

Current and Future Functional Genomic Analysis of Grass Cell Wall Synthesis

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(courtesy with Chem/Biochem and CBME)
University of Oklahoma**





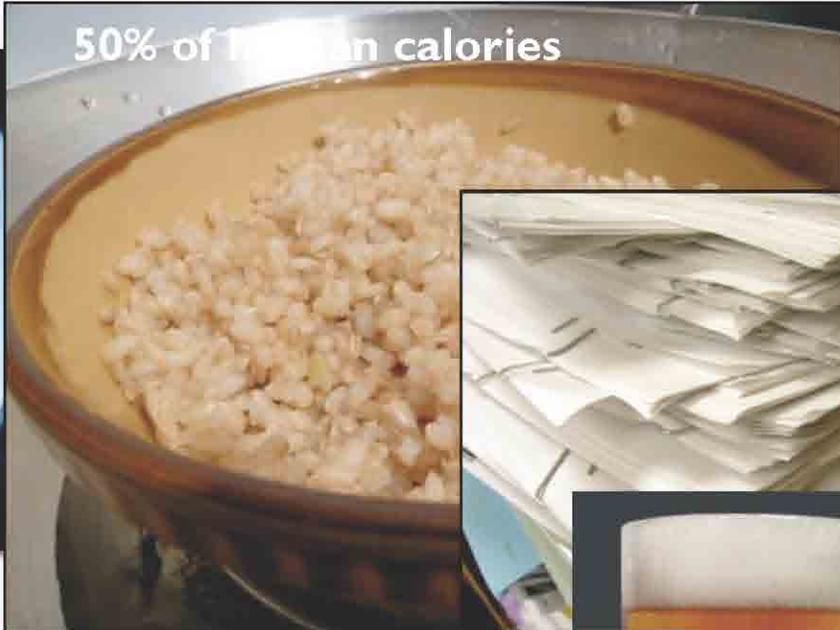
Coming Up:

- Why study grass cell walls?
- Recent Wall Projects
 - Time course of rice cell wall development and gene expression
 - Reverse genetics analysis of a grass-specific cell wall crosslink
- Future Directions
 - Acyltransferase biochemistry and mutant analysis
 - Identifying other enzymes involved in phenylpropanoid incorporation into grass walls
 - Genetic and physical analysis of wall-wall adhesion in grass cell walls
 - Switchgrass genome diversity

Feed



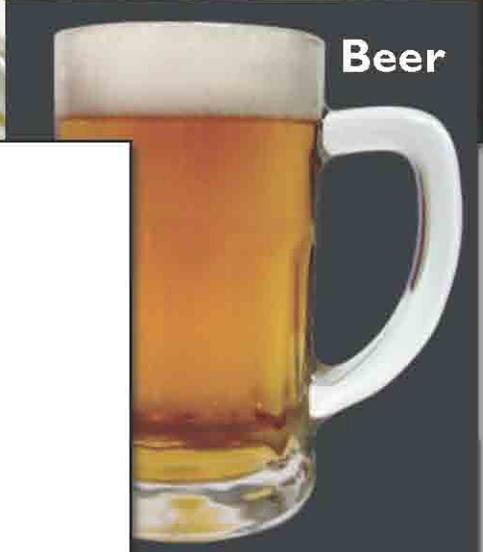
50% of Human calories



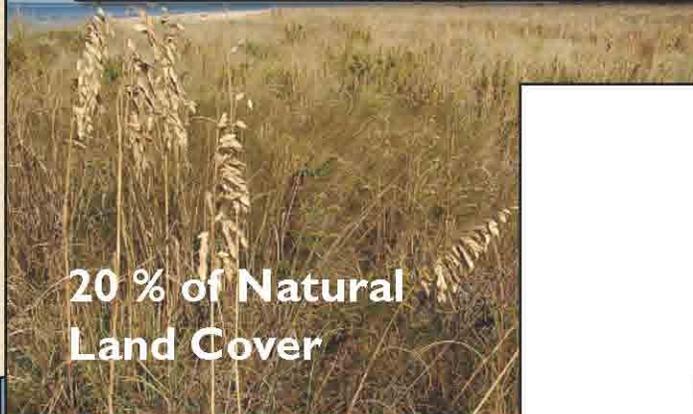
Paper



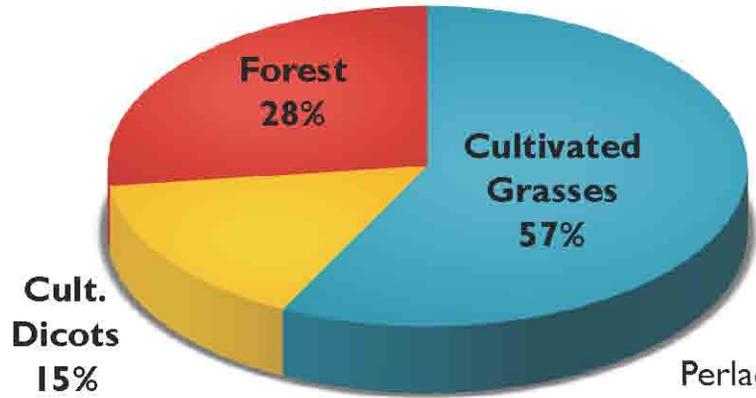
Beer



20 % of Natural Land Cover



The "Billion Ton" Vision

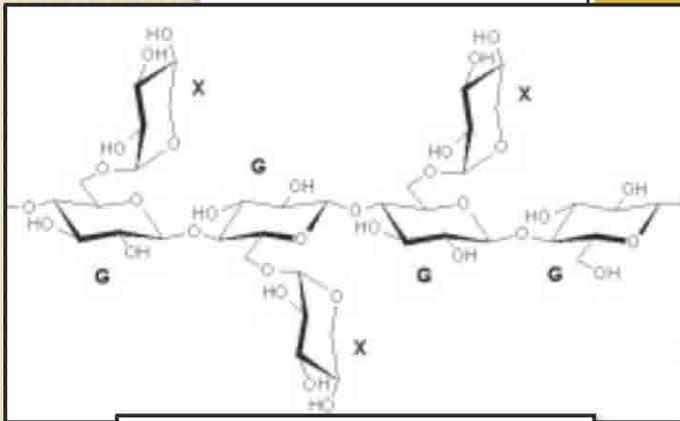
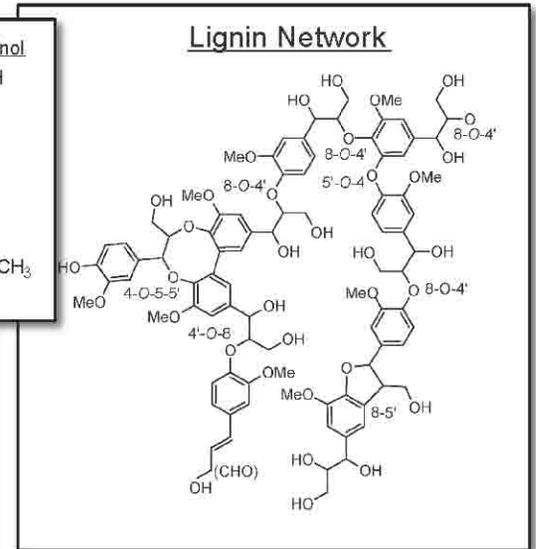
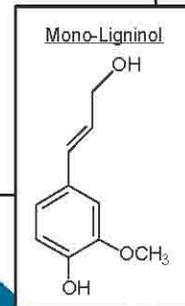
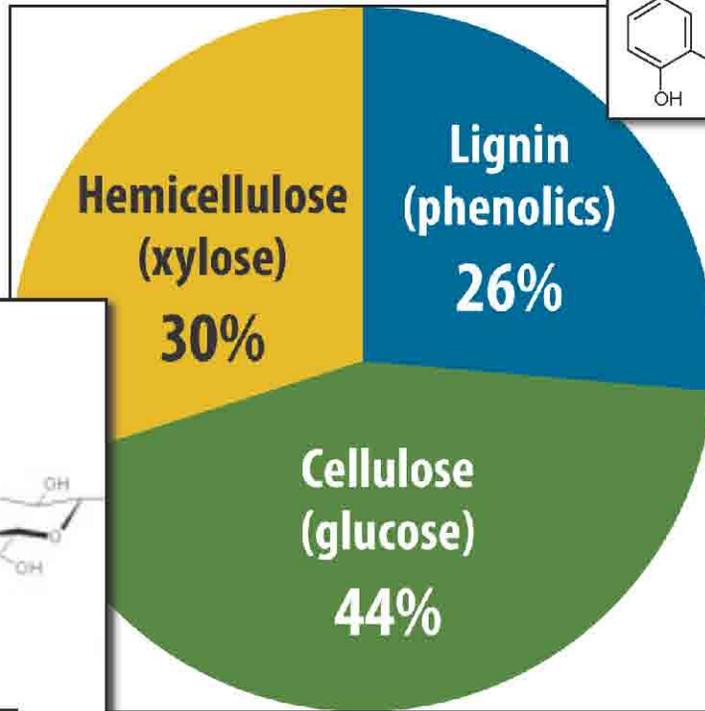


Perlack 2006

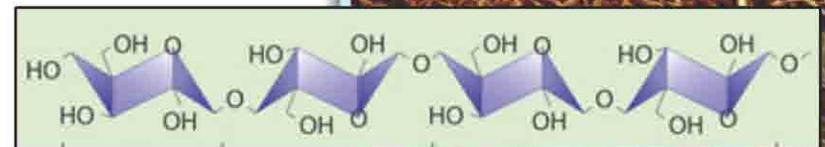
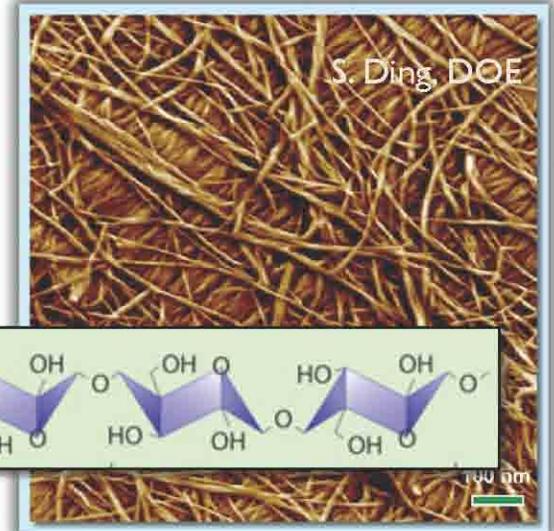
Biofuels



