



Application of Biotechnology in Forestry: Current Status and Future Perspective

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ABSTRACT

Biotechnology provides important tools for the sustainable development of agriculture, fisheries and forestry, and can be of significant help in meeting an indispensable part in the rise of human civilization. It is indeed often considered as one of the fields of scientific research in which the most rapid advances have been made in recent years. It includes the unique roles and functions that trees, major structural constituents of forest ecosystems have, their special biological characteristics, and their importance in the provision of environmental, social and economic goods and services. Forest trees have unquestionably entered the genomic era. Biotechnological tools viz., transgenic technology, RNA interference, functional genomics, marker assisted selection, QTL and tissue culture etc. have paved road for successful exploitation and integration of scientific fields with an increased sense of urgency for delivery of cutting edge research in tree biotechnology, both in academia and industry. Advances and integration of such fields will have a great impact in many respects, and will continue to provide new information, thereby offering exciting prospects for future tree improvement programs worldwide.

INTRODUCTION

Establishing forest plantations to meet the ever-increasing demand for tree products has been a long standing tradition in the tropics (Evans 1999). Apart from alleviating the pressure on the valuable primary forests, plantations offer continuous production of wood materials through intensive management practices. Besides the direct economic benefits, the ecological dimensions of plantation forestry have attained greater importance in the recent times in view of the invaluable contribution they provide in regulating atmospheric CO₂ emissions and thereby playing a dominant role in mitigating climate change (IPCC 2007). Forestry has been enormously benefited from the development and implementation of improved silvicultural, forest management practices and breeding techniques, which have contributed significantly to the improvement of forest tree species in the past, and will continue to have a substantial impact on the genetic gain and productivity of economically important tree species by providing better germplasm and improved management practices for plantation forests. Although good progress has been made in breeding trees for altered xylem-fibre lengths and lignin content, which is valuable to the paper and pulp industries (Turnbull 1999, Heilman 1999), much less progress has been made in improving timber quality, precisely because the wood formation is so poorly understood (Lev-Yadun & Sederoff 2000, Plomion et al. 2001). It is probably one of the most com-

plex phenomena facing plant biologists today, with perhaps 40,000 genes being involved (Lorenz & Dean 2002), so without biotechnological tools to gain a better understanding of the process, markers for wood quality traits will remain a distant prospect.

Traditional breeding methods are often constrained by the long reproductive cycles of most tree species and the difficulty in achieving significant improvements to the complex traits such as wood properties, disease and pest control, and tolerance to abiotic stresses. The state of food and agriculture reported that biotechnology is more than genetic engineering (FAO 2004). In fact, 81% of all biotechnology activities in forestry over the past ten years were not related to genetic modification (Wheeler 2004).

Genetic engineering has the potential to boost global wood production in many ways (Sutton 1999, Sedjo 2001). Applications currently under consideration, for plantation forests, include resistance to biodegradable herbicides, altered lignin properties for reduced downstream processing costs or improved burning, resistance to selected pests, altered reproductive mechanisms for faster breeding or genetic containment, phytoremediation of polluted sites, and the production of novel chemicals or pharmaceuticals (Strauss 1999, Strauss et al. 2001, Yanchuck 2001). It might also be possible to manipulate wood-quality traits, photosynthetic efficiency, and tolerance to abiotic stresses such as drought.

The term “biotechnology” broadly defined, it is anything that combines technology and biology. More narrowly defined, modern biotechnology focuses on such things as DNA-level analyses, transfer of single genes from a different species, and cloning by somatic embryogenesis. Biotechnology is an adjunct to the long-established traditional tree improvement practices and one that utilizes fundamental discoveries in the field of plant tissue culture for clonal forestry, gene transfer techniques, molecular biology, and genomics. These new discoveries now provide an extended platform for the improvement of traits that have previously been considered impractical via conventional breeding methods. Biotechnology provides exciting opportunities to further expand our understanding of genome organization and functioning of genes associated with complex value-added traits, and to transfer such genes into economically important tree species. This will lead to the development and deployment of trees ready to meet the future demand of the world’s ever-increasing population for timber and other forest products, while preserving natural forests for future generations.

