

# Genetic Modification of Plant Cell Walls for Enhanced Biomass Production and Utilization

## Investigators

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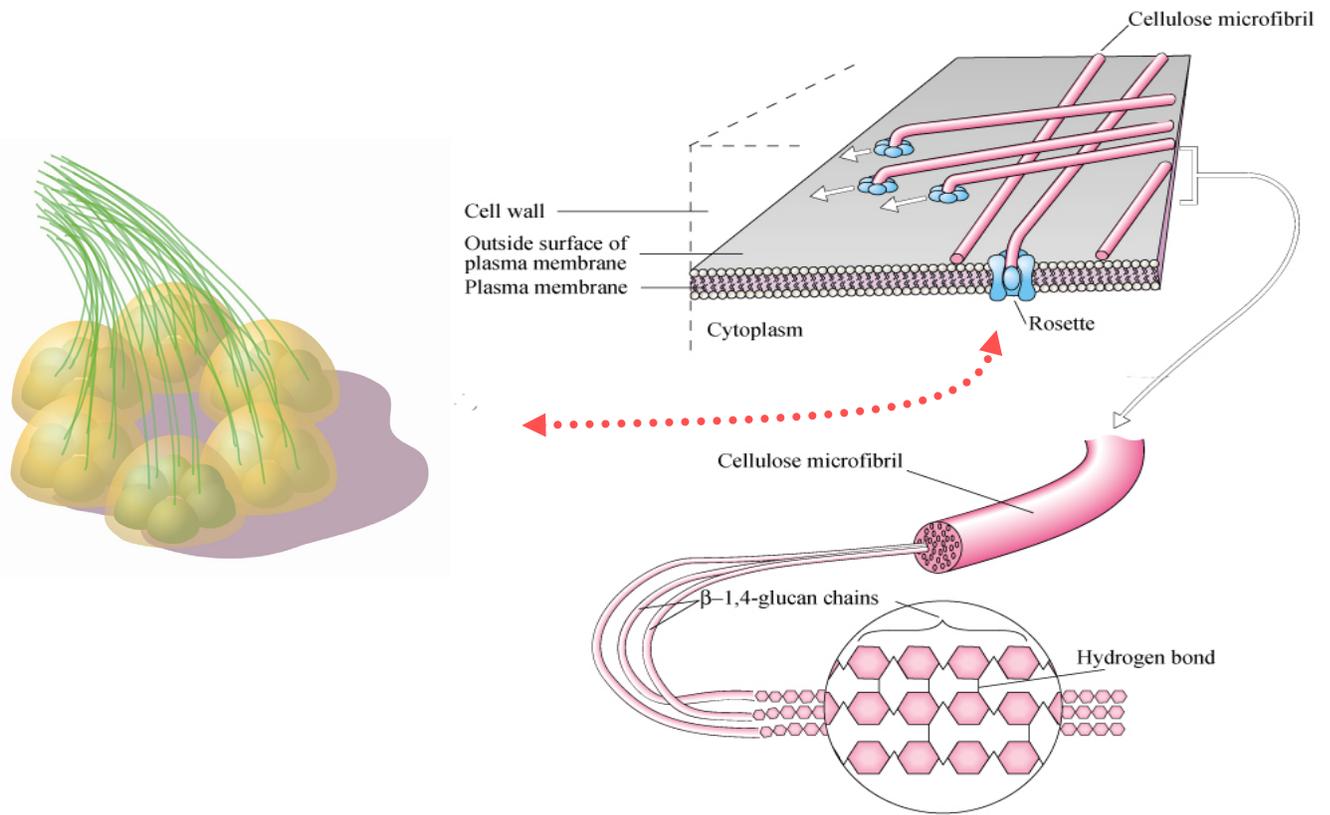
## Introduction

Plant cell walls are the main component of terrestrial biomass. Optimization of biomass-based sources of energy will involve modifying cell wall composition to maximize the recovery of useful components of plant cell walls. One of the principle components of plant cell walls is cellulose, a polymer of  $\beta$ -(1-4) linked glucose. This can be hydrolyzed to glucose and fermented to ethanol. By modifying the normal biological control of cellulose synthesis, we think it possible to develop plants that accumulate more fixed carbon per unit land area per unit time. The percentage of cellulose to other wall polymers may also be increased, potentially making processing of the biomass more efficient.

