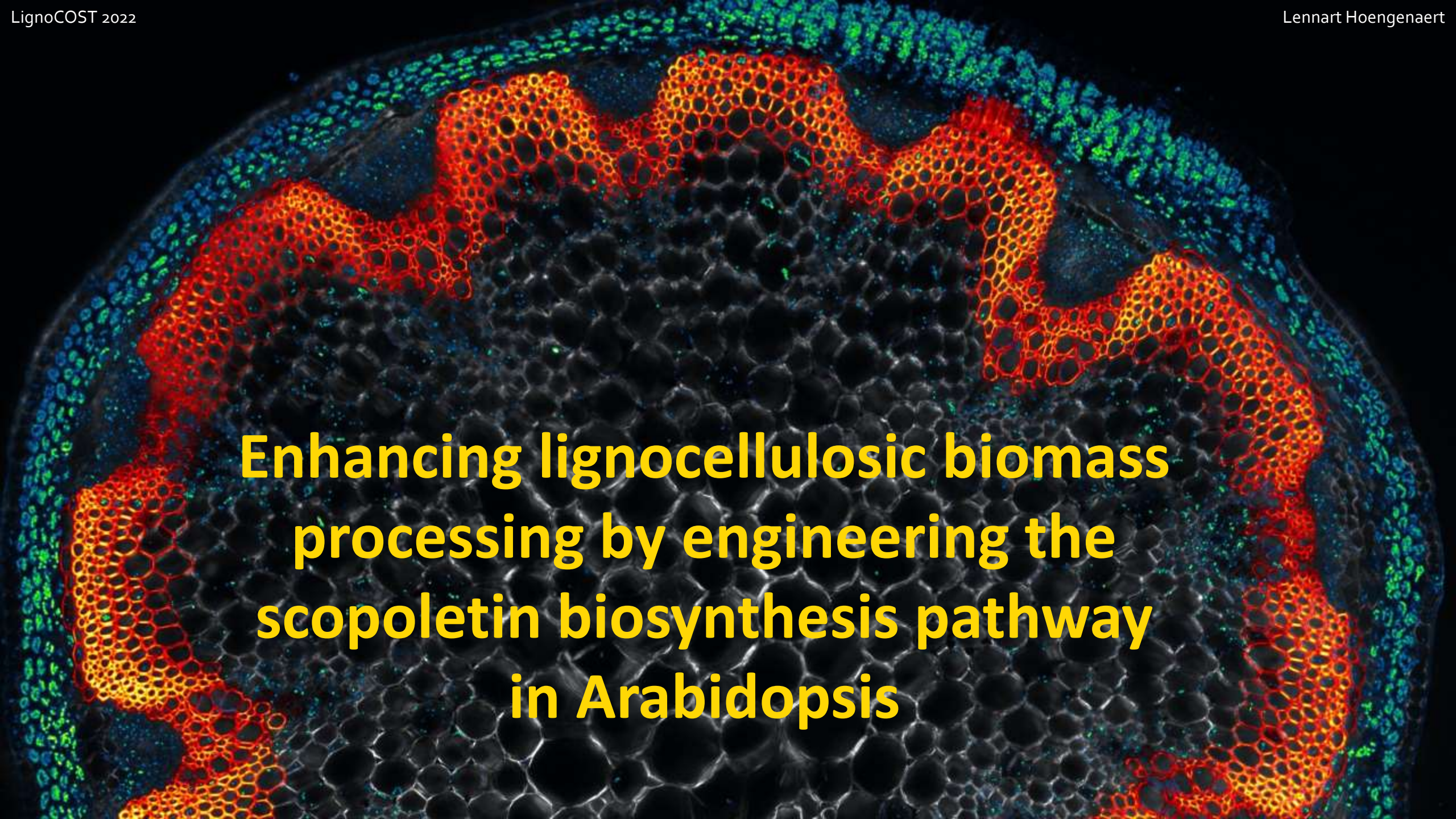
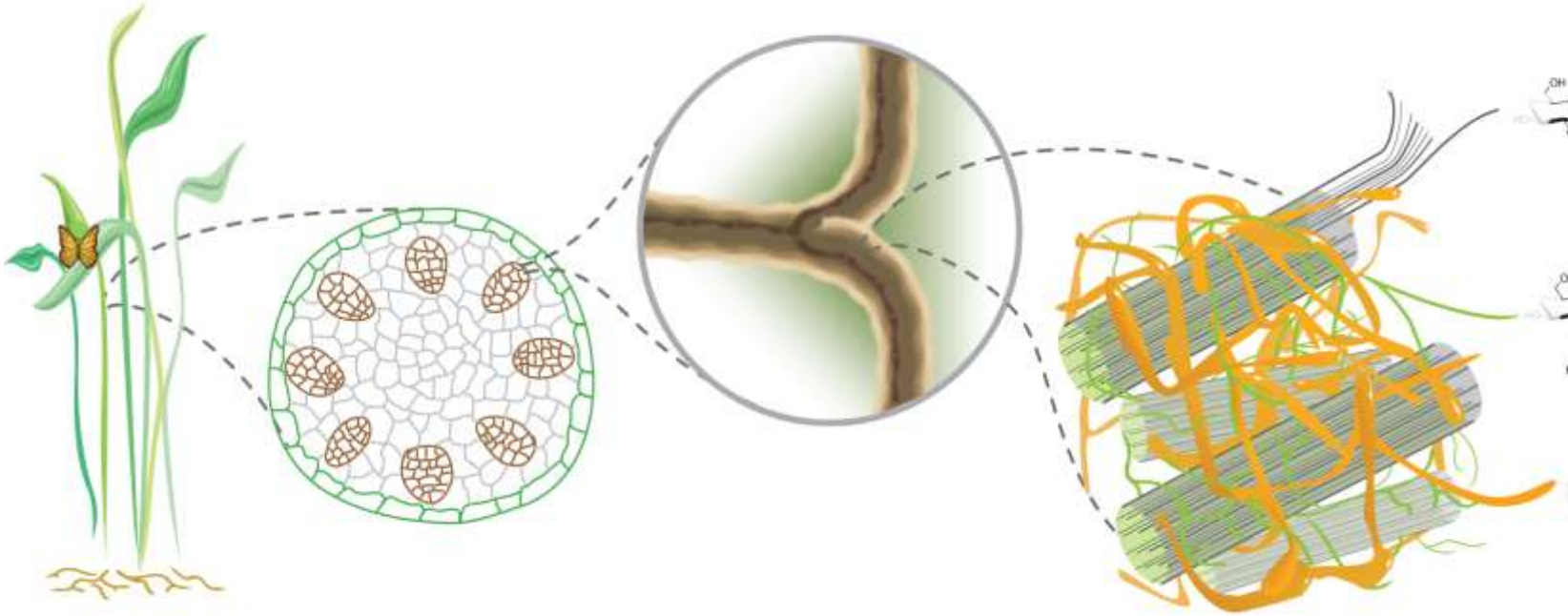
Lennart Hoengenaert

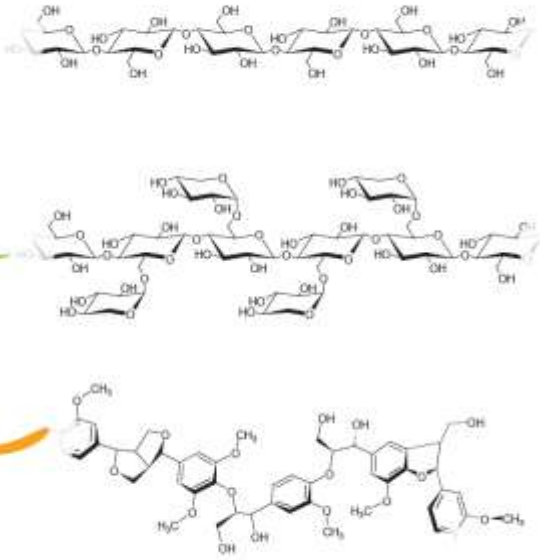


Enhancing lignocellulosic biomass processing by engineering the scopoletin biosynthesis pathway in Arabidopsis

