TREE ENGINEERING AT ARACRUZ CELULOSE - PRESENT PERSPECTIVES

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ABSTRACT

Aracruz Celulose has been developing an intensive research program on *Eucalyptus* tree improvement since 1973, going through the introduction, evaluation, selection and recombination of superior trees. Significant increase on productivity and wood quality have been achieved since then. Furthermore, the more recently enhanced competitiveness in the pulp and paper industry has demanded increased integration among tree improvement, forest management and product development programs, which, in turn, has pushed the concept of "engineered forests" for different products and market segments much closer to reality.

In our company this discussion has been part of the most important strategic decisions. This paper intends to illustrate how the integration of results , achieved via different technological processes, promotes the generation of more "engineered forests", in terms of productivity and desirable wood properties. There are still many challenges ahead, but recent results indicate that previous objectives are being actually achieved.

INTRODUCTION

Traditional tree improvement programs and the use of best forest management practices have begun to pay dividends, especially to the pulp and paper industry in Brazil. Breeding has long been applied to tree improvement, and is a powerful tool for manipulation of plant populations, based on the statistical extension of Mendelian genetics. Tree selection, tree breeding and statistical analysis of genetic data have led to significant progress in tree improvement in the past, and will continue to be useful [1, 2].

More recently, the intellectual excitement and great power of cell culture and molecular genetics, and its practical biotechnology tools, have attracted substantial interest and support in the forestry research community [3]. It appears that forest genetics, molecular biology and tree physiology are coming of age at about the same time in the field of forest biotechnology, and are synergistically interacting to produce mutual successes. This synergy has been illustrated quite frequently in the literature [4, 5, 6, 7, 8, 9, 10].

Besides the opportunities proportioned by the applied genetic tree improvement, the development of new systems and techniques in silviculture and forest management are significantly increasing the technical horizons, as well as, the business of many forest products. This is specifically true in case of fast-growing tree species, like *Eucalyptus* spp, where the technological frontiers are being expanded, allowing the rupture of paradigms, considered unquestioned few years ago. Important examples of this are stand density, rotation age, nutrient distribution and cycling, utilization of process-based growth models, forest hydrology and tree water relations, which tends to apply techniques commonly used in agronomic short rotation crops, as well as, increasing the precision of forest management planning, by adding new perspectives and concepts of intensive forestry.

The integration of traditional genetic tree improvement, applied techniques in tree physiology and tree biotechnology, and novel techniques in intensive silviculture management, has allowed the development of a new model, more closely related to an "agribusiness", aiming at a speedier connectivity among wood, process and final products. However, this new paradigm, of great potential for the pulp and paper industry, will still need some time, until it can be realized in the sector. The current practice, in the majority of the companies, is still focused on the tentative adjustment of processes and products to what's available, in terms of forests, and by doing so, significant opportunities are lost, because of the difficulty to align resources, from the tree seedling at the nursery, to the final product for the client.

In our company this discussion has been part of the most important strategic decisions. This paper intends to illustrate some of the recent results obtained at our company, in different field of study, in a more recent version of our last publication regarding this issue [11]. We would like to show how the integration of results, achieved via different technological lines, promotes the generation of “engineered forests”, in terms of productivity and desirable wood properties.

“ENGINEERED FORESTS”: WHAT DOES IT MEAN EXACTLY?

Considering our current strategy and the objectives of this paper, it is important to point out that "forest tree engineering" in our concept has been characterized by the technological development in four major areas of applied forest science:

Genetic Improvement: focus is on understanding basic genetic and physiological processes on trees (mainly *Eucalyptus*), as well as, the interaction between trees x environment, allowing continuous improvement of planting stock with regard to productivity, tree and wood quality.

Tree Biotechnology: focus is also on the tree quality and function, and the work developed here is complementary to the first area. Current biotechnological approaches, which involve a range of laboratory-based techniques, now provide important results to long-established plant breeding protocols and strategies.

