POTENTIALITIES OF GENETICALLY MODIFIED TREES FOR THE EUCALYPT KRAFT PULP INDUSTRY

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ABSTRACT

Applications of biotechnology in forestry can prove highly beneficial from both the industrial and environmental perspectives. By using biotechnology to reduce total lignin or alter the composition of lignin in eucalyptus trees, the final yield in pulp mills can be greatly enhanced. These traits will also lead to reductions in chemicals and energy needed for the process. These direct benefits can also translate into lessened environmental impacts from the production process and/or reduced plantation areas. Experimental results have demonstrated lignin reductions and increased S:G ratio in research-scale tests. The very first field trials with elite-clones established in Brazil by ArborGen in association with local partners aim to demonstrate that that also can be achieved in tropical environments.

Keywords: eucalyptus, lignin reduction, pulp yield, biotechnology, wood consumption, transformation, clonal, transgenic.

INTRODUCTION

The benefits of biotechnology are well known to industrial agricultural crop growers throughout the world. The best demonstration of this is the pace at which agricultural products improved through biotechnology have been adopted worldwide, from semi-experimental areas planted in 1995 to over 90 million hectares in 2006, of which approximately 10% are planted in in Brazil alone (Figure 1).

Country	**Hectares planted with bioengineered crop species – 2005**
USA	49,8 million
Argentina	17,1 million
Brazil	9,4 million
Canada	5,8 million
China	3,3 million
Paraguay	1,8 million
Índia	1,3 million
South Africa	0,5 million
Uruguay	0,3 million
Australia	0,3 million
Mexico	0,1 million
Romania	0,1 million
Filipines	0,1 million
Spain	0,1 million

Figure 1 – "Mega-Countries" in biotechnology adoption: Countries with over 50,000 hectares planted with transgenic crop species
Source: Clive James, Global Knowledge Center on Crop Biotechnology, 2005 (http://www.isaaa.org/kc/).

It is only natural to expect that South American Countries such as Brazil, Argentina and Uruguay will similarly benefit from the utilization of similar technologies being applied to their large-scale forest plantation programs. In these countries, forestry practices closely resemble those in agriculture, as trees are intensively managed in large plantations through relatively short rotations. This production system has evolved due to the highly favorable climatic conditions found in tropical to sub-tropical areas, common to specific regions of those cited countries, as well as intensive breeding and research and development programs developed by local public and privately funded institutes and companies.

The use of large-scale clonal forestry in South America, and particularly in Brazil, provides an ideal situation for the utilization of trees improved through biotechnology. The elite commercial clones currently in use provide a strong platform for genetic transformation, further improving already known commercial traits. The fact that clones of proven performance can be further enhanced for industrial use, with an additional upside of being improved ecological fitness, provides a compelling opportunity to create a competitive advantage for companies that adopt this technology.

Among the potential products is eucalyptus with a lower total lignin content and eucalyptus with a higher ratio of syringyl to guaiacyl lignin.

OVERVIEW OF ARBORGEN

ArborGen is a global leader in the research, development and commercialization of applications in biotechnology that will improve forest sustainability and productivity. Established in 2000 in Summerville, SC, USA, by combining the forest biotechnology efforts of three companies, ArborGen is now recognized as the largest venture of its kind in the world. ArborGen currently employs scientists and professionals in the US, New Zealand and Brazil.
ArborGen's South American branch was formally established in October 2004 and is located in the city of Campinas, State of Sao Paulo, Brazil. By the end of 2006 ArborGen has amongst its customers and collaborators several the major forest-products companies currently active in South America. The company is currently developing traits that enhance

volumetric growth, increase stress tolerance as well as several different approaches related to the improvement of wood-quality and its various properties.
ArborGen also is a world leader in the propagation of pine species, a technology that enables the benefits of clonal forestry, already demonstrated in eucalyptus, to be realized by pine growers as well.

ArborGen established its first trials with eucalyptus improved through biotechnology for enhanced pulping characteristics in mid-2005 in the State of Sao Paulo, the first test of its kind in South America (Figure 02).

Figure 02 – View of a line-selection clonal trial with transformed eucalyptus trees for improved pulping traits (Brazil, mid 2005)

The trials were established with elite commercial clones improved through biotechnology from a customer in Brazil with over 200 transgenic lines tested for different gene constructs.

These initial trials are already reaching initial sampling age and results from wood quality analysis are set to be available by early 2007. ArborGen is also set to establish new trials in the 2007-2008 timeframe in South America, making for the largest regional field trial network with eucalyptus trees improved through biotechnology. In addition to plans for South America, ArborGen and its partners have conducted extensive field tests in the United States and have generated the world's largest dataset available on field performance of tree species improved through biotechnology.

THE POTENTIAL BENEFITS OF TREES IMPROVED TRHOUGH BIOTECHNOLOGY

Enhancing wood quality properties is one of the main targets of tree breeding programs in South America. Arguably, though, the majority of the latest groundbreaking achievements in the area of conventional tree breeding – particularly in Brazil – were in the areas of total volumetric production and site adaptation.