Transition from a fossil based to a biobased economy

Research @ bioenergy group



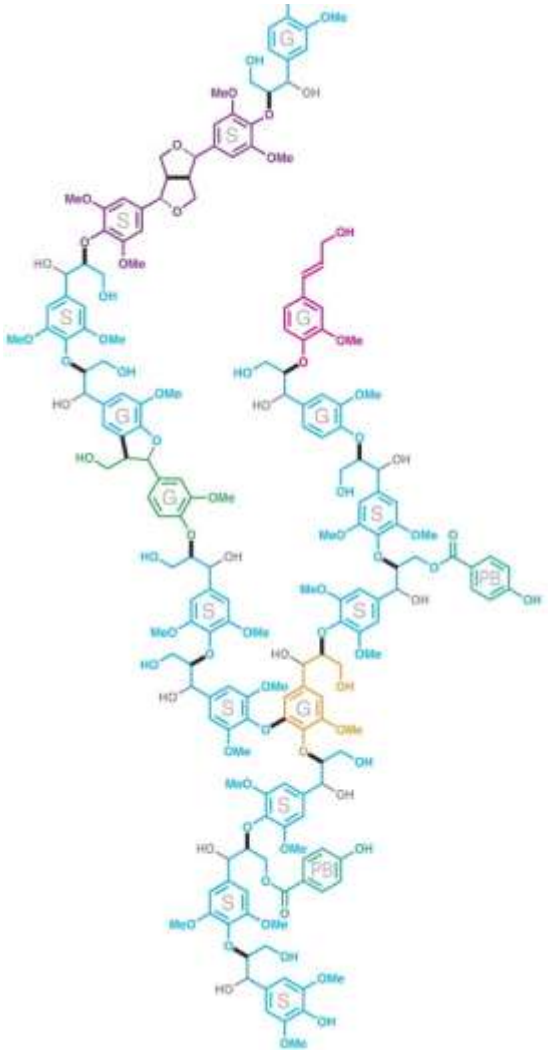


Cellulose

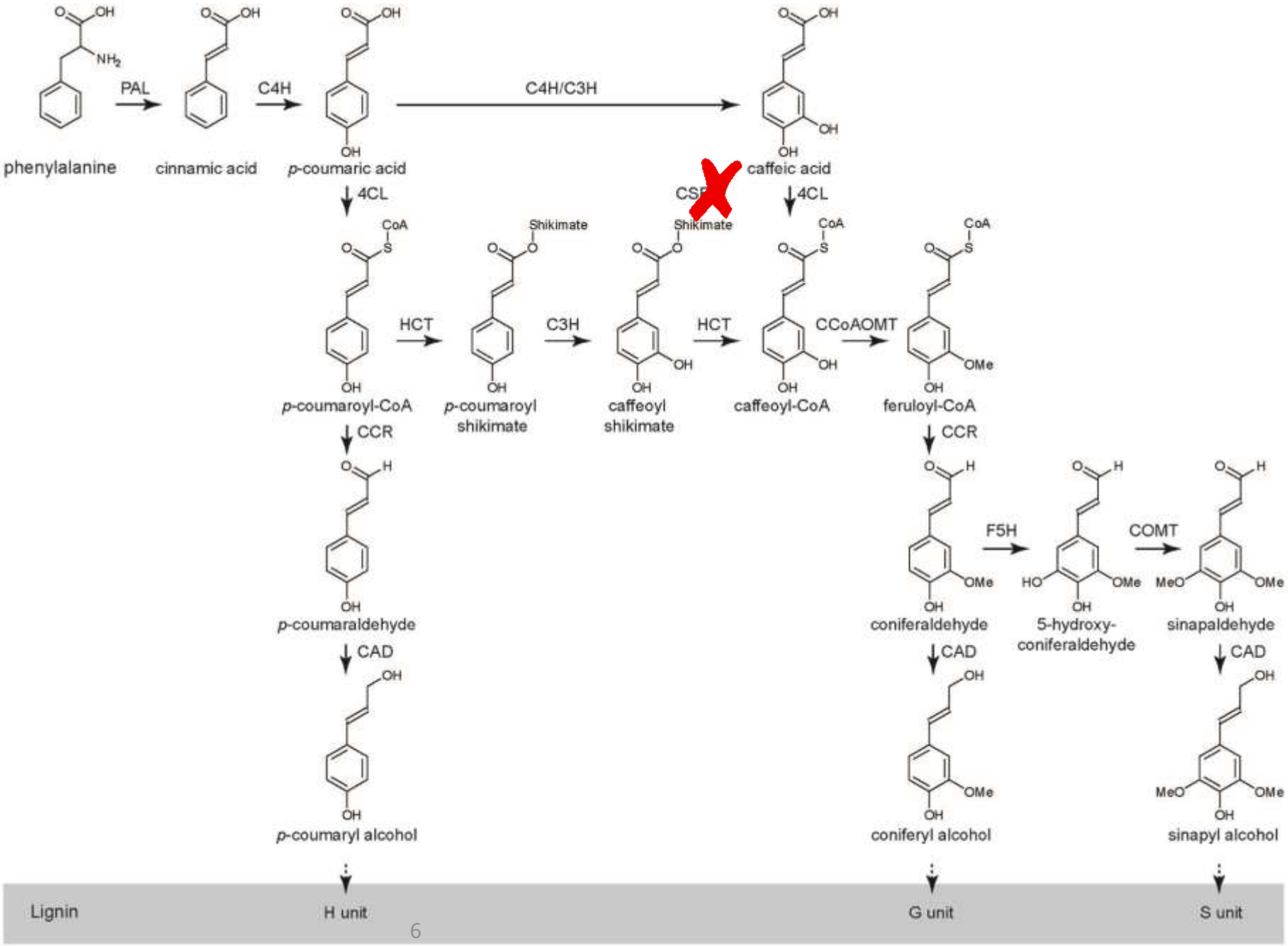
Hemicellulose

Lignin

Lignin biosynthesis



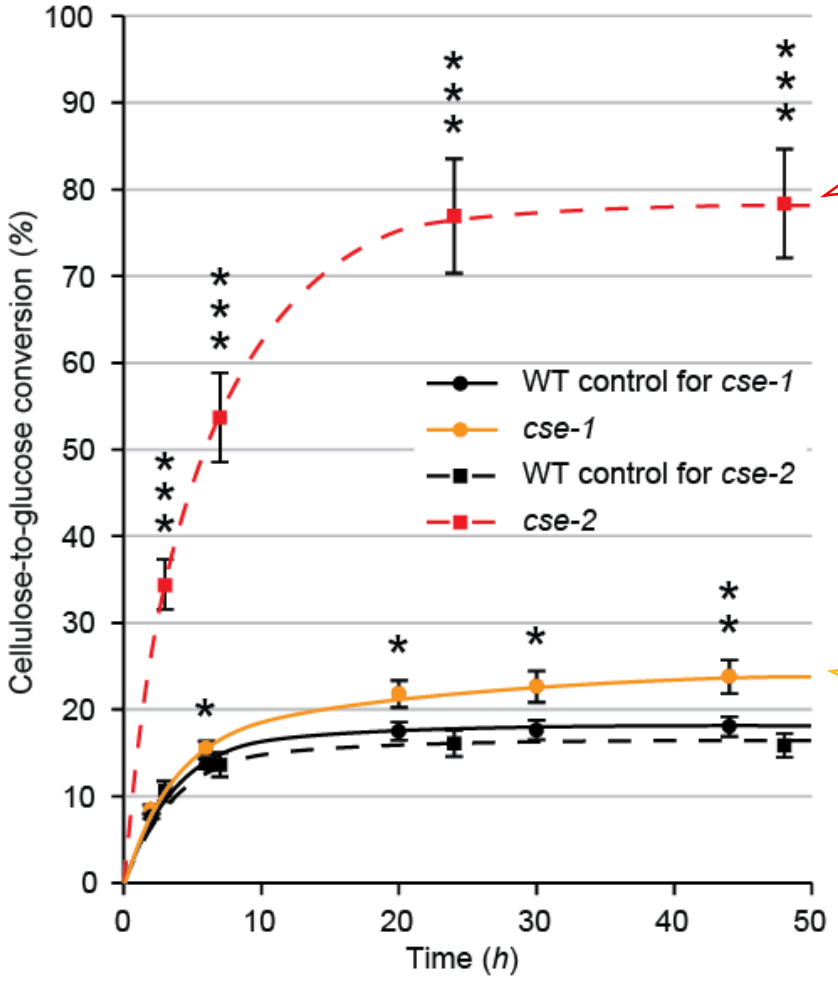
Stewart *et al.* (2009), Plant Phys.



Miedes *et al.* (2014), Front. Plant Sci

80% cellulose to glucose conversion in *cse* mutants, without pretreatment

Vanholme *et al.*, *Science* (2013)



KNOCK-OUT

✓ 4 x improved saccharification efficiency (80% cellulose converted)

KNOCK-DOWN

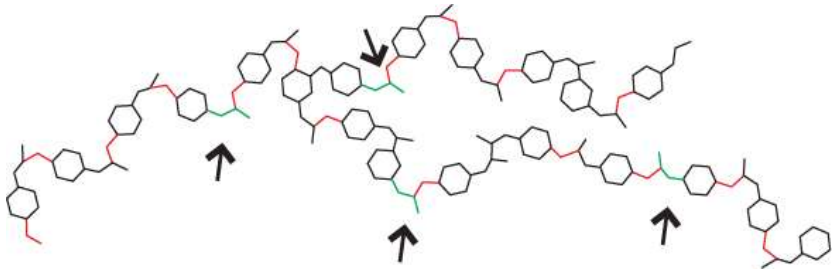
✓ 30% increase in saccharification efficiency (25% cellulose converted)

