

# More cellulose per square centimeter

Transgenic eucalyptus has 20% higher productivity than conventional eucalyptus

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PUBLISHED IN FEBRUARY - 2013

By all appearances, the small (2.2 hectares) eucalyptus plot on a farm in the municipality of Angatuba in São Paulo State is nothing out of the ordinary. But something unusual is happening in the cells of these trees, into which a gene from the species *Arabidopsis thaliana*, a model plant often used in genetic experiments, has been introduced. The new gene enables the trees to produce 20% more wood than their unaltered *Eucalyptus* congeners. This small forest of transgenic eucalyptus trees is one of four test plots of genetically modified trees planted by FuturaGene, a company whose mission is to improve the productivity and sustainability of forests cultivated for the cellulose, bioenergy and biofuels markets. The goal is to assess the biosecurity of the transgenic plants and determine whether they have any impact on the environment or other plants.

FuturaGene, originally called CBD Technologies, was founded in Israel in 1993 as a protein engineering spin-off from the Hebrew University of Jerusalem. In July 2010, the enterprise was purchased by the Brazilian company Suzano Papel e Celulose. The experiments currently being conducted in inland São Paulo State and the states of Bahia and Piauí are a necessary step toward the commercial release of the genetically modified plants, as required by the National Biosecurity Commission (CTNBio), the Brazilian agency responsible for evaluating transgenic products. "The four plantings we did in 2012 totaled nine hectares," says Eugenio Ulian, FuturaGene's vice president of regulatory affairs. "The goal is to make observations and collect data so as to



comply with the biosecurity law, and to submit an application to CTNBio in the future for commercial release. The expectation is that this product could be approved for commercial use in approximately four years.”

The gene that was introduced into the eucalyptus trees codifies endoglucanase, one of the enzymes that play a role in the chemical makeup of cellulose. “By expressing the *Arabidopsis thaliana* gene for that enzyme in the plants, FuturaGene discovered a way to alter the structure of the cell wall (which is made of cellulose) of the transgenic trees,” Ulian says. “The exogenous gene causes the cells to deposit more cellulose during formation of the cell walls of the tree, which, in the case of species like the eucalyptus, results in a larger volume of wood.”

The cell walls of a plant consist primarily of cellulose, a polymer composed of glucose units, which is embedded in other complex polymers such as hemicellulose and lignin. The molecules form a rigid structure around the plant cell that relaxes only to enable growth and division. “The FuturaGene technology makes it possible to produce species with modified cell walls that can speed up this relaxation and reconstitution process during normal tree growth,” Ulian explains. “Introduction of the new gene into eucalyptus results in accelerated growth and higher yield.” These results make the technology particularly attractive to the paper and energy industries. The cellulose extracted from the cell walls

Six-year-old genetically modified eucalyptus in Angatuba, in inland São Paulo State

## The company uses plantings with seeds from crosses between transgenics and conventional plants to test productivity

of the plant is the source of all industrial fiber used in the production of paper, boards and wood. In addition, cellulose provides the raw material for a multitude of more advanced forestry and agricultural products, including the sugars used to produce second-generation ethanol and the chemicals used in bioplastics. The transgenic eucalyptus developed by FuturaGene not

only produces 20% more cellulose than normal plants, which produce an average of 45 cubic meters per hectare, but also yields 30 to 40% more wood for use in applications such as bioenergy.

The company's journey to successful genetic modification of this plant has been long. The initial research studies that led to the transgenic eucalyptus began soon after the firm was established

at the Hebrew University of Jerusalem. "Many studies were done using various genes involved in cell wall formation that were cloned and introduced for superexpression in model species such as *Arabidopsis*, in poplar and in eucalyptus," Ulian recalls. "The endoglucanase gene was chosen for ongoing study because it yielded the best results."

FuturaGene has now planted 12 test plots with transgenic eucalyptus. The first crops were grown in 2006 and 2007 in Israel and Brazil. The work continued after the company was acquired by Suzano, with new plantings in Brazil. In 2012, in addition to nine hectares planted with the original genetically modified plant, another six were planted with seeds from crosses between the transgenic line and the conventional parents to develop and select improved clones with the increased-yield trait.

Since 1998, Suzano has also been developing projects in partnership with Professor Carlos Alberto Labate from the Genetics Department in the Luiz de Queiroz School of Agriculture at the University of São Paulo (Esalq/USP). "The work is focused on biotechnology and the functional genomics of eucalyptus," Labate says. "We already have two projects financed by FAPESP's Partnership for Technological Innovation (PITE) that have been approved, and now we are on the third one." The objective of the first PITE project was to develop the methodology for the genetic transformation of eucalyptus. "My doctoral student Esteban Roberto González was hired by Suzano, and today he manages research and development at FuturaGene," notes Labate, who became director of the Brazilian Bioethanol Science and Technology Laboratory (CTBE) in January. "The method we developed was patented, and all the knowledge we created was in some manner transferred to the company. In addition, to this day we hold frequent meetings and staff training sessions at FuturaGene, which gives us the opportunity for very good interaction with the company."