Cellulose, synthase, the multi-subunit enzyme complex that makes cellulose in the model plant *Arabidopsis thaliana*, is composed of 36 protein subunits (Figure 1). Each subunit makes one glucan chain and their proximity to one another in the plasma membrane leads to the formation of a strong, inextensible microfibril that is extruded into the cell wall. Our goal is to increase the amount of cellulose that is deposited by altering the amount of cellulose synthase that is produced. This project will test the hypothesis that cellulose synthesis is controlled at the level of gene expression by increasing expression of the genes that encode the components of the cellulose synthase. We will place the *CESA* genes that form the cellulose synthase of secondary cell walls under the control of a chemically inducible promoter in *Arabidopsis* and express them throughout the plant.

## Background

The leaf cell walls of a dicot species such as *Arabidopsis*, contain three major classes of polysaccharides: cellulose, hemicellulose, and pectins. Cellulose is present as long unbranched fibrils composed of approx 30-36 hydrogen bonded chains of  $\beta$ -(1-4) glucose. Hemicelluloses are branched polysaccharides containing backbones of neutral sugars that can form hydrogen bonds to cellulose fibrils. Pectins are defined by the presence of uronic acids as major components. The amounts of the various polymers vary from one cell type to another for largely unknown reasons. Notably, the amount of cellulose may vary many-fold from one cell type to another. This is most readily evident in vascular tissue where the xylem cells exhibit “secondary cell walls” that form a second layer of deposition within the “primary wall”. Secondary walls are only deposited in cells that have stopped dividing and expanding.



**Figure 1:** Schematic of cellulose synthase. The 36 subunit complex is embedded in the plasma membrane. Each protein subunit catalyzes the formation of a glucan chain. The 36 chains interact to form a microfibril that is extruded to the extracellular space and becomes incorporated into the cell wall.

In *Arabidopsis*, the leaf mesophyll cells have a composition such that cellulose comprises only 14% of the total wall mass. By contrast, in xylem cells, cellulose appears to form the majority of the mass. From these studies on *Arabidopsis* it appears that the amount of cellulose deposited is under developmental control and increased amounts of cellulose are not necessarily incompatible with cellular function.

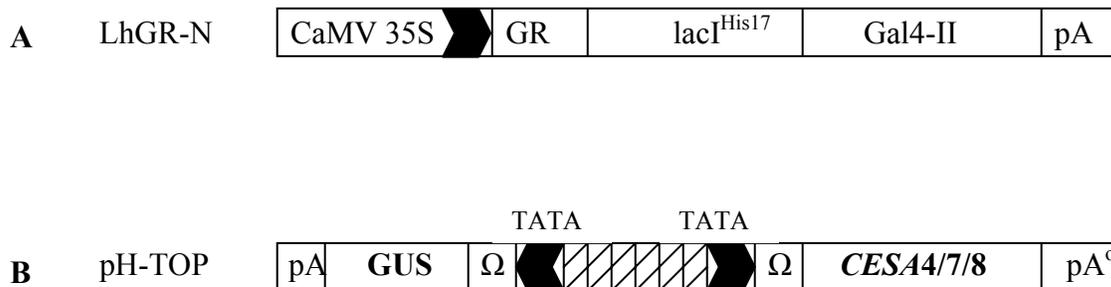
A family of genes encoding the catalytic subunit of higher plant cellulose synthase, termed *CESA*, was identified by genomic methods on the basis of weak homology to bacterial cellulose synthase. The molecular characterization of mutants with defects in cell wall biogenesis confirmed the participation of the *CESA* proteins (Arioli et al., 1998; Fagard et al., 2000; Taylor et al., 1999). *Arabidopsis* has 10 *CESA* genes. Analysis of the tissue specificity of expression of various *CESA* genes combined with knowledge of the mutant phenotypes for several of the genes has led to the proposal that three or more different *CESA* proteins are required for formation of a functional cellulose synthase complex (Taylor et al., 2003). Direct evidence for formation of mixed complexes has been obtained by immuno-precipitation experiments (Taylor et al., 2000).

Co-expression analysis of the *CESA* genes has revealed further genes that may be involved in and required for cellulose synthesis (Persson et al., 2005). The mutant phenotypes of plants with knockouts in these genes are consistent with those of the *CESA* mutants, having xylem cells with walls that have collapsed inwards. Some of these mutants have reduced cellulose compared to wild type plants. Experiments are underway to characterize these mutants.

