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Assembly of a lignin modification toolbox

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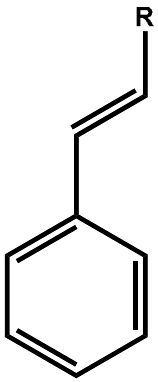
Overview



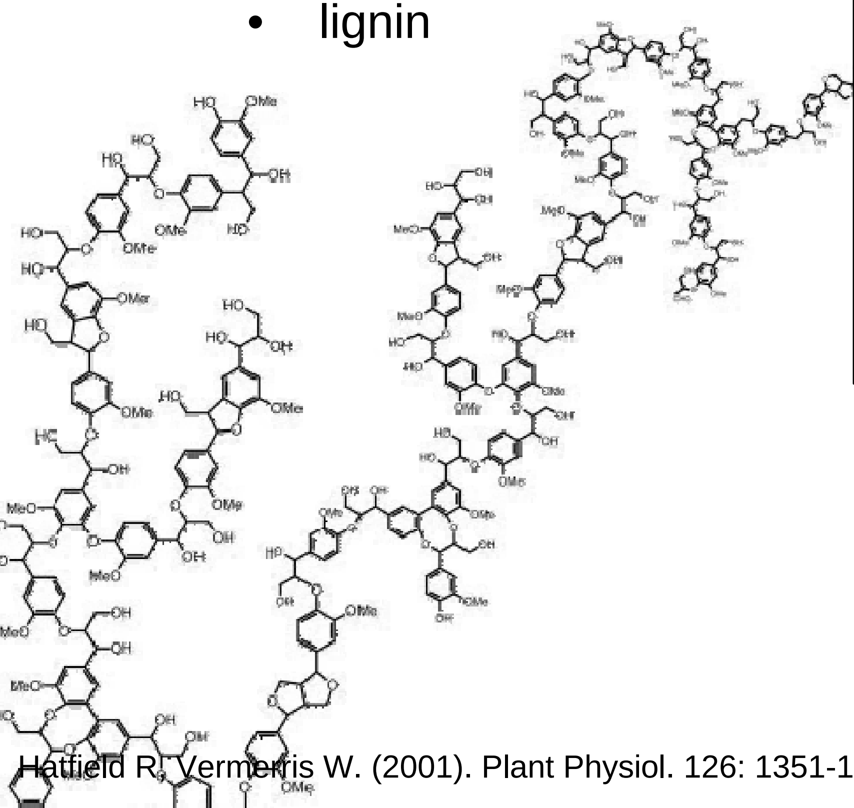
- Phenylpropanoid metabolism, plant survival, and the lignin / biofuel dilemma

- Arabidopsis phenylpropanoid mutants: a few lessons learned about modifying lignin

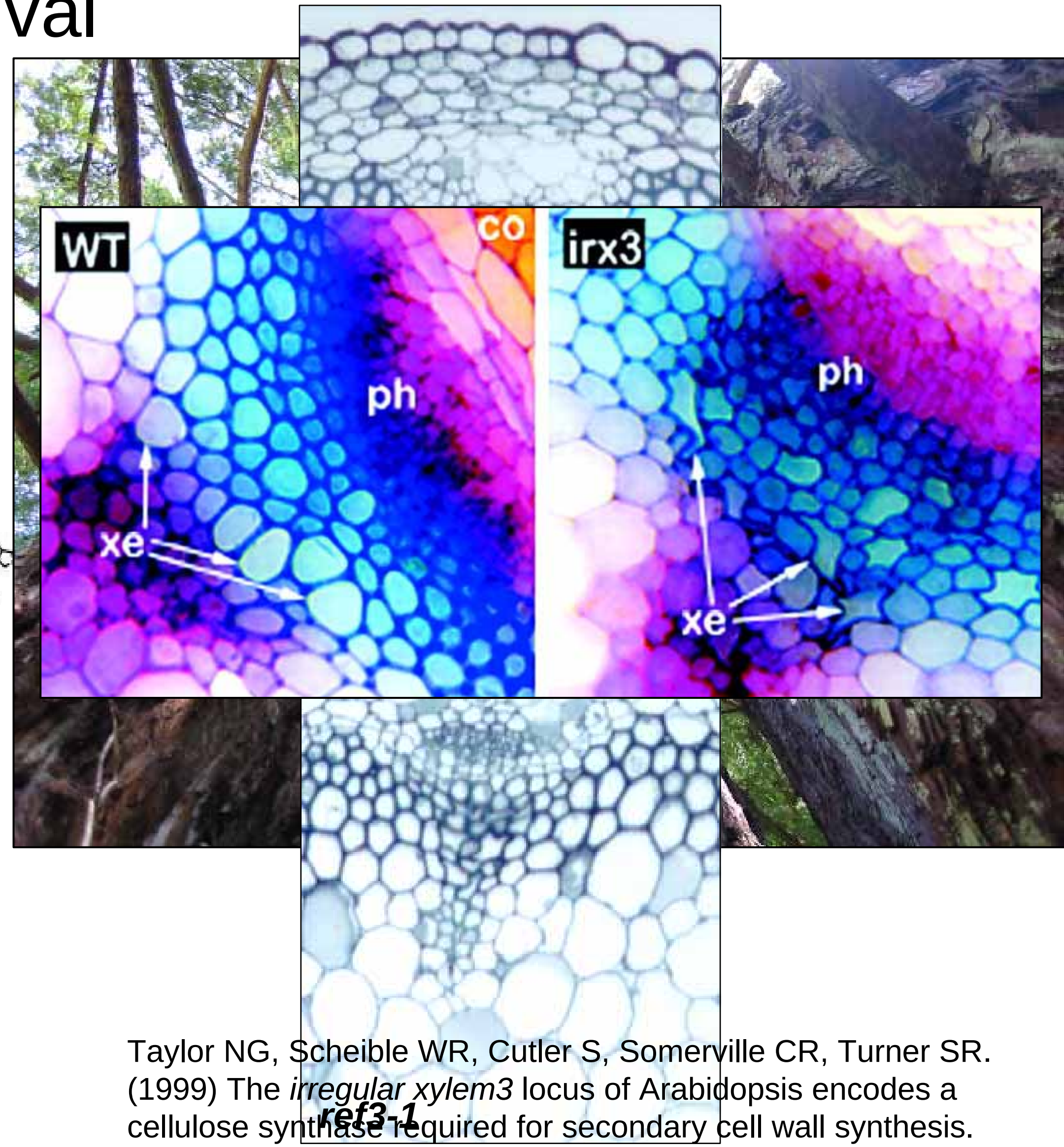
- Can we assemble a lignin modification toolbox with which we can modify lignin without impacting plant performance?

Phenylpropanoid metabolism is critical for plant survival

- UV resistance
  - soluble compounds
- structural support and water transport
  - lignin



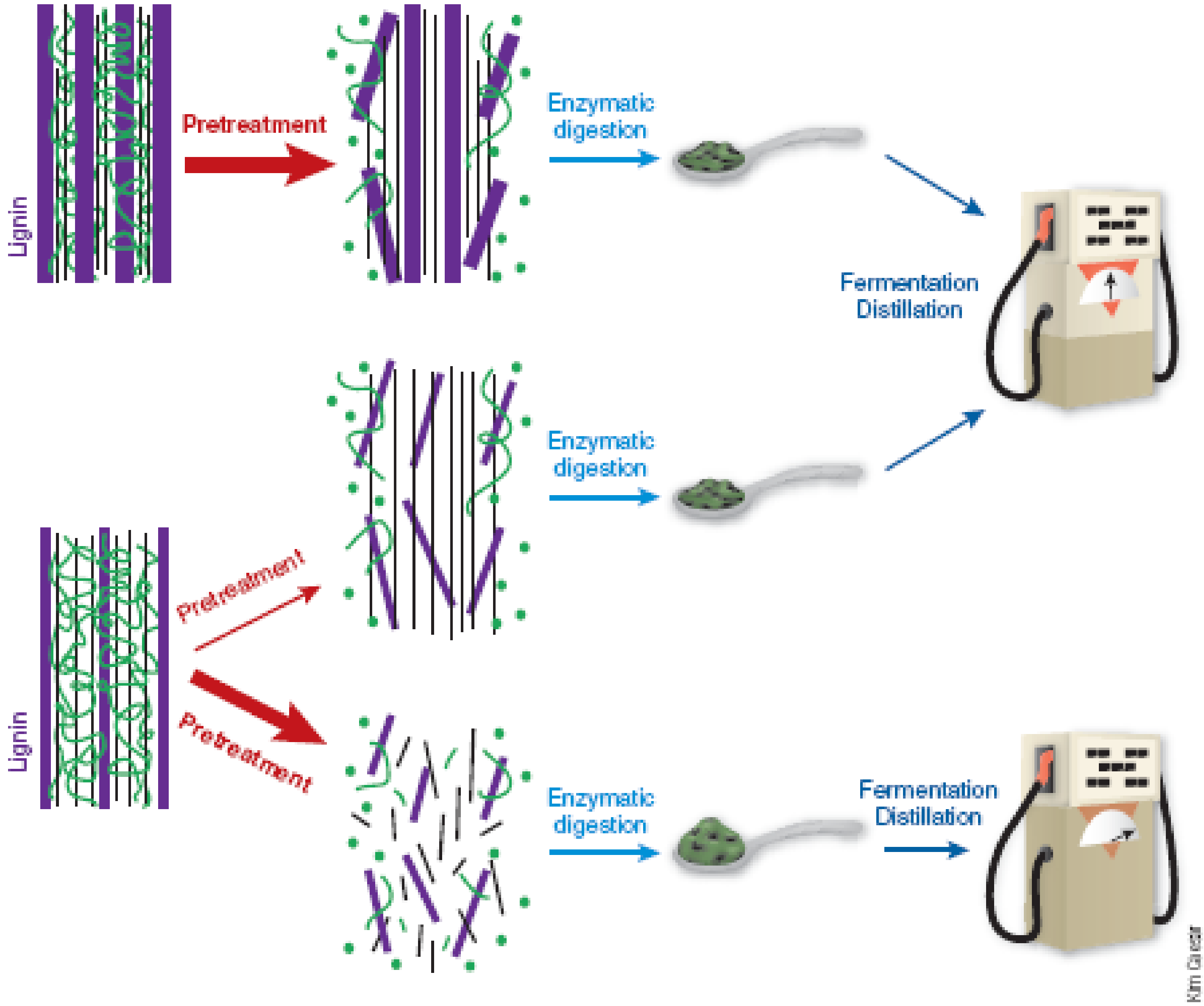
Hatfield R, Vermerris W. (2001). Plant Physiol. 126: 1351-1357