### SUGAR CONTENT

In the second PITE project, Professor Labate developed PITE several transgenic eucalyptus plants with altered expression levels of genes related to synthe-



1 Biotechnology laboratory at FuturaGene in the city of Itapetininga (SP)

2 Manipulation of eucalyptus leaves for replanting

3 Two phases of transgenic planting in Angatuba



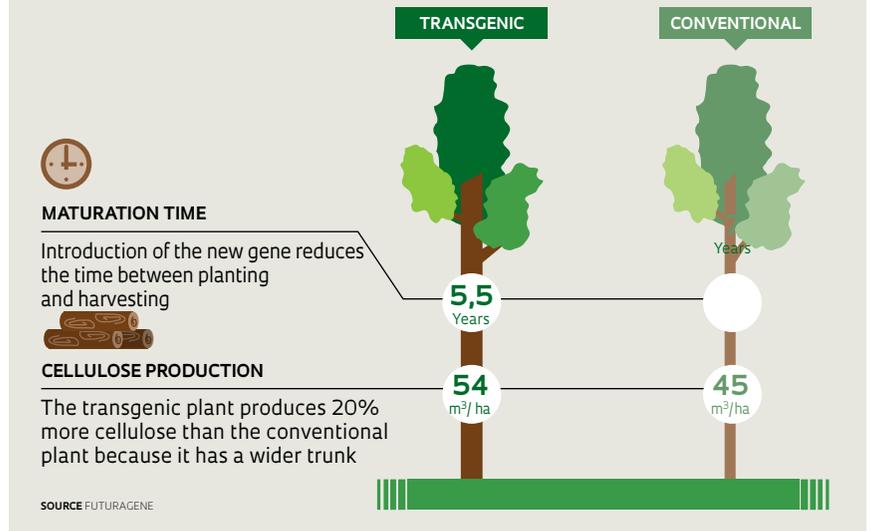
sis of carbohydrates. “The goal of the project was to increase the amount of xylans in eucalyptus wood,” he explains. “Those transgenic plants are at FuturaGene, where field tests are to be conducted.” Xylan is a hemicellulose, a polymer of xylose (one of the sugars present in wood), and the compound plays an important role in the bleaching of cellulose pulp and in the properties of paper. Modifying the concentration of this sugar in the plant can increase production and differentiate the properties of the pulps and papers produced from the wood.

FuturaGene plans to plant 30 hectares for testing genetically modified eucalyptus in 2013. “The objective is to test new genetic alterations that could potentially lead to other products with the same increased-yield trait as the first transgenic eucalyptus, but with different genes or improved genetic structures,” says Eduardo José de Mello, vice president of Brazilian operations and manager of forest improvement at FuturaGene. “That is why we think this year’s experiments will help in selecting new products.” Beyond these efforts, the company’s laboratories are developing species that are resistant to pests and diseases and that promote improved weed control and wood quality. In addition to its biosecurity focus, FuturaGene is also testing the behavior of transgenic eucalyptus grown at different crop spacings. “That information will be important for planning future plantations tailored to their end use, such as energy production, boards or cellulose, for example,” Mello says. “Owing to the high yield of the transgenic plant and depending on the end use of the biomass, harvesting can be done earlier, at five and a half years.” Conventional eucalyptus reaches that level of productivity at seven years.

According to Mello, Brazil is now the world’s most productive eucalyptus-growing nation owing to its favorable climate and efforts toward technological development. “Conventional genetic improvement through selection and propagation of the best individuals has played an important part in the productivity gains, but these gains are becoming increasingly difficult to top,” Mello observes. “Biotechnology through the use of transgenics will be an important tool for

## Accelerated growth

Transgenic eucalyptus requires shorter growth times and provides greater productivity for the paper and conventional cellulose industries



## Genetic manipulation of plants could play an important role in the maintenance and preservation of native forests

Brazil to remain on the forefront of productivity and continue to be competitive in the market for eucalyptus wood and its by-products.” Suzano is currently the world’s second-largest producer of eucalyptus cellulose. The company posted net revenues of R\$4.8 billion in 2011, with over 50% of its sales in the export market.

The genetic manipulation of plants could also play an important role in maintaining and preserving native forests around the world. According to data from the United Nations Food and Agriculture Organization (FAO), the planet has approximately four billion hectares of forest coverage, roughly 27% of its land area. The current global consumption of wood is estimated at approximately 3.4 billion cubic meters per year and is expected to increase 25% by 2020. To meet this demand, native forests are being cut down at a rate of 12 million hectares each year. According to a study by the Center for Environmental Risk

Assessment (CERA), a group at the ILSI Research Foundation that brings together research institutions from around the world, planted forests accounted for just 5% of the Earth’s total forested area in 2000 but contributed approximately 35% of its harvested wood. The global area planted in tree species currently stands at 264 million hectares, representing 6.6% of the world’s forests. Since the late 1980s, when the first transgenic plants were released for commercial cultivation, more than 800 field tests have been conducted throughout the world using genetically modified trees from approximately 40 species. ■

### Project

*Functional genomics applied to the discovery of genes resistant to eucalyptus rust* – No. 2008/50361-1; **Grant mechanism** Partnership for Technological Innovation (PITE); **Coordinator** Carlos Alberto Labate (USP); **Investment** R\$330,195.78 and US\$242,235.41 (FAPESP) and R\$1,376,000.00 (Suzano).