Engineering the lignin polymer

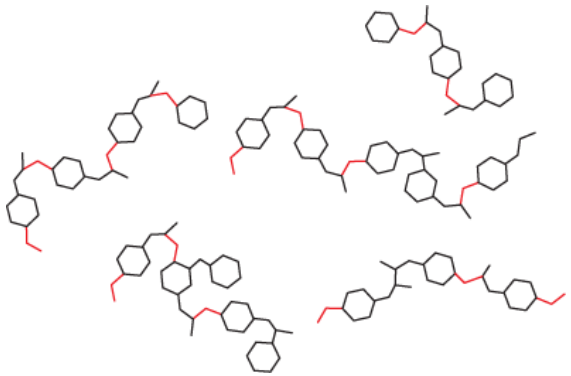
Lignin amount

De Vries *et al.* (2021), Plant Biotech. Journal

Lignin composition

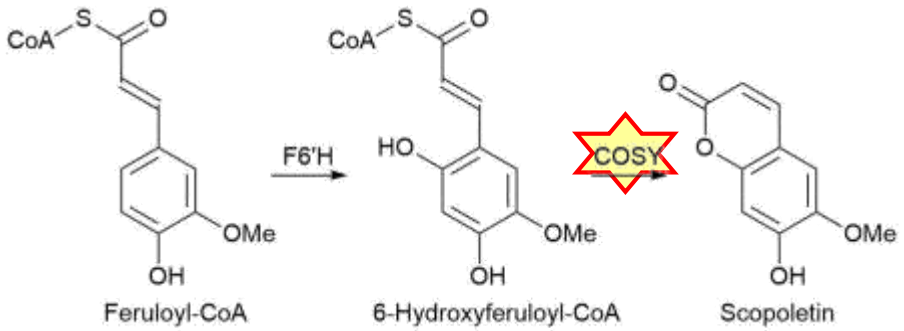


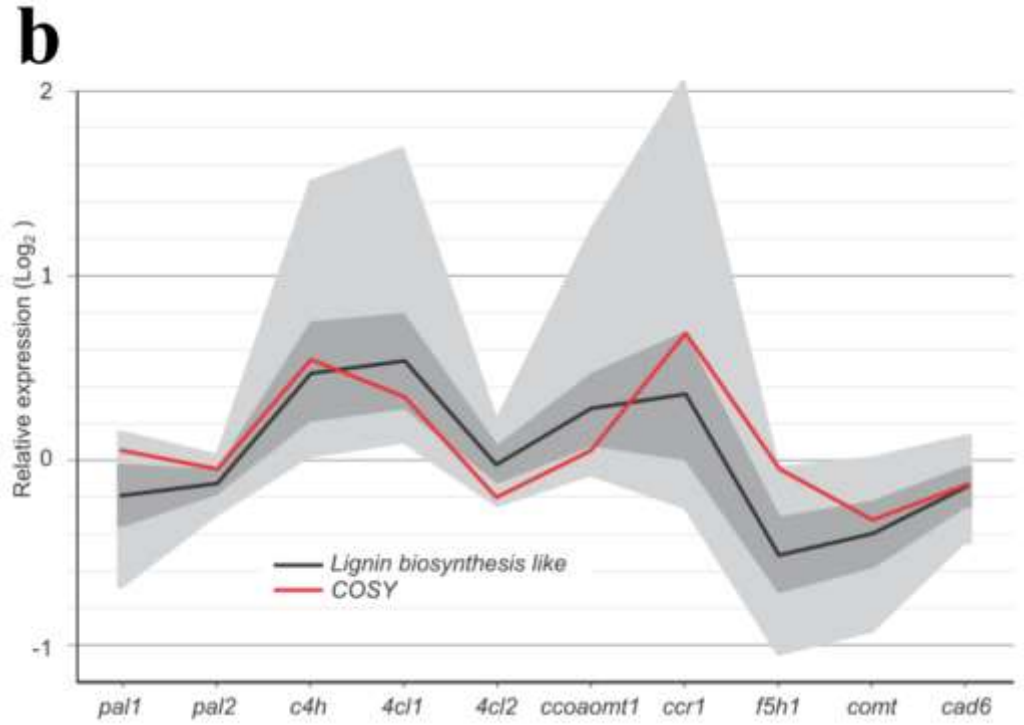
Soft conditions



Gene discovery @ bioenergy

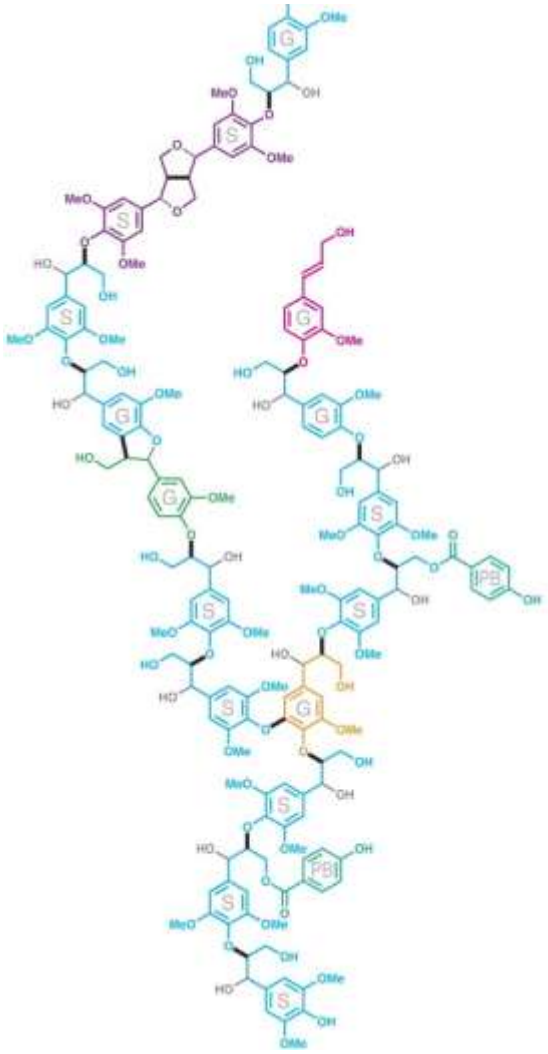
- Co-expression analysis designed to identify genes that are involved in lignin biosynthesis.
- *COSY* was selected
- COSY catalyzes *trans-cis* isomerization and lactonization in the biosynthesis of coumarins