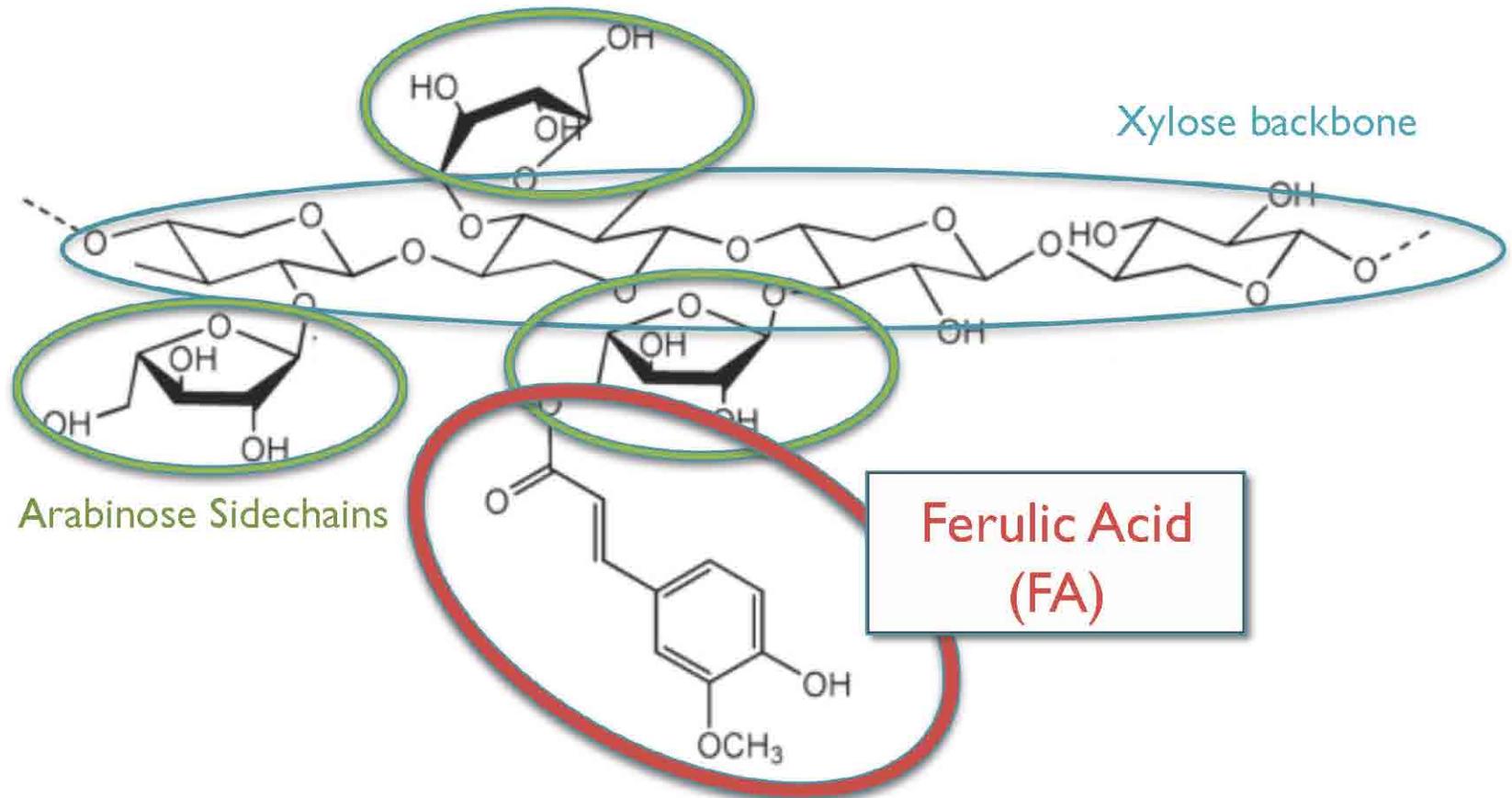
Cell Wall Components...



Matrix Polysaccharides

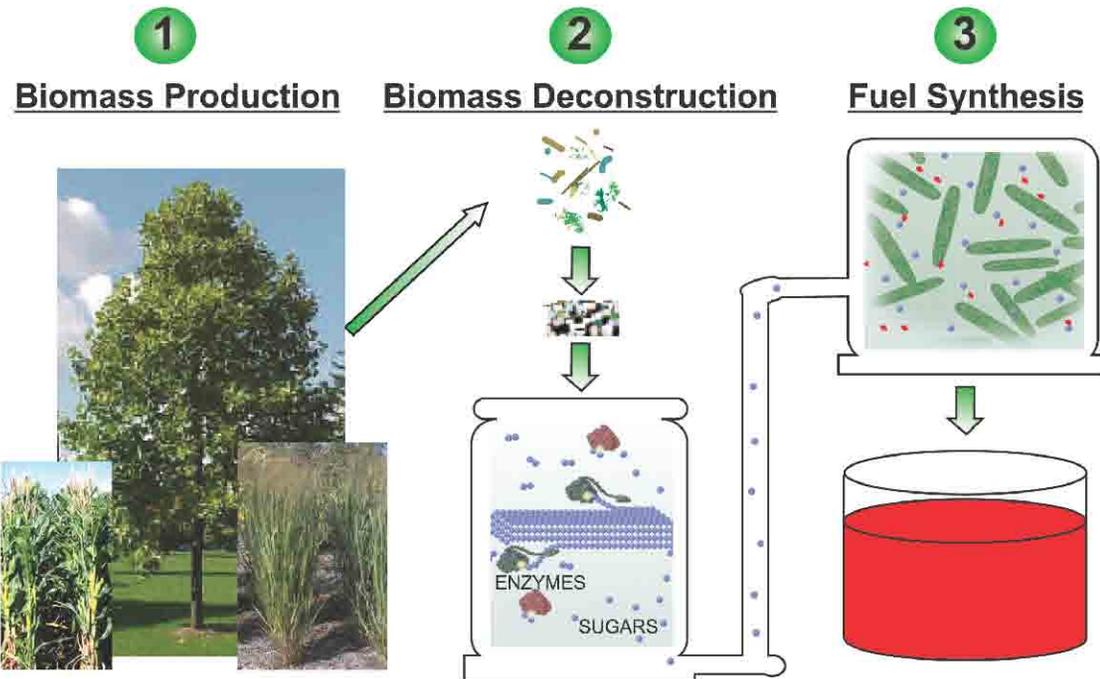
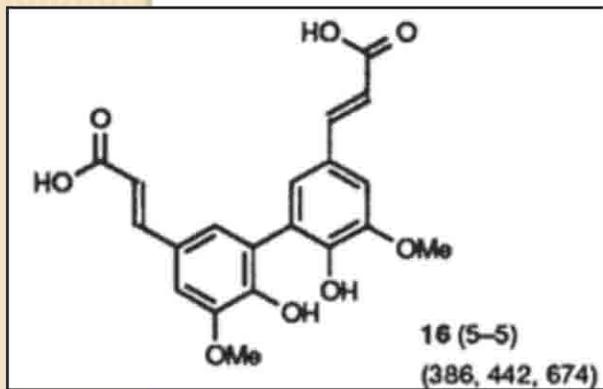


Grass Specific Polysaccharide: Feruoylated-Arabinoxylan



Buanafina 2009 *Mol Plant* 2:861-872

FA Crosslinks Xylan Strands to Each Other and Lignin...



...blocking cell wall digestion...inhibiting yeast growth.

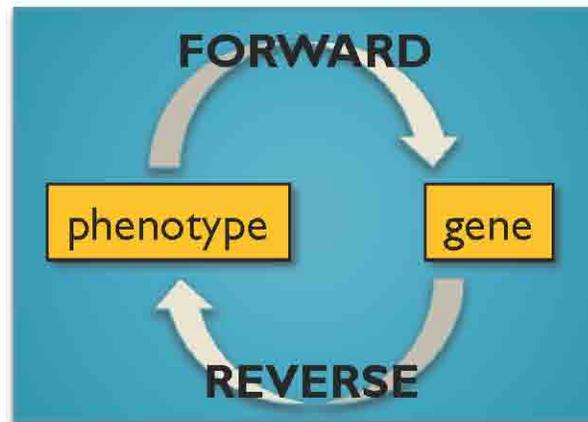
What effects on thermochemical processes?

Questions about Ferulic Acid



- What is the pathway for incorporation of FA into arabinoxylan of grass cell walls?
- Do modified FA levels in cell walls effect wall digestability?
- What are the biological functions of wall FA?

Method to understand grass cell walls and improve them for deconstruction.



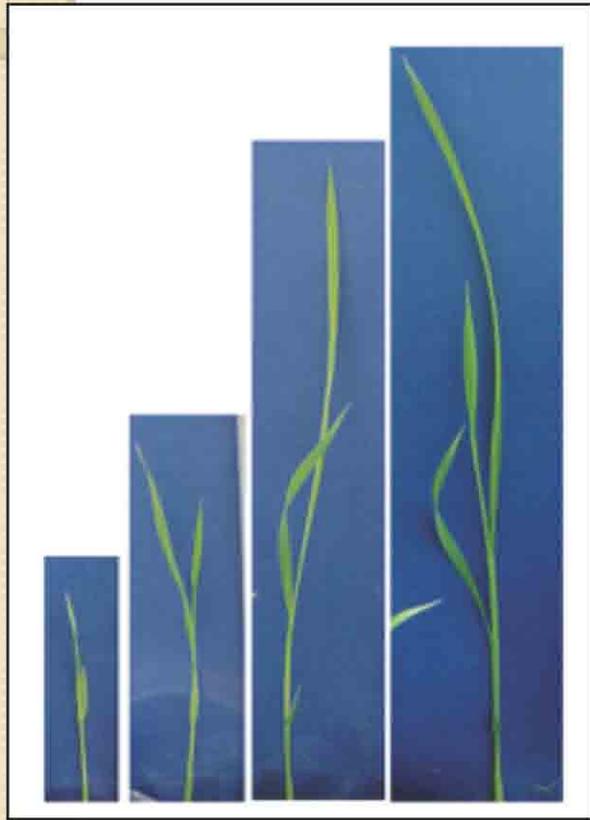
Reverse Genetics Framework

When and where? (Developmental Time Course)

Hypothesis building: bioinformatics.

Testing hypotheses with mutants.