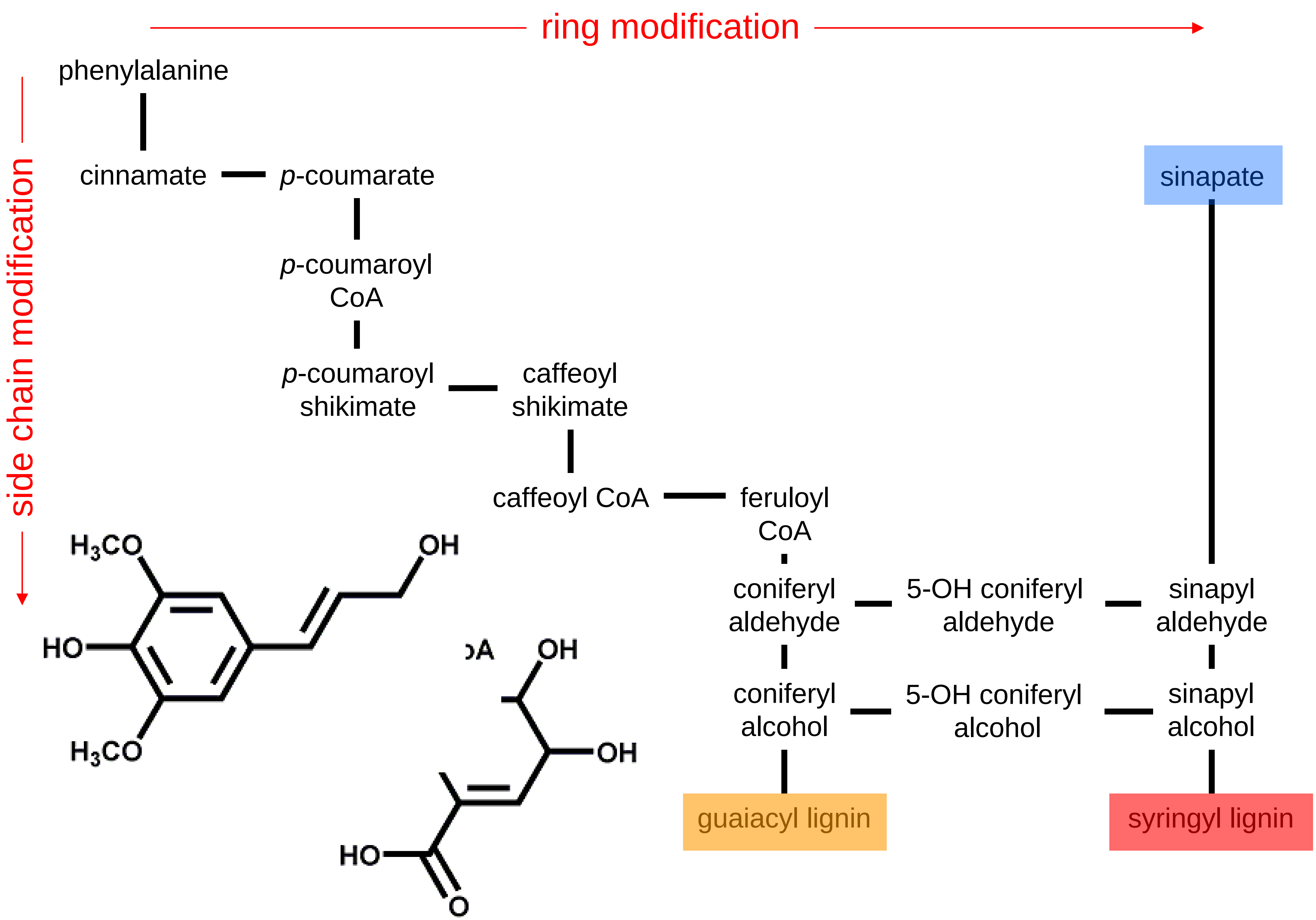
Taylor NG, Scheible WR, Cutler S, Somerville CR, Turner SR. (1999) The *irregular xylem3* locus of Arabidopsis encodes a cellulose synthase required for secondary cell wall synthesis. Plant Cell 11: 769-780

http://givingspace.org/benlomond/up%20redwoods.jpg

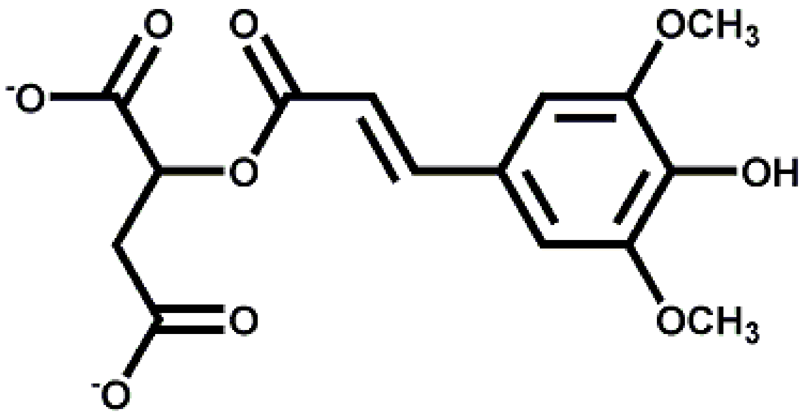
Lignin modification may decrease the need for pretreatment



The lignin biosynthetic pathway



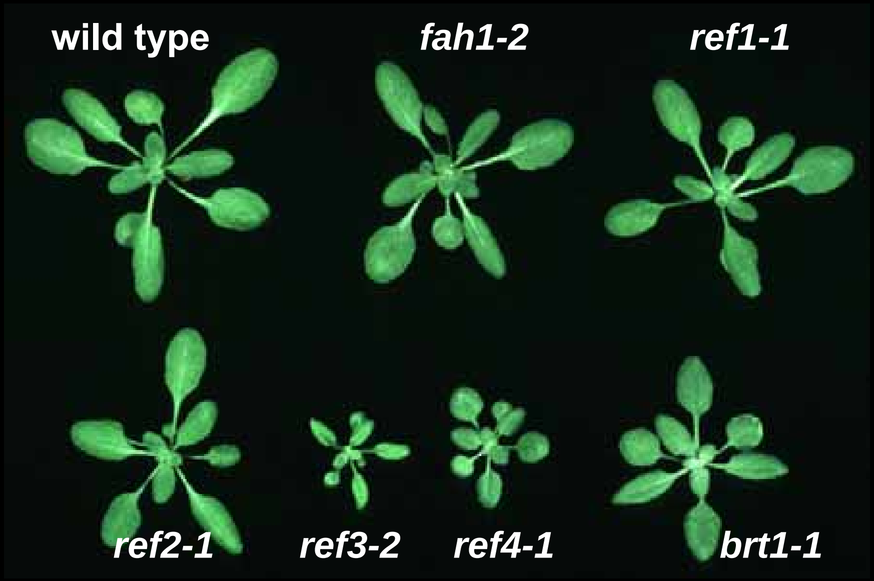
Arabidopsis accumulates sinapoylmalate as a UV sunscreen



