Biotechnology and economic productivity of pulp wood

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ABSTRACT

The application of biotechnology to the productivity of pulp wood holds great promise. Molecular biology does have much to offer for gain in a reasonably short period of time. There are still barriers to be crossed before these methods could be applied to trees. Basic knowledge of mechanisms of the important biological processes in trees or about the tree genomes is not of adequate level. Then identification of gene and a reliable vector for inserting the altered DNA will be required for actual transformation in a tree in a desirable way, finally the means of regenerating the whole tree from the cell. What we require at the moment is to create interest in scientific community to tackel the research questions and decipher the puzzle.

INTRODUCTION

Biotechnology is broadly defined as the use of biological organisms, systems and processes for practical or commercial purposes It includes such diverse activities as fermentation, immobilized-cell and enzyme technology, cell and tissue culture, and monoclonal antibody techniques. In recent years, however, biotechnology has been increasingly associated with manipulation of important biological processes at the DNA level. In pulp and paper industry, it includes many aspects of the growing trees, facets of processing wood and pulp and the utilisation of byproducts and received much attention in recent past. Such applications cover a range of operations; pulping, bleaching, effluent treatment and waste management (1-10).

This paper, however, addresses the application of biotechnology to an area now often discussed in the technical circles of the pulp and paper industry—the Growing of trees for pulp production. The issue of increased economic productivity of pulp wood, possessing, desired uniform chemical composition (cellulose, hemicelluloses, lignin and extractives) and anatomical characteristic (fibre length, fibre diameter, wall thickness of fibre and orientation of cellulose microfibrils in S_2 layer of secondary cell wall) are crucial. Current level of biomass production per unit area is very low and diversity in the chemical composition and anato mical characteristics is also very wide. Variation in these features compel for frequent changes in process parameters which ultimately effect the quality and quantity of end product. Variation in sp.gr., alone, poses problems in pulping. Wood chips of different sp. gr., yield heterogenous pulp of different degree of delignification under identical conditions of pulping (11). Thus the pulp produced from wood of different sp, gs. would require variance in bleaching, stock preparation, paper making parameters and finally effect the end product; as it would be difficult to optimise single set of suitable parameters for pulping, bleaching, stock preparation and papermaking similarly, amount of cellulose, its degree of polymerisation and anatomical charecteristics of the fibre are also directly related with pulp yield and optimisation of various paper

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making parameters and finally with the properties of paper. Thus, the trees possessing comparatively uniform chemical composition and anatomical characteristics, would make the paper making operation more easy and yield consistent end product quality.

THE ADVENT OF BIOTECHNOLOGY

Classical Genetic Techniques

Classical genetic and silvicultural techniques are already being used to improve several species, Improved straightness, vigour disease resistance and wood/pulp properties have been obtained though selection and propogation of superior trees. In the context of pulp wood production the success achieved by Aracruz Cellulose in Brazil with eucalypts is an example for citation; and could be seen from their data recorded in Table I.

Table—1

Results of Aracruz's Forestry Programme on eucalypts.

	Initial forest (7 yrs)	Todays forest (7 yrs)	Change by quality	% Change
Yield, (m^3/ha/yr)	33	70	+37	+112
Characteristics				
Density range (kg/m^3)	300-900	500-600	—	—
Average density (kg/m^3)	460	575	+115	+25
pulp yield,%	48	51	+ 3	+6
Pulp Content (kg cellulose/m^3)	238	293	+55	+23
Specific consumption (m^3/ton cellulose)	4.20	3.41	—0 79	—19
Forest productivity (ton cellulose/ha/yr)	7.85	18.45	+10.6	+135

Although these are effective, standard breeding and selection techniques are expensive, long term prospects, requiring generation of trees. Biotechnology, Research and Development promises to decrease the time required for identifying and propagating selected trees. Presently, traditional vegetative propogation by various cuttings is used to clone trees with desirable traits. The success rate of vegetative propogation varies greatly with species or varieties. The major problem with vegetative propogation is the selection of superior trees must come after the trees have reached maturity and at that time propogation becomes problematic (12-14).

Plant tissue culture may provide an alternative means to clone superior trees and has received much attention in recent past (15-17). Plant cells excised from meristem tissue of many plants can be grown as cell suspension or as callus in laboratory. Either spontaneously or under the inducement of appropriate plant growth hormones, the cultured cells become organised and form plantlets. The problem is not so much with establishing callus cultures as with the subsequent differentiating of the tissue and regeneration of plantlets. Another potential problem is genetic instability of rapidly grown cells (16). Therefore, cloning via tissue culture needs more attention both in laboratory and in field. Tissue culture methods are micropropogation, organogenesis and somatic embryogenesis. Somatic embryogenesis although more difficult to accomplish, seem to have the most promise for use with forest trees because (i) when appropriately employed, it can be a true mass producer (ii) the approach can be used efficiently with several genetic engineering techniques. Major gain in volume growth, wood quality, disease and insect resistance and improved climatic adaptability are anticipated when used in combination with genetic engineering.

