

# IMPROVEMENT OF WOOD DENSITY, CELLULOSE CONTENT AND FIBER LENGTH BY EXPRESSING CANDIDATE GENES IN A XYLEM-PREFERRED MANNER

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## INTRODUCTION

*Eucalyptus* trees represent the largest source of fibers used globally in the paper industry. The major advantage of the *Eucalyptus* tree is its very high growth rate and ability to grow in a wide range of conditions, both tropical and temperate (Verhaegen and Plomion, 1996).

Fiber characteristics are controlled by a complex set of factors and are difficult to be improved by classical breeding methods. Through traditional forest tree breeding it is possible to achieve some modification of fiber characteristics, but the developments in gene technology can reduce significantly the time required to produce a new variety of plant and allow closer targeting of traits considered desirable by the forest and pulp industries in specific trees species.

Thus far, genetically engineering plants to produce useful traits requires the availability of promoters that would allow the genes of interest to be expressed in a tissue- and time-specific manner. Thus, isolation and characterization of tissue-preferred, particularly cambium/xylem-preferred, promoters that can serve as regulatory regions for expression of heterologous nucleotide sequences of interest in a tissue-preferred manner is essential for the genetic engineering of plants exhibiting particular traits.

By the use of promoters that drive expression of particular genes in the lignifying tissues in plants, we were able to obtain plants with altered cellulose content, increased fiber length and reduced vessel elements.

## Alteration in the cellulose content

Wood formation is a fundamental biological process with significant economical interest. During wood formation, most glucose arising from carbohydrate metabolism is channeled to cellulose synthesis in the secondary walls (Djerbi *et al.*, 2004). Cellulose is a fibrous polymer consisting of linear chains of  $\beta$ -(1,4)-linked glucose molecules. These linear glucan chains crystallize to form microfibrils that impart the characteristic flexible strength of cellulose. Cellulose is synthesized in higher plants by

large multimeric plasma membrane-bound complexes that form rosette structures at the ends of microfibrils (Somerville, 2006).

Although the great importance of the cellulose molecule in fiber industry and in plant morphogenesis, its biosynthesis pathway is poorly understood at the molecular level. Few genes affecting the cellulose synthesis were isolated by the characterization of several cellulose-deficient mutants of *Arabidopsis*, such as genes of the CesA gene family, and genes encoding proteins for N-glycan synthesis and processing (Nicol *et al.*, 1998).

The cellulose biosynthetic pathway is a useful target for metabolic engineering, because cellulose is valuable as pulp, fiber and as starting point for the synthesis of commercially important polymers. Besides enhancing the cellulose deposition, alterations in its biosynthetic pathway is likely to have a repressive effect on lignin deposition (Hu *et al.*, 1999), which is also desirable, as the industrial production of cellulose and chemical removal of lignin is costly and represents an enormous environmental challenge. The chemical removal of lignin from wood during the pulping process makes use of large amounts of concentrated chemicals. For high-quality paper production, residual lignin needs to be further removed by an additional bleaching step involving the use of extremely hazardous substances. For this reason, reducing lignin content in woody plants, typically trees, is expected to lessen the chemical and energy demands of these highly expensive extraction processes and should also reduce the amount of effluent material, a major potential environmental pollutant that is both difficult and expensive to process (Campbell *et al.*, 1996).

Thus, genetic engineering of cellulose biosynthesis can provide a strategy to augment cellulose quality and quantity, while reducing lignin content in transgenic plants, bringing considerable economic benefit. In particular, production of transgenic plants with altered cellulose and/or lignin content is a highly desirable objective.

## Alteration in the fiber length

The forestry industry has different requirements concerning the quality of the starting material. Notwithstanding with these specific demands, fiber uniformity and strength are common requirements for most industrial uses. In pulp manufacture, for example, strength characteristics are determined in part by fiber length. Long fibers are ideal for papermaking due to their strength and bonding properties, which contribute to the production of a strong paper, increase pulp yield and decrease consumption of alkali.

The fiber length is controlled by endogenous regulation of cell elongation, which appears to be related to cell wall mechanics. This process is a result of the interaction between internal turgor pressure and the mechanical strength of the cell wall, but its mechanism and genes involved have not until recently been totally discerned.

Xylem fiber cells develop from already much-elongated fusiform initials located within the vascular cambium. They increase in diameter by extension of their radial walls, and, in addition, developing fiber cells elongate by intrusive tip growth, which results in up to a several fold increase in cell length (Gray-Mitsumune *et al.*, 2004).

In tip-growing cells, expansion occurs over a small area of the cell surface, which results in tubular, elongated cells. For example, poplar fibers elongate intrusively in the radial-expansion zone in the xylem, reaching 150% of their initial cell length at the average when fully differentiated. (Hussey *et al.*, 2006; Mellerowicz *et al.*, 2001).

The rapid expansion of fiber cells may be achieved by concerted action of pushing against the cell wall exerted by turgor and loosening of the cell wall. In cotton fibers, the phase of cell elongation follows a significant rise of turgor, resulted from the observed accumulation of malate, sugars, and K<sup>+</sup>, the major osmoticum, hence the influx of water and the generation of high turgor in the fiber cells. (Ruan *et al.*, 2004).

If trees could be produced with longer fibers, this would be a considerable advantage to the paper industry, increasing the quality of the raw materials for pulp and paper synthesis.

### Alteration in wood density

Wood is essentially a matrix of cell walls and cellular air spaces from secondary xylem. In this sense, wood density would be affected by the cell wall thickness, the cross-sectional area of the lumen of the vessels and the number of the vessels involved in water transport through the stem (Preston *et al.*, 2006). In general wood density is negatively correlated with hydraulic conductivity and the cross-sectional area of the vessels in *Eucalyptus camaldulensis* (Thomas *et al.*, 2004). Wood density and vessel characteristics are functionally interrelated. In a comparative study of 51 angiosperm species it was shown that the mean vessel lumen area and vessel density were negatively correlated with wood density (Preston *et al.*, 2006).

The influence of vessels on wood density can be decomposed into two components – vessel area (the transverse lumen area of individual vessels) and vessel density (number of vessels per transverse area). Vessel lumen area strongly affects the capacity of wood to

conduct water (Zimmermann, 1983). By contrast, the number of vessels in a given transverse area should have a relatively small effect on sapwood conductance. These component traits, vessel area and vessel density, contribute to wood density in parallel by affecting the amount of lumen space in the wood. It is therefore expected both traits to vary inversely with wood density.

Since wood density has a major effect on both yield and quality of fibrous and solid wood products and considerable influence on strength, machinability, paper yield and properties, the improvement of basic density is a key factor in the profitability of kraft pulp production.

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