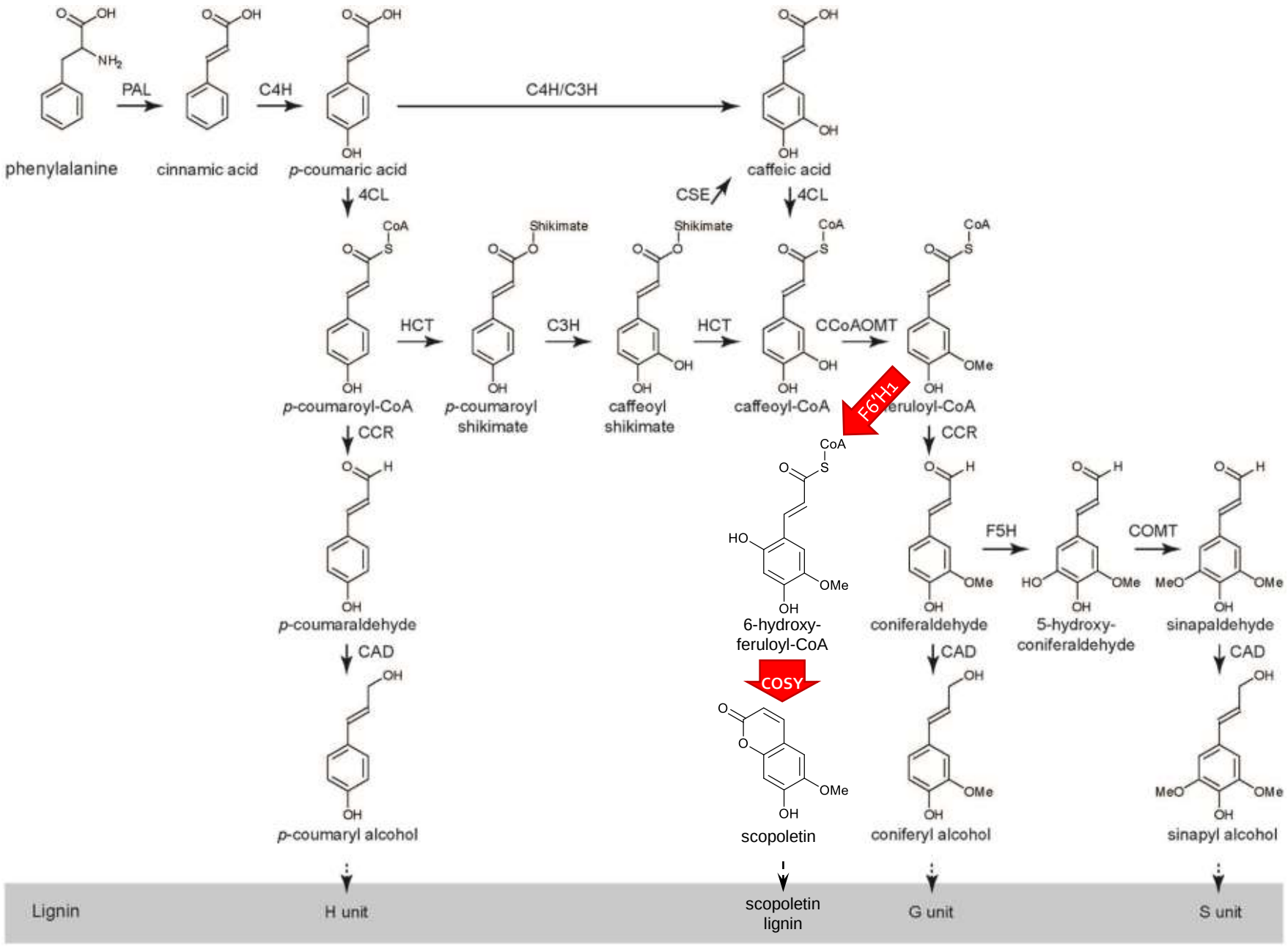


Vanholme *et al.* (2019), Nature Plants

Lignin engineering

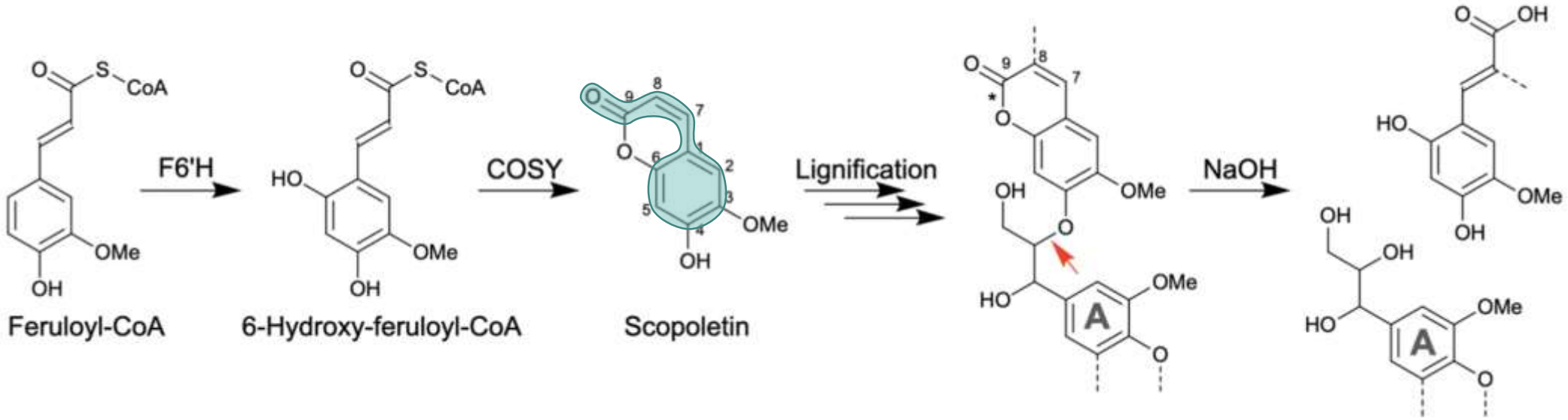


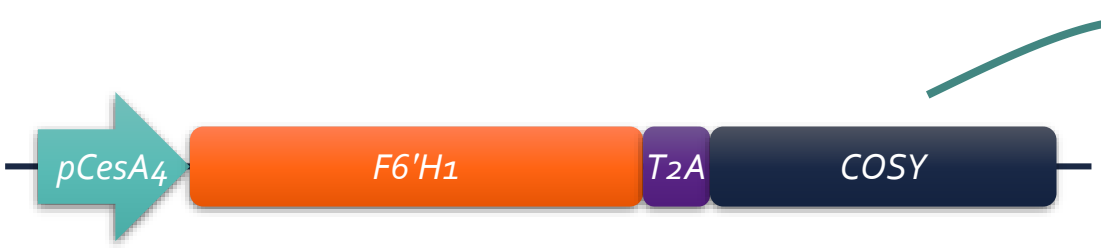
Stewart *et al.* (2009), Plant Phys.