Recombinant DNA TechnologY

Traditional methods of manipulation have relied on the broad natural variability of genotypes within tree populations (17) or on the introduction of altered genes through mutagenesis. New methods include the transfer of spcific genes into the host plant, which involves introducing foreign DNA in to the host cells. Several methods are being developed for the same (18. 19.). Through protoplast fusion, multiple gene transfer is also possible and is expected to become in creasingly important over the next few decades in improving forage legumes, vegetables, and tree crops to desirable traits.

Genes are segments of DNA with the code for the production of proteins. These proteins might be key enzymes in important metabolic processes; they might be vital binding proteins that anchor complexes of mole cules to membrane surfaces in such a |way that they can function properly : or they might be structural proteins

improtant to the mechanical integrity of the cell. Because these proteins are vital to the functions and performance of the cells, seemingly insignificant changes at the DNA level can bring about profound changes in the whole organism or plant.

The technology for genetic manipulation at this level is relatively new. But already the technology has created a new generation of products for human health care. veterinary medicine, animal husbandry, industrial enzymes, food processing and now promise to bring about an agricultural "second green revolution" with greater yields, better nutritional characteristics, herbicide, resistance and with a broader range of tolerance to drought and envionmental stresses. Hence, same technology could be used in forestry.

In fact. for forestry. manupulation at the DNA level may be the best way to make improvements in an acceptably short period of time. If a transformed tree can be regenerated through successful manipulation at the DNA level, then that ttree could be clonally propagated and offspring could be put in the field for testing in about one third the time required for the normal breeding cycle. In addition it should be necessary to go through the cycle only once to effect a significant gain rather than having to wait for successive cycles to pool desirable genes. However. the applicatson of DNA technology involves a number of unique perequisistes. Indeed; for application to trees, there are experimental barriers that may require some time yet to overcome.

Understanding the molecular mechanism of the trait af interest

The first problem one encounters in attempting to apply DNA manipulation techniques to trees is the lack of basic information. Only a few of the important biological processes that influence productivity improvement have been studied at the molecules level. The lack of information about the biochemistry of the many metabolic and regulatory steps operating in trees has hindered progress towards control of tree growth and quality. Fortunately, several of these important processes have been studied in other plant systems ; so we do have some point of departure for resarch on trees.

Finding the gene controlling or affecting the trait

There are three general strategies for finding a gene (20.)

- Isolate the protein whose code is contained in the gene of interest and determine its amino acid sequence. Then prepare a piece of synthetic DNA with which to probe the plant genome for the gene itself.
- Isolate the messenger RNA (m RNA), which is the first mature product of gene experession. Then using an enzyme called reverse transcriptase, prepare a piece of complementary or copy DNA (c DNA) with which to probe the genome.
- Use a gene previously isolated from another plant organism.

Suitable probe is, then, reacted with DNA fragments isolated from the tree genome, and the complementry of DNA used to find the matching sequences. Molecular biology techniques are then used to isolate and clone these fragments of desired DNA in order to built up a sufficient quantity for further manipulation, Unfortunately, it is much easier to summarize these steps than it is to carry them out. Finding genes in trer genomes is complicated by the complexity of the genome itself But despite these complexities, an encouraging number of key genes affecting the biologicai processes for manipulation have already been identified and isolated in studies of plant systems These genes, listed in Table-II, could thus be used as probes to find and isolate the similar genes from tree genomes.

Transfer the DNA into the recipient plant cell

A piece of DNA containing the gene for the trait of interest, or the DNA fragment altered in order to change an existing trait in some desirable way has to be introduced in host plant cell, by attacking the DNA fragment to a suitable vector (A) vector is self-replicating carrier, such as bacterial plasmid or a virus, that operates by exchanging some of its DNA with the DNA in the host plant cell. Most of the successful plant transformations reported to date have used the Ti plasmid of the soil bacterium. Agrobacterium tumefaciens as the vector. The Ti plasmid contains a piece of DNA (called T-DNA) that is incorporated into the host cell genome when plant cells are co-cultured with the bacterium. Several laboratories with an interest in forest biotechnology are working on the Ti plasmid as a vector system for shuttling foreign DNA into conifers. The problem with this approach is that no body

Table-II

Plant genes isolated via molecular biology approaches and their potential value for tree improvement (7)

Process studies	Genetics	Systems studied	Possible value for tree improvement
Photosynthetic efficiency	Some target proteins and genes well characterized rate	Angiosperms	Increased photosynthetic efficiency; improved CO fixation; growth
Phytochrome control	Gene cloned	Oats	growth and dormancy cycle control
Protein structural component of system II complex	protein and gene well characterized	many plants	herbicide resistance; competing vegetation control in young stands
Extension (primary cell wall protein)	gene fragment cloned	carrot	Implicated in synthesis of primary cell well.
Microtubule protein	Several tubulin genes cloned	Green algae	Primary cell wall synthesis; also plant form, structure branching ("body plan").
Cellulose synthesis	biochemical synthesis complex isolated; characterization in progress	mung bean	secondary cell well synthesis; cambial production of wood
Lignin formation	gene for key enzyme in path way characterized.	celery, bean	secondard cell well synthesis; cambial production of wood
Osmoregulation	Osmotic tolerance compounds formed by plants; gene cloned from E. coli	many plants	drought resistance; salinity tolerance
PIIF respones mechanism insect attack	m-RNA isolated and characterized; complementary DNA prepared	tomato pea	defense against insect infestation and damage

has yet succeded in regenerating whole plant from conifer cell cultures. However, Ti plasmid transformation system that works with surface sterilized leaf discs of plants such as petunia, tobacco, and tomato have been developed recently (21). The inoculated leaf discs are taken through a more or less standard tissue culture procedure from which transformed plants are regenerated.