Forest Management: focus is to adapt management systems to guarantee maximum productivity, environmental stability and wood quality, at lowest possible cost (soil and nutrition, pests and diseases, climatic aspects and their effects on the growth rates and silvicultural practices).

Product Development: the focus is on the product, selecting and/or developing trees with fiber characteristics and relative concentrations of wood components, that can provide optimized combinations of pulp properties (besides pulping yield), according to different market segments.

In all these fields of science we have been conducting relevant work, and delivering important products, from several types of approaches and lines of thoughts. A brief discussion of the most recent strategic directions and results is described in the following topics.

GENETIC IMPROVEMENT

Research Background

Forest breeding can be described as the application of different techniques related to quantitative genetics, to the continual generation, evaluation and selection of productivity, and wood quality of superior clones and varieties, for the several environmental conditions and purposes of the company.

In 1973 we started a genetic improvement program with the aim of producing better adapted materials to the Brazilian environment, more specifically to the region of Espirito Santo State. The first step was the introduction of 55 different *Eucalyptus* species from Australia and Indonesia. After field evaluations, *E. grandis* and *E. urophylla* were identified as the most suitable, due to their fast growth and adequacy to the pulping process. From that time onwards new introductions of these species were accomplished, and by the early 1980s improved pure and hybrid varieties were produced for our own use, as well as for sale, on a commercial basis to other companies.

At the same time the company developed the cloning process at a commercial scale, and great care and attention was given to the selection and propagation of superior trees, both from the improvement program and also from the already-established commercial plantations. In the later case, the majority of the superior trees were natural hybrids from the *E. grandis*. The clonal forests obtained from that period onwards provided significant improvements in wood productivity, uniformity and quality, and also gave the company a distinct competitive edge in the worldwide market.

Recent Results

Nowadays, several recurrent selection methods are used in order to produce advanced generations of *E. grandis*, *E. urophylla* and their hybrids, as well as, of the natural hybrids of the past. Special attention has been devoted to the wood quality improvement, aiming at maximization of pulp yield per unit of area, and also at differentiation of products, which can be used to maximize the company competitiveness in the various market segments. In this context, other species with better pulping attributes have been tested in hybridization with our main genetic materials. Besides, many basic studies have been carried out, seeking fundamental parameters for the definition of selection methods to be used in the breeding program. These studies may be summarized as follows:

Definition of traits of interest in the breeding program: this work comprises several stages, from the identification of the main properties demanded by the pulp mill and the market, to the knowledge of genetic correlations among different properties, and also the development of methods which allow evaluation of a large number of trees in the field, in a rapid, cost-effective and, preferably, non-destructive way [12, 13]. All of these issues have been continuously investigated, enabling the identification of a reduced number of important wood quality traits to be considered in the breeding program.

Evaluation of environment effects on eucalypt wood quality: since the plantations have a wide geographic distribution, it becomes essential to evaluate eucalypt wood quality as affected by environment, as well as, the response of different genetic materials to environmental variation – genotype (G) x environment (E) interactions. The results from these studies have shown that both environment and G x E interaction significantly affect wood quality, inducing improvements in forestry and industrial planning operations, and in experimental procedures, such as the number of environment to be used in the establishment of clonal tests.

Evaluation of age effects over eucalypt wood quality: one of the major difficulties observed in the conduction of forest breeding programs is the long time required to the completion of selective cycles when evaluation is accomplished at rotation age. Hence, it becomes extremely important to determine early selection efficiency for wood quality traits, as it had already been done for silvicultural ones [14]. This is a long-term study (seven years) and we are currently starting wood properties evaluation for age four years.

APPLIED BIOTECHNOLOGY

Research Background

The general objective of this work in our company is to develop and to establish a high efficiency genetic transformation system and a solid and reproducible methodology for high frequency *in vitro* plant regeneration system, applicable to our best elite and adult *Eucalyptus grandis* hybrid genotypes.