Miedes *et al.* (2014), Front. Plant Sci

Objective

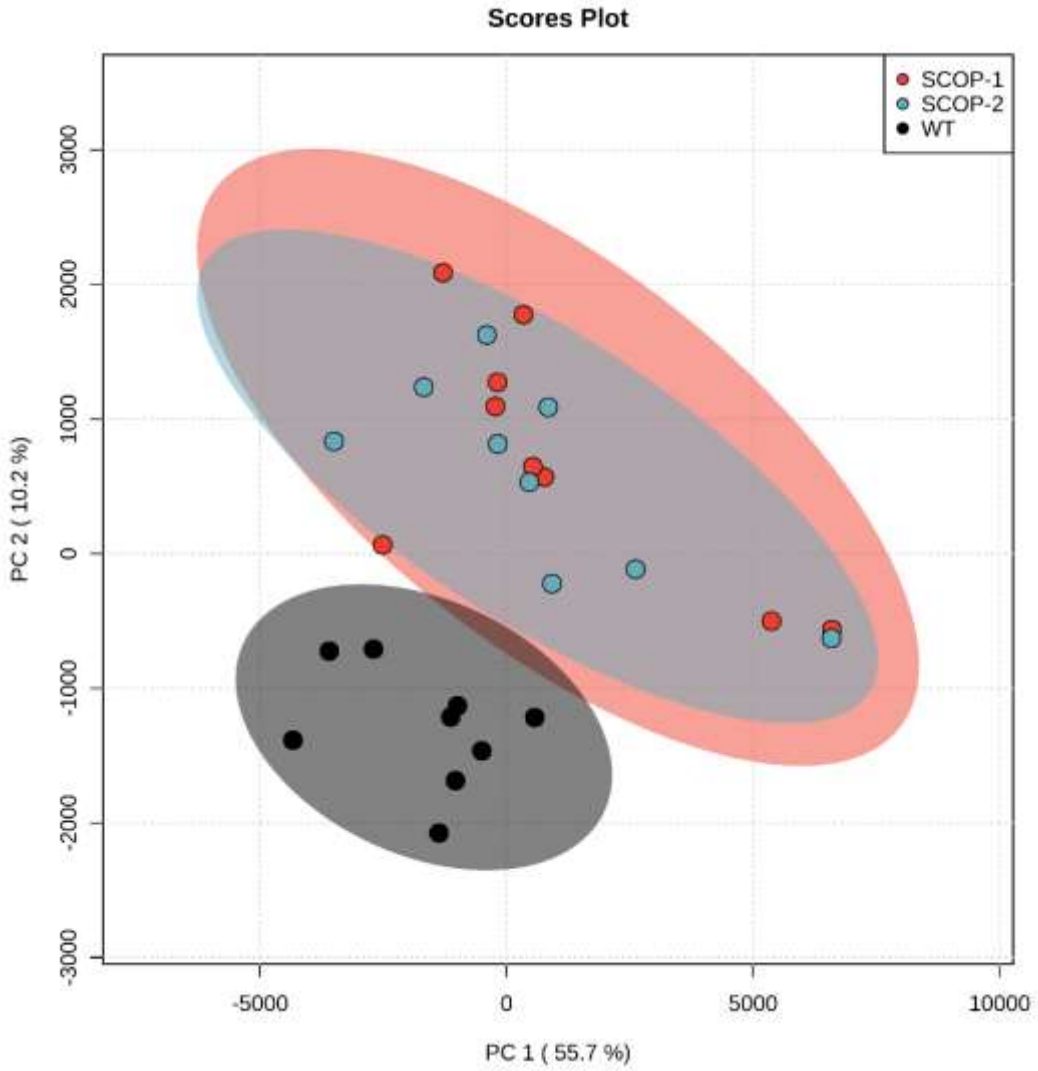




2 Ho lines were selected

SCOP-1 *SCOP-2*

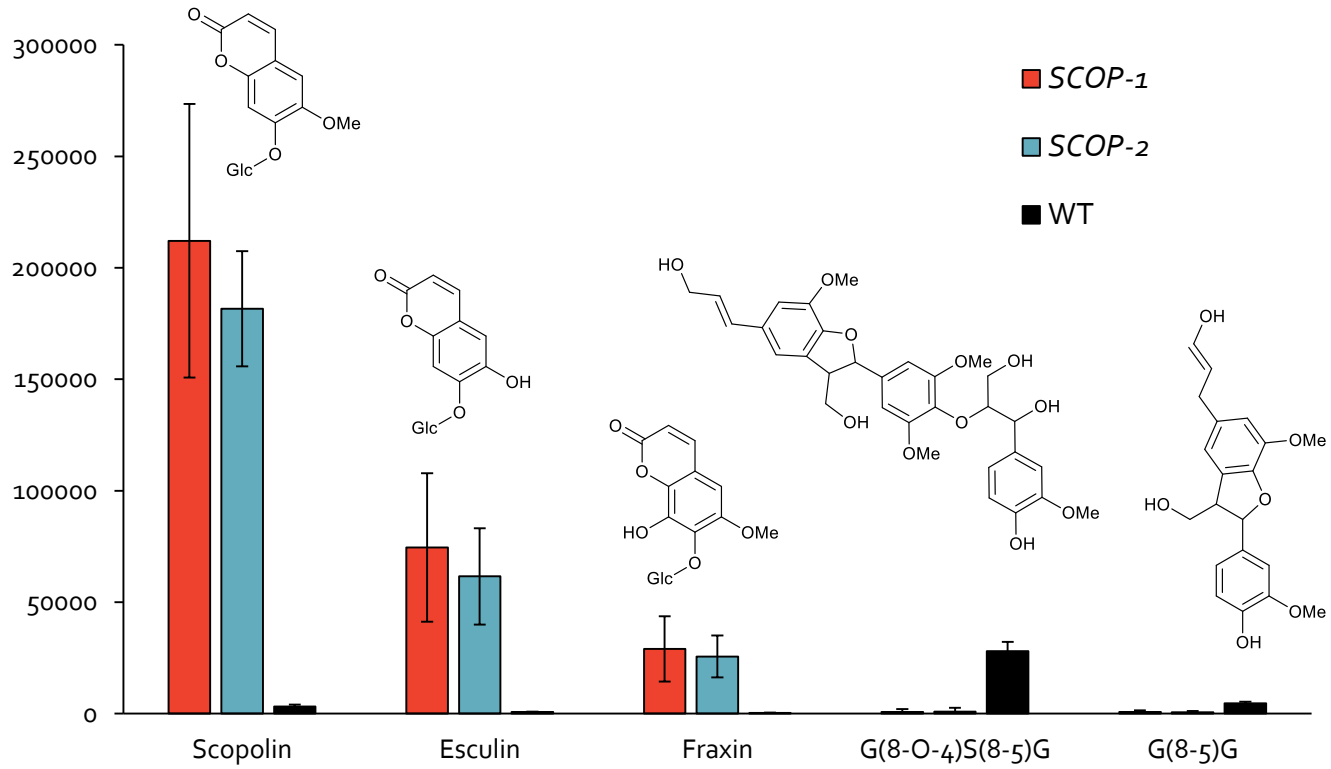
Phenolic profiling



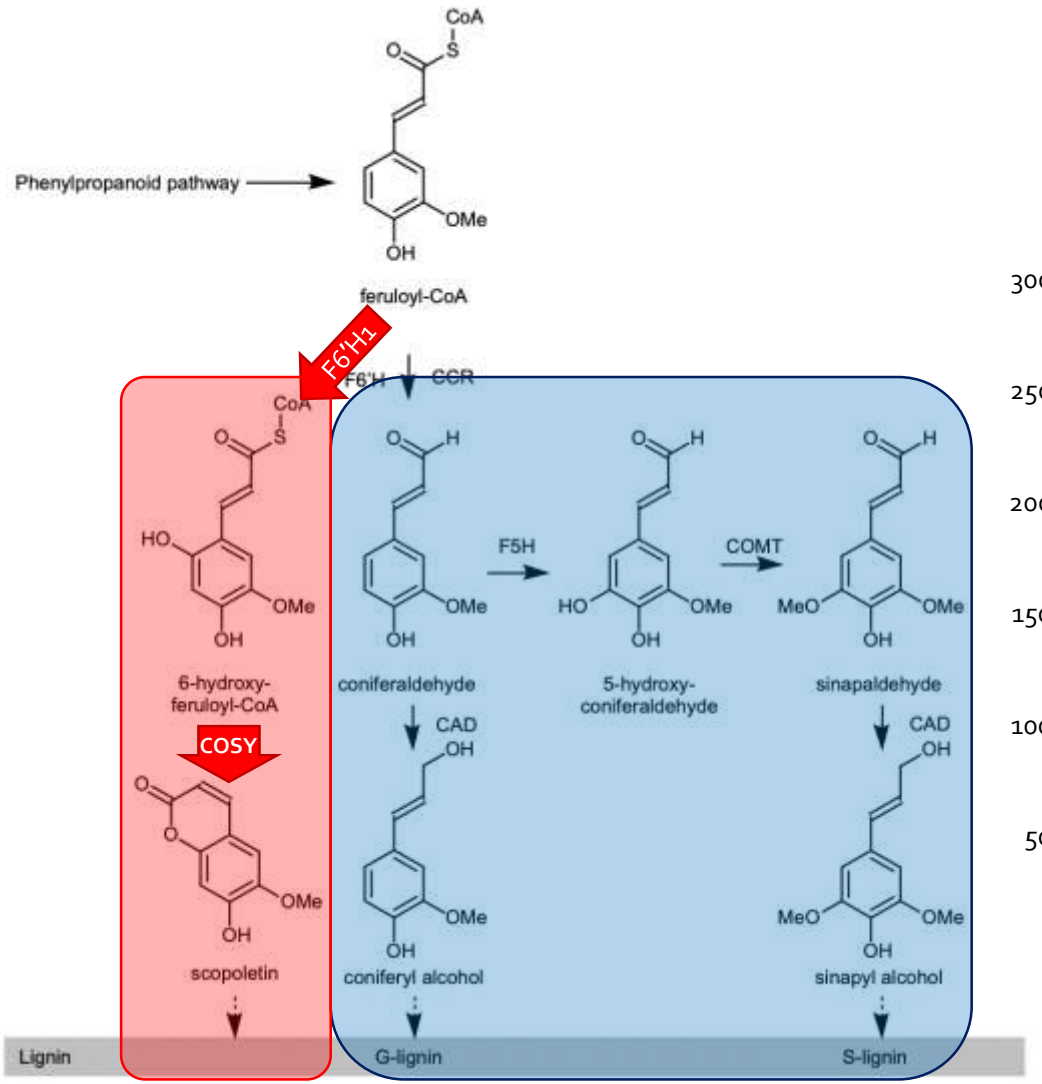
Main results:

Major peaks present in *SCOP* lines: coumarins

Major peaks lacking in *SCOP* lines: oligolignols



Phenolic profiling



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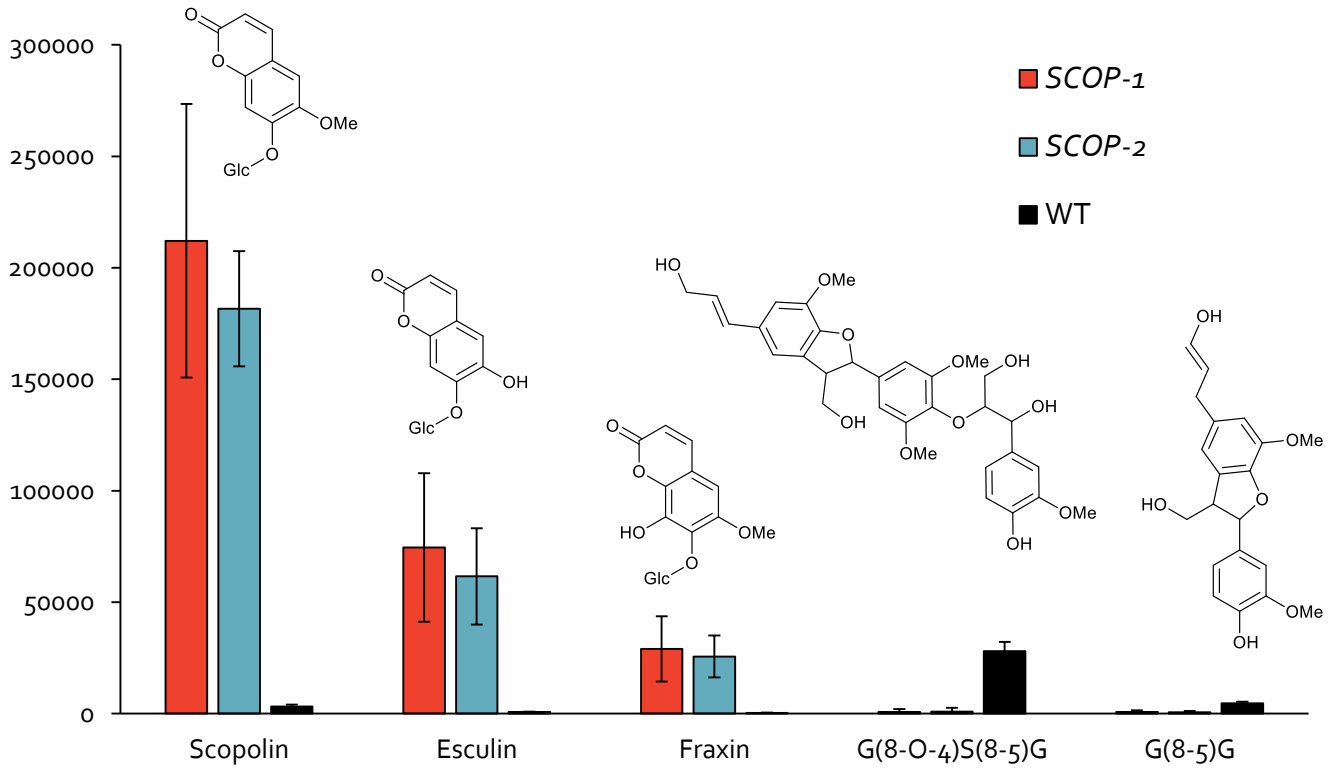
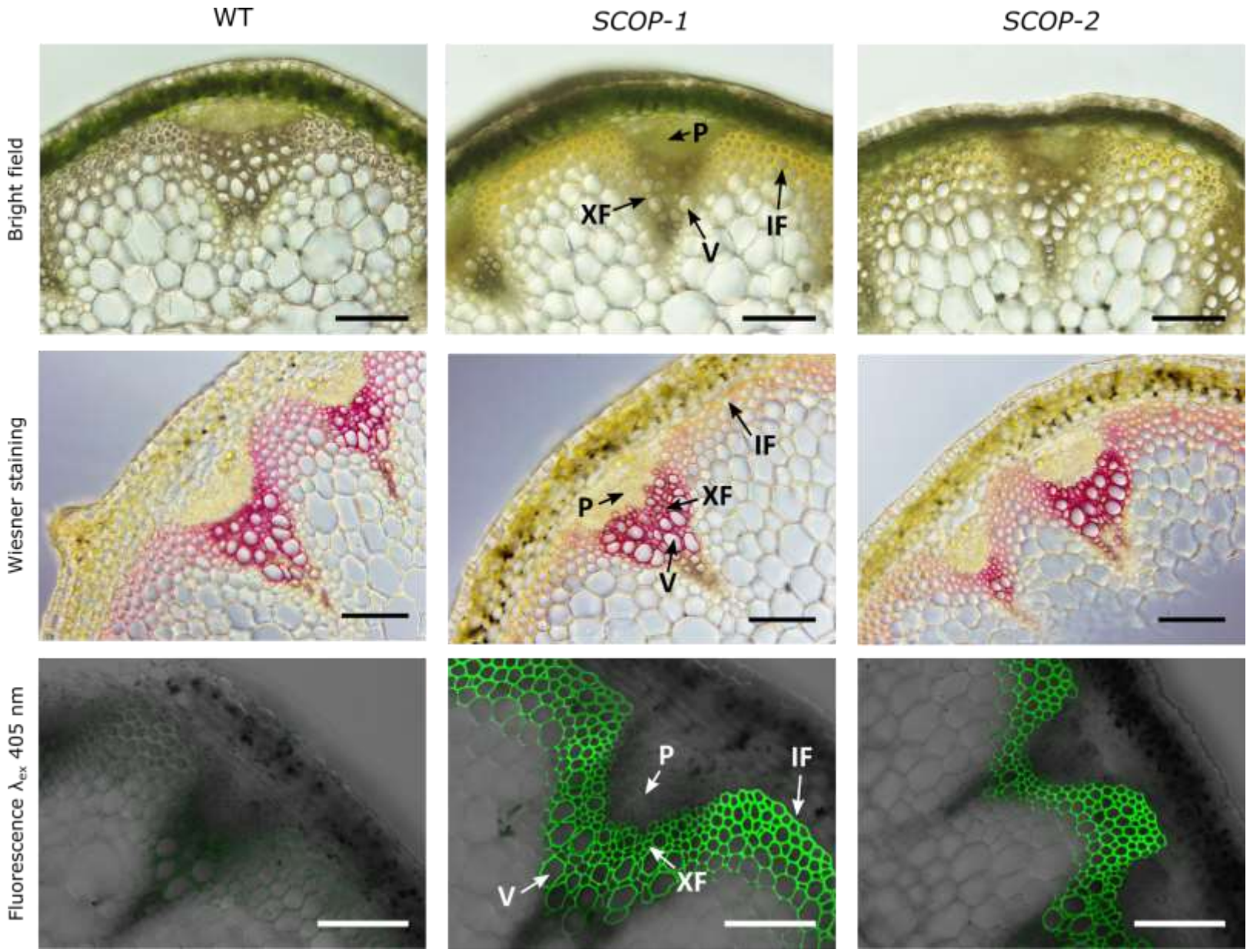