The Brazilian industry has experienced a continuous increase in volumetric production performance derived mostly from breeding, identification, propagation and large-scale deployment of clonal varieties adapted to the Country's wide range of climatic and edaphic conditions. Although most clones currently planted for large-scale pulp production in Brazil are of high industrial yield, in general they do not differ significantly in wood quality properties – namely lignin content and pulp yield – from what could be observed in the immediate previous generation. That is due to the fact that, among other reasons, breeding programs are restricted to populations that perform well in the dominant local climatic conditions, and show a somewhat limited variability for the wood quality traits of interest.

The *Eucalyptus* species planted in Brazil in particular show, on average, higher lignin content than comparable temperate species of the same genus, such as *Eucalyptus globulus*. Coupled with other variables such as basic density and S:G Ratio, this characteristic translates into comparatively lower pulp yields for kraft pulp processes (Figure 03).

Species	Pulp yield, %	Kappa #	ClO_2 cons., % / pulp
E. globulus	**58.7**	18.9	-
	55.6	16.4	4.4
E. urograndis	49.0	18.4	-
	49.6	15.7	5.3
E. grandis	50.6	16.1	5.4

Figure 03 – Pulping and ECF bleaching conditions/results. Source: Colodette, 2006 UFV.

Only recently the efforts of some research programs have shown some degree of success in introducing, via hybridization, characteristics from temperate species into tropical and subtropical driven breeding programs (Assis, 2004).

To address the need for more rapidly improving wood quality traits on existing high-volumetric performance commercial clones, ArborGen's first products for the South American market – and initially in Brazil – include lignin modifications. These modifications comprise reductions of total lignin content and/or the modification of the ratio between syringyl and guaiacyl lignin molecules ("S:G Ratio"). These products will improve the industrial and environmental capabilities of BEKP and EKP producers that use fast growing tropical and subtropical *Eucalyptus* species.

Early results obtained from wood quality analyses run in transformed elite tropical eucalyptus clones demonstrated that the lignin content can indeed be significantly reduced when compared to the original controls (Figure 04)

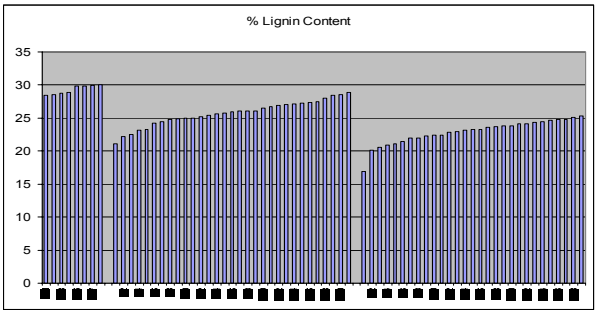

Figure 04 – Total lignin reduced x 02 distinct plasmids (gene constructs). Results obtained from analysis of wood quality run by pyrolysis molecular beam mass spectrometry (pyMBMS). Source: ArborGen, LLC.

Reduction in lignin content can potentially improve mill economics and efficiencies through the increase of pulping yield and decrease of the recovery load. This in turn will lead to increased mill throughput coupled with decreased wood and chemical consumption (Colodette, 2006).

There are a number of research results that demonstrate the efficacy of transgenic trees in delivering higher potential industrial yields. In one particular case, Kraft pulping of tree trunks of Poplar lines improved through biotechnology showed that the lines with reduced lignin had improved processing characteristics, thereby allowing for easier delignification, using smaller amounts of chemicals, while yielding more high-quality pulp (Pilate, 2002). It is important to point out that direct environmental benefits also can be realized from the use of biotechnology. It has been observed that the reduction in chemical use could also have cascade effects on all associated operations, leading directly to reduced environmental impacts. (Chiang, 2002).

It is expected that a reduction of total lignin by more than 10% can significantly impact the economics of the process in a mill. A similar level of reduction could only be achieved through conventional breeding after long multi-year and multi-generational testing programs. Any lignin reductions above the threshold of 15% in existing breeding populations are highly improbable to achieve to using conventional means in a reasonable timeframe.

Reduction of lignin is not the only trait that can be delivered through biotechnology with potential industrial and environmental benefits. Modification of existing lignin can also play an important role in addressing commercial needs. For instance, wood with a higher S:G ratio is considered, in general, to be easier to delignify (Carvalho, 2002).

ArborGen has obtained results from early wood quality analysis, showing that the S:G Ratio can be significantly increased by the introduction of a gene that encodes a key enzyme in syringyl lignin biosynthesis (Figure 05).

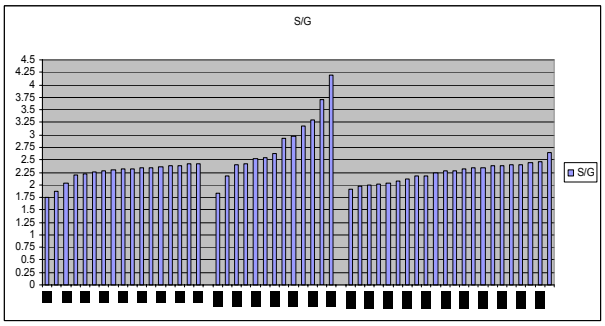

Figure 05 – S:G Ratio x plasmid. Results obtained from analysis of wood quality run by pyrolysis molecular beam mass spectrometry (pyMBMS). Source: ArborGen, LLC.