Only limited studies are available on the role of biotechnology in plantation forests (Fenning & Gershanson 2002), economic benefits resulting from the introduction of forest biotechnology (Sedjo 2001) and ecological issues associated with the deployment of genetically modified forest tree species (Van Frankenhuyzen & Beardmore 2004). In this article, we cover recent innovative technologies, which will provide the basis for acceleration in the improvement of forestry through biotechnology. Over the past few decades, conventional tree improvement programs, which involve selection, genetic crosses and recurrent testing, have been used around the world to improve plantation forestry yields and have certainly proven useful. Unfortunately, the recognition of the potential of biotechnology in the forest sector is much more limited. However, it is becoming an increasingly important component of the processing sector, such as pulp and paper production, and it also plays an important role in various stages of the production chain, from planting to harvesting. One of the first applications of biotechnology in forestry was the inoculation of seedlings with symbiotic organisms (specifically mycorrhizae) with the objective of increasing seedling growth. Since then, tremendous progress has been made in the field of forest biotechnology, which currently focuses on main areas such as propagation, genetic transformation, transgenic approaches, *In vitro* culture, abiotic stress resistance, biotic stress resistance, modification of lignin, RNAi interference, marker assisted selection and QTL mapping and future directions in forest tree genomics research.

PROPAGATION

Plant cloning has been used for centuries for tree breeding and propagation using grafts and cuttings. Chinese fir (*Cunninghamia lanceolata*) has been propagated by cuttings for clonal forestry in China for more than 800 years (Li & Ritchie 1999) and Japanese cedar (*Cryptomeria japonica*) has been propagated clonally by cuttings in Japan for plantations since the beginning of the fifteenth century (Toda 1974). Some tree species are easier than others to propagate by cuttings. Easy to root hardwood species, such as poplars (*Populus* spp.), willows (*Salix* spp.) and some eucalypt (*Eucalyptus*) species, and conifer species, such as spruces (*Larix* spp.), redwood (*Sequoia sempervirens*), and some pines (*Pinus* spp.), are widely planted as cuttings in family or clonal plantations (Ritchie 1991, Ahuja & Libby 1993, Assis et al. 2004, Menzies & Aimers-Halliday 2004). In the future, the use of vegetatively propagated trees for intensively managed, high yielding plantations are expected to increase in all regions of the world. While the main use of propagation technologies has been for forest establishment of genetically-improved families or clones, there is also a conservation use for those species that are at risk, rare, endangered or of special cultural, economic or ecological value (Benson 2003). Integrating traditional methods such as *in situ* conservation and seed storage with biotechnologies such as micropropagation and cryopreservation can provide successful solutions.

GENETIC TRANSFORMATION

Biotechnology has tremendous potential in tree improvement. Genetic transformation not only complements the conventional selection and breeding of superior trees, but also makes a major contribution to overcome constraints like long breeding cycles, species barriers and narrow genetic pools in cultivated commercial tree crops. The first successful transformation in trees was achieved by Fillatti et al. 1987. Since then progress has been slow but steady and has witnessed many new inventions and techniques over the past decade, which have been reviewed extensively (Merkle & Dean 2000, Pena & Seguin 2001, Herschbach & Kopriva 2002, Diouf 2003, Gallardo et al. 2003, Gartland et al. 2003). The main theme to attempt genetic transformation in trees is the improvement of productivity and quality. The potential of production of trees with novel traits is one of the most distinct benefits of genetic transformation. The idea of using several dozen species, most of which belong to the genera *Eucalyptus*, *Pinus*, *Picea*, *Populus* and Rubber for molecular farming of desired products is also gaining momentum.

