

Increasing Cellulose Accumulation for Enhanced Biomass

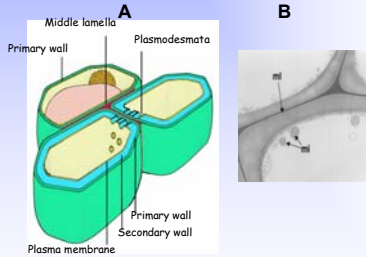
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ABSTRACT

Plant cell walls are the primary source of terrestrial biomass. Any attempt to develop and use biomass-based sources of energy must involve optimized production and utilization of cell walls. One of the principle components of plant cell walls is cellulose, a polymer composed of β -1-4 linked glucose. The amount of cellulose varies from one cell type to another within the various tissue types in a plant. Two main types of cell wall, primary and secondary, are known to exist in plants, (FIGURE 1). Secondary walls by definition are those laid down after cell division and expansion have ceased. These cells can have thickenings of cellulose and other polymers that increase their width by more than 10 times compared to primary walls. The Cellulose Synthase that makes the cellulose in these secondary wall thickenings is known to require three different protein subunits to make a functional complex. We propose to test the concept that the production of cellulose can be increased by increasing the expression of the genes encoding the components of Cellulose Synthase.

Plants have two main types of cell wall



From Taiz & Zeiger Plant Physiology 1991

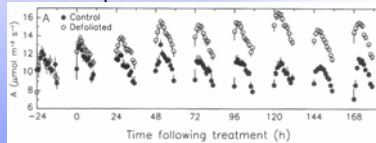
FIGURE 1: Schematic (A) and Electron micrograph (B) of a cross-section of plant cells showing location and thickness of primary and secondary walls. In B, ml is the middle lamella.

GOAL

Our goal is to increase the amount of cellulose produced per cell without changing the number of cells that accumulate per unit of ground area per unit time. Studies suggest that photosynthesis is not the primary limitation to plant growth and that there is excess carbon flux available to support increased cellulose synthesis (FIGURE 2). Plants make enough cellulose for mechanical integrity and could potentially make more based on the available carbon. We will test the hypothesis that cellulose synthesis is controlled at the level of gene expression by producing transgenic plants in which the genes that control secondary cell wall synthesis are expressed in cells that do not normally produce secondary cell walls.

Photosynthesis is "sink limited"

Sour cherry tree experiment



Plants can fix more carbon than they currently do –
What happens if we increase the DEMAND for Carbon

Layne & Flor J Am Hort Sci 120:583

FIGURE 2: Removal of leaves increases photosynthetic rate, suggesting plants have the capacity to fix more carbon and that photosynthesis is not the primary limitation.

Each *cesA* gene has been placed under the control of an inducible promoter

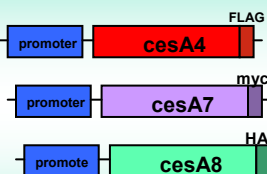


FIGURE 7: The promoter sequence is blocked by a protein complex before the addition of DEX, preventing gene transcription. Each CESA protein will carry a different tag (FLAG, myc, and HA) for identification by antibodies and determination of protein levels.

INTRODUCTION

Cellulose Synthase is a multisubunit complex embedded in the plasma membrane, (FIGURE 3). In the model plant *Arabidopsis thaliana* (FIGURE 4), the *cesA* genes encode the proteins that are the main catalytic subunits of the complex (FIGURE 5). Three of these genes *cesA4,7*, and *8* encode the protein subunits that are required for the functional cellulose synthase (FIGURE 4) that makes secondary cell wall cellulose in stems of *Arabidopsis*.

Cellulose is synthesized at the plasma membrane

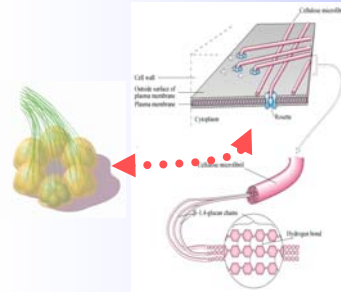


FIGURE 3: Schematic of Cellulose Synthase. The 36 subunit complex is embedded in the plasma membrane. Each protein subunit catalyzes the formation of a glucan chain. The 36 chains interact to form a microfibril that is extruded to the extracellular space and becomes incorporated into the cell wall.

The model plant: *Arabidopsis thaliana*



FIGURE 4: *Arabidopsis thaliana* has many technical advantages over other plants for experimental work. The genome is fully sequenced and a large literature and research community exist. This plant has a lifecycle of only 6 weeks, is easy to transform and wild type plants have a well characterized phenotype making it easy to observe the effects of insertion or deletions of genes. Knowledge gained from basic research with *Arabidopsis* can be readily translated to other plants.

Cellulose synthase is composed of at least three related proteins

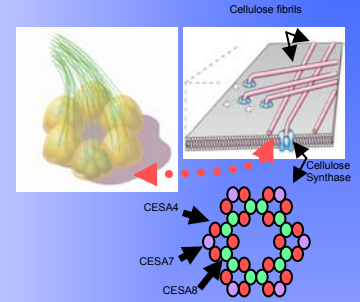


FIGURE 5: The CESA proteins are arranged in a 36 subunit complex. Each CESA is required for a functional complex although the exact arrangement of subunits is not yet known.

Plants with a mutation in an individual *cesA* gene have reduced cellulose and as a result have the same collapsed xylem phenotype, (FIGURE 5A). Plants with a decrease in lignin compared to wild type also show this phenotype (FIGURE 5B), suggesting that cellulose and lignin both contribute to the mechanical properties of the cells within the stems.

Irregular xylem (*irx*) mutants are deficient in cellulose

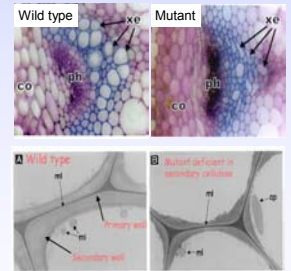


FIGURE 6: Transverse sections of stems of *Arabidopsis* wild type and mutant plant stained with toluidine blue, and imaged with a light microscope (top) and Electron Micrograph (bottom). The irregular xylem (*irx*) mutants have reduced cellulose or lignin by up to 30% compared to wild type. The collapsed xylem cells (*xe*) are a result of pressure from transpiration of water through the xylem elements. With a reduction in cellulose/lignin, the walls of these cells are thinner than wild type and cannot withstand the high pressures and collapse inwards.

Experimental Design

Each *cesA* has been placed under the control of an inducible promoter in vectors that also carry antibiotic resistance genes for selection (FIGURE 7) and these have been transformed into *Arabidopsis*. Plants resistant to antibiotic carry the inserted gene which remains silent until induced with dexamethasone (DEX).

A simple colorimetric assay identifies plants which express the *cesA* gene under these conditions and accumulation of the CESA proteins can be identified by antibodies. Plants expressing all three genes that show accumulation of protein will be selected and screened for altered phenotypes/increased cellulose.

Cellulose Synthesis will be induced after cell division and cell expansion have

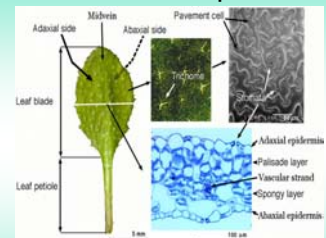


FIGURE 8: Images of an *Arabidopsis* leaf showing the different cell types. Epidermal cells and mesophyll cells (Spongy layer) have only very thin primary walls. To prevent adverse effects on cell division and plant growth of extra cellulose deposition, gene expression will be induced in adult plants after cell division and cell expansion have ceased.