Potential benefits from a higher S:G Ratio are somewhat more difficult to assess than total lignin reduction since less is known about isolated impacts of higher S:G Ratio in pulping. Species of *Eucalyptus* that show high S:G Ratio, such as *Eucalyptus globulus*, tend to also contain lower overall lignin contents and higher basic densities. In addition, associated fiber morphology characteristics particular to different species complicates the evaluation of specific direct impacts in pulp throughput from singled-out S:G Ratio modifications. Studies with *Eucalyptus tereticornis and Eucalyptus deglupta* indicated that wood having lignin with high S:G Ratio requires less effective alkali to reach a given kappa number and produce higher yields (Collins, 1990). Another study has conversely shown a moderate correlation of S:G Ratio with pulping yield for *Eucalyptus nitens*, a weak correlation for *Eucalyptus camaldulensis* and no correlation for *Eucalyptus globulus* (Hibbert, 1999).

Although more definitive conclusions on the relation between S:G Ratio and pulp yield will be available in the future, it is possible to estimate the potential benefits based on what is known about the properties of the two molecules.

One benefit of increasing the S:G ratio relates to the increased throughput of the pulp mill. If the recovery load is the bottle neck in the pulp mill, an x% decrease in recovery load means an increase of approximately the same order of magnitude in throughput. This would be similar to the potential results that could be found in total lignin reduction.

An expected penalty of increasing S:G ratio is the slight decrease in black liquor heating value because syringyl lignin contains less carbon than guaiacyl lignin. However, the higher pulping yields obtained with high S:G lignin may compensate for such loss

since less carbohydrate loss means black liquor with higher heating value (Colodette, 2006). Furthermore, the lower effective alkali demand (see Figure 06) required for cooking high S:G lignin wood decrease causticizing loads and decrease the white liquor production costs (Colodette, 2006).

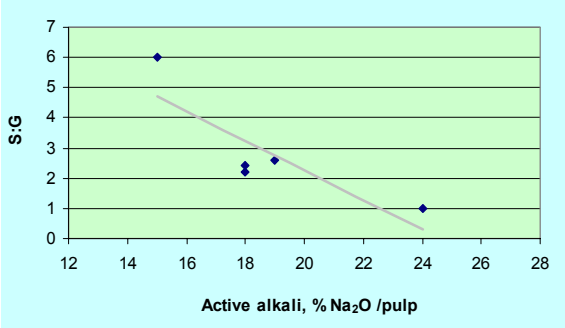

Figure 06 – Impact of wood lignin S:G ratio on effective alkali demand to achieve 16-18 kappa number during kraft pulping. First point on the left is for *E. globulus* wood and next two points are for *E. grandis and E. urograndis.* Source: Prof. Jorge Colodette, 2006 UFV.

Finally, wood containing a higher S:G ratio leads to kraft pulps containing higher S:G ratio as well. Pulps having higher S:G ratio consume less chlorine dioxide to reach full brightness (Figure 07).

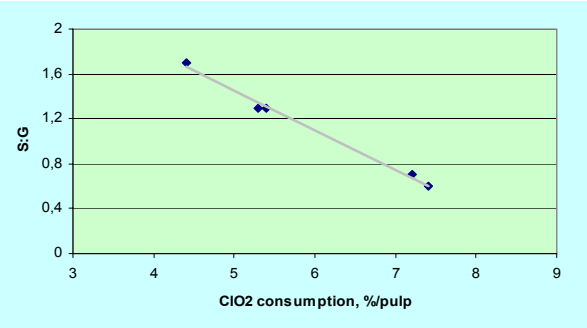

Figure 07 – Impact of kraft pulp (kappa 16-18) lignin S:G ratio on ClO2 demand (expressed as % active chlorine) to achieve 90% ISO brightness. First point on the left is for *E. globulus* wood and next two points are for *E. grandis and E. urograndis.* Source: Prof. Jorge Colodette, 2006 UFV.

In addition to the potential benefits each product offers pulp manufacturers, there is the possibility of combining these traits in a "stacked" product where both traits – reduced total lignin and higher S:G Ratio – would deliver additive benefits from both products, further improving efficiencies in the pulping process.

CONCLUSIONS

The potential benefits of biotechnology to improve eucalyptus plantation forestry are numerous, and its derived benefits to manufacturing for the wood products industry are significant. Genetic improvement through the application of biotechnology is a valuable tool that is widely utilized in many agricultural crops and is now becoming increasingly available in tree species. The results from already established field trials in Brazil will help provide a more accurate estimate of the actual economic opportunities for the pulp and paper industry in Brazil and South America.

The deployment of such tools on an industrial scale will provide new opportunities for the South American pulp and paper sector to gain a competitive advantage in the global marketplace from the social, environmental and industrial viewpoints.

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