Developmental Time Course For Cell Wall Content and Transcript Abundance



→ 30 organs/stages with biological replicates



Cell Wall Content

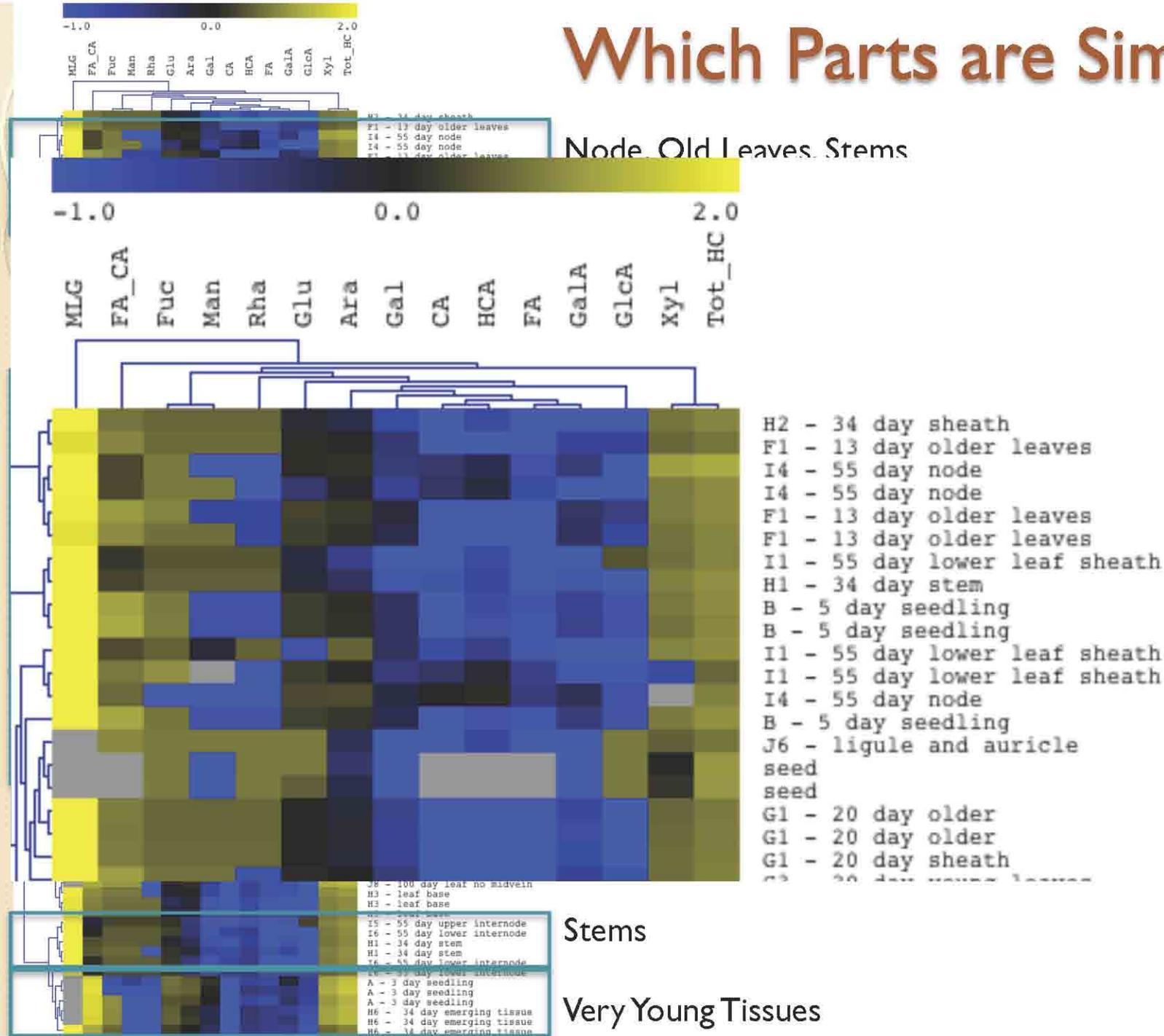
Spectroscopic Analysis



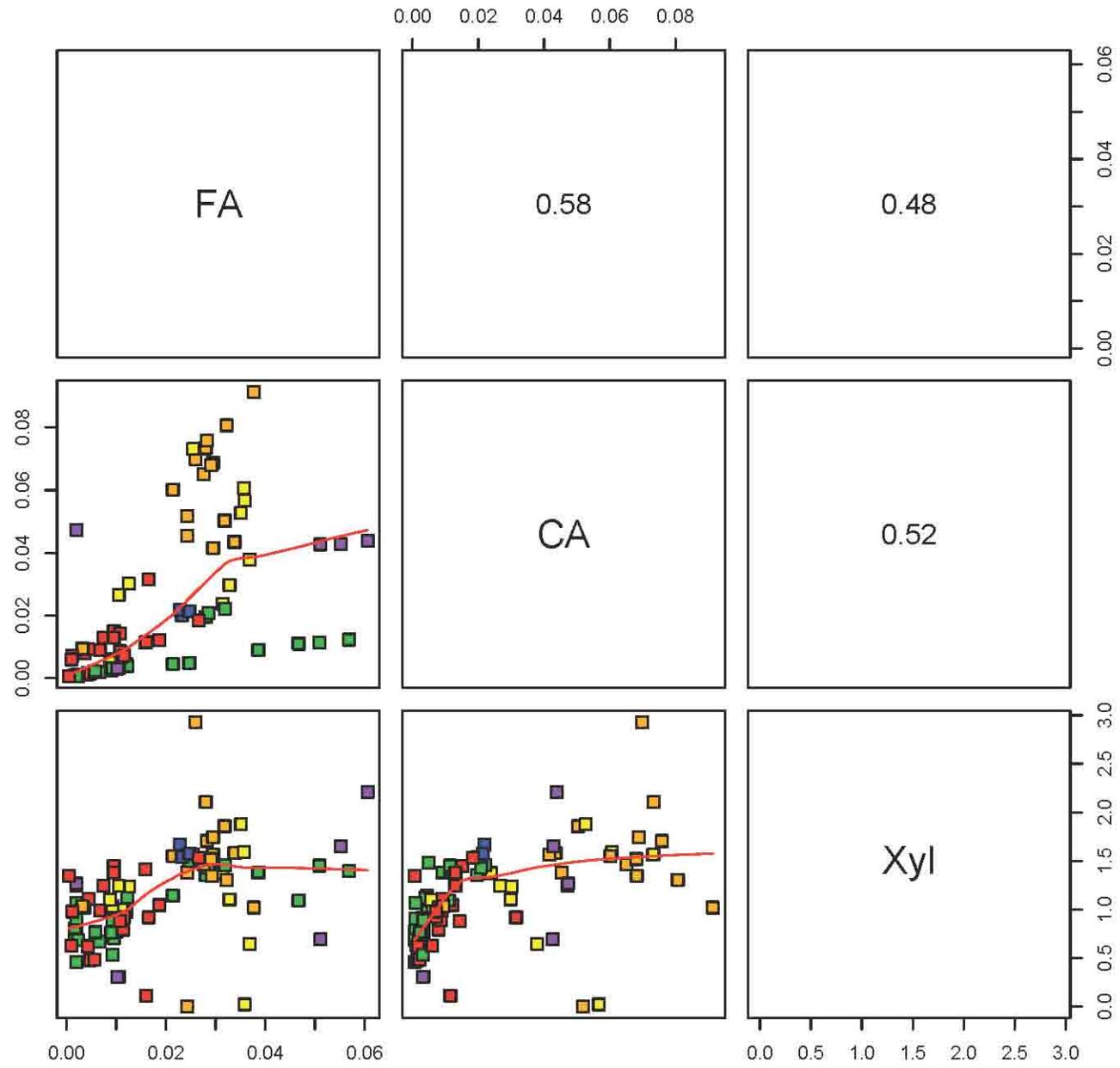
qRT-PCR

with Dawn Chiniquy, Brian Conlin, Brian Williams, and Jenna Fall

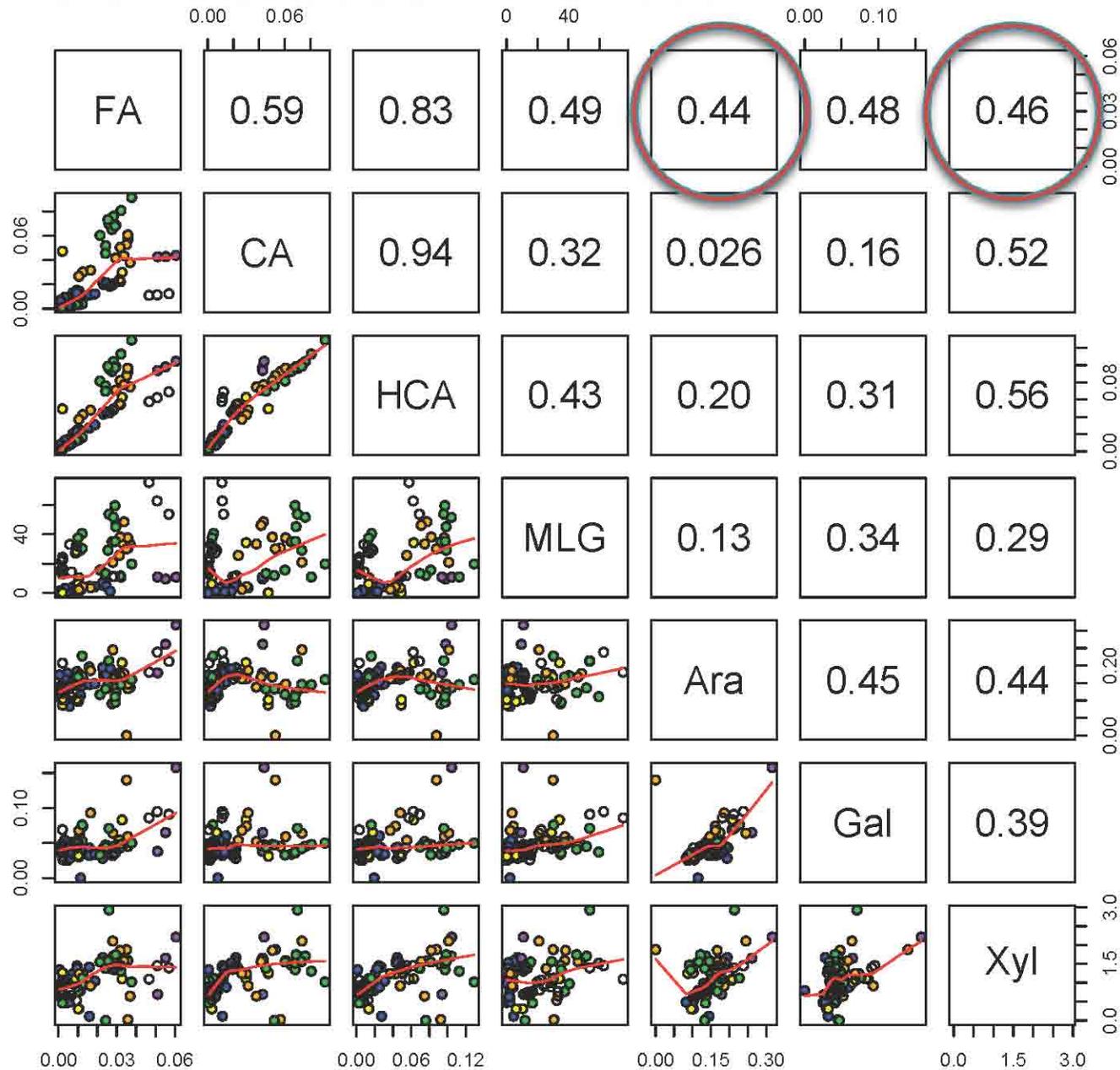
Which Parts are Similar?

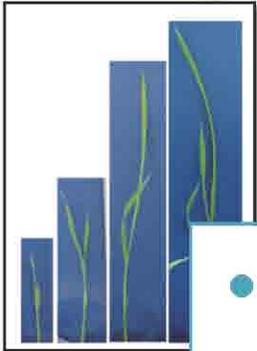


Cell Wall Content (umoles/mg): Pearson's Correlation



FA Correlates with...