Although considerable research effort has been devoted to the genetic engineering of eucalypts [15], it has lagged behind advances made in agronomic crops (rice, wheat, cotton, etc) and other woody plants (poplar, pines, sweetgum, etc), due to the recalcitrant nature of highly selected elite eucalypts for pulp and paper to *in vitro* transformation and regeneration. Only a few eucalypt species, mostly with juvenile explants, have been amenable to whole-plant regeneration through either organogenesis or somatic embryogenesis [16, 17, 18, 18, 20, 21, 22, 23].

Moreover, when used in conjunction with *Agrobacterium*-mediated genetic transformation, these systems normally lose their ability to regenerate plants from transformed tissues. In particular, the inadequacy of these systems to both select transformed shoots and to initiate roots from these transformed shoots represent the major obstacle to the success of regenerating whole transgenic plants. In this context, we strongly believe that reproducible methods for genetic engineering of elite eucalyptus with economically important genes for commercial purposes still need additional research and subsequent field trials.

Over the past few years, several methods for micropropagation and plant regeneration by organogenesis of elite and mature eucalypt clones has been tested in the company, as well as, in collaboration with our research partners, which has resulted in the production of rapidly producing regenerated shoots and plantlets. This method has brought us closer to a workable and consistent procedure for genetic transformation of our elite eucalypt clones. We have chosen to focus first on the process of organogenesis as an approach to identify competent cells in the explants with the ability to respond to phytohormones signals of *de novo* organ induction, then to develop an efficient system for *Agrobacterium*-mediated genetic transformation accomplished by cocultivation of leaf explants of elite clones and then *in vitro* regeneration of transgenic shoots.

Recent Results

Recent advances in the tissue culture and transformation of our elite genotypes through *Agrobacterium* have demonstrated that plant regeneration and gene transfer is possible. Several different elite genotypes of mature eucalypt clones have been tested for the production of organogenetic cultures from mature leaf explants. Reliable shoot regeneration protocols have been developed in order to produce morphogenic cultures capable of regenerating adventitious and axillary buds and subsequent rooted plantlets.

Attemps to regenerate buds from either excised young developing leaves from both *in vitro* grown shoot cultures and from *ex vitro* grown plants were performed on a range of culture media and culture environment, containing various combinations of growth regulators, in various of elite clones and varieties of *E. grandis* x *E. urophylla* hybrids. At least four clones could be reliably regenerated on several occasions from among the fifteen clones tested. One of these clones exhibited more than 69% of the cultures, derived from *in vitro*-grown leaves, producing adventitious multiple shoots after 70 days of culture. Regeneration in this clone was confined to the whole portion of the original explant, and it has been subsequently used routinely for transformation experiments. Shoots from *in vitro*-grown leaves of an elite variety also regenerated at a much higher frequency than the elite clone, with more than 79% of the cultures producing adventitious multiple shoots after 70 days of culture.

Conditions leading to bud regeneration from *ex vitro*-grown leaves are currently being tested to acquire organogenic competence to respond to phytohormone signals of *de novo* organ induction. *De novo* shoot formation from calli induced on young leaves from *ex vitro*-grown plants have been observed, however, yielding a low frequency of regenerated buds.

The influence of a variety of parameters have been investigated on *Agrobacterium*-mediated transient *gus* expression in leaf-derived calli, in order to develop and to optimize conditions for suitable transient gene expression. Transformation was accomplished through cocultivation of leaf explants with *A. tumefaciens* (AGL1:p35SGUSINT) carrying the chimaric neomycin phosphotransferase (NPT II) and β-glucoronidase (GUS) genes. When these explants were transfered to bud induction medium containing kanamycin, morphogenic callus was observed within 6 weeks after cocultivation. Formation of putative transformed calli was visible at the edges of leaf sections on a selection medium within one month after cocultivation, but average putative transformation rate was low. No adventitious shoots have been regenerated from the putative calli yet of these highly selected genotypes for pulping characteristics.

