

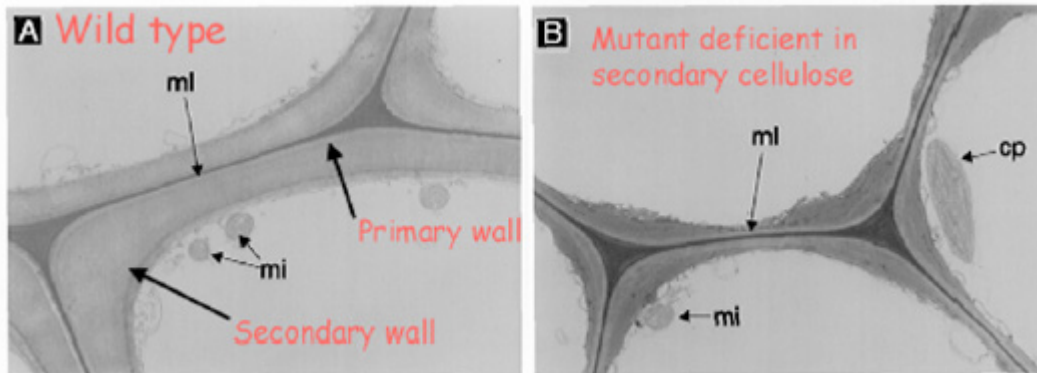
## Genetic Engineering of Cellulose Accumulation

**Investigator:** Christopher Somerville, Professor, Department of Biological Sciences, Stanford University

**Sponsor:** Global Climate and Energy Project

**Objective:** The production of biomass as an energy crop can be optimized through breeding and genetic modification. Plant cell walls are the primary component of terrestrial biomass that is used for energy production through direct combustion, gasification, pyrolysis and enzymatic conversion to ethanol or other volatile organics. Any attempt to develop biomass-based sources of energy must involve optimized production and utilization of cell walls and its principle component, cellulose. The objective of this proposal is to test the concept that the production of cellulose can be increased by increasing expression of the genes encoding the components of cellulose synthase.

**Background:** The proposed experiments are focused on experiments with the model plant *Arabidopsis thaliana*. The leaf cell walls of a dicot species such as *Arabidopsis* contain three major classes of polysaccharides: cellulose, hemicelluloses, and pectins. Cellulose synthase is located in the plasma membrane. The amount of cellulose may vary many-fold from one cell type to another. This variance is most readily evident in vascular tissue where the xylem cells exhibit “secondary cell walls” that are evident as a second layer of deposition within the “primary wall” (Figure 1). An important aspect of secondary cell walls that is relevant to this proposal is that they are not thought to be expandable. Thus, secondary cell walls are only deposited in cells that have stopped dividing and expanding.



**Figure 1:** Electron micrographs of sections through the xylem of *Arabidopsis*. (A) Wild type, (B) a mutant deficient in the synthesis of secondary wall cellulose. Note that the primary wall appears normal but in the mutant the non-cellulosic polymers are deposited in a disorganized manner (Turner and Somerville, 1997).

**Approach:** 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. Analysis of where and when the various CESA genes are expressed is generally compatible with the idea that two of the genes (CESA1, CESA3) are expressed in all

cells and participate in synthesis of the primary cell wall (Scheible *et al.*, 2001). Two other CESA genes that have been implicated in primary wall biosynthesis have developmentally regulated patterns of expression and mutant phenotypes that indicate they are alternate partners to the CESA1 and CESA3 pair. Three additional genes (CESA4, 7, 8) are only expressed in cells that undergo secondary cell wall thickening (Taylor *et al.*, 2003). A variant of the KOR gene is also co-expressed with these three genes. Thus, the evidence indicates that there are two types of CESA genes – those required from primary wall synthesis and those required for secondary wall synthesis.

The goal is to express the three CESA genes (CESA4, 7, 8) that make secondary cell wall cellulose in cells that do not normally have secondary wall thickening. Because it is likely to be deleterious to induce extra cellulose synthesis in cells that need to divide and expand to support normal growth and development, the genes must be placed under transcriptional control of a promoter that is active at a time that is compatible with normal development. We will approach this technical issue in two ways. First we will use a chemically inducible promoter (dexamethasone or a similar promoter) so that we can induce transcription by exogenous application of an inducing chemical. This technique will allow production and propagation of the transgenic plants and will also facilitate studies of the consequences of inducing expression of the CESA genes at specific times and places and to different degrees.

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. The purpose of this experiment is to determine the effects on growth and development of increasing cellulose deposition in mature cells. Such plants may also allow the formulation of expectations for increased cellulose production in energy crops based on similar strategies.

The transgenic plants containing the ectopic CESA genes will be analyzed for cell wall composition (*e.g.*, cellulose and other polymers) and for effects on growth and development. Additionally, in order to examine whether the transgenic plants sense and respond to the abnormal expression of the CESA genes we will examine effects on gene expression using whole genome DNA chips.

If the experiments described above are promising, it would be useful to proceed directly to implementation in an advanced switchgrass cultivar. In order to implement the approach in switchgrass, it will almost certainly be possible to use the Arabidopsis cellulose synthase genes. By contrast, it will probably not be possible to use the same promoters. Therefore, if time permits and preliminary results with Arabidopsis are promising, we will begin work on identification of a suitable promoter from switchgrass that could be used to induce secondary cellulose deposition.

## References

1. Turner S. and Somerville C. (1997). Collapsed xylem phenotype of Arabidopsis identifies mutants deficient in cellulose deposition in the secondary cell wall. *Plant Cell* 9, 689-701.

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3. Taylor N., Howells R., Huttly A., Vickers K. and Turner S. (2003). Interactions among three distinct CesA proteins essential for cellulose synthesis. Proc. Natl. Acad. SCI. USA, 100, 1450-1455.

**Contact:** Chris Somerville, [crs@stanford.edu](mailto:crs@stanford.edu)