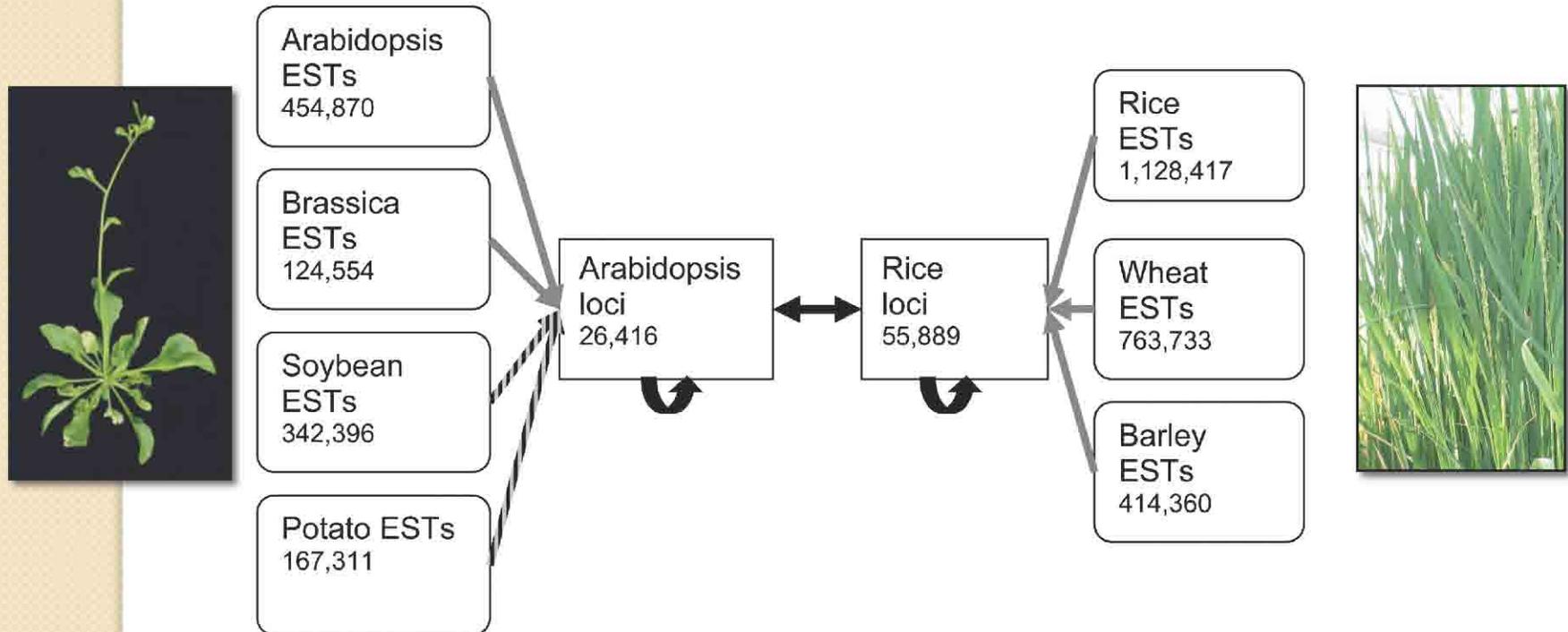




Time Course: Observations and Future

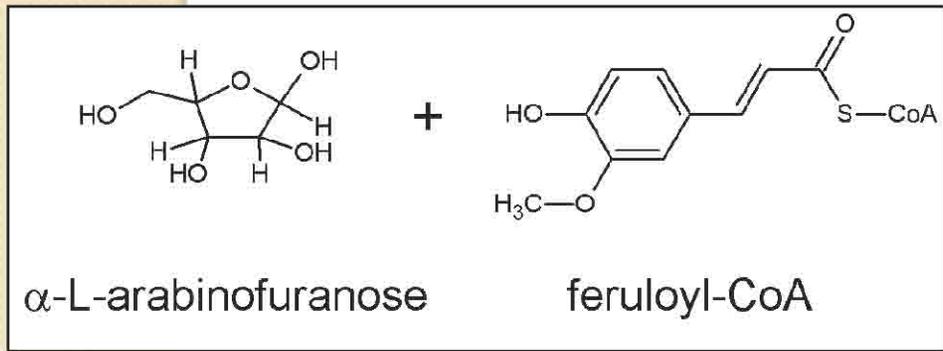
- Wall ferulic acid levels correlate significantly with xylan and arabinose but not highly.
 - Ara:Xyl:FA ratios may vary in arabinoxylan.
 - Ara and Xyl may be part of other polymers.
- Cross correlations with gene expression will support hypothesis development regarding gene function. (Martin *et al.* 2007 *Hepatology*)
- Correlations may reveal developmental signatures and regulatory units.

Hypothesis: A Group of CoA Acyltransferases Highly Expressed in Grasses Relative to Dicots May Incorporate FA



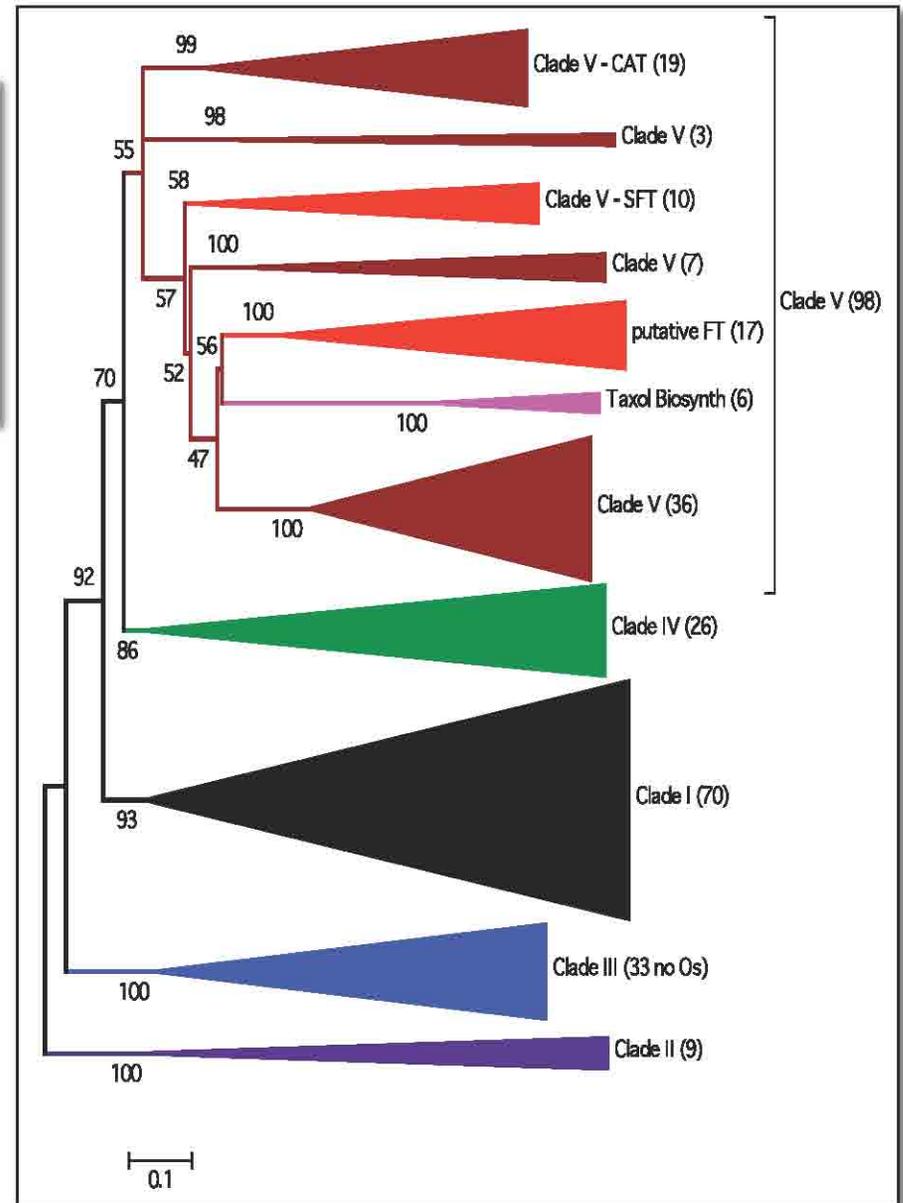
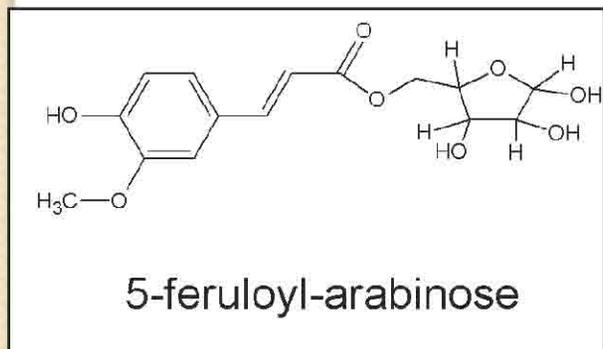
Mitchell et al. 2007 Plant Physiol. 144:43-53

BAHD CoA Acyltransferases

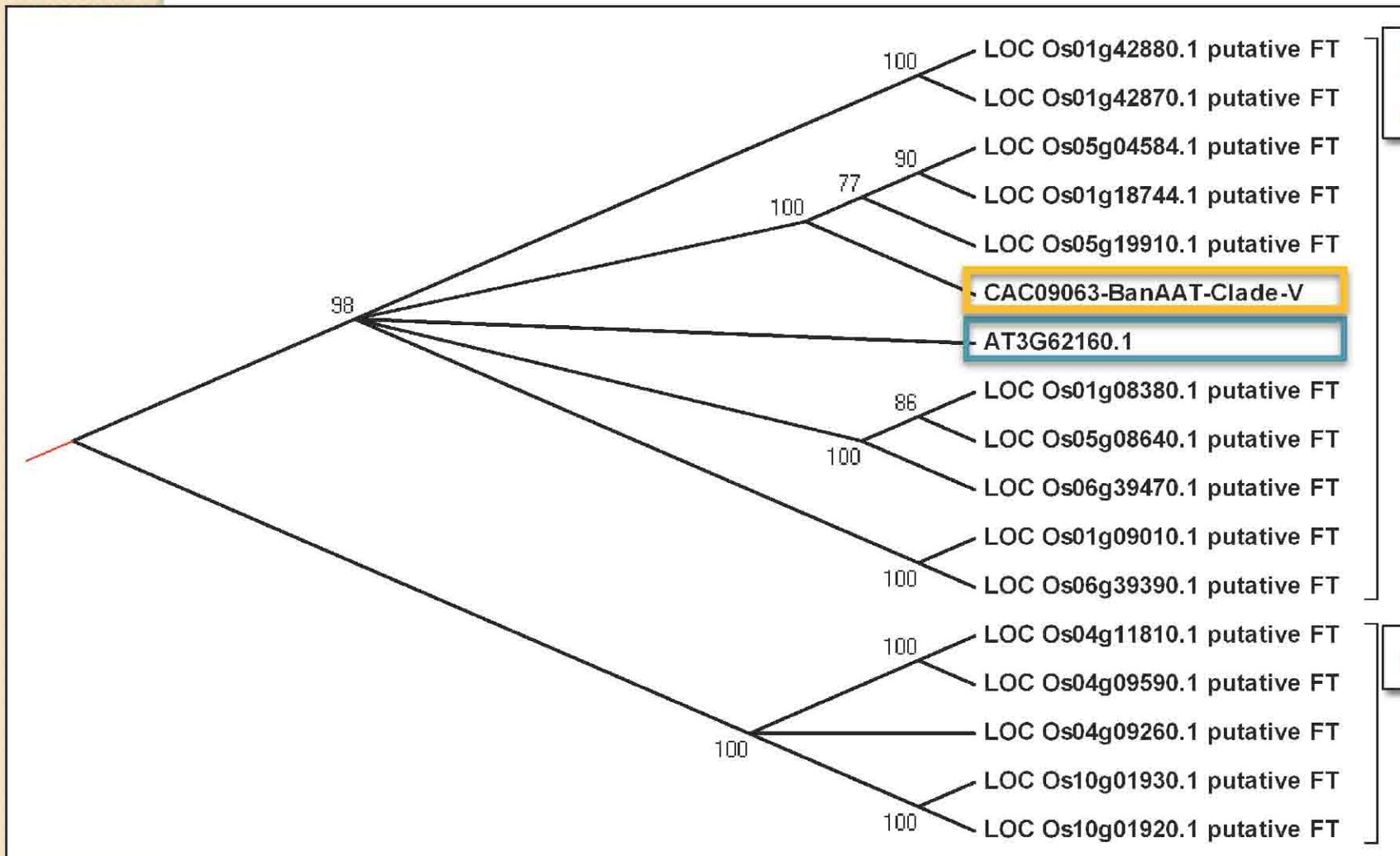
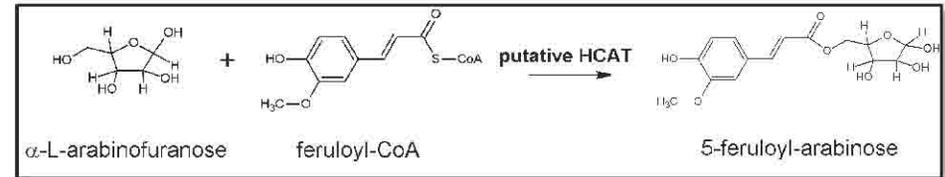