These preliminary studies on transformation show that large-scale regeneration of transgenic eucalypts requires improvement of the selection regime employed for screening putative transformed shoots, but there are several good indications that it is possible to obtain a reliable and stable transformation system for some of these selected adult clones.

Therefore, in the next couple of years, research will continue towards the objective of improving our new transformation method, which will improve transformation efficiency and optimize the conditions for transgenic plant regeneration. Mastering transformation in selected eucalypt hybrids would open new and very important prospects for improving these species, by integrating genes encoding useful traits.

FOREST MANAGEMENT

Research Background

We have been using sustainable management of the harvested forest ecosystems in a way that is likely to maintain and improve species diversity and structure, silvicultural systems and methods, stand dynamic processes and the functioning and biological productivity of the ecosystem, for a foreseeable future.

Since we started our operations, 33 years ago, the company has striven to be in a forefront position of an innovative and proprietary approach, regarding the use of advanced forest management practices. Presently, all pulp is produced from wood planted forests, managed through the principles of sustainable forest management.

Research improvements is being applied in a number of different areas, such as processes in intensive vegetative propagation system, process-based and empirical forest productivity models, intensive silvicultural management systems, soil fertility and plant nutrition, and integrated pest management systems. The principal theme among these areas has been the modeling of processes of carbon and water exchange between eucalypt forest canopies and the atmosphere by interacting environmental variables. This comprehensive study covers the estimation of fluxes of carbon and water from canopies, processes of carbon assimilation at the scale of leaves and shoots, carbon dynamics in forest stands, carbon modeling approaches, and aspects of water use efficiency at scales from individual trees to catchments.

Recent Results

Intensive management of eucalypt plantations grown on short rotation is a complex process, that requires an understanding of the basic physiological mechanisms and processes of productivity, which contributes to individual tree growth. Therefore, the company has decided to study the hydrological impact of eucalypts plantations, under the conditions of interest, especially regarding the control of transpiration and its relationship with carbon assimilation [24].

In 1992 we started an important project aiming to study these points in a watershed, in order to better evaluate the environmental effects of our forest operations. It is increasingly obvious that the management of fast-growing plantations for optimum utilization of available water resources of the ecosystem requires a precise knowledge of the patterns of demand and water use efficiency, throughout the course of a rotation.

The results have shown that our fast-growing clonal plantation has very high rates of carbon assimilation, and that canopy transpiration is controlled mainly by stomatal conductance, which are very susceptible to variations in the water content of superficial layers of the soil, where the major concentration of active roots occur [25].

In general, in a mature eucalypt clonal forest growing under a short and intensive rotation regime , water vapour exchange is strongly dominated by the regional vapour pressure deficit and that canopy transpiration is controlled mainly by stomatal conductance. On a seasonal basis, stomatal conductance and canopy transpiration were mainly related to predawn leaf water potential and, thus, to soil moisture content and rainfall. These studies have demonstrated that commercial eucalypt species have an efficient stomatal control of transpiration when soil moisture levels are low and evaporative demand is high and that instantaneous water use efficiency was dependent to predawn leaf water potential and vapor pressure deficit [26].

Other results indicate that leaf gas exchange are susceptible to seasonal changes in rainfall and in the water content of upper layers of the soil, where the major concentration of roots occurs. Multiple linear regression analysis indicated that photosynthetic active radiation (Q), vapor pressure deficit (D), atmospheric CO_2 molar fraction (C_a) and leaf water potential (ψ_{pd}) were the most important factors controlling stomatal conductance for water vapor (g_s), whereas, Q and D were the main microclimatic variables controlling net photosynthetic rate (A) [27].

PRODUCT DEVELOPMENT

Research Background

As eucalypt pulp is becoming more and more a specialized commodity, it has become necessary to improve it in many ways, to satisfy different market segments. In this context, our product development process has been developed into an interactive process, involving intensive inputs from different parts, such as: the market in general, customers, research institutions, etc., all aligned with the company business strategy. During last 19 years, many studies have been carried out, as part of a continuous effort to improve and develop new fibers, that fully satisfy or exceed our customers expectations.