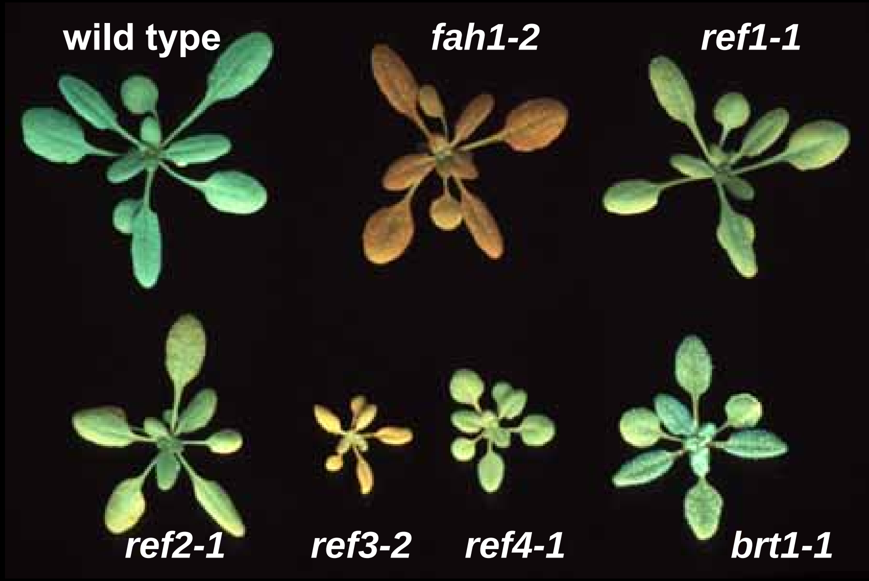
SINAPOYLMALATE

Phenotype of the *fah1*, *ref* and *brt* mutants under UV

VISIBLE

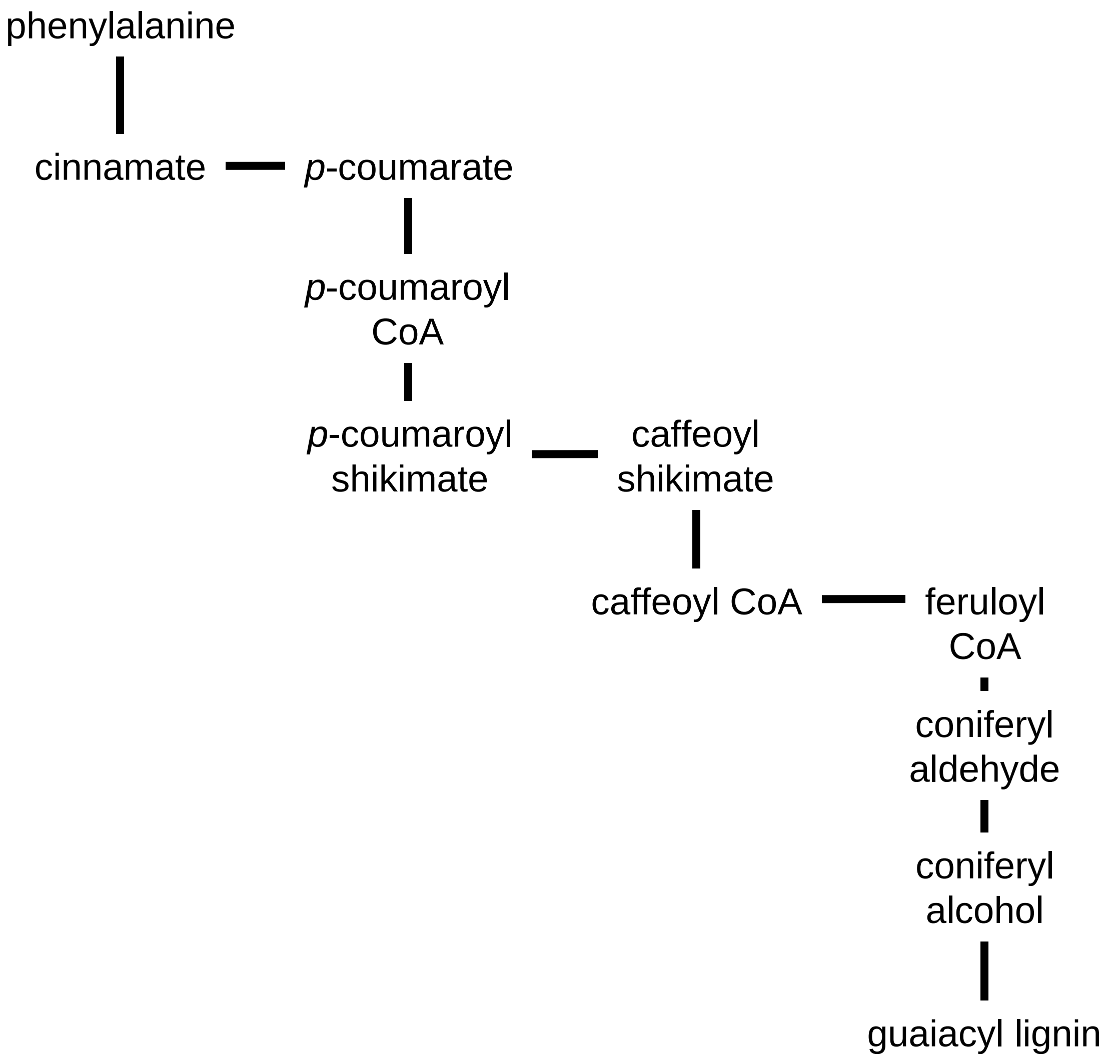


UV

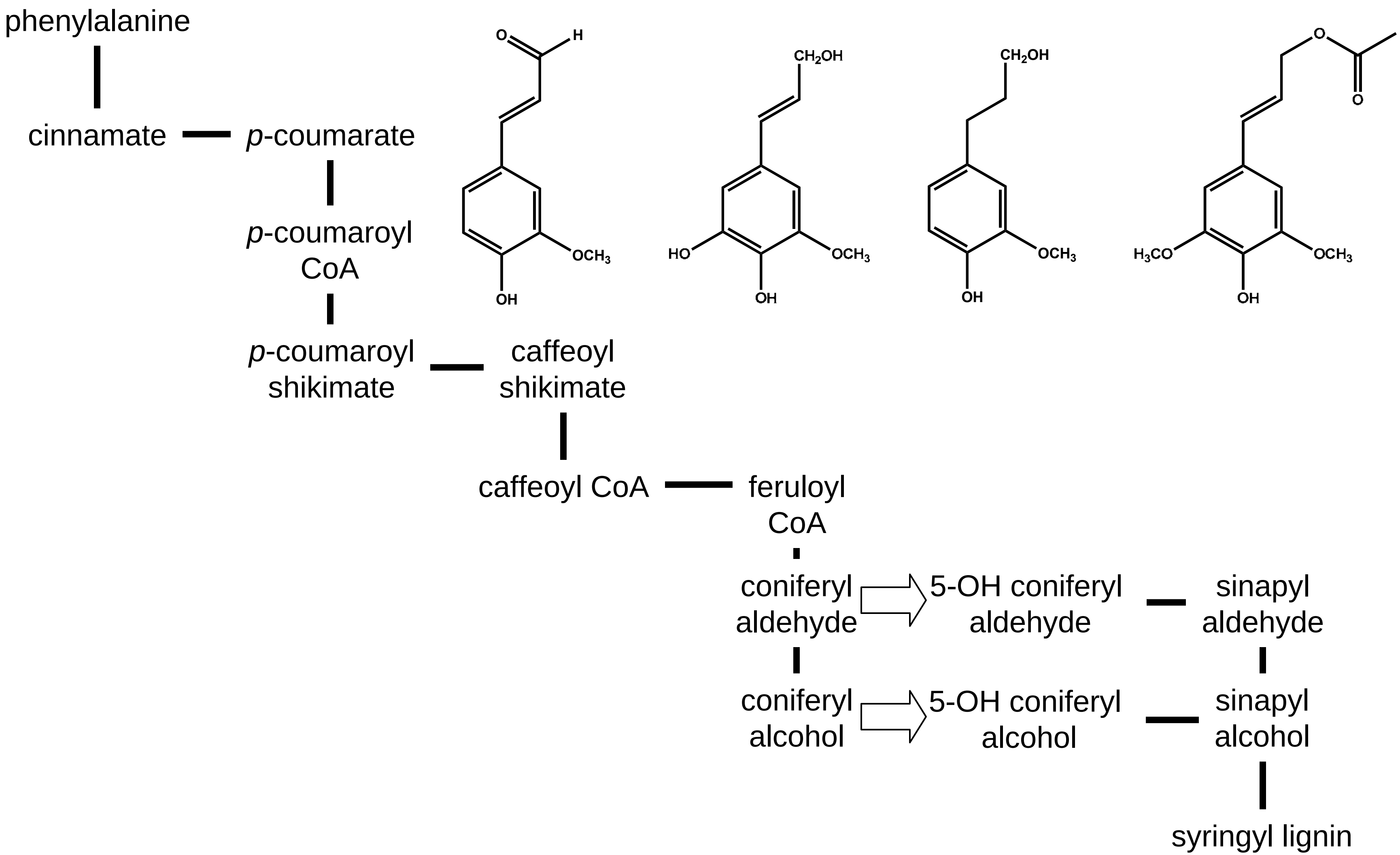


- genetics
- sequenced genome
- advanced genomic resources

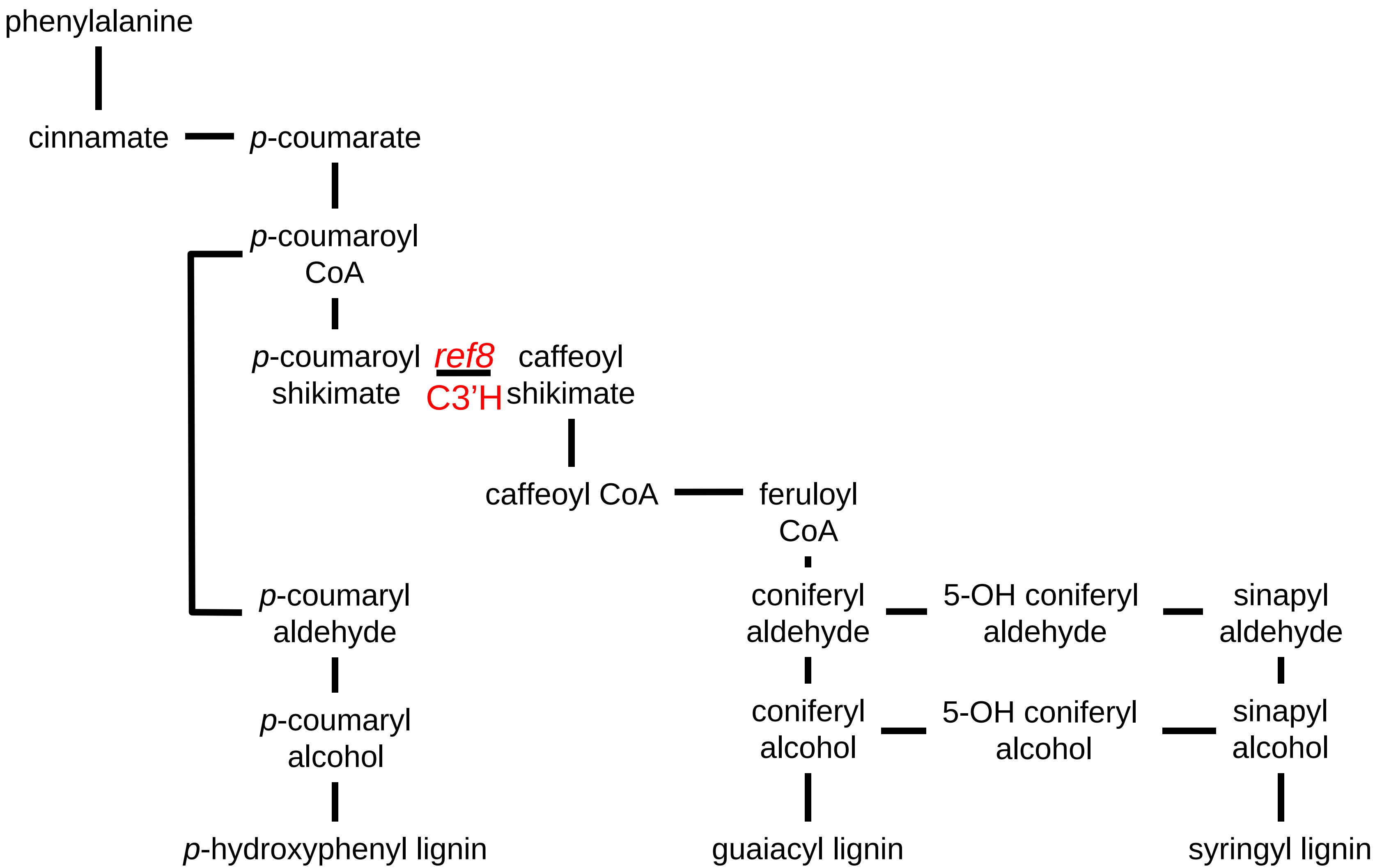
The *fah1* mutant deposits only G lignin