TRANSGENESIS

A wide diversity of sources of transgenes and regulatory elements, and intended traits, have been tested, including expression of reporter genes; insect, disease, and herbicide resistance; modified wood properties; modified flowering and fertility; and modified growth rate and stature (Viswanath et al. 2011). Procedures for genetic transformation of forest trees differ little from those for other plant species and are mainly confined to the use of *Agrobacterium*, with a few reports on particle bombardment-mediated transformation. Differentiation of transformed cells is a prerequisite to obtaining transgenic plants and two systems are being used in forest trees: organogenesis and embryogenesis. Such transformation procedures, including the use of selectable markers and screening methods, are well established. It is possible to introduce one or more perfectly characterized new characters without, in theory, adversely affecting the overall genetic make-up of the plant. This approach also offers the possibility of overcoming the genetic barrier between species, in a relatively shorter time frame than through conventional tree breeding. The major obstacles to efficient production of transgenic trees are: (i) difficulties in plant regeneration from *Agrobacterium*-infected or particle-bombarded explants; (ii) incomplete development beyond the *in vitro* stage of rooted plants for establishing field trials; and (iii) transgene instability during the long life-span of forest trees, including transgene silencing and somaclonal variation (Harfouche et al. 2011). Once transgenesis is performed at the cell level, *in vitro* culture techniques can be used to regenerate the entire tree.

IN VITRO CULTURE

This technique involves propagating plant tissues (units as small as a cell) in a controlled environment free of microorganisms. Approximately 34% of all biotechnology activities reported in forestry over the past ten years related to propagation (Chaix & Monteuis 2004, Wheeler 2004). An entire tree can be regenerated from a single cell. *In vitro* culture can be used to reproduce seedlings and to cryopreserve cell lines from which it will be possible to regenerate other copies of the same seedlings in the future. In *in vitro* plant culture, regeneration occurs via two main pathways: organogenesis and somatic embryogenesis. Organogenesis is the regeneration of plants through organ formation on an explant or from cell masses, and for somatic embryogenesis it is done through the formation of embryo-like structures. Organogenesis has been the method of choice for species such as poplar and eucalyptus, and embryogenesis has been used very successfully with conifers (Park et al. 1998). Both processes provide the means to clonally

propagate large numbers of elite trees for research and reforestation. One drawback of somatic embryogenesis is that it is fully applicable only using juvenile material as initial explants (embryos but difficult to carry out with needles). To capture maximum gains a two-step procedure must be established. Firstly, while testing new lines produced with replicated clonal trees, tissue lines must be cryopreserved. Secondly, once the best clone has been identified after a few years of testing, cryopreserved tissue of the best lines are put back into *in vitro* culture for tree multiplication and propagation. *In vitro* culture is also essential to genetic engineering or transgenesis work because it provides the material on which the technology can be carried out.

ABIOTIC STRESS RESISTANCE

Drought, which is often associated with osmotic or salinity stress, is a major factor involved in the decrease in forest productivity. Enhancing drought and salinity tolerance is of particular importance when reforesting marginal arid and semi-arid areas, which are prone to degradation. Molecular control of plant response to abiotic stress is complex, usually involving coordinated expression of several genes. The use of known abiotic-stress-associated genes from other species to enhance tolerance in forest trees has been limited. However, recent studies in genomics, transcriptomics and proteomics in several forest tree species, as well as release of the draft *Eucalyptus grandis* genomic sequence (www.eucagen.org), have provided new tools for improving abiotic stress tolerance in trees (Harfouche et al. 2011). Over expression of a pepper ERF/AP2 transcription factor, CaPF1, in eastern white pine resulted in a significant increase in tolerance to drought, freezing and salt stress (Tang et al. 2007b). The increased tolerance was associated with polyamine biosynthesis. Moreover, over expression of the choline oxidase (*codA*) gene from *Arthrobacter globiformis* resulted in increased tolerance to NaCl in several lines of *Eucalyptus globules* (Yu et al. 2009).

BIOTIC STRESS RESISTANCE

The use of genetic engineering to improve tree resistance to insects and microbial pests has been the subject of investigation in several laboratories. For example, at Natural Resources Canada (NRC), researchers introduced the gene corresponding to the toxin *Bacillus thuringiensis* (B.t.) into white spruce (*Picea glauca* [Moench] Voss). White spruce is susceptible to spruce budworm (SBW), an insect that has caused the defoliation of large areas in Canada. The transgenic trees obtained were tested for their toxicity to SBW and complete resistance was observed in several trees (Peña & Séguin 2001). This research is part of the development of a research model and is not aimed at the commer-

cialization of such a product. Similarly, in several parts of the world, fungal and bacterial infestations cause substantial forest losses. These losses are very often underestimated, as compared to the damage caused by insects, because the damage is less visible. However, it is possible to induce resistance by introducing genes associated with the production of antifungal or antibacterial proteins (e.g., endochitinase, PPO) (Séguin 1999). Various approaches, which are currently in the experimental stage, will be used to assess the effectiveness of these strategies for forest trees (Peña & Séguin 2001).

