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Agricultural biotechnologies in developing countries: Options and opportunities in crops, forestry, livestock, fisheries and agro-industry to face the challenges of food insecurity and climate change (ABDC-10)

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Current Status and Options for Forest Biotechnologies in Developing Countries

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Acronyms and Abbreviations

Bt = *Bacillus thuriengensis*

CBOL = Consortium for the Barcode of Life

EMBL = European Molecular Biology Laboratory

GM = Genetically modified

GMO = Genetically modified organism

MAS = Marker-assisted selection

NCBI = National Center for Biotechnology Information

QTL = Quantitative trait locus

R&D = Research and development

SE = Somatic embryogenesis

SNP = Single nucleotide polymorphism

VAM = Vesicular-arbuscular mycorrhizae

1. Introduction

In recent decades, forest biotechnology has grown into a dynamic portfolio of tools, moving beyond research into global trade and development. This portfolio concept is consistent with the *sensu lato* definition of biotechnology put forth in the Convention on Biological Diversity's Article 2 "*…any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products and processes for specific use*." This is the definition used in this and in the other FAO documents being prepared for ABDC-10.

This introduction gives a brief description of the state of the world's forests and some factors shaping forestry and forest biotechnology.

1.1 Forest and Tree Resources Management - State of the World's Forests.

Forests and other wooded areas perform key economic and ecological functions. Not only do they provide goods and livelihoods but they also protect soils, regulate water and absorb carbon. Forests also shelter much of the world's biodiversity. FAO's most recent review on the overall status of forest resources, the Global Forest Resources Assessment (FAO, 2006), indicates that the world has just under 4 billion hectares of forests, covering about 30 percent of the world's land area. It also revealed that production of wood and non-wood forest products is the primary function for 34 percent of the world's forests, and that more than half of all forests are used for wood and non-wood production in combination with other functions, such as soil and water protection, biodiversity conservation and recreation. Only 5 percent of forests in the world are in plantations, with the balance found in natural or semi-natural, largely unmanaged and undomesticated forest stands. Planted forests are expanding and their contribution to global wood production is approaching 50 percent of the total. In 2004, the production of industrial roundwood was 1.6 billion cubic meters, representing some 45 percent of the global wood production, and forest products trade reached a total value of US$327 billion. More than half the wood biomass consumed globally – and well over 80 percent in developing countries – is burned as fuel. About 1.6 billion people rely heavily on forest resources for their livelihoods (World Bank, 2001). Sixty million indigenous people living in the rain forests of Latin America, Southeast Asia and West Africa depend heavily on forests; 350 million people living in, or next to, dense forests rely on them for subsistence or income; and 1.2 billion people in developing countries use trees on farms to generate food and cash. Forest and tree resources are managed in different main types of systems, which are presented in Table 1. The intensity of management varies very much between primary natural forests and productive industrial plantations.

FAO (2006) indicates that the world's forested area is shrinking, particularly at tropical latitudes (Table 2). Only a few countries have seen a net increase in forested land area, and these include China, Vietnam, Cuba, Uruguay, Chile, United States and most of Europe, west and east. Forested land area is not increasing in tropical regions where biodiversity and growth rates per hectare (not shown) are highest. This table points to a few of the factors shaping the forest biotechnology opportunity.

Table 1. Characteristics of main types of forest and tree resources management

Naturally regenerated forests			**Planted forests**			**Trees outside forests**
Primary	**Modified natural**	**Semi-natural**		**Plantations**		
		Assisted natural regeneration	**Planted component**	**Productive**	**Protective**	
Forests of native species, where there are no clearly visible indications of human activities and the ecological processes are not significantly disturbed	Forests of naturally regenerated native species where there are clearly visible indications of human activities	Silvicultural practices by intensive management: • Weeding • Fertilizing • Thinning • Selective logging	Forests of native species, established through planting or seeding intensively managed	Forests of introduced and/or native species, established through planting or seeding mainly for production of wood or non-wood goods	Forests of introduced and/or native species, established through planting or seeding mainly for provision of services	Stands smaller than 0.5 ha; tree cover in agricultural land (agroforestry systems, home gardens, orchards); trees in urban environments; and scattered along roads and in landscapes

Table 2. Forested areas and annual rates of change for the world's forested land cover by region (from FAO, 2006).

Forest area	Land area (1 000 hectares)	% Land area	Annual change rate 2000-2005 (%)	Forested countries with highest net increase
Africa	635 412	21.4	-0.62	Rwanda, Egypt
Asia and Pacific	734 243	25.8	+0.09	China, Vietnam, New Zealand
Europe	1 001 394	44.3	+0.07	Bulgaria, Spain
Latin America and Caribbean	859 925	47.3	-0.51	Uruguay, Chile, Cuba
North America	677 464	32.7	-0.01	United States
West and Central Asia	43 588	4.0	+0.03	Uzbekistan
World	3 952 025	30.3	-0.18	

Rapid loss of the forested areas is coming from changes in land use. In addition to deforestation, existing forests are being degraded by pathogens and pests, fire, atmospheric pollution, extreme weather events, climate change and unsustainable forest management practices.