F5H over-expression increases syringyl lignin content



The *ref8* mutation reroutes lignin biosynthesis

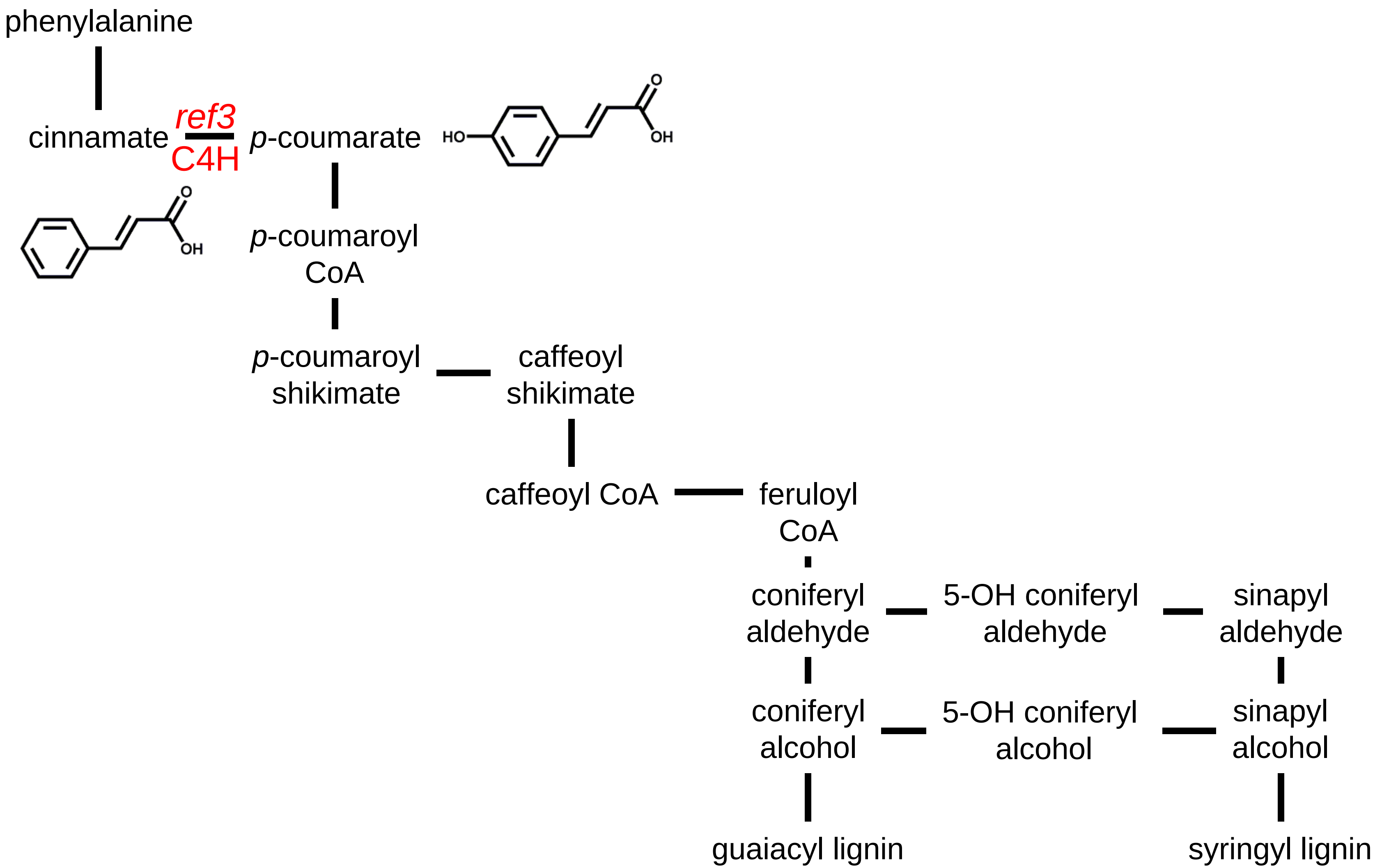


ref8 mutants are strongly dwarfed

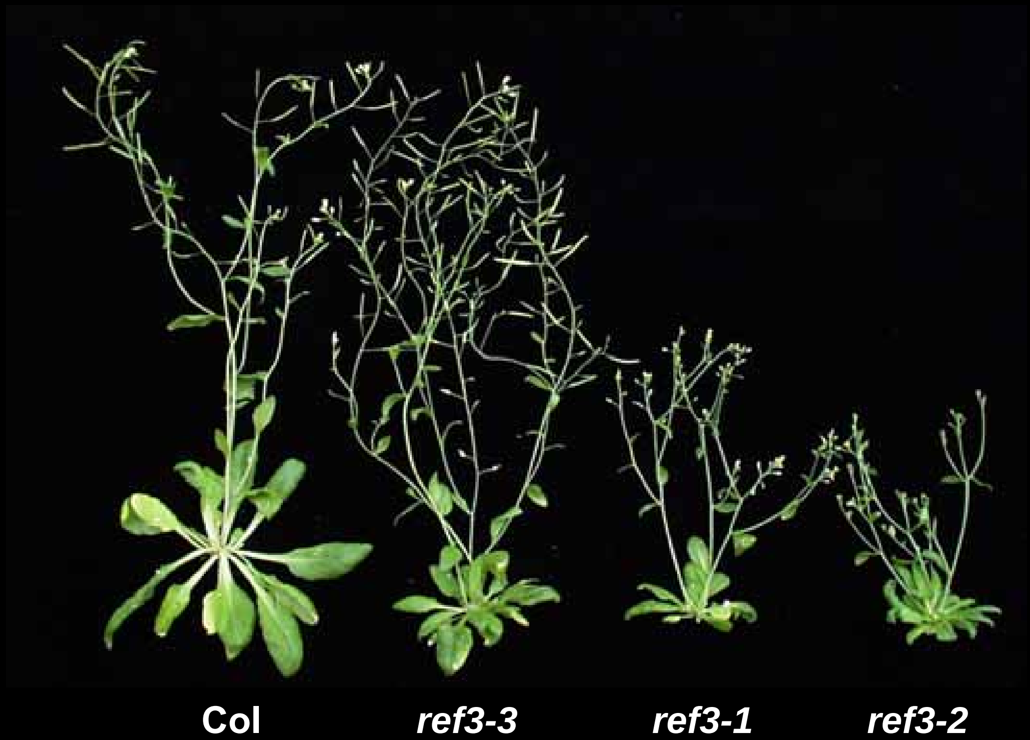
wild type ***fah1*** ***ref8***

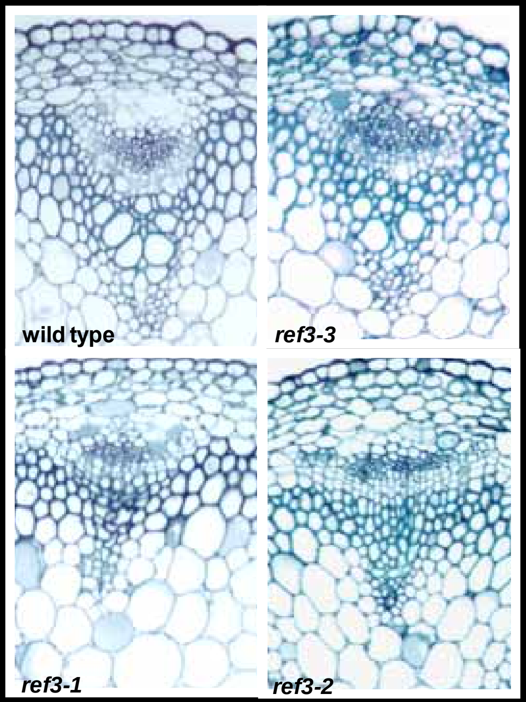
- Altered subunit composition
- Decreased lignin content
- Toxicity of accumulated upstream intermediates
- Absence of downstream products

The *ref3* mutant is defective in C4H



REF3 is required for normal plant development

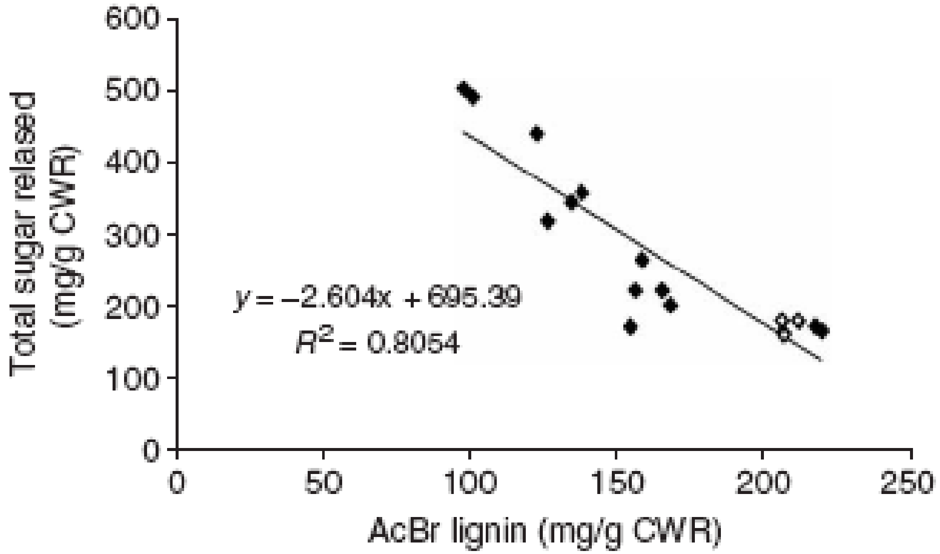


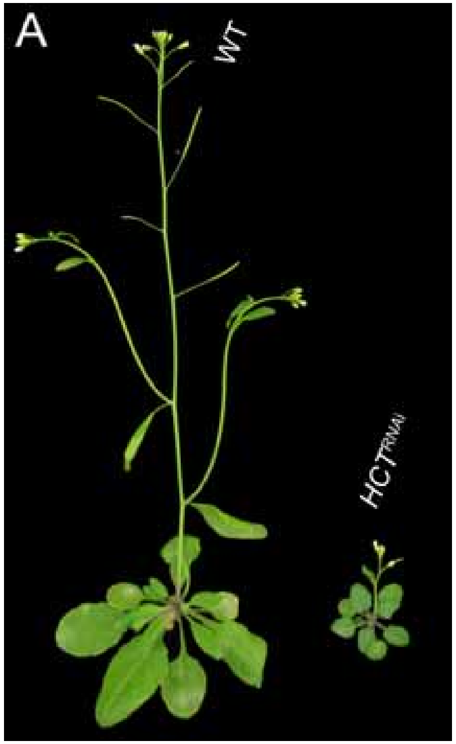


- Altered subunit composition
- Decreased lignin content
- Toxicity of accumulated upstream intermediates
- Absence of downstream products

Plant CANNOT tolerate large changes in lignin content

- Large decreases in lignin content may improve saccharification efficiency but are likely to incur a yield penalty





- The origin of dwarfism in lignin-deficient plants remains to be conclusively determined

Plant CAN tolerate large changes in lignin monomer composition

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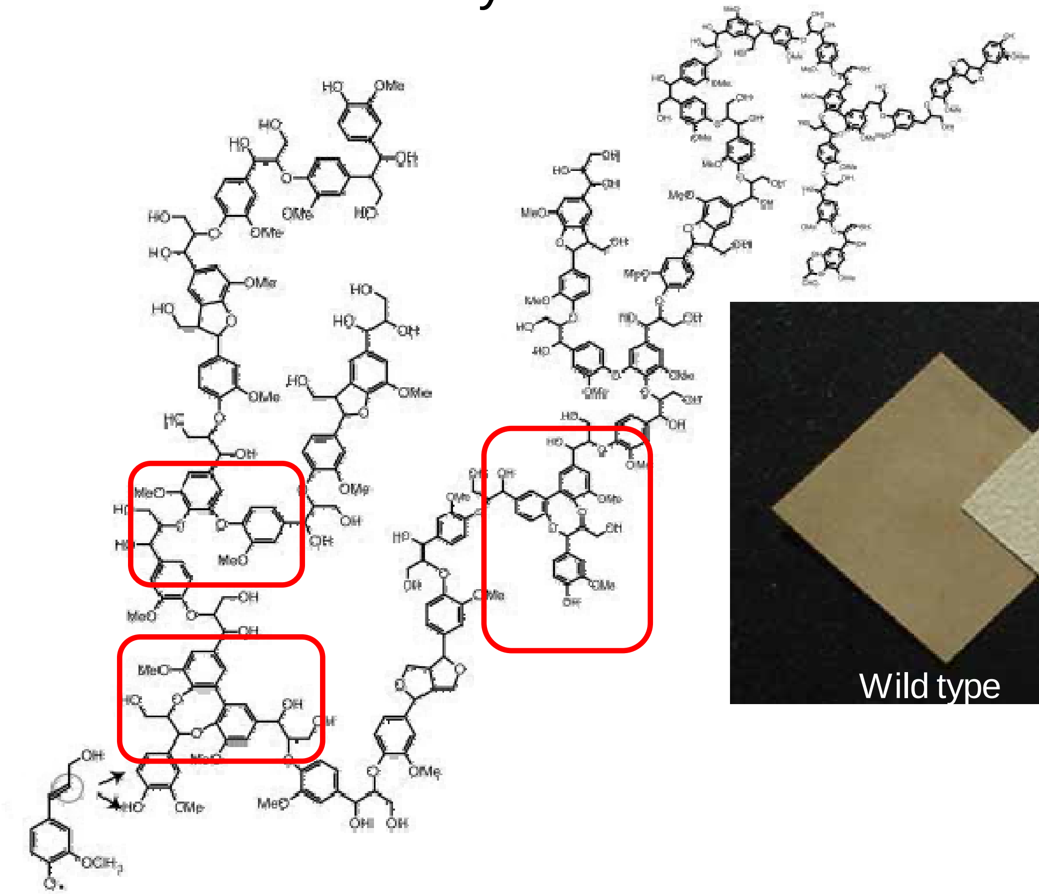
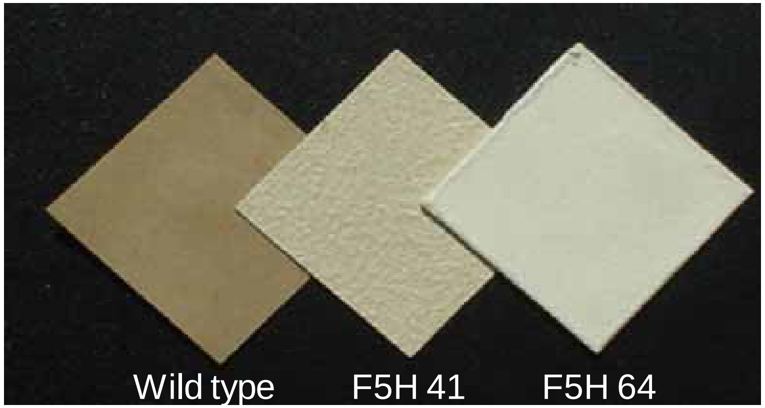
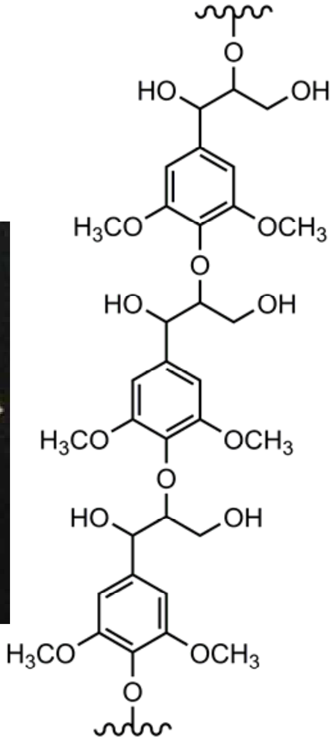
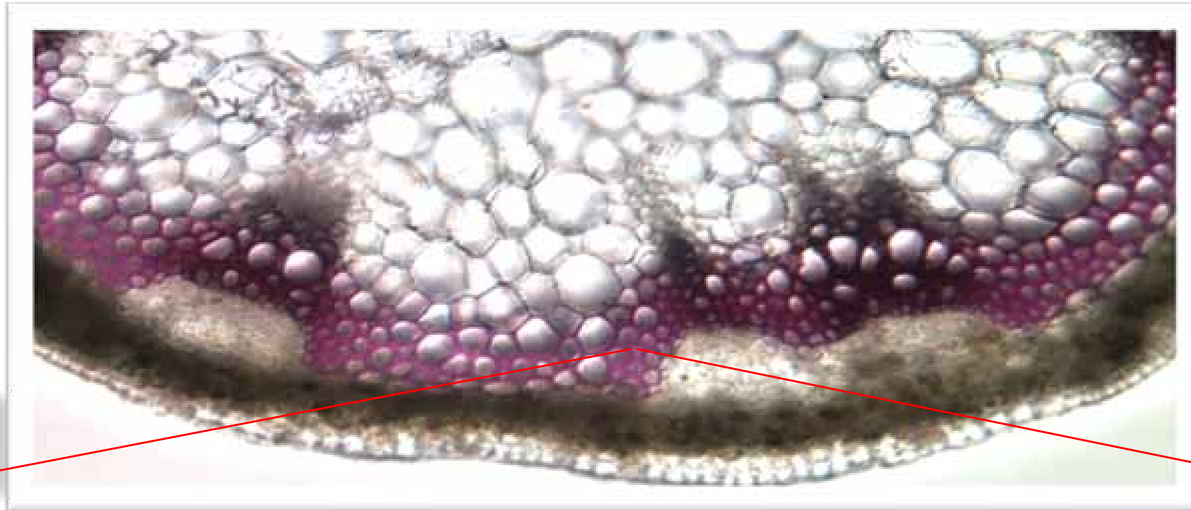
- Changes in lignin G and S content impact lignin architecture and extractability