Genetically engineered insect resistance can be environmentally beneficial because of the reduced need for synthetic insecticides. Hybrid triploid poplars [(*Populus tomentosa* × *P. bolleana*) × *P. tomentosa*] transformed with a cowpea trypsin inhibitor gene (CpTI) exhibited resistance to three defoliating insects: forest tent caterpillar (*Malacosoma disstria*), gypsy moth (*Lymantria dispar*) and willow moth (*Stilpnotia candida*) (Zhang et al. 2005). Forest trees play host to a wide range of fungal, bacterial and viral pathogens. Trees engineered for disease resistance can provide both environmental and commercial benefits. Enhanced disease resistance has been achieved using a variety of genes derived from plants and microorganisms, with varying degrees of success. For example, Chinese white poplar (*P. tomentosa*) expressing a chitinase gene from *Beauveria bassiana* (Bbchit1) exhibited increased resistance to a pathogenic fungus (*Cytospora chrysosperma*) (Jia et al. 2010). Testing for disease resistance in a natural setting is imperative and multi-year field trials will be needed to verify the durability of resistance against ever-evolving pathogen populations.

MODIFICATION OF LIGNIN

During chemical pulping of wood, one of the most expensive and environmentally hazardous processes is to separate lignin from cellulose and hemicellulose (Pilate et al. 2002). The production of plant material with lower contents of lignin would mean a significant reduction of cost and pollution to the paper industry. It is now possible to develop transgenic trees that have lower lignin content, but do not have unfavourable physiological characteristics. Biochemical pathways in lignin synthesis have been the subject of numerous investigations, and several genes responsible for the enzymes involved have been characterized (Tzfira et al. 1998, Merkle & Dean 2000). One of the approaches to obtain reduced lignin forest trees has been the down regulation of lignin biosynthesis pathways (Hu et al. 1999). The main genes involved with genetic transformation targeting lignin reduction are 4-coumarate: coenzyme A ligase (Pt4CL1) (Hu et al. 1999), cinnamyl alcohol

deshydrogenase (CAD-the final enzyme in the biosynthesis of lignin monomers) (Baucher et al. 1996) and caffeate/5-hydroxyferulate O-methyltransferase (COMT-enzyme involved in syringyl lignin synthesis) (Lapierre et al. 1999). After cellulose, lignin is the most abundant organic compound in the biosphere and makes up 15 to 35% of the dry weight of trees. By manipulating the expression of these genes, it has been possible to modify the lignin content or structure.

Tremendous efforts have been devoted to the current tree biotechnology emphasis on low lignin quantity must be expanded to include greater lignin reactivity and, ultimately, a combination of low and reactive lignin traits. Lignin quantity and reactivity [which is associated with its syringyl/guaiacyl (S/G) constituent ratio] are two major barriers to wood-pulp production. To verify our contention that these traits are regulated by distinct monolignol biosynthesis genes, encoding 4-coumarate-CoA ligase (4CL) and coniferaldehyde 5-hydroxylase (CALD5H), we used *Agrobacterium* to cotransfer antisense 4CL and sense CALD5H genes into aspen (*Populus tremuloides*). Trees expressing each one and both of the transgenes were produced with high efficiency. Lignin reduction by as much as 40% with 14% cellulose augmentation was achieved in antisense 4CL plants; syringyl/guaiacyl ratio increases as much as 3-fold was observed without lignin quantity change in sense CALD5H plants. Consistent with our contention, these effects were independent but additive, with plants expressing both transgenes having up to 52% less lignin, a 64% higher S/G ratio, and 30% more cellulose. A S/G ratio increase also accelerated cell maturation in stem secondary xylem, pointing to a role for syringyl lignin moieties in coordinating xylem secondary wall biosynthesis. The results suggest that this multigene cotransfer system should be broadly useful for plant genetic engineering and functional genomics (Li et al. 2003). The antisense 4CL (4-coumarate: CoA ligase) gene was transformed into triploid Chinese white poplar (*Populus tomentosa*) mediated by *Agrobacterium tumefaciens* (Lee et al. 1997). PCR and Southern blot analysis indicated that antisense 4CL gene had been integrated into the genome of the transgenic Chinese white poplars and found repression of 4CL expression could result in a remarkable reduction of lignin content in transgenic poplars, with most reduction of 41.73% compared with that of wild type (Caihong et al. 2004).