 Putative
 Feruloyl
 Transferase
 (FT)



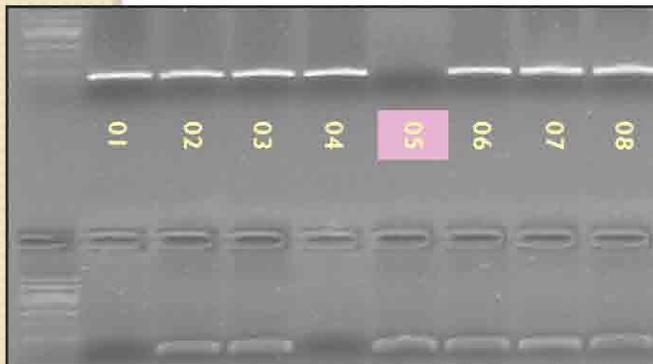
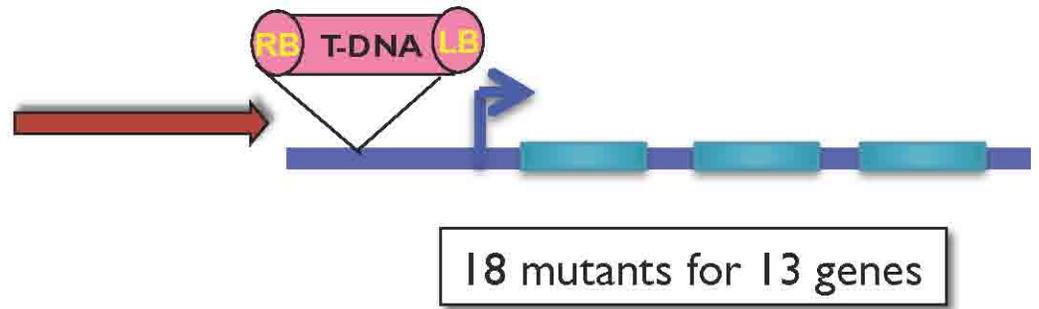
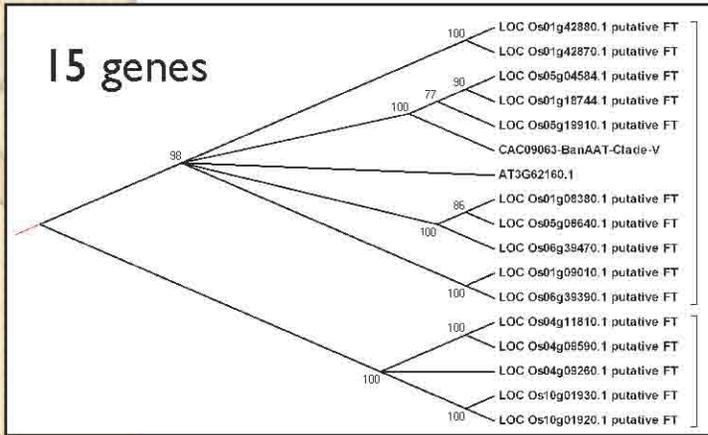
A Rice-Expanded Clade



Mitchel et al.