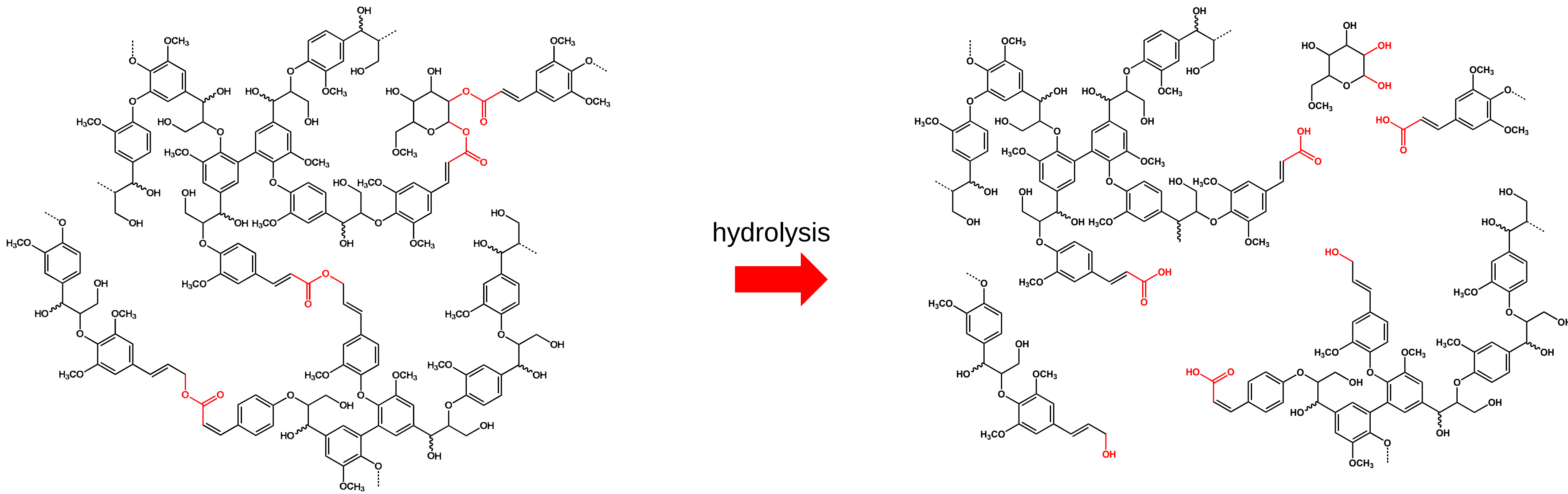






Can the plasticity of lignin monomer composition be exploited?

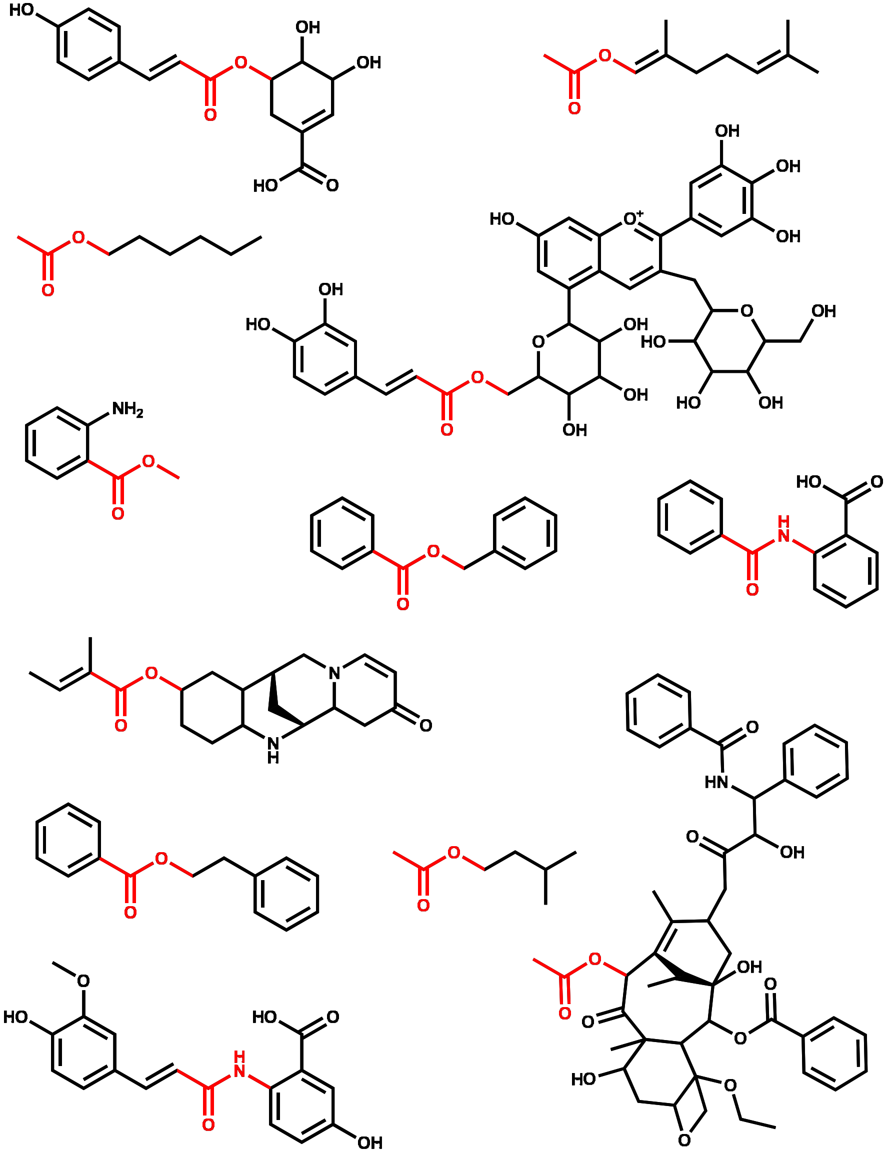


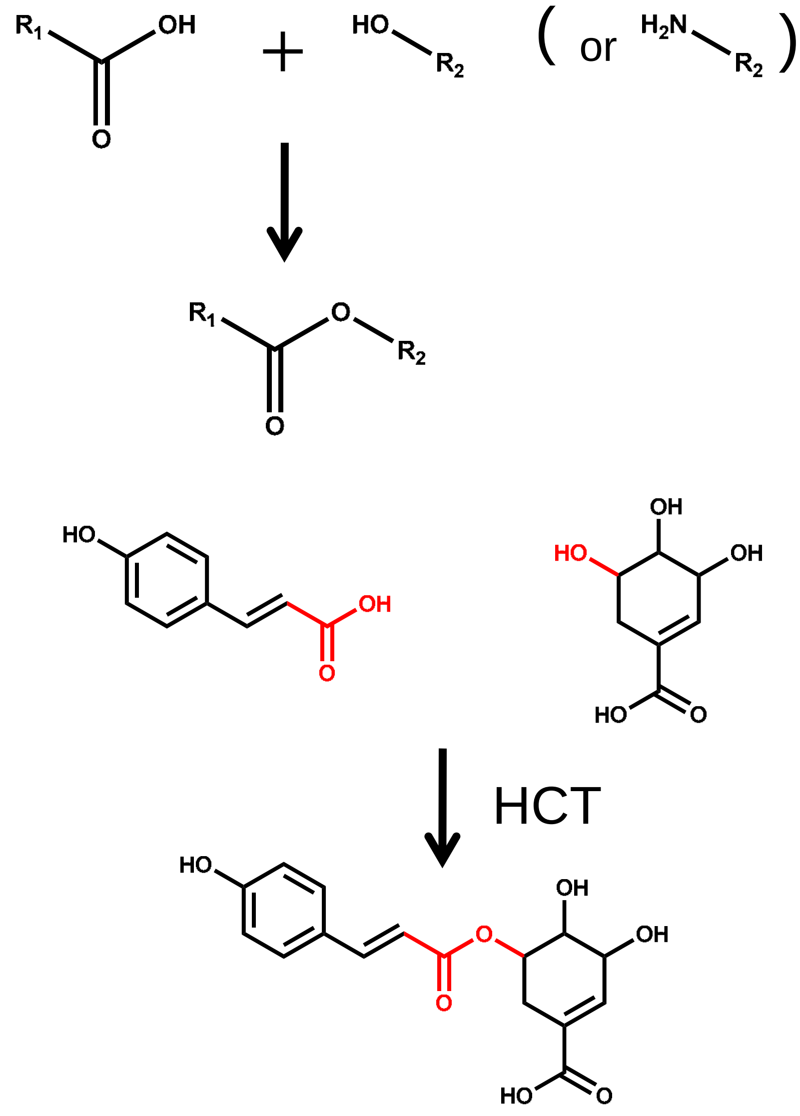


A toolbox for engineering novel lignins

- “Bioprospecting” for BAHD acyltransferases and serine carboxypeptidase-like (SCPL) acyltransferases
  - Hydrolysable lignin
- Cytochrome P450-dependent monooxygenases
  - Lignins with novel properties
- Protein engineering via chimerigenesis
  - Enzymes with novel properties

BAHD acyltransferases are involved in the synthesis of many plant metabolites





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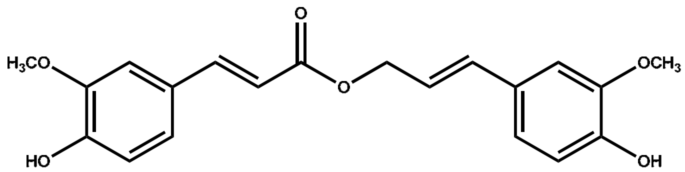
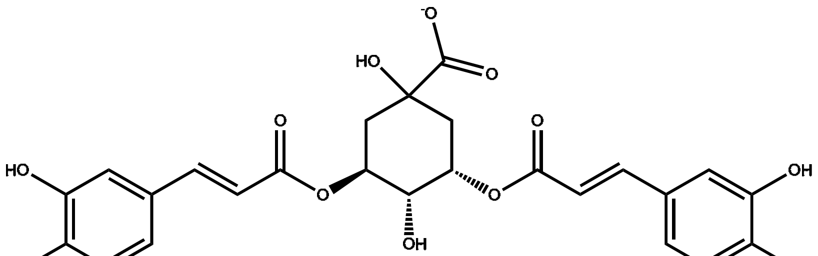
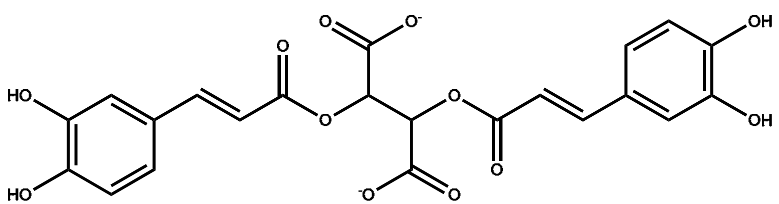