Microscopy

- Plants look phenotypically normal
- Yellow stain in secondary thickened cells → scopoletin
- Healthy vessel architecture
- Lignin strongly fluoresces in *SCOP* lines



Lignin content

Gravimetry based (Klason)

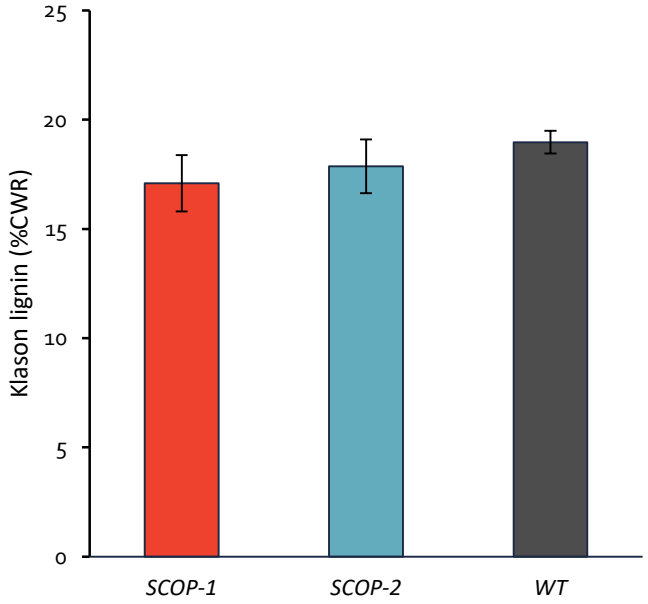
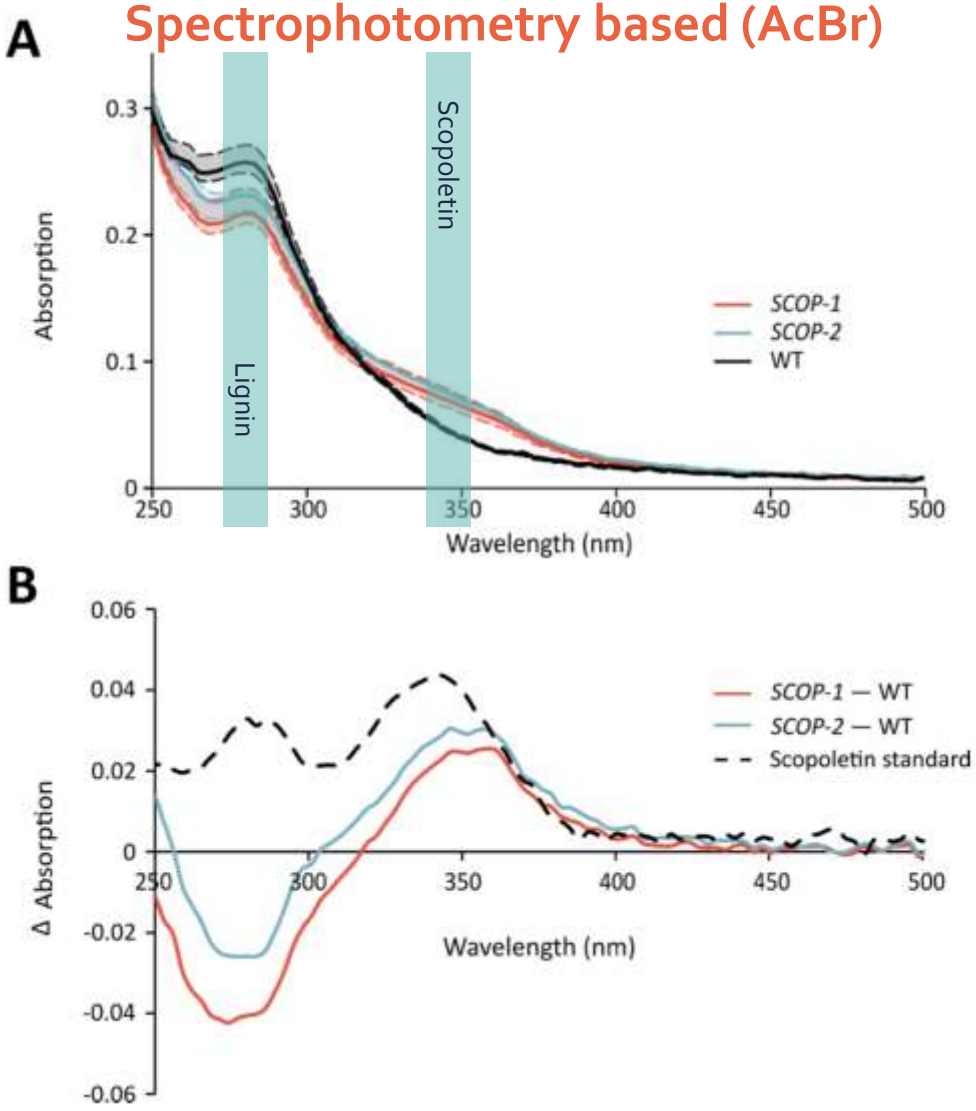


Table 1. Lignin content and composition

Line	CWR (%)	Klason	ASL	AcBr
WT	80.7 (2.0)	19.0 (0.5)	2.4 (0.04)	13.1 (0.7)
SCOP-1	81.2 (1.9)	17.1 (1.3)*	2.5 (0.3)	11.6 (1.1)*
SCOP-2	77.9 (1.9)***	17.9 (1.2)	2.3 (0.3)	12.1 (1.3)•

→ ~ 10% decrease in lignin quantity

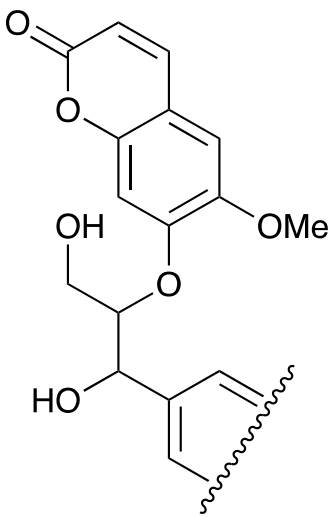
Spectrophotometry based (AcBr)