RNA INTERFERENCE

RNA silencing is a novel gene regulatory mechanism that limits the transcript level by either suppressing transcription (transcriptional gene silencing [TGS] or by activating a sequence-specific RNA degradation process

(posttranscriptional gene silencing [PTGS]/RNA interference [RNAi] (Agrawal et al. 2003). Although there is a mechanistic connection between TGS and PTGS, TGS is an emerging field while PTGS is undergoing an explosion in its information content (Baulcombe 2000, Matzke et al. 2001). Double-stranded RNA-mediated gene suppression, also known as RNA interference (RNAi), was first reported in *Caenorhabditis elegans* a decade ago (Fire et al. 1998). It is currently the most widely used methods to down-regulate gene expression. It can be used to knock out all copies of a given gene, thus providing insight into its functionality. However, it does not always result in complete inhibition of a gene's expression. Recent advances in targeted gene mutagenesis and replacement using the yeast RAD54 gene (Shaked et al. 2005) or zinc-finger nucleases (Lloyd et al. 2005, Wright et al. 2005) may eventually lead to efficient methods for engineering null alleles in trees.

The natural function of RNAi is referring to the mechanism involved in cellular defence against viruses, genomic containment of retrotransposons, and post-transcriptional regulation of gene expression. RNAi can specifically silence individual genes, creating knockout phenotypes, either in transformants that can produce the required hairpin RNAs, or upon infection with recombinant RNA viruses that carry the target gene (VIGS, viral-induced gene silencing) (Tenea 2009). The mechanism of RNAi was reviewed and then some of its applications in plants discussed (Table 1).

The efficiency and stability of RNA interference (RNAi) in perennial species, particularly in natural environments, are poorly understood. Li et al. (2008) studied 56 independent poplar RNAi transgenic events in the field over 2 years. A resident BAR transgene was targeted with two different types of RNAi constructs: a 475-bp IR of the promoter se-

quence and a 275-bp IR of the coding sequence, each with and without the presence of flanking matrix attachment regions (MARs). RNAi directed at the coding sequence was a strong inducer of gene silencing; 80% of the transgenic events showed more than 90% suppression. In contrast, RNAi targeting the promoter resulted in only 6% of transgenic events showing more than 90% suppression. Results suggest that RNAi can be highly effective for functional genomics and biotechnology of perennial plants. RNAi technology has enabled the creation of varieties of Coffee that produces natural coffee with low or very low caffeine content, thus bypass the need of extraction (Van Uyen 2006)

MARKER ASSISTED SELECTION AND QTL MAPPING

The primary goal of tree breeding is to increase the quantity and quality of wood products from plantations. Major gains have been achieved using recurrent selection in genetically diverse breeding populations to capture additive variation. However, the long generation times of trees, together with poor juvenile-mature trait correlations, have promoted interest in marker-assisted selection (MAS) to accelerate breeding through early selection. MAS relies on identifying DNA markers, which explain a high proportion of variation in phenotypic traits. Genetic linkage maps have been developed for most commercial tree species and these can be used to locate chromosomal regions where DNA markers co-segregate with quantitative traits (quantitative trait loci, QTL). MAS based on QTL is most likely to be used for within-family selection in a limited number of elite families that can be clonally propagated. Limitations of the approach include the low resolution of marker-trait associations, the small proportion of phenotypic variation ex-

Table 1: Application of RNAi interference in plant systems.