coniferyl ferulate

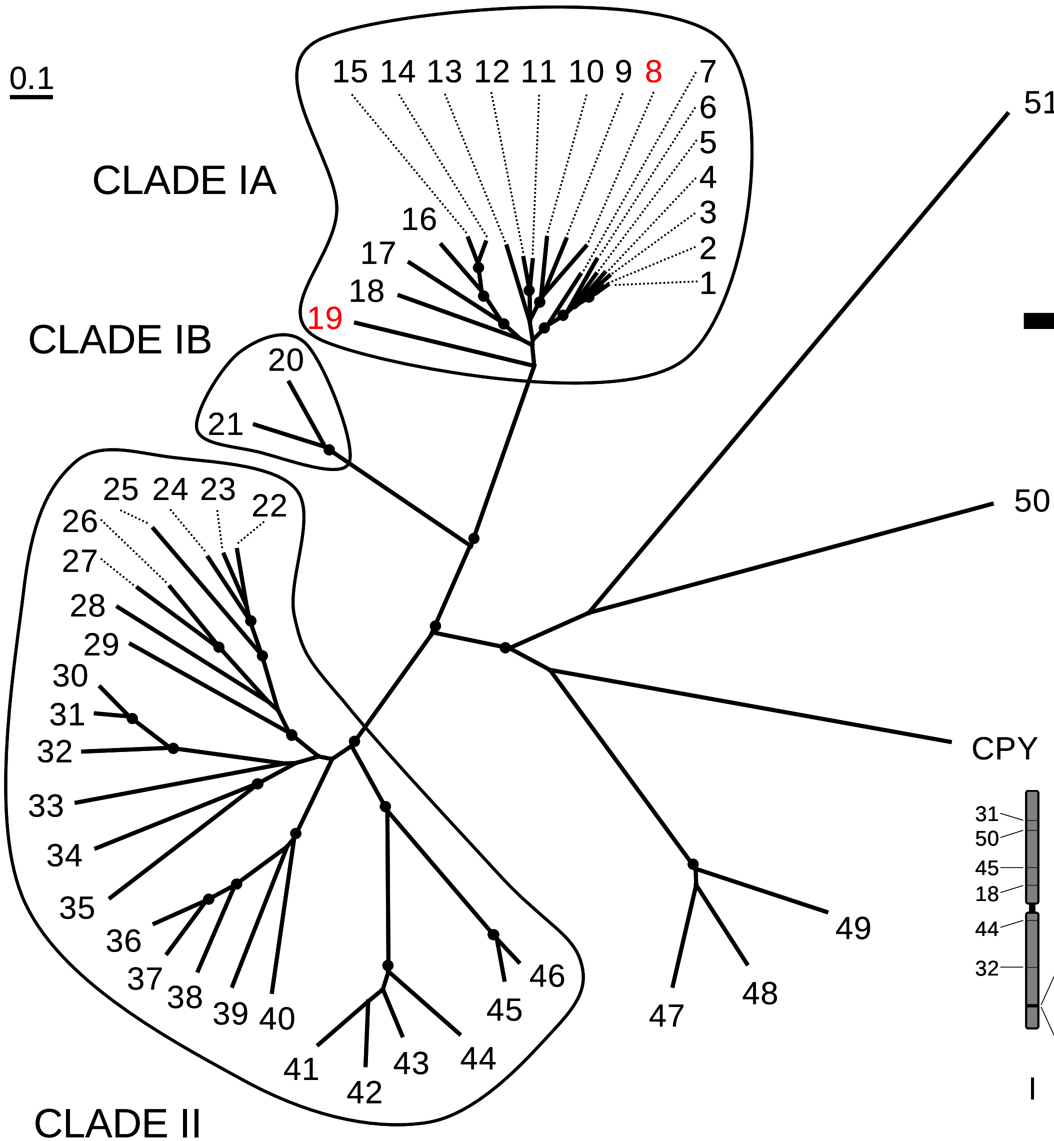


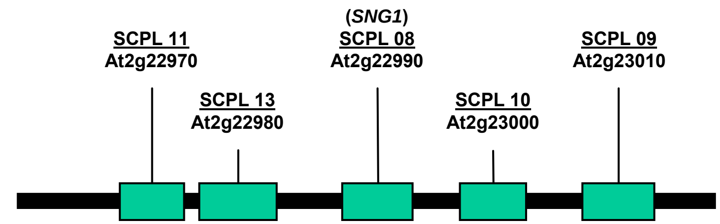
isocholorogenate (dicaffeoyl quinate)

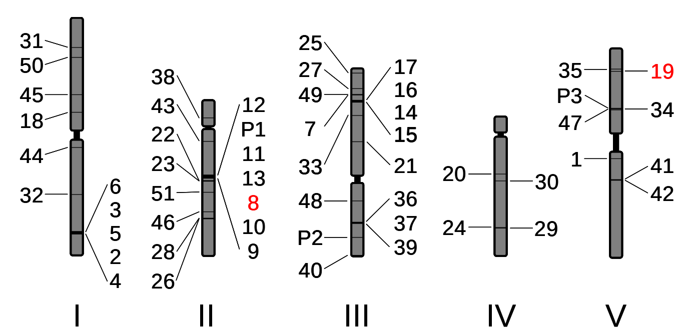


chicorate (dicaffeoyl tartrate)

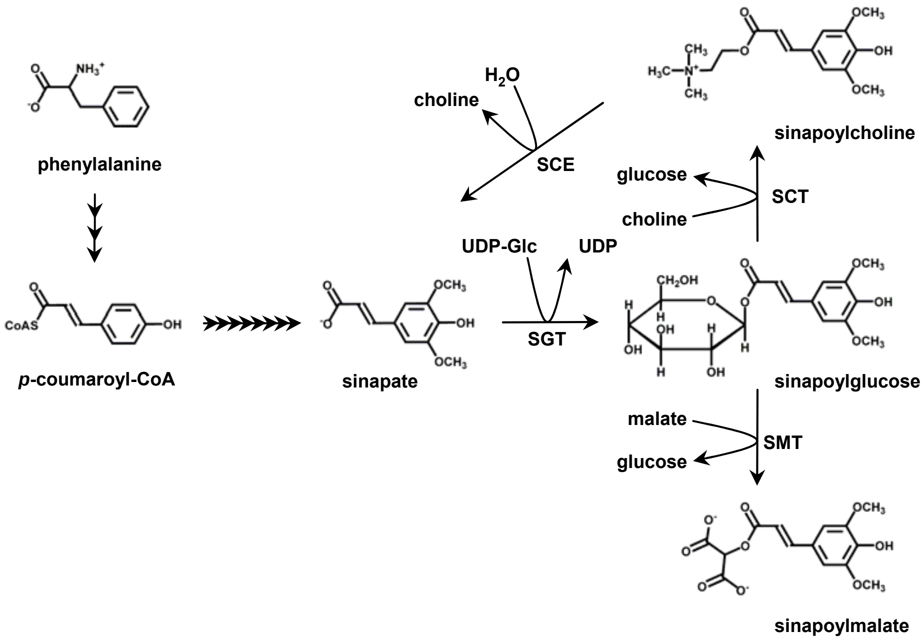
The Arabidopsis genome encodes 51 SCPL proteins







1,2-disinapoylglucose is a candidate molecule for hydrolysable lignin synthesis



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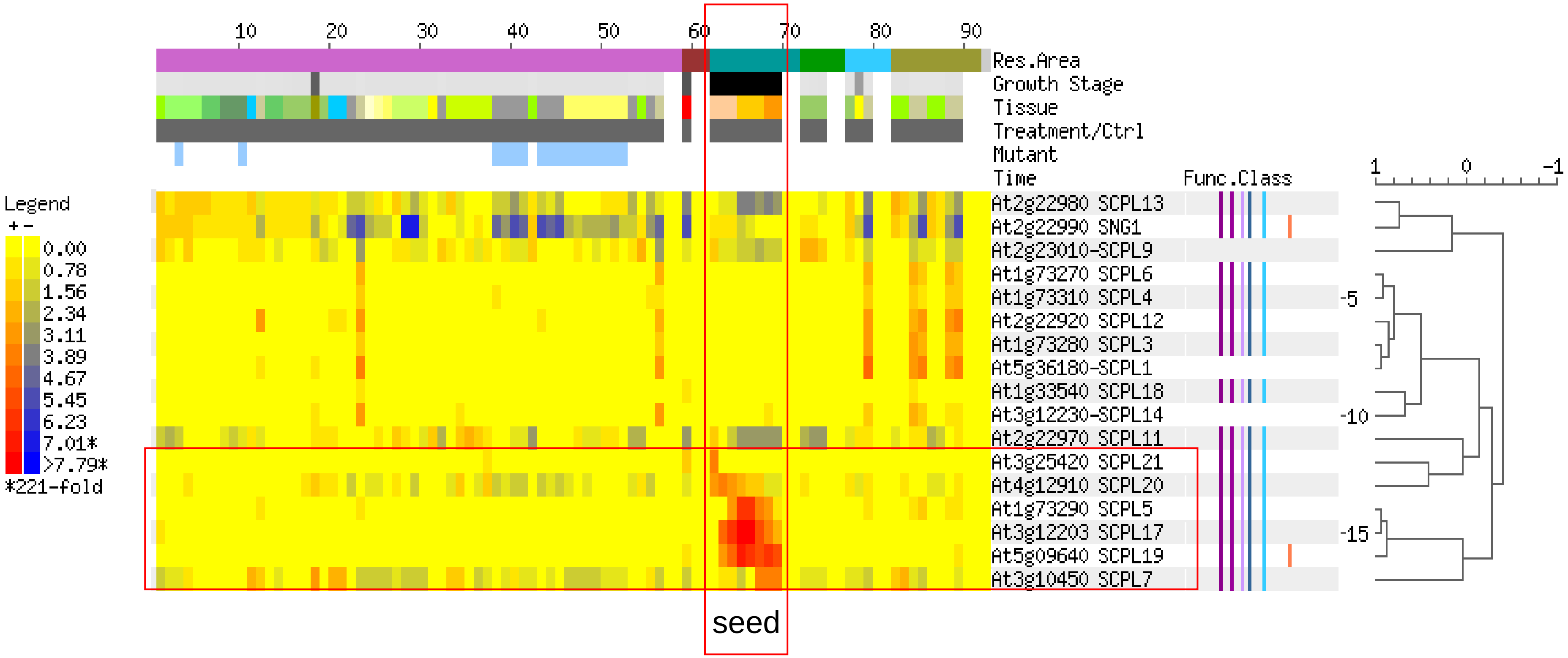
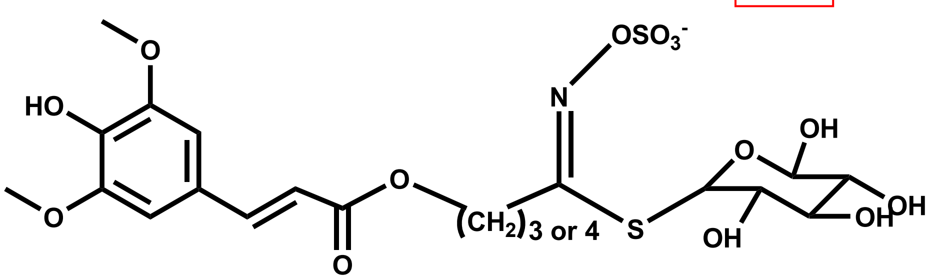
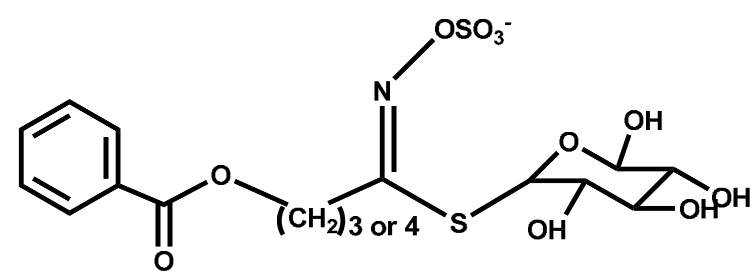
Reverse genetics has identified the function of other SCPL acyltransferases