New fiber development efforts have been concentrated in the three broad market segments that use the company's high quality bleached eucalyptus pulp: tissue, printing & writing and specialty papers. Depending upon an specific market requirement, the development has been made to adapt or modify pulp characteristics in order to provide customers with a fiber that enables them to produce more efficiently, and at reduced costs, while improving paper quality. Considering the many challenges to meet different desirable pulp characteristics, studies on product development have been made focusing on wood characteristics, considering its chemistry and morphology, pulpmaking process technologies, which can modify the physico-chemistry properties of the fibers, and fiber applications, involving several paper processes.

Recent Results

Great effort has been applied to get a better understanding of eucalypt fiber uses. Results have continuously demonstrated that eucalypt has unique properties (morphology and chemistry), with very interesting combinations of paper properties. Increased knowledge has continuously been applied, in developments with some of our customers. As an example, some joint refining studies have shown that the eucalypt pulp, with very small floc size, must receive a very specific treatment (gentle refining), in order to reach a maximum strength development, while preserving other important paper properties for fine papers, such as bulk, opacity, smoothness and porosity [28, 29].

The definition of key wood properties for optimum paper quality continues to be a major challenge, specially when considering all impacts of other aspects already discussed in this article. Despite all these aspects, the impact of wood density has been demonstrated, over and over to be a key property, when considering different uses of eucalypt fiber. Other fundamental fiber characteristics, such as coarseness and the amount and type of hemicelluloses have been important, when trying to meet different paper properties requirements [30, 31, 32].

More recently, other results have shown that many different pulpmaking strategies may significantly impact pulp characteristics. Depending on the kind and intensity of pulp delinignification and bleaching technologies, pulp properties may change significantly [33, 34, 35].

INTEGRATING ALL AREAS OF RESULT: THE WOOD QUALITY DILEMMA

During last 10 years, we have observed an important evolution, in terms of understanding and establishing the most important characteristics in forest tree improvement programs, when applied to the development of novel products to the pulp and paper industry. Some of the examples of this perceived evolution during this period were mentioned by several authors [36, 37, 38, 39].

However, it is perceived that the wood quality dilemma is still with us, mainly due to our unsteadiness in keeping the pace between changes in the needs of papermakers and the biological possibilities of actually being able to change the forests. The rate of changes ahead of us is very likely stepping up, as e-commerce grows and new printing technologies put increasing pressures upon the fast development of new paper grades. The "fiber of the future" is starting to look as a material with unique combination of properties, which in turn can be changed in a fast cycle, in order to adapt to ever changing demands!

More recently, with the arrival of modern biotechnological techniques, tree breeders have new opportunities to generate engineered genetic materials for specific purposes. Many of these new biotechniques are still being developed to their full potential, but if we consider the new green revolution taking place in agronomic crops, we can easily perceive that we are dealing with a wave which most certainly is likely to change our business as well.

These new technological opportunities are breaking the traditionalism of conventional genetic tree improvement programs for many forest genera, currently being used for pulp and paper production. As a result of this rupture in the *status quo*, companies need to urgently rethink their way of producing and selecting new improved trees and, moreover, to convert this into economically viable plantations for specific products.

CONCLUDING REMARKS

The use of genetic material yields high-productivity and high-quality wood, state-of-the-art technology to continuously improve the management system and production capacity in forested areas and the complete integration of the forest science with the product development and marketing areas.

We understand that the pressure for the definition of the most important forest attributes for each type of product has increased somewhat, notably in the last four years. Consequently, today's postponed decisions by a given company may become the critical competitive advantage for another. The technical obstacles for many objectives are gradually being removed, and previous “wishes” can actually be applied, with the introduction of modern biotechnological tools into classic genetic tree improvement programs. In summary, a new business model, based on the integration of wood, production processes and final products is already a reality.

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