Sr. no.	Application	Case study	Authors
1.	Increasing the level of lysine	Reduction of lysine catabolism and improving seed germination generating a dominant high-lysine maize variant by knocking out the expression of the 22-kD maize zein storage protein	Zhu & Galili (2003), Tang et al. (2007b)
2.	Coffee	RNAi technology has enabled the creation of varieties of Coffee that produces natural coffee with low or very low caffeine content	Davuluri et al. (2004)
Flower color modification			
3.	Gentian	Producing white flowered transgenic gentians by suppressing the chalcone synthase (CHS) gene using antisense technology	Van Uyen (2006)
4.	Pest control	Combining Bt technology with RNAi would both enhance product performance and further guard against the development of resistance to Bt proteins	Goldstein et al. (2009)
5.	Wood and fruit quality	Down regulation of lignin biosynthesis pathways. Producing transgenic hypoallergenic apples and a possible solution for the undesirable separation of juice into clear serum and particulate phase by using RNAi	Hu et al. (1999), Teo et al. (2006) Amancio José de Souza et al. (2007)

plained by QTL and the low success rate in validating QTL in different genetic backgrounds and environments. This has led to a change in research focus towards association mapping to identify variation in the DNA sequence of genes directly controlling phenotypic variation (gene-assisted selection, GAS). The main advantages of GAS are the high resolution of marker-trait associations and the ability to transfer markers across families and even species (Guimarães et al. 2007). Association studies are being used to examine the adaptive significance of variation in genes controlling wood formation and quality, pathogen resistance, cold tolerance and drought tolerance. QTL mapping in forest trees has been applied to numerous species and traits for more than 20 years. The traits that have been most intensively studied are complex and are usually related to the fitness of trees. Regardless of species, they fall into four categories: growth and biomass, apical bud phenology, resistance to biotic or abiotic stresses, and wood properties (Neale & Kremer 2011). The lack of resolution in mapping candidate genes and QTL alleles can be overcome by association genetics, using natural populations in which the long evolutionary history has broken up the linkage between markers and genes (Neale & Savolainen 2004). A prerequisite is the presence of large allelic variation in the population. Sequencing alleles in a range of candidate genes in eucalyptus, pine and aspen demonstrate that such variants, including single nucleotide polymorphisms (SNPs) and indels, can readily be found (Poke et al. 2003, Gill et al. 2003, Brown et al. 2004, Ingvarsson 2005). In the loblolly pine germplasm, linkage disequilibrium (LD) decays in the order of the physical length of a gene, and in European aspen LD extends only a few hundred base pairs, indicating the potential of association genetics to identify genes responsible for variation in the trait (Brown et al. 2004, Ingvarsson 2005). Single nucleotide polymorphisms (SNPs) in these gene sequences that are significantly associated with trait variation can then be used for early selection. Markers for SNPs can be transferred among individuals regardless of pedigree or family relationship, increasing opportunities for their application in tree breeding programmes in developing as well as developed countries. Significant reductions in genotyping costs and improved efficiencies in gene discovery will further enhance these opportunities.

TISSUE CULTURE

The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt, German Academy of science in 1902 on his experiments on the culture of a single cell. The term plant tissue culture (micropropagation) is generally used for the aseptic culture of cells, tissues, organs and their components under defined chemical and physi-

cal conditions *in vitro*. The basic concept of the plant body can be dissected into smaller part termed as "explants" and any explants can be developed into a whole plant. Tissue culture is a process that involves exposing the plant tissue to a specific regimen of nutrients, hormones, and lights under sterile, *in vitro* conditions to produce many new plants, each a clone of the original mother plant, over a very short period of time.

Advantages of Tissue Culture Planting Material

- True to the type of mother plant under well management.
- To produce Free for viral, fungal and bacterial pathogen of quality seedlings.
- Uniform growth and increases yield (20-30%).
- Shorter crop rotation with minimum cost of cultivation-maximum land use is possible in a low land holding country like India.
- New varieties can be introduced and multiplied in a short duration.
- Planting possible as seedlings are made available throughout the year.
- High multiplication rate per unit area.
- A quick way for breeding and production of excess plants.
- Homogeneity in plant growth and timing of flowers and fruits.
- No staggered harvesting.
- Faster diffusion of improved varieties.
- Easy transfer of seedlings and availability throughout the year.