Chimeric proteins can be selected for enhanced or novel activities

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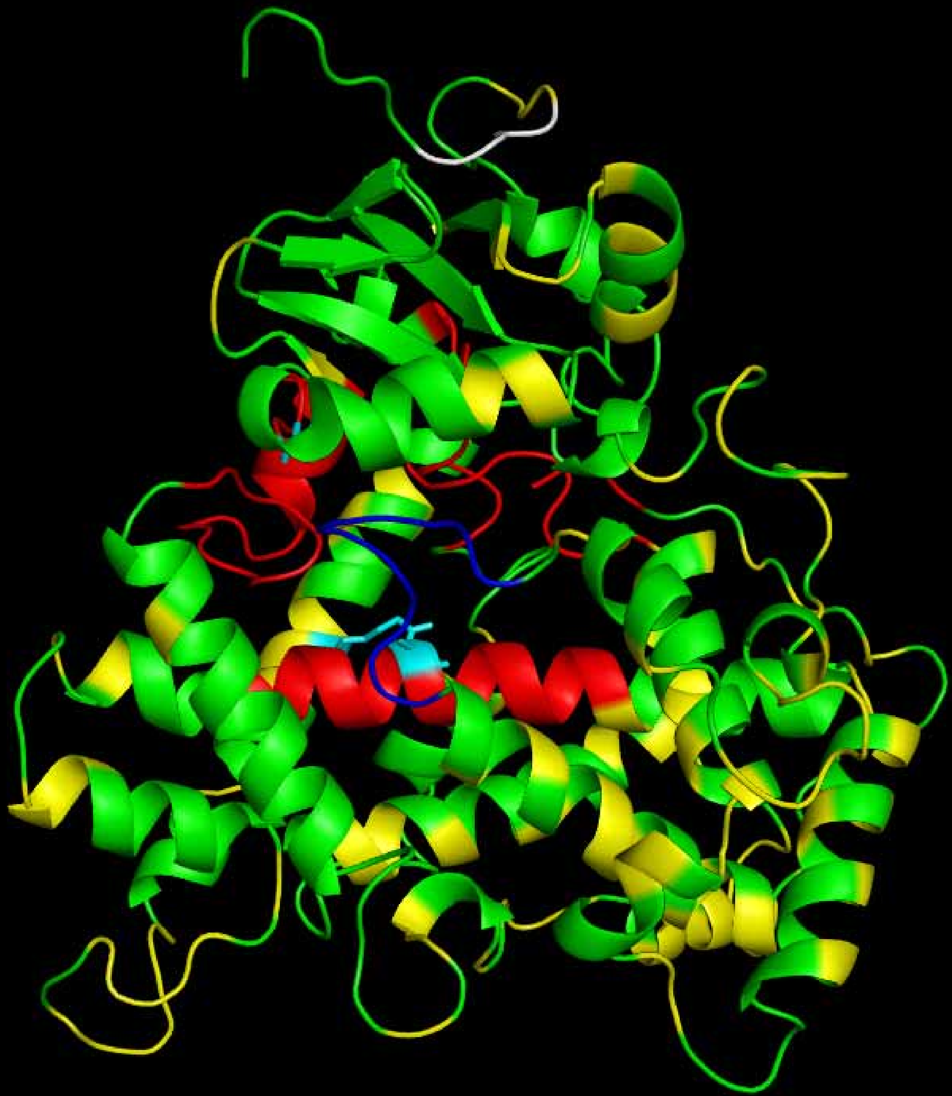
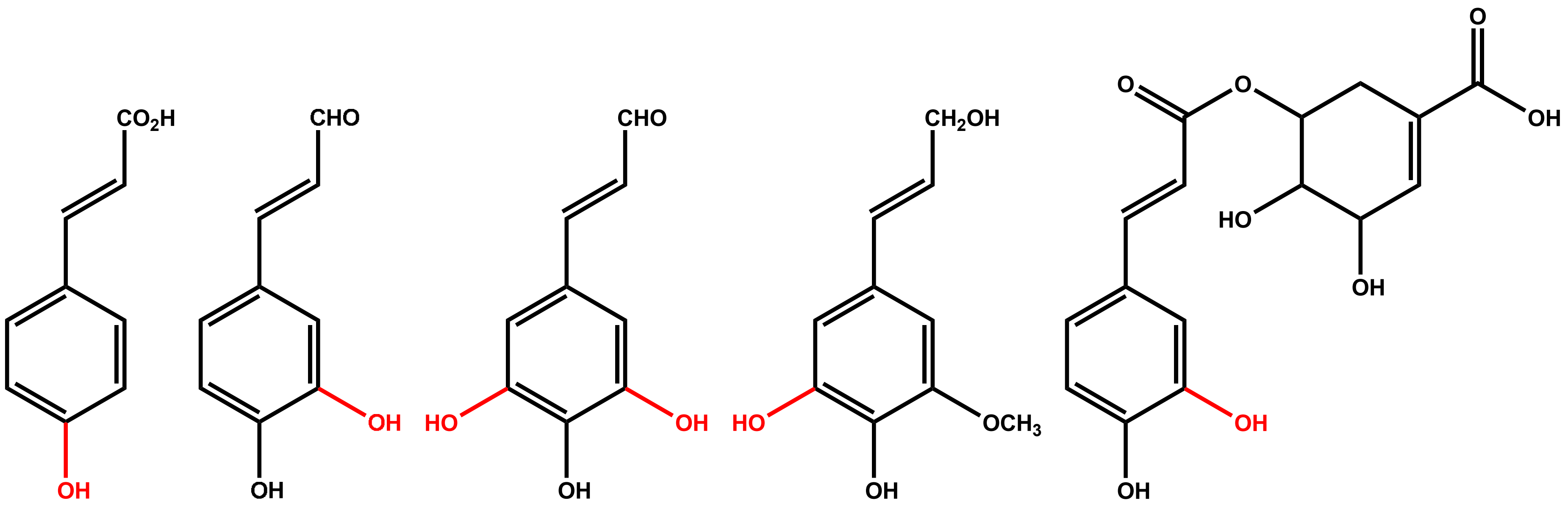


P450 chimeras may show novel substrate or regioselectivity



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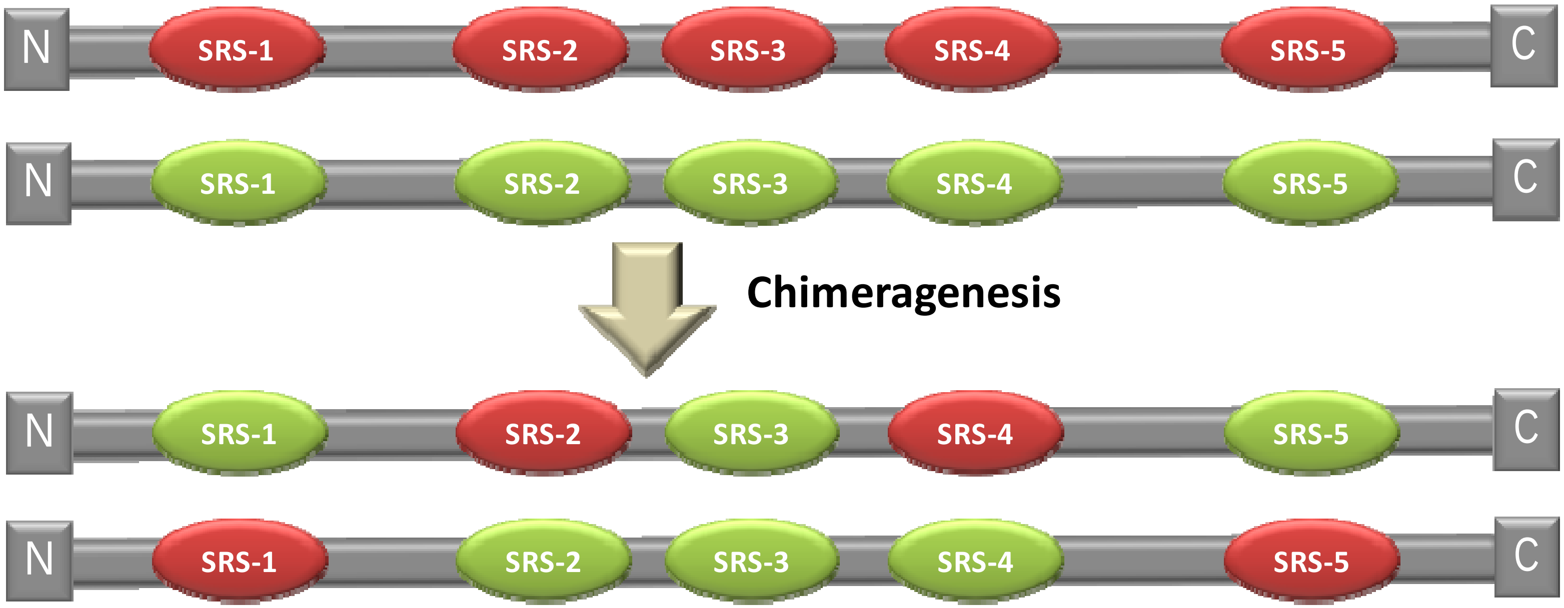
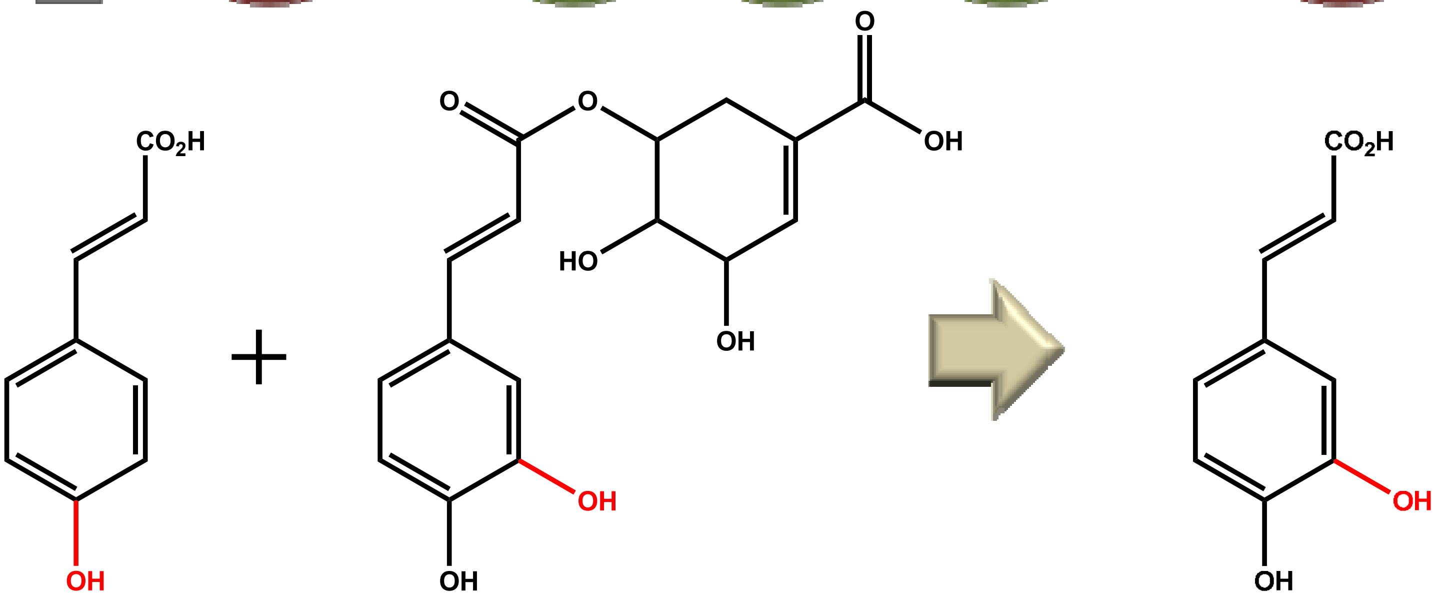
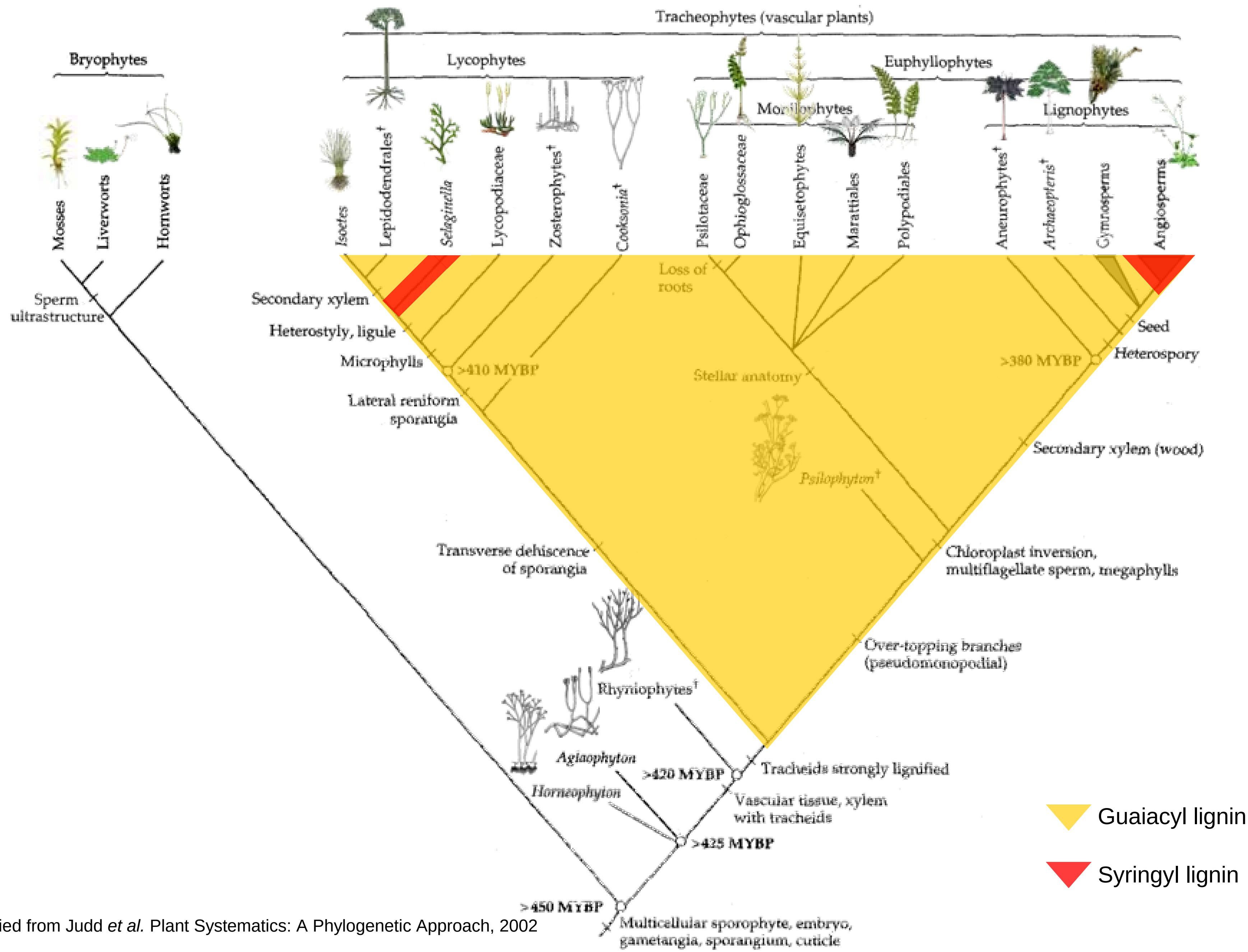