## Results

Primers were designed for amplification of each *CESA* gene with restriction enzyme sites for cloning into the expression vector pH-TOP (Figure 2, B) (Craft et al., 2005), and with the addition of a TAG sequence. *CESA4* was cloned into the multiple cloning site of pH-TOP using unique restriction sites XhoI and KpnI; *CESA7* using HindIII and XhoI; and *CESA8* using Sall. A FLAG TAG was added to the 5' end of *CESA4*, a myc TAG to *CESA7*, and HA TAG to *CESA8*.

Each TAG will allow identification of the expressed proteins by specific antibodies on a Western Blot. The constructs containing *CESA4*-FLAG and *CESA7*-myc have been sequenced, errors introduced by PCR corrected, sequenced again and transformed into plants. *CESA8*-HA will be transformed into plants in the near future.



**Figure 2:** Schematic diagrams of DNA constructs.

Relevant expression cassettes only are shown. All constructs represent binary T-DNA vectors with the right T-DNA border located to the right of the diagram. The reporter construct, (B) carries a pNOS::HPT selectable marker at the left border and activator construct, (A) carries kanamycin resistance (LhGR) marker at the left border (Moore et al., 1998). Activator construct expresses LhGR from the CaMV35S promoter (Moore et al., 1998). LacI<sup>His17</sup> indicates residues 1-330 of the Y17H mutant of lac repressor (Lehming et al., 1987); Gal4-II indicates transcription activation domain II (residues 768-881) of *Saccharomyces cerevisiae* Gal4p; GR indicates the LBD (residues 508-795) of a rat glucocorticoid receptor at the amino terminus (LhGR-N). pA indicates the CaMV35S polyadenylation signal.

Reporter construct, pH-TOP (B), carries six copies of a 52-bp repeat that contains an ideal lac operator (hatched rectangles). GUS and *CESA* indicate the coding sequences of the *Escherichia coli* uidA and *Arabidopsis thaliana* *CESA* coding regions respectively.

pA, CaMV35S polyadenylation signal. Contains TMV  $\Omega$  translation enhancer; pAo, octopine synthase polyadenylation signal; MCS, multiple cloning site for insertion of *CESA* genes; unique recognition sites for enzymes HindIII, Sall, XhoI, KpnI, SmaI, BamHI, only. The selectable marker cassette in pH-TOP has a polyadenylation signal from nopaline synthase and has no elements in common with the cassettes shown here.

Selection of the plants containing *CESA* 4 and 7 under the inducible promoter is now underway. Plants are being plated out on standard growth medium supplemented with the antibiotic, hygromycin. Only plants carrying the resistance gene and the *CESA* gene will grow normal roots. An untransformed control and transformed control are being compared with the *CESA* transformants to check for the behavior of the plants under our laboratory conditions.

### **Future plans**

Resistant plants will be further screened using the GUS assay. This gene is coupled to the same promoter as the *CESA* genes. Application of dexamethasone to the plants will induce expression of both *CESA*'s and the gene that causes blue color to develop in the tissue in the presence of substrate.

Lines expressing each gene will be crossed to give all three genes inducible in the same plant. Several lines of each will be chosen to work with so that expression levels of each gene can be optimized. These plant lines will be further screened for accumulation of the *CESA* proteins by Western Blot using antibodies specific to each TAG.

Plants containing all three genes that are successfully expressed will be analyzed for cell wall composition and for effects on plant growth and development. If quantitative differences are observed, transmission electron microscopy will be used to determine the morphology of the cellulose. Other techniques such as immuno-labelling using specific antibodies to other polysaccharides will be used to determine the composition of the new wall material. In order to determine how the plants respond to the abnormal expression of the *CESA* genes we will examine the effects on gene expression using whole genome DNA chips.

If increased cellulose is obtained from chemical induction of the genes, we will test the feasibility of engineering enhanced cellulose under the control of developmental stage specific promoters. A number of suitable promoters have been identified through analysis of tissue specific gene expression for other purposes. Suitable promoters for expressing the *CESA* genes will be those that show high expression in non-dividing and non-expanding cells and no expression in expanding cells.

### **References**

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