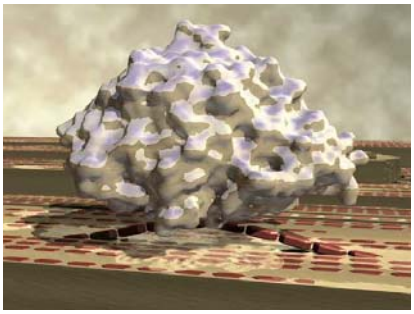
4. Transgenic trees for bio-ethanol

Lignocellulose to ethanol

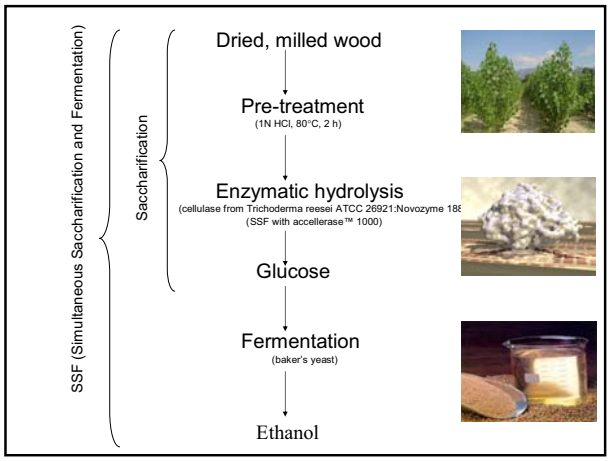


Wood C. Somerville Polymerised glucose

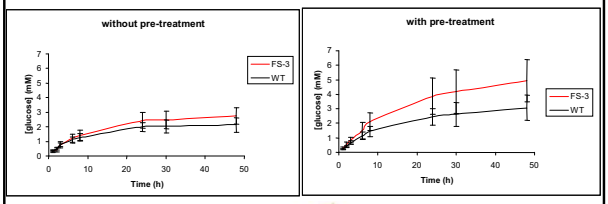
Lignocellulose to ethanol



Cellulase NREL



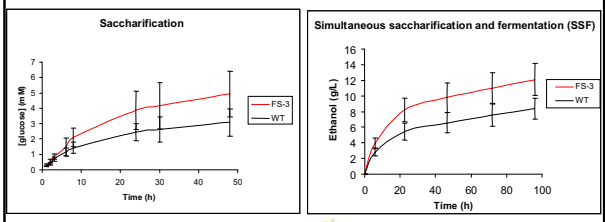
Saccharification of FS-3 and WT
(with and without acidic pre-treatment)



-> 28 % more glucose released by CCR-down-regulated poplar

-> 60 % more glucose released by CCR-down-regulated poplar

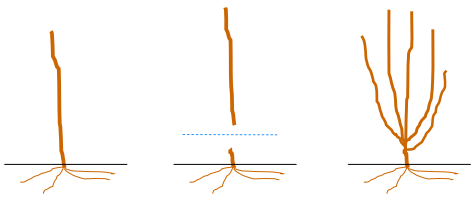
Saccharification and SSF of FS-3 and WT
(with acidic pre-treatment)



-> 60 % more glucose released by CCR-down-regulated poplar

-> 50 % more ethanol released by CCR-down-regulated poplar

Short Rotation Coppice



Year 1 End of year 1 Year 2-4

Short Rotation Coppice (SRC)

INBO; Belgium

Short Rotation Coppice (SRC)

INBO; Belgium

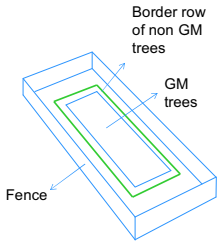
- 15,000 cuttings/ha
- 3-year rotation for 15-20 years
- ~12 ODT/ha/y -> 30 ODT/ha/y

5. Science and society

VIB's field trial with CCR-downregulated poplar

- Planting distance: 0.75 en 1.50 m
- Distance between rows: 0.75 m
- Density of 15,000 trees/ha
- WT and 2 CCR downregulated lines
- 250 trees/line
- Low input of fertilizer
- Slight herbicide treatment
- Harvest after three years

- No flowering
- No food



Science and society

Public consultation -> public is worried about safety

Biosafety Advisory Council -> field trial is safe!

Greenpeace asks ministers not to sign

-> waiting for signature ministers

Acknowledgements

- Kris Morreel
- Geert Goeminne
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