Modified from Judd *et al.* Plant Systematics: A Phylogenetic Approach, 2002

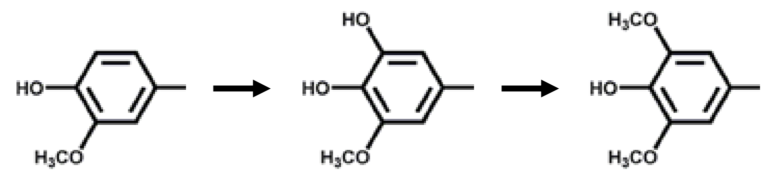
S lignin in Selaginella indicates it must possess a phenylpropanoid 5-hydroxylase

phenylalanine

cinnamate — *p*-coumarate

p-coumaroyl CoA

p-coumaroyl shikimate — caffeoyl shikimate



caffeoyl CoA — feruloyl CoA

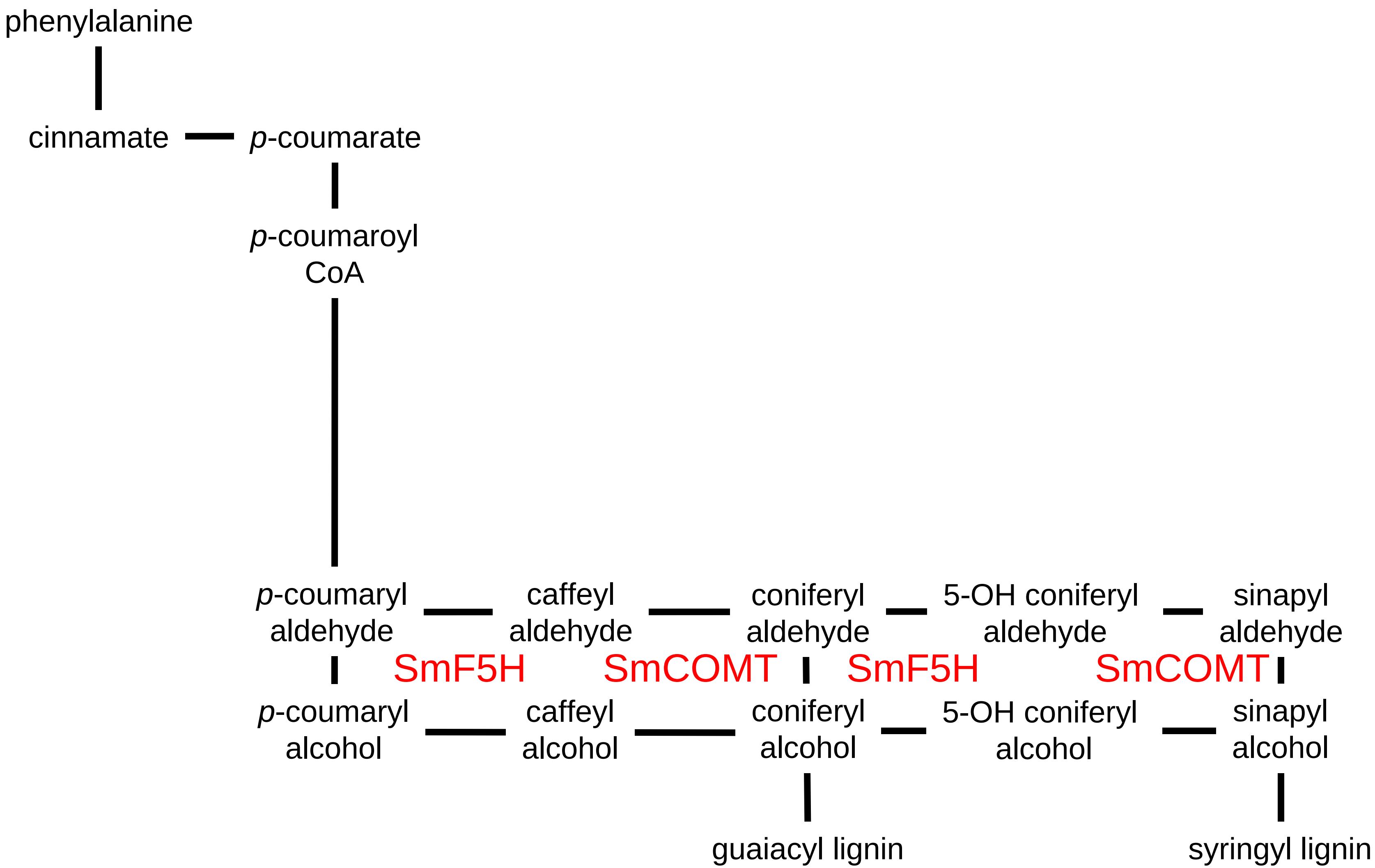
coniferyl aldehyde — 5-OH coniferyl aldehyde — sinapyl aldehyde

coniferyl alcohol — 5-OH coniferyl alcohol — sinapyl alcohol

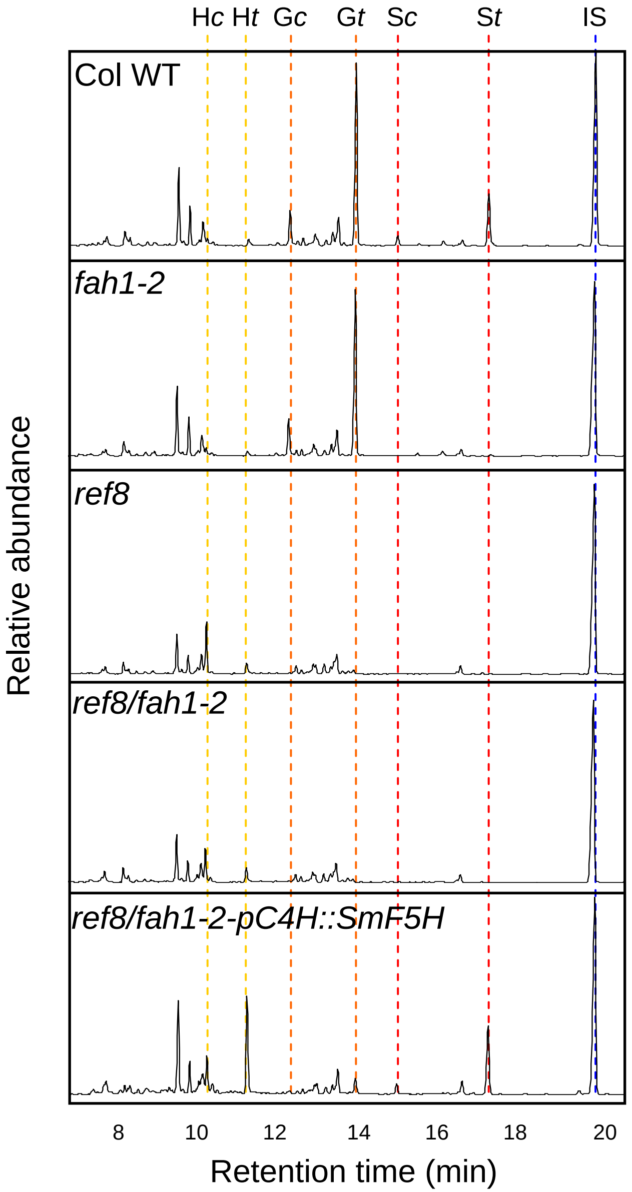
guaiacyl lignin

syringyl lignin

Lignin biosynthesis in Selaginella bypasses several steps found in angiosperms

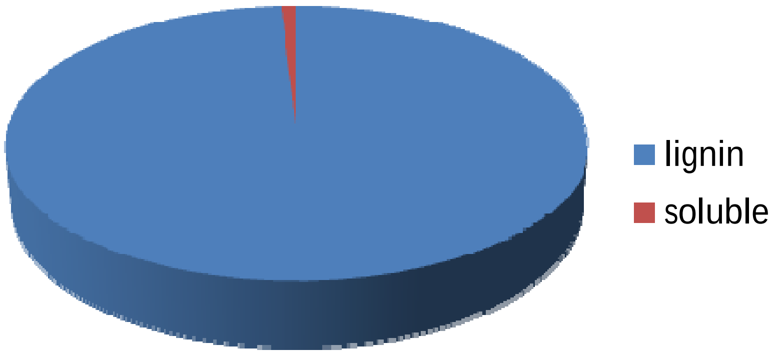


ref8 / *fah1* C4H::SmF5H transgenics exhibit an unusual lignin composition



Challenges for engineering novel lignins

- Identification of suitable monomers/dimers that can be incorporated into the lignin polymer
- Identification of genes/enzymes for monomer synthesis
- Redirection of carbon flux from lignin biosynthesis



- Export of lignin modifying molecules to the cell wall
- Incorporation into lignin in planta
- Evaluation of efficacy
- Plant viability and resistance in the field

Related posters

- Poster 6
  - Development of a systems based lignification archetype using unconventional monomer substitutes
  - Ress, Toimatsu, Rehbeck, Kim, Lu, Ralph
- Poster 12
  - Engineering new types of lignins for efficient biomass conversion using the best monolignol substitutes
  - Ress, Toimatsu, Kim, Lu, Ralph

Conclusions

- Lignin synthesis can be modified to improve biomass feedstocks for biofuel production
- Lignin synthesis is not template-directed like other biopolymers
- There are probably limits to the extent to which lignin can be reduced in plants but its composition can be altered significantly
- Dimeric lignin modification molecules may permit the generation of hydrolyzable lignins that would enhance biofuel production efficiency

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