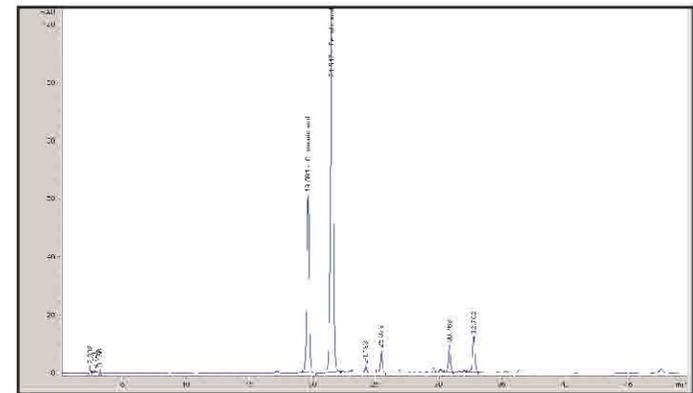
related

Hypothesis Testing with Indexed Rice Mutants

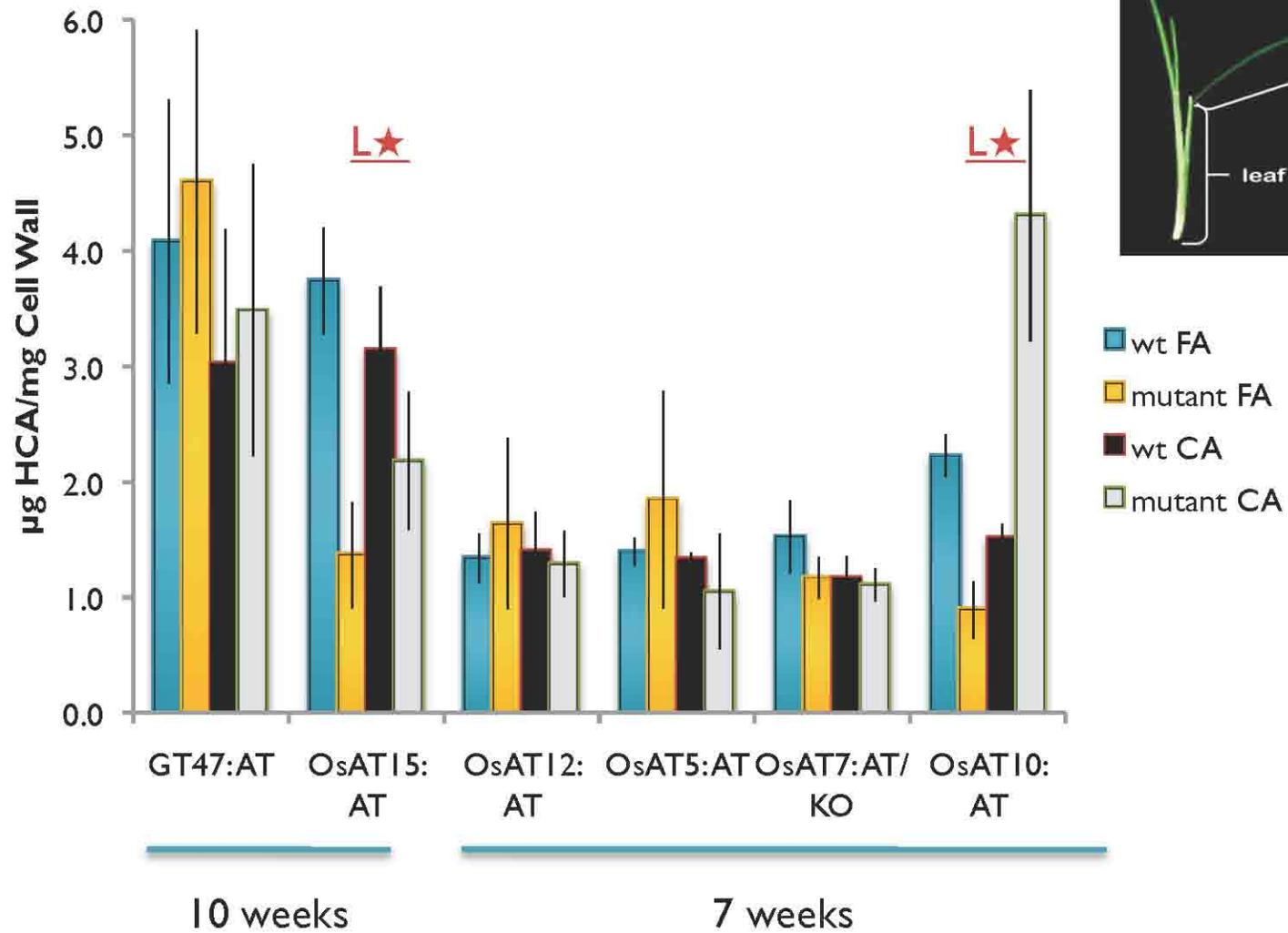


11/18 mutants with confirmed insertions

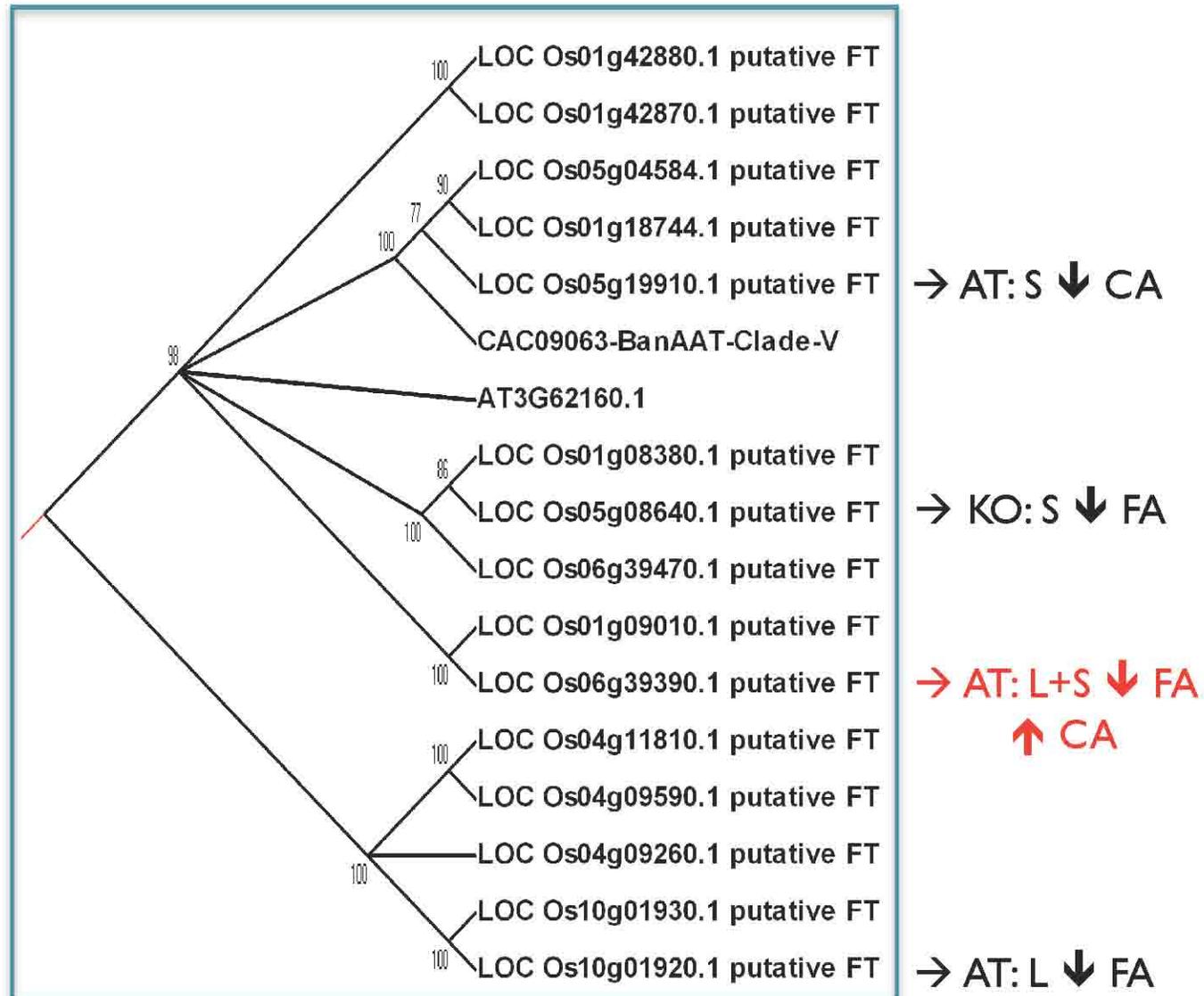
With Sung-Ryul Kim and Gynheung An,
Khyung Hee University, Korea



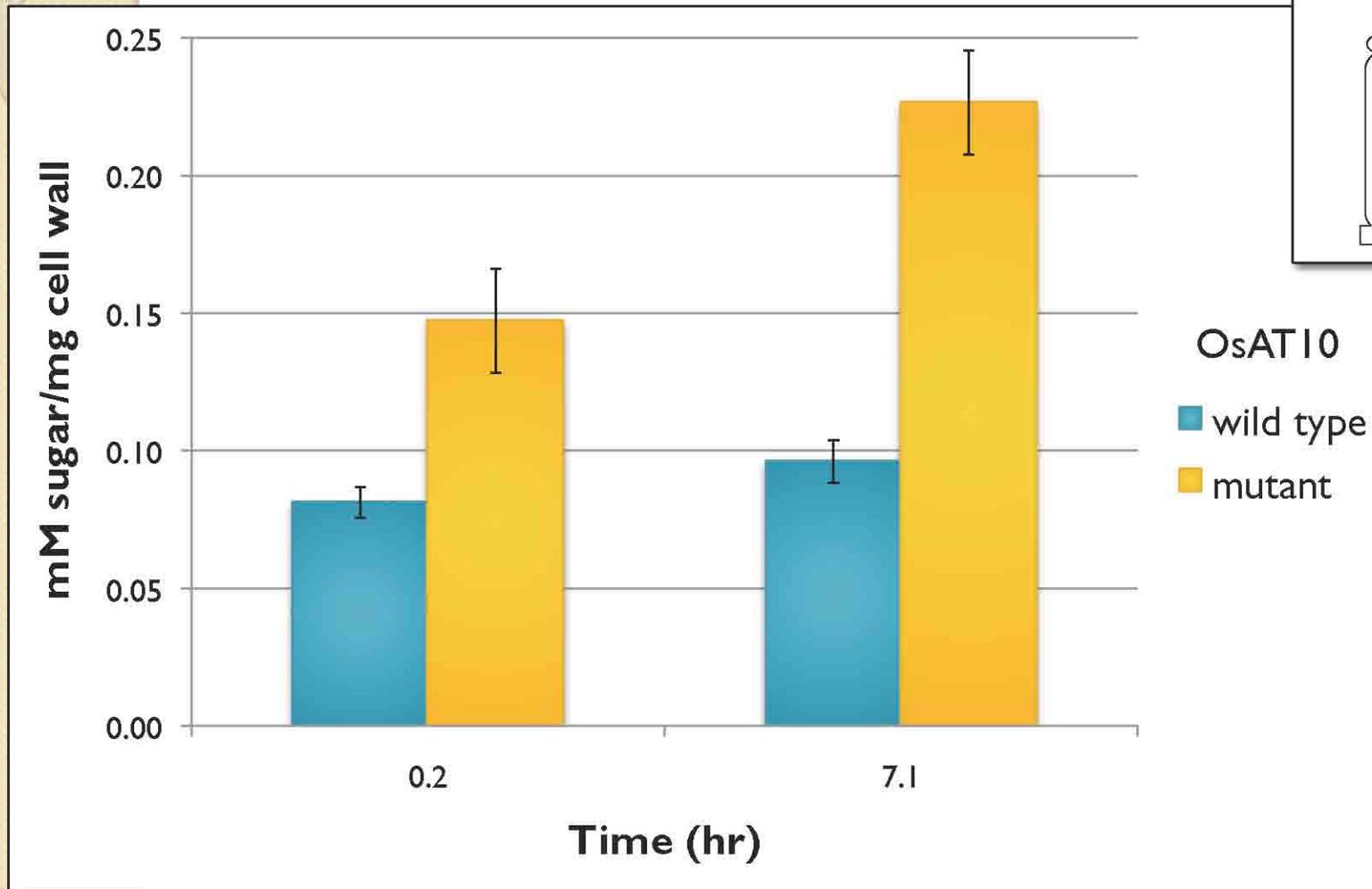
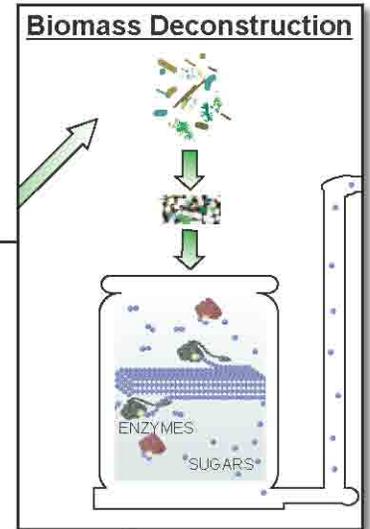
Two Leaf Phenotypes



Four Acyltransferase Cell Wall Mutants

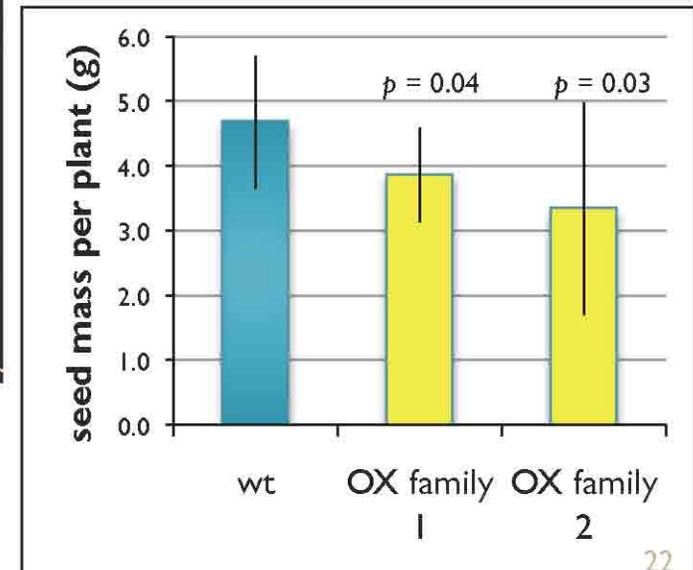
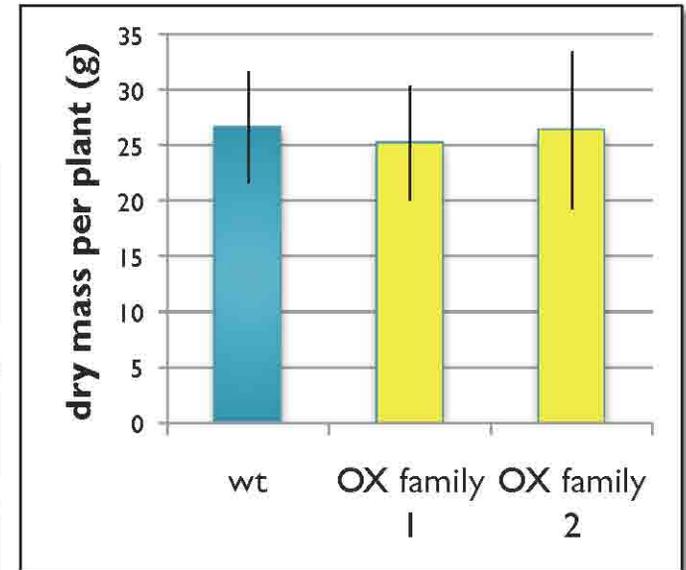


More Sugar Released!