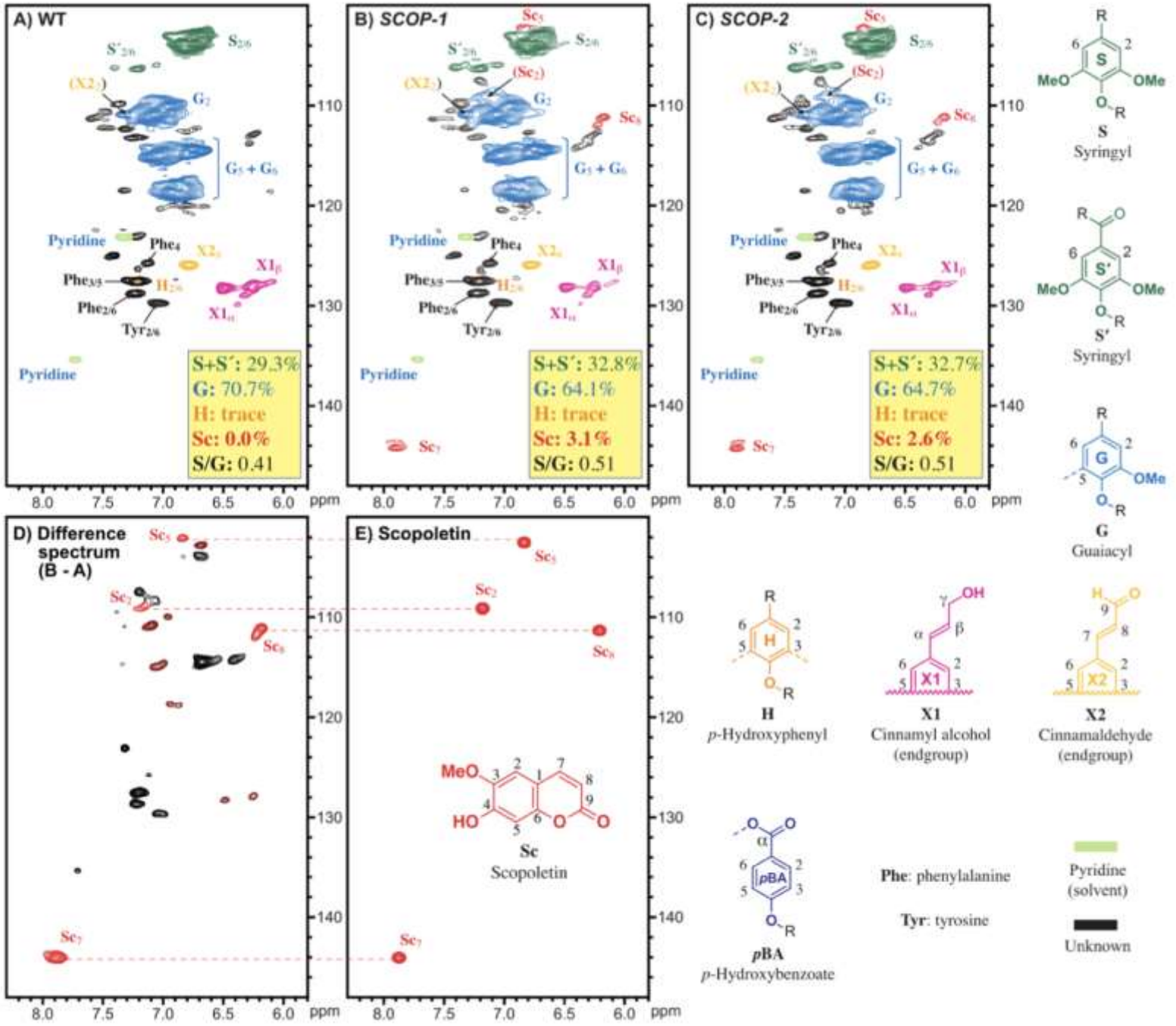


Did scopoletin incorporate into the lignin polymer?

NMR analysis

- Up to 3.1% incorporation
- Endgroup – starting unit of the polymer





DFRC analysis

Derivatization Followed by Reductive Cleavage

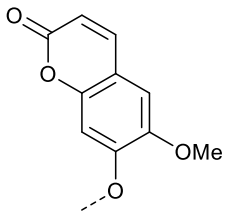
WT

Detection of H/G/S

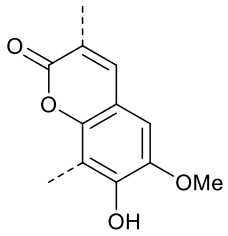
SCOP-1 & SCOP-2

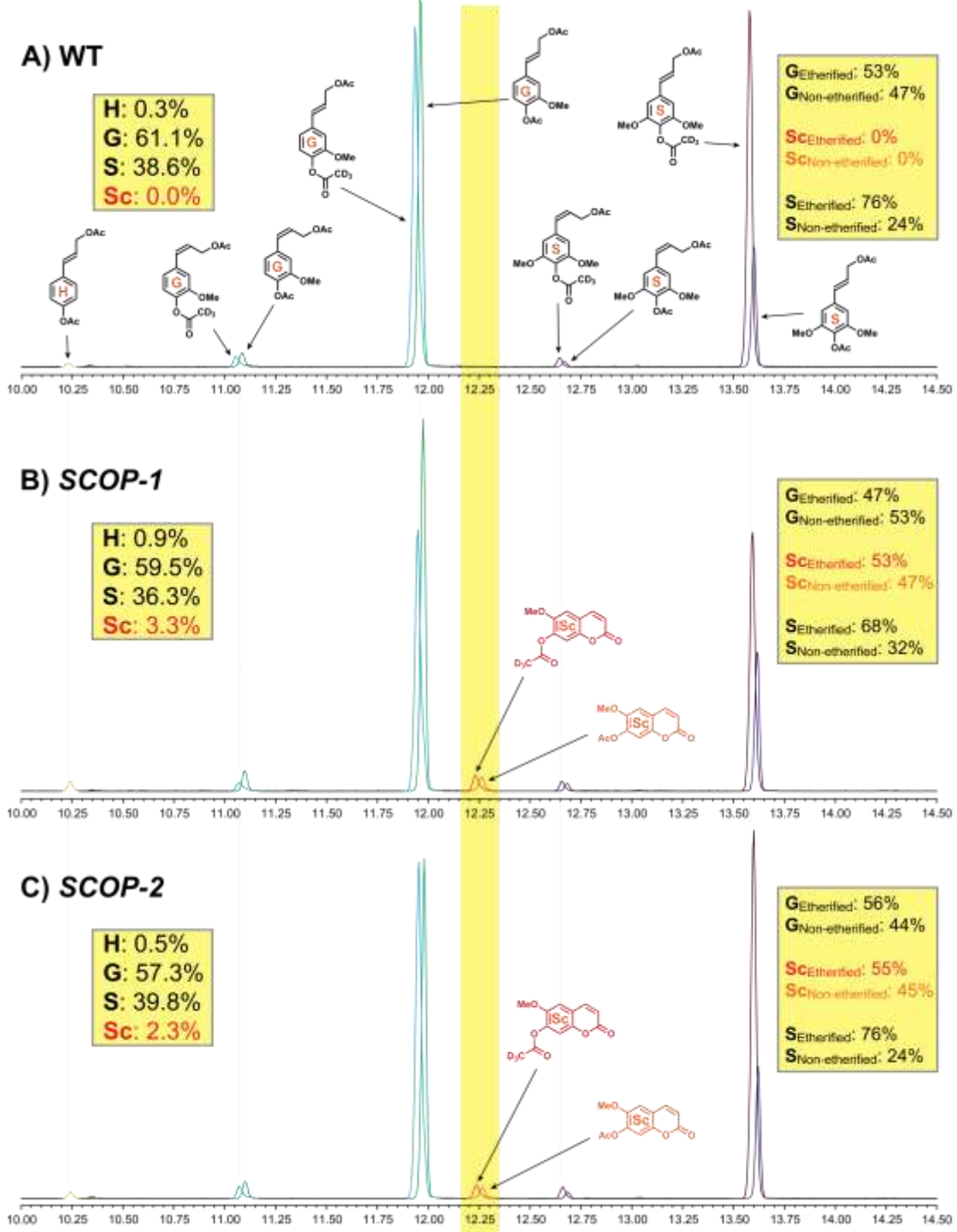
Detection of H/G/S + both etherified a non-etherified scopoletin are detected.