1.2 Factors shaping forests, forestry and forest biotechnology

The following factors shape global opportunity, condition investment decisions and drive research priorities for forest biotechnology. They also point to important differences in the use of biotechnologies compared with the crop or livestock sectors.

- Forest trees are highly heterozygous, long-lived perennials with late sexual maturity and a lengthy regeneration cycle which places high priority on retaining genetic diversity as an insurance policy against rapid change (Namkoong, Barnes and Burley, 1980).
- Most forest tree species have narrow, regional adaptation, so species numbers used for planting are orders of magnitude higher than those for food crops (Pautasso, 2009).
- Forest trees serve as keystone species in dynamic ecosystems, so managing against loss translates into more than tree survival (Whitham *et al*. 2006). Survival for colonizing forest tree species often depends on the presence of specific symbiont microbial species (Bonfante and Anca, 2009).
- Forest trees are largely undomesticated although a few species have had some population-level improvement for one to four generations.
- Most of the world’s forests have public ownership (Agrawal, Chattre and Hardin, 2008).
- A forest tree is utilized for multiple purposes, not a single product. A single log can be used for sawtimber, paper and pulp. Waste products from papermaking are sold in secondary markets. Pulping waste is a rich source of industrial solvents, livestock feed, lubricants and consumer products such as artificial vanillin and medication.

Within this context, this document’s purpose is to review the state of biotechnology and its impact on forest activities. It addresses this first by looking at the past (Section A) then by looking forward (Section B). Under stocktaking, Section 2 provides an overview of the history and status of application of forest-specific conventional technologies with special attention to developing countries. Section 3 documents the current status of application of biotechnologies in developing countries. Section 4 provides an analysis of successes and/or failures of forest biotechnologies in developing countries, while Section 5 presents a small number of case studies. Under looking forward, Section 6 addresses key issues in the sector where the forest biotechnologies could be useful. Section 7 identifies options for developing countries, and Section 8 presents priorities for action for the international community.

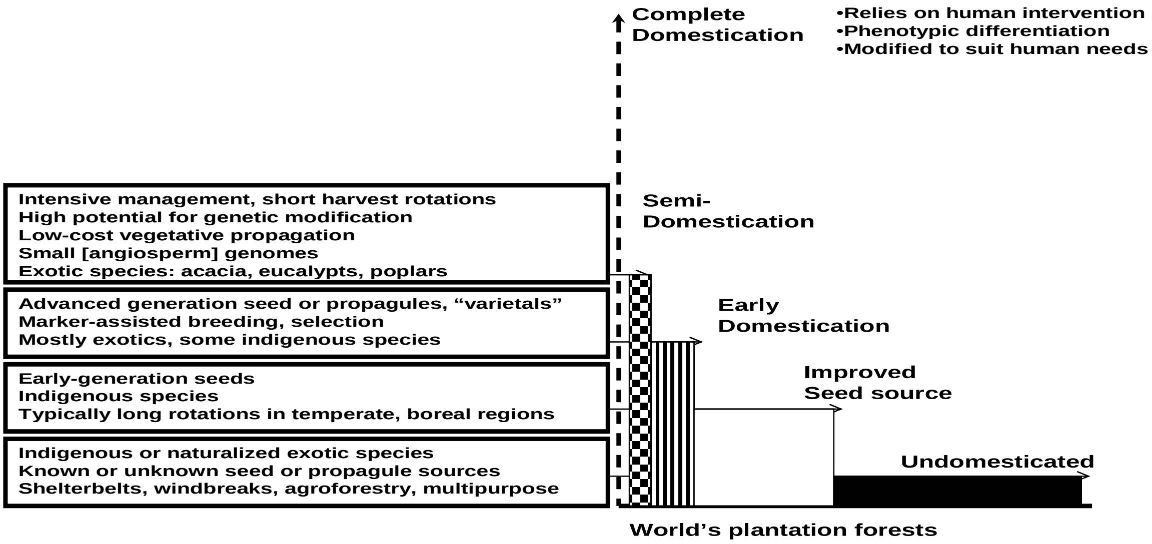
A. Stocktaking: Learning from the Past

2. Overview of forest-specific conventional technologies in developing countries

Oddly, planted forests are not domesticated forests. To explain this, consider the definition of domestication put forth by Allard (1960), who states: “Domestication is the bringing of a wild species under the management of humans.” Another definition of domestication is when a plant or animal is modified for human use to the point where it relies solely on human intervention for its survival. Under either definition, even the most intensively managed forests are only semi-domesticated (Figure 1