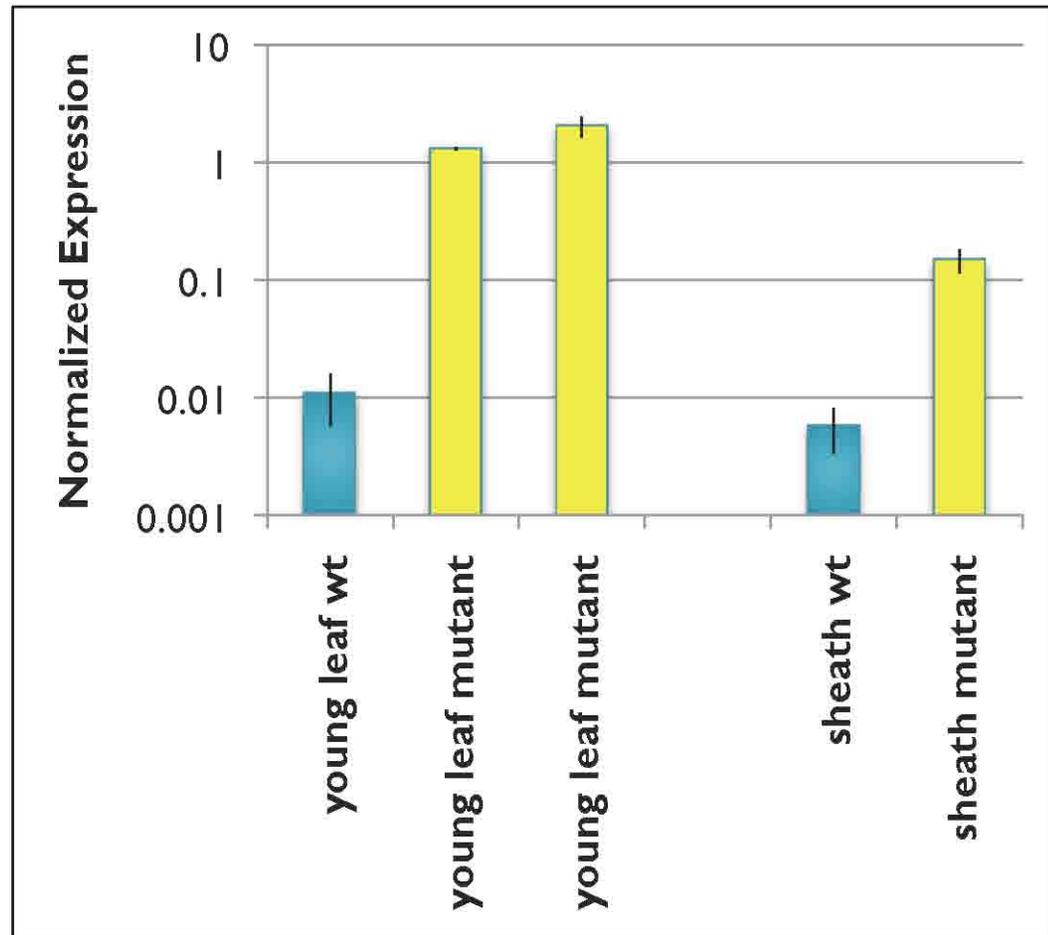
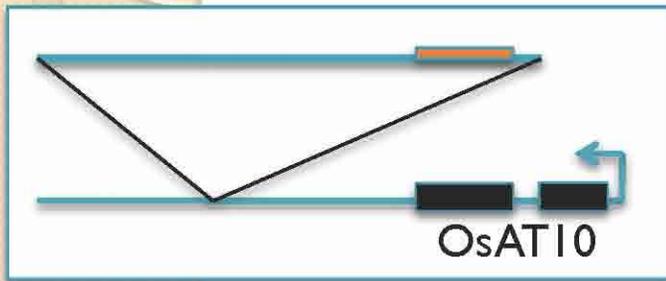


No Major Growth Defect

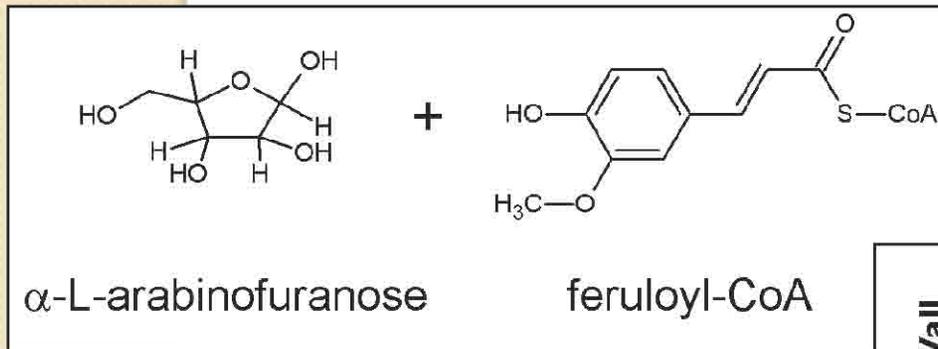
OsAT10

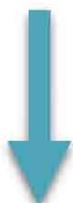


Increased Expression of OsAT10 in Activation Tagged Mutant

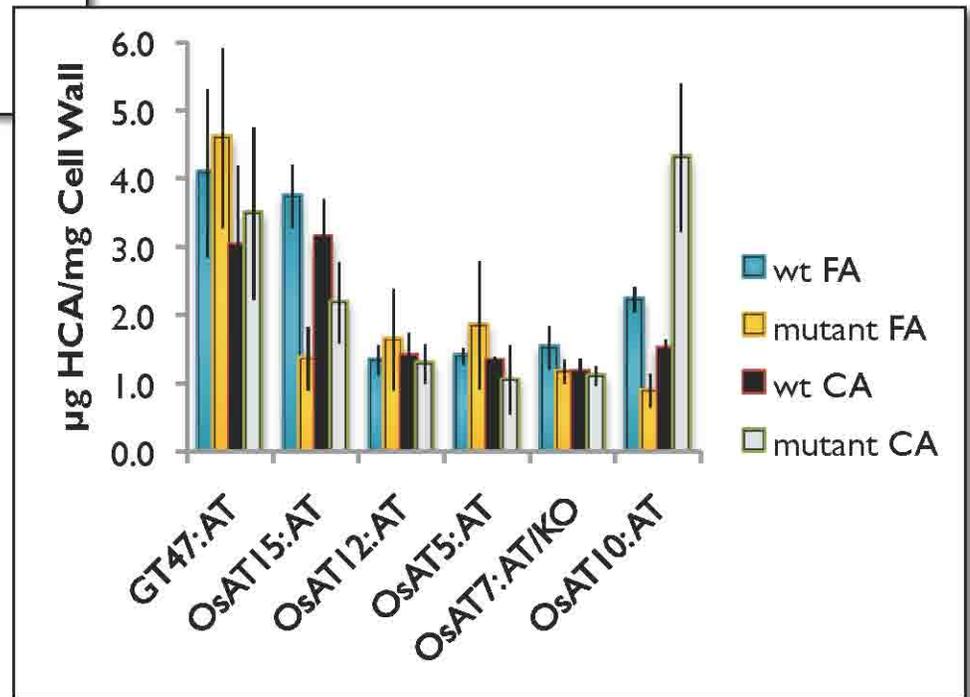
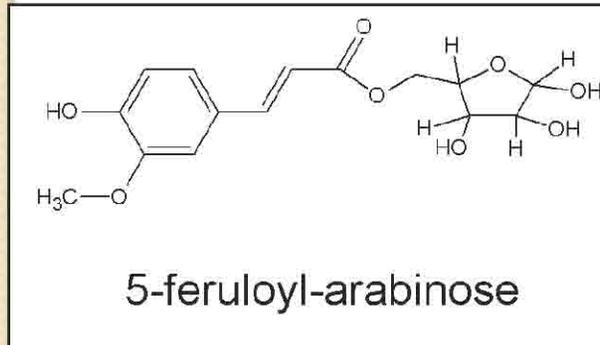


Likely NOT Feruloyl Acyltransferases

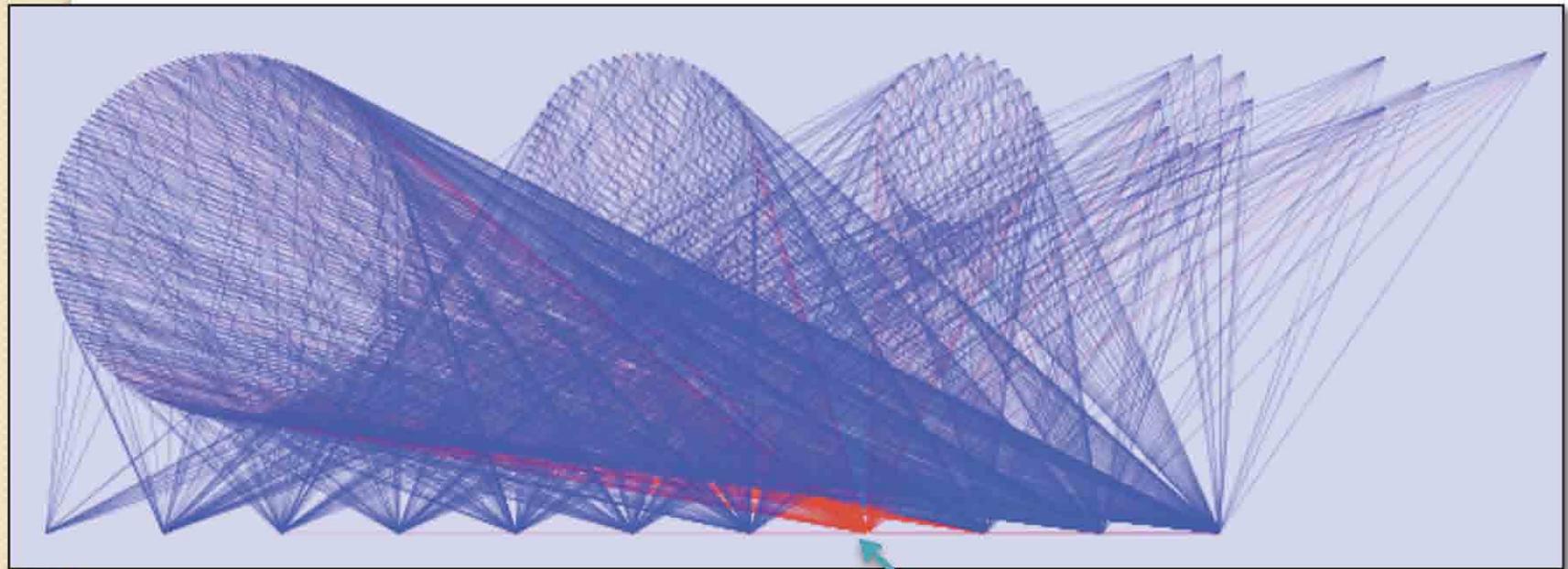
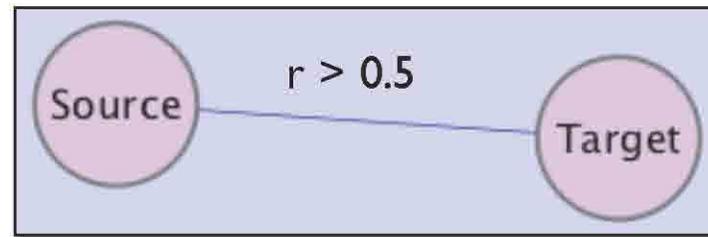




 Putative
 Feruloyl
 Transferase
 (FT)



OsATs are Coexpressed with a Large Number of Other Genes



OsAT10

Testable Clues to Function

# of Correlated Acyl Transferases	Putative Function
11	Os 4-Coumaryl Ligase 3: Synthesize HCA-CoA
10	Germin-like protein 3: ROS synthesis Involved in Defense

Questions about Ferulic Acid



- What is the pathway for incorporation of FA into arabinoxylan of grass cell walls?
- Do modified FA levels in cell walls affect wall digestibility?
- What are the biological functions of wall FA?