Half of the detected scopoletin is etherified via the phenolic function



Half of the detected scopoletin is coupled to the lignin via other means





Lignin composition Thioacidolysis

GC-MS

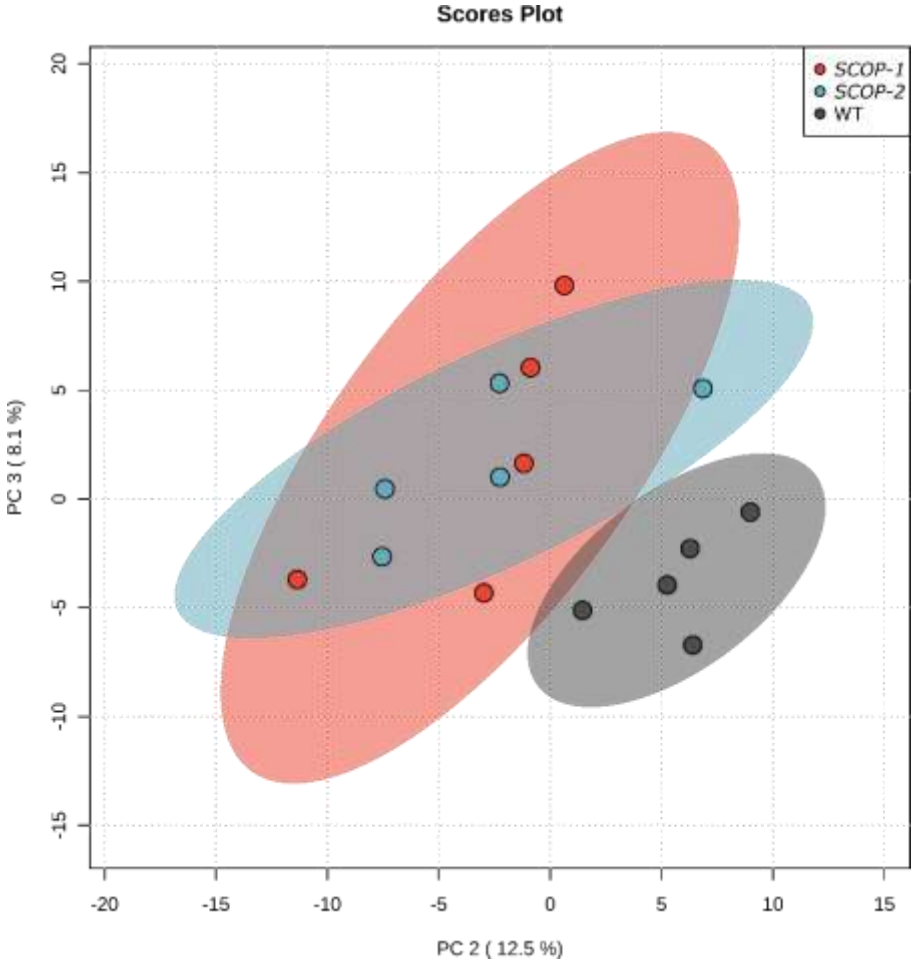


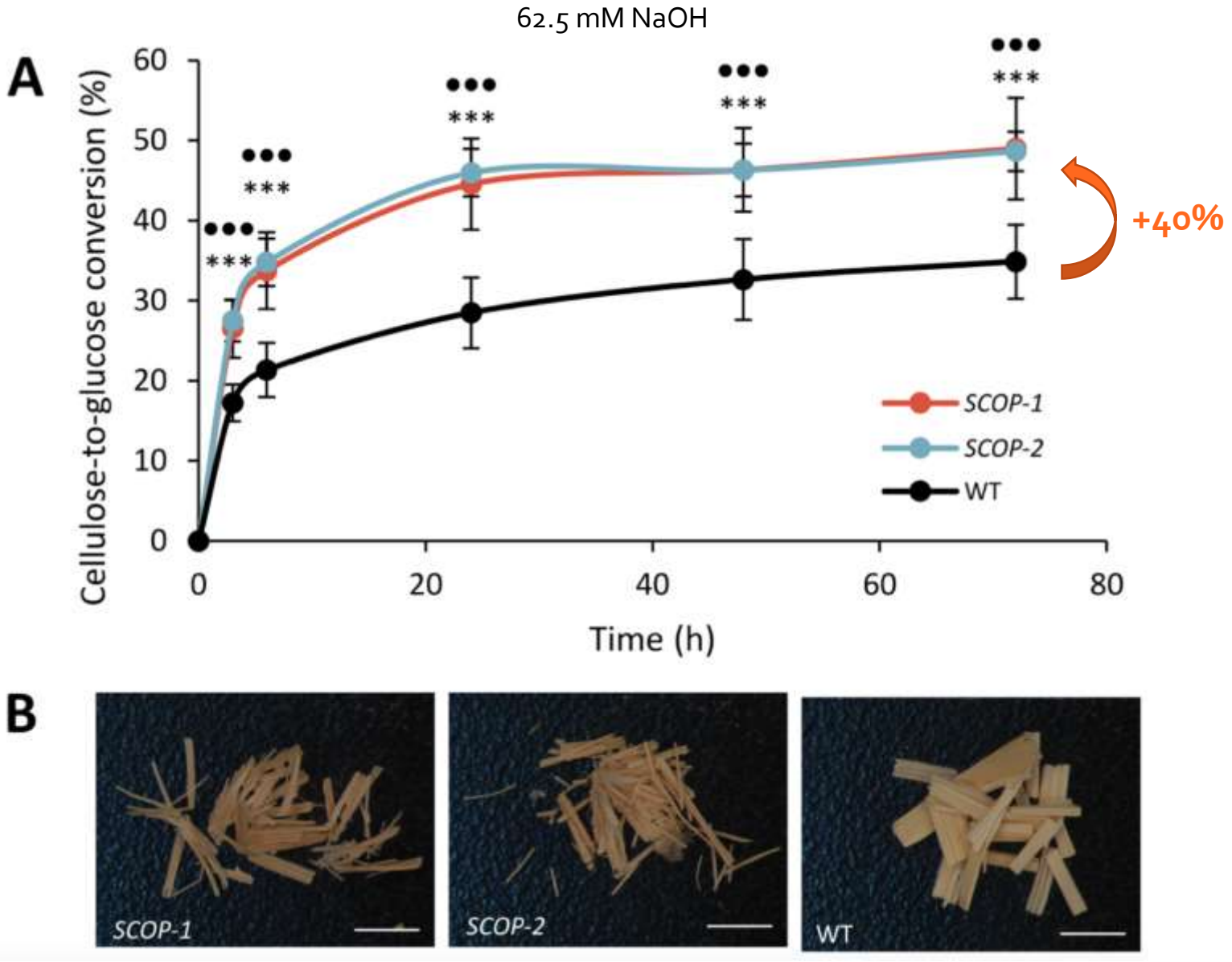
Table 1. Lignin content and composition

Line	CWR (%)	Klason	ASL	AcBr	H (%)	G (%)	S (%)	S/G
WT	**80.7 (2.0)**	**19.0 (0.5)**	**2.4 (0.04)**	**13.1 (0.7)**	**0.99 (0.08)**	**79.36 (0.74)**	**19.21 (0.64)**	**0.24 (0.01)**
SCOP-1	81.2 (1.9)	17.1 (1.3)*	2.5 (0.3)	11.6 (1.1)*	1.53 (0.44)*	74.67 (1.34)***	19.96 (0.79)	0.27 (0.01)
SCOP-2	77.9 (1.9)***	17.9 (1.2)	2.3 (0.3)	12.1 (1.3)•	1.36 (0.14)	74.53 (1.57)***	20.15 (1.29)	0.27 (0.02)*

Conclusions

- Subtle shift in lignin composition
- 3% incorporation of scopoletin >>> %H in WT lines

Cellulose-to-glucose conversion



Hoengenaert *et al.* (2022), Science Advances (accepted)

Translation

Translation is currently ongoing in the bioenergy crop poplar and followed-up by a new PhD student

Take home messages

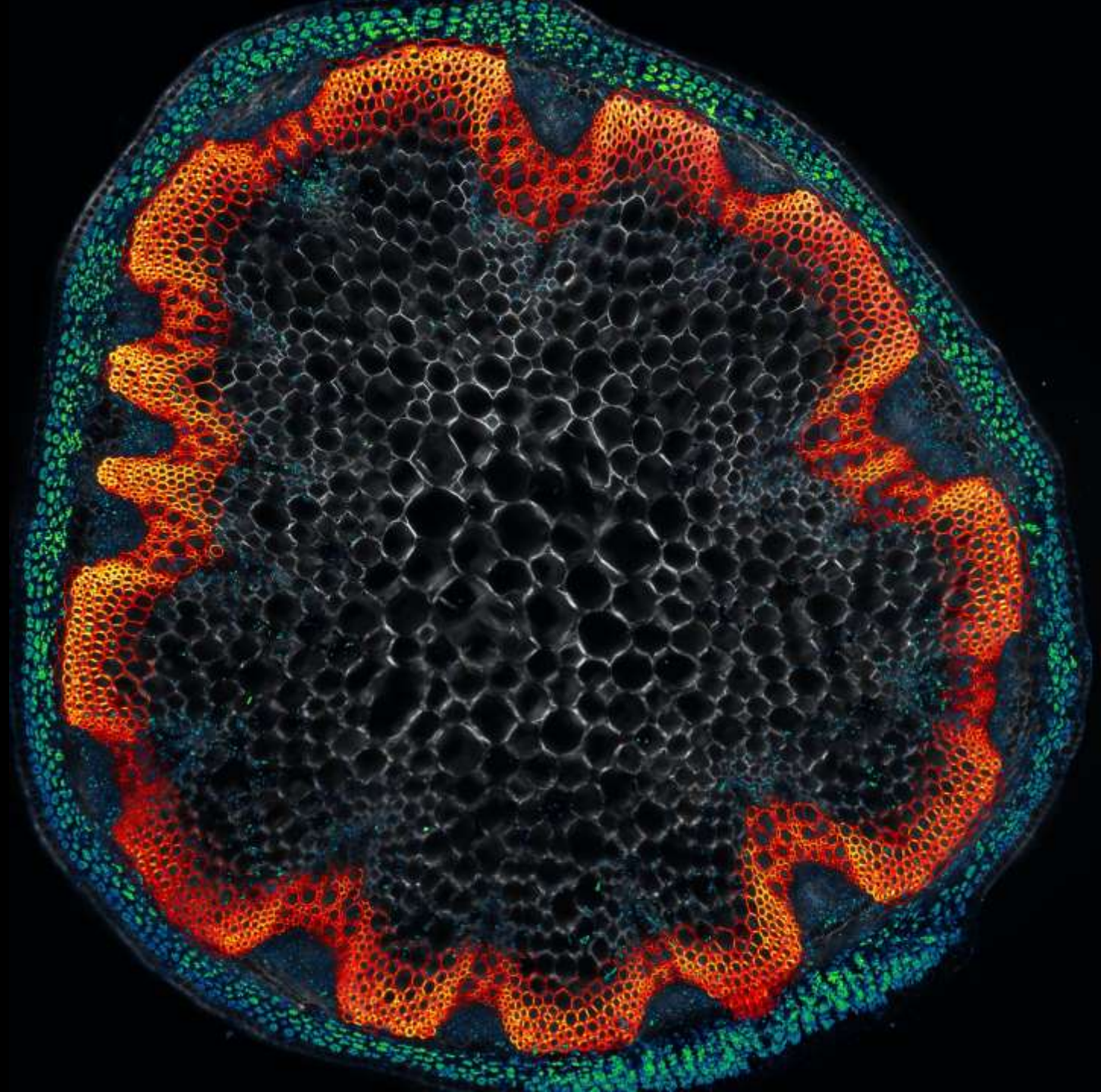
Aim: engineer the lignin polymer of Arabidopsis with the coumarin scopoletin, to enhance the biomass processing efficiency.

Overexpression of *F6'H1* and *COSY* led to:

- The successful production of scopoletin in lignifying cells
- The **incorporation of scopoletin into the lignin** polymer
- A minimal decrease in lignin content
- A **40% increase in cellulose-to-glucose conversion**
- Without affecting the phenotype of the plant

Translation

- Ongoing – promising!



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