Plant tissue culture techniques are of tremendous potential value to forest tree improvement. The technology is envisaged as playing a complementary role to traditional methods through exploiting spontaneous or induced genetic and epigenetic variability in culture, by use of haploidy and by the use of protoplasts. Clonal propagation is an integral part of any tree improvement program, and in addition can play an independent role in reforestation, clonal orchard establishment and in energy foresting. We have not given up on the possibility of growing haploid tissues of pines and hardwoods because of the extremely important application of these procedures to forest genetics. With the production of haploid tissues, followed by chromosome doubling, and the formation of plantlets (roots and buds), a homozygous line would be effectively produced. Geneticists have always attempted to create genetic options for the future. If we look carefully at where these current and future biotechnologies are heading in forestry, it is obvious that the research and development will largely be used in support of advancing clonal forestry. Therefore, it is in the context of appropriate

clonal forestry that most of the issues need to be examined and evaluated.

FUTURE PERSPECTIVE

Biotechnology application in forestry has made tremendous strides in the past decade. Many tree species engineered for expression of a variety of traits are already under extensive cultivation in many parts of the world. The status of biotechnology in India is very encouraging and the future back on with many opportunities. All modern biotechnologies require large research and development investments. The allocation of funds, through either private or public agencies, needs to achieve a balance between building scientific capabilities and knowledge and supporting more applied, well proven forestry technologies. From a genetic perspective, concerns that biotechnology is "unnatural" ignore the dynamic changes in the genetic code that occur within and across species genomes through modification of transposable genes or elements by virus vectors and through mutation. Tissue culture or genetically modified trees will be substantial managers with time to evaluate many of the issues being faced in agriculture, the economic realities of relatively long generations will continue to be a major challenge for investors in biotechnology in forest trees. It appears that genetic modification will therefore become a reality only for particularly novel and valuable traits in short-rotation species in intensively managed plantations. Other forest management decisions with potentially more serious ecological consequences, including large-scale species introductions or inappropriate use of provenances or improved trees from even conventional breeding, need to be evaluated by foresters, managers and regulatory agencies in the same way as the products of modern biotechnology. However, while those in the forefront of any technology will promote its potential benefits, in the end it will be the economic and regulatory systems of governing bodies at the national and global levels that must evaluate the technology's relevance and appropriateness.

CONCLUSION

This article has sought to address the main issues that should remain relevant to the use of any modern biotechnology in forestry, now and in the future. Many cultures have a powerful attachment to forests, and concern over their destruction has driven much of the growth of environmental groups. Their activities have done much to highlight the problems of deforestation worldwide, but the solutions they offer for stabilizing the world's wood supply have sometimes been less than helpful. For the world to be supplied with the wood it needs on a long-term sustainable basis, it needs to invest much more in the development of high-yielding, short rota-

tion plantation forests. Biotechnology is essential to achieving this goal. The alternative is that the world's remaining natural forests will continue to be degraded, probably at an accelerating rate, and/or pollution from wood substitutes will increase. Those who oppose plantation forests either in any form, or the application of biotechnology to their development, need to be clear what the choices really are, rather than what they might like them to be. The logic of plantation forests is so strong that they will undoubtedly play a major role in achieving global sustainability. The only real question is how much more damage will be done to Earth's natural forests before the essential contribution of plantation forests is fully recognized. Genetic engineering is becoming a routine method in forestry. Although the possibilities in agriculture are much better because of the broader knowledge available and background breeding information, forest trees are also clearly in the focus of research. The ability to transfer genes into the genome of trees offers ample opportunities in the field of breeding research. Based on the Indian government's aim to enlarge the total area covered by forests to 23% by 2010 and to 33% by 2020, biotechnology is going to play a central role to tackle specific challenges. Trees are long living organisms and often surrounded by their wild relatives, which causes a risk of gene flow from transgenic stands into natural populations. Research is still needed to investigate the problems related to transgenic trees, e.g. concerning all aspects of biosafety including efforts to prevent the escape of transgenes into natural populations. Approval for the commercial use of transgenic trees and their easy vegetative propagation by cuttings (e.g. in poplar, *Eucalyptus*, casuarinas and *Salix*) can cause a rapid distribution of transgenic plant material in the near future.

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