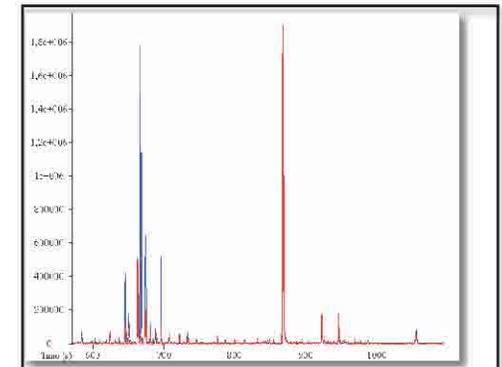
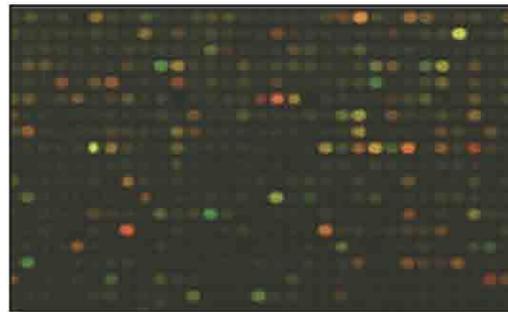
How is FA Incorporated into Grass Walls?

- Further analysis of public gene expression data.

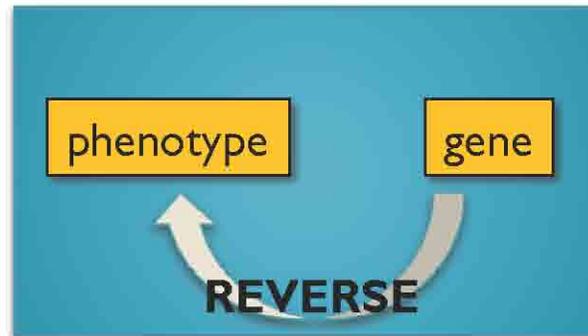


- Metabolic profiling (LC-MS and GC-MS) of mutant tissue, cells, and immobilized recombinant proteins.

- Transcript profiling of mutants.



Test Hypotheses of Genes Involved in Wall FA Incorporation



Reverse Genetics Framework

When and where? Redundancy and gene expression.

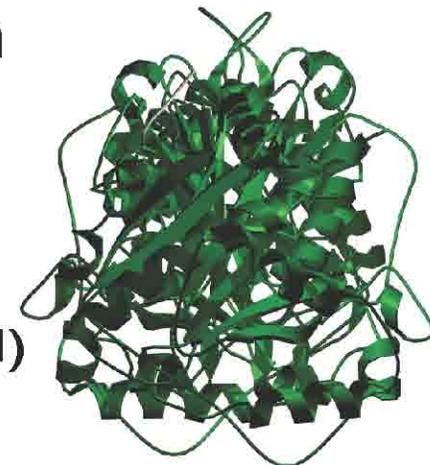
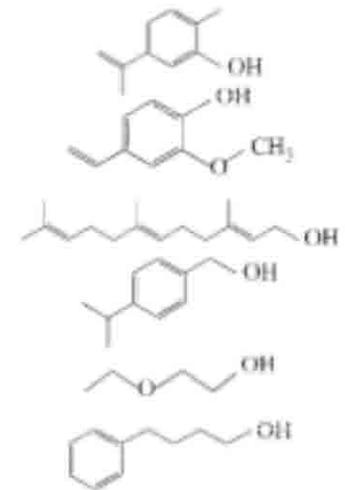
Testing hypotheses with mutants in cell wall content.

Detailed phenotypic analysis: vegetative and reproductive development, disease phenotype, leaf mechanical strength, detailed cell wall analysis.

Can we modulate cell wall HCA content?

Thermodynamic and Kinetic Framework and Structural Biology of Putative FTs

- Express in *E. coli* with tag and purify.
 - 2 of 4 Cloned into Gateway System
- Do different ATs have quantitatively different substrate specificities and catalytic efficiencies?
- Collaborate to crystallize and obtain structure(s) +/- substrates.



Vinorine Synthase (Clade III)

Ma et al. JBC 2005

Adhesion and Separation are Fundamental Properties of Multicellular Organisms



Abscission

Shattering



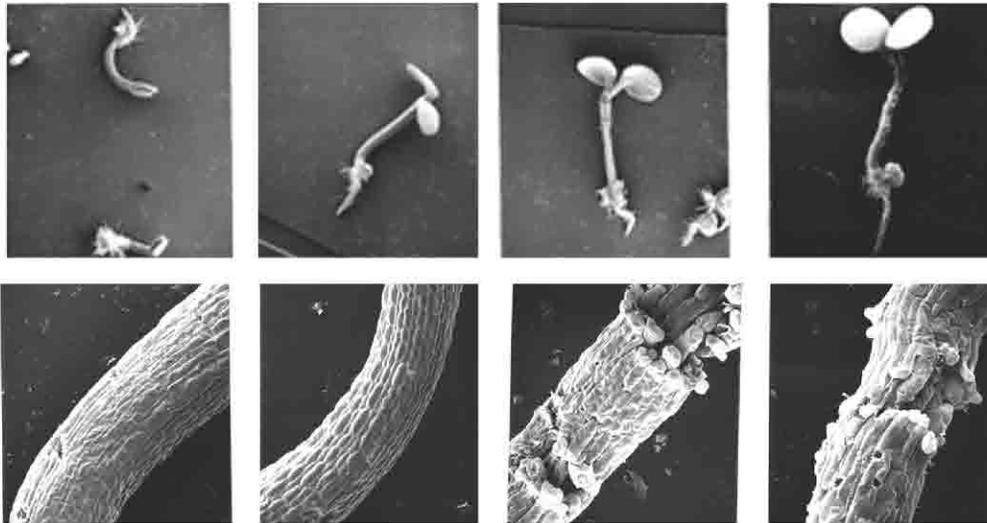
Pollen Dehiscence

Root Cap Function



Lateral Root Development

Known Wall Adhesion Components Are Scarce in Grasses



things fall apart I:
Courtesy of Sara Patterson
(Univ. of Wisconsin)

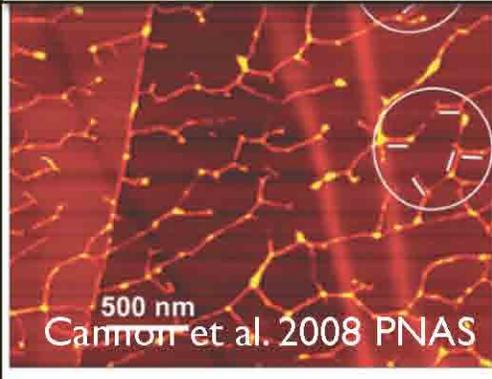
1 day

2 day

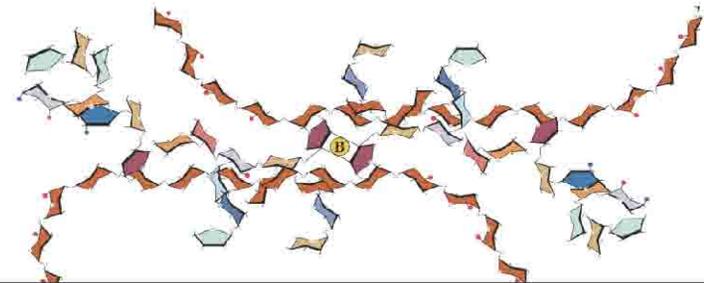
3 day

4 day

Proteins: 10% in dicots, 1% in grasses



Carpita & McCann 1996 Biochem & Mol Bio of Plants



Pectins: 30% in dicots, 5% in grasses

Switchgrass Genomics

- API3
 - Alamo cultivar
 - Parent of mapping population
(Missaoui *et al.* 2005)
- Outcrossing, Tetraploid
($1n = 2C_x = 1400$ Mbp)
- Strategy:
 - Next generation shotgun sequencing
(5-30X)
 - Paired-end reads from long clones
 - Align to Foxtail Millet
 - Shotgun sequence of mapping population



How diverse are the switchgrass chromosomes? What are they similar to?

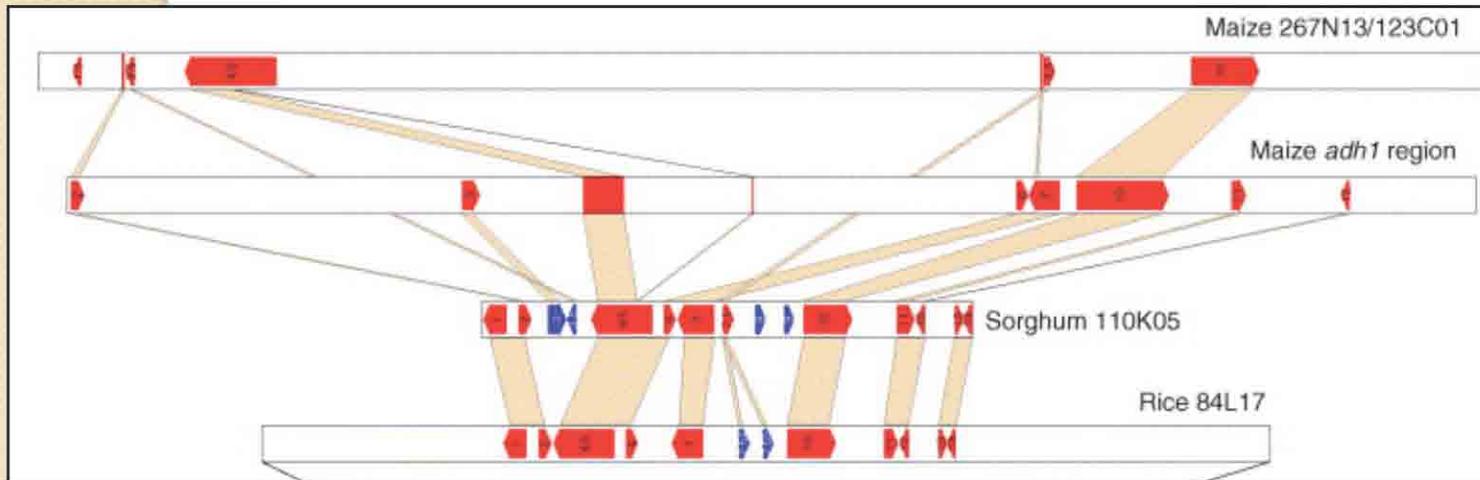
Adh1 Locus

**Genome
Size (In)**

2300 Mbp

730 Mbp

390 Mbp



Devos, KM. 2005. *Cur. Op. in Pl. Biol.* 8:152-161

- Other Gene Targets of High Interest:
Xa21 and highly expressed, conserved members of the *Cs1F* and *GT61* clades

Many Thanks To:

JBEI: Grass Genetics

Pamela Ronald

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Peijian Cao

Elias Marvinney

Dawn Chiniquy

Brian Conlin

JBEI: Cell Wall Biosynth.

Henrik Scheller

Chithra Manisetti

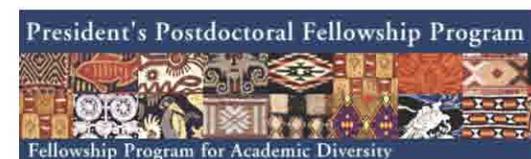
Kyunghee University

Gyn An

Sung Ryul Kim



U.S. DEPARTMENT OF
ENERGY



Experimental Program to Stimulate Competitive Research