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


http://givingspace.org/benlomond/up%20redwoods.jpg
Lignin modification may decrease the need for pretreatment
The lignin biosynthetic pathway

**Ring modification**

- Phenylalanine
  - Cinnamate → p-coumarate
    - p-coumaroyl shikimate
      - Caffeoyl CoA
        - Feruloyl CoA
          - Coniferyl aldehyde
            - 5-OH coniferyl aldehyde
              - Sinapyl aldehyde
                - Sinapyl alcohol
                  - Syringyl lignin
          - Coniferyl alcohol
            - 5-OH coniferyl alcohol
              - Sinapyl aldehyde
                - Sinapyl alcohol
                  - Syringyl lignin

**Side chain modification**

- Caffeoyl CoA
  - Phenylalanine
    - p-coumaroyl shikimate
      - Caffeoyl shikimate

Arabidopsis accumulates sinapoylmalate as a UV sunscreen.
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

phenylalanine

\[ \text{cinnamate} \quad \rightarrow \quad p\text{-coumarate} \]
\[ \text{p-coumaroyl CoA} \quad \rightarrow \quad \text{p-coumaroyl shikimate} \]
\[ \text{shikimate} \quad \rightarrow \quad \text{caffeoyl shikimate} \]

\[ \text{caffeoyl CoA} \quad \rightarrow \quad \text{feruloyl CoA} \]

\[ \text{coniferyl aldehyde} \quad \rightarrow \quad 5\text{-OH coniferyl aldehyde} \]
\[ \text{coniferyl alcohol} \quad \rightarrow \quad 5\text{-OH coniferyl alcohol} \]

\[ \text{sinapyl aldehyde} \quad \rightarrow \quad \text{sinapyl alcohol} \quad \rightarrow \quad \text{syringyl lignin} \]
The *ref8* mutation reroutes lignin biosynthesis

- **phenylalanine**
  - **cinnamate** — **p-coumarate**
    - **p-coumaroyl CoA**
      - **p-coumaroyl shikimate**
        - **referred**
        - **C3’H shikimate**
  - **p-coumaroyl CoA**
    - **caffeoyl CoA** — **feruloyl CoA**
      - **p-coumaryl aldehyde**
        - **p-coumaryl alcohol**
          - **p-hydroxyphenyl lignin**
      - **coniferyl aldehyde**
        - **coniferyl alcohol**
          - **guaiacyl lignin**
    - **5-OH coniferyl aldehyde**
      - **5-OH coniferyl alcohol**
        - **syringyl lignin**
      - **sinapyl aldehyde**
        - **sinapyl alcohol**
          - **syringyl lignin**
ref8 mutants are strongly dwarfed

- 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

- Changes in lignin G and S content impact lignin architecture and extractability
Can the plasticity of lignin monomer composition be exploited?

hydrolysis
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 (dicafeoyl quinate)
- Chicorate (dicafeoyl tartrate)
The Arabidopsis genome encodes 51 SCPL proteins
1,2-disinapoylglucose is a candidate molecule for hydrolysable lignin synthesis.
Reverse genetics has identified the function of other SCPL acyltransferases.
Chimeric proteins can be selected for enhanced or novel activities
P450 chimeras may show novel substrate or regioselectivity
P450 chimeras may show novel substrate or regioselectivity
S lignin in Selaginella indicates it must possess a phenylpropanoid 5-hydroxylase.
Lignin biosynthesis in Selaginella bypasses several steps found in angiosperms

phenylalanine

\[ \text{phenylalanine} \rightarrow \text{cinnamate} \rightarrow p\text{-coumarate} \rightarrow p\text{-coumaroyl CoA} \rightarrow \text{p-coumaryl aldehyde} \rightarrow \text{SmF5H} \rightarrow \text{SmCOMT} \rightarrow \text{p-coumaryl alcohol} \rightarrow \text{guaiacyl lignin} \]

\[ \rightarrow \text{caffeyle aldehyde} \rightarrow \text{SmF5H} \rightarrow \text{caffeyle alcohol} \rightarrow \text{SmCOMT} \rightarrow \text{coniferyl aldehyde} \rightarrow \text{coniferyl alcohol} \rightarrow \text{SmF5H} \rightarrow \text{5-OH coniferyl aldehyde} \rightarrow \text{sinapyl alcohol} \rightarrow \text{syringyl lignin} \]
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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