Regnerate the transformed tree

To regenerate the transformed tree is the final experimental barrier preventing the application of DNA technology to forestry. Some plants regenerate readily from cell or protoplast culture (22); but in some tree species (particulary in conifers) while it is possible to work and maintain cells and protoplasts in culture, the regeneration of whole functioning plant from these cultures has thus far eluded all researcher. However, despite of many experimental problems, the feasibility of this entire process has demonstrated. Model plant system such as petunia and tobacco, have successfully regenerated whole plants from transformed cells bearing foreign "Marker" Genes (23). A group of researchers have succeeded regenerating tabacco plants

and poplar planting with an artifically introduced resistance to the herbicide glyphosate (24).

POTENTIAL FOR ECONOMIC YIELD IMPROVEMENT VIA MOLECULAR BIOLOGY

The following examples highlight potential area. where DNA technology could be used to improve economic productivity.

Manipulation of tree form and structure

The "body plan" of the tree has an obvious impact on the amout of wood a stand of such tree will produce and on the quality and consequent value of that wood at final harvest. The narrow crown phenotype is one morphological variant found in nature that leads to direct improvement in yield and should lead to direct improvement in yield and should lead to improved wood quality as well(25, 26). This phenotype appeares to be controlled by a single dominant gene (20). Trees of the narrow-crown Phenotype grow strongly in height and thickness, but the grown of branches is much slower. Gain in stem yield and height is some time as high as 40.50% and 20-25% , respectively, and their average stem is correspondingly smaller 25).

The gene responsible for the narrow-crown phenotype has not yet been identified and isolated- However, plant harmones such as auxins, cytokinins, and gibberelline are believed to be involved in the biological processess leading to narrow-crown characteristics (27). Manipulation of these function at DNA level may allow to alter tree from and structure so that these potential gains can become a reality.

Photosynthetic efficiency

There is a direct relationship between plant productivity and CO_2 assimilation via photosynthesis(28). Photosynthesis is a two-step process that involves light harvesting and electron transport via two photosystems and their associated auxiliary pigments and proteins. The purpose of photosynthesis is to produce chemical energy and reducing power and then to use this chemical energy and reducing power to fix CO_2. Light absorption in a closed-canopy commercial wood stand is already a very efficient process but what trees do with this absorbed light is not efficient (29) Photosynthesis rate of trees is very low as compared to agriculture crop (30, 31).

Whatever be the reasons for this, it is clear that if the rate of photosynthesis could be improved to the level of agriculture crop the direct relationship between CO_2 assimilation via photosynthesis and plant productivity continues to hold-then tree productivity could presumably be very high. Scientists working at the DNA level with agricultural crops hope to improve the intrinsic efficiency of the key CO_2 fixing enzyme in the process or to reduce or eliminate the photorespiration cycle that occurs in agriculture crop.

Formation of wood

Most of the quality and end use performance aspects of forest economic productivity can be traced directly to the physical, structural, and chemical characterstics of the xylem cell wall, praticularly the secondary cell wall, since this is where the bulk of the wood substance is concentrated. There are a number of possible ways that these processes could be manipulated to our advantage at the DNA levl.

Manipulating the function of the microtubules

In andition to the role the microtubules play in helping to define tree form and structure, the orientation of the microtubules within the cell also appears to play a role in orienting the newly formed cellulose microfibrils as they are deposited in the secondary wall The cellulose-synthesizing terminal complexes move through the lipid membrane, along channels defined by the microtubules. Tubulin, the key protein component of the microtuboles, is a well studied protein whose genes has been cloned (table II) and is thus available for genetic manipulation experiments on this aspects of secondary cell wall formation.

Manipulating the mechanism and apparatus for cellulose synthesis

very little is known at present about the genetic and regulatory aspects of cellulose formation itself but several reasearch groups have these questions under active investigation.

Manipulating lignin formation

The majority of aromatic constituents in higher plants is generated through the well-known shikimic acid pathway, and it is now commonly accepted that this is also the route to lignin. Many of the important enzymes in this pathway have already been isolated from higher plants, and DNA probes for many of the genes involved have been prepared (Table-II). With these probes and techniques at hand, we can begin to consider the possibilities of manipulating lignin biosynthesis in order to tailor lignin specifically for our purposes. For instance, in pulp wood species it might be desirable to develop tree strains of lower lignin eontent (especially in softwoods), higher springyl-to-guaiacyl ratios for ease of processing. It is even possible to consider deleting or mutating specific enzymes in the pathway and thus produce lignins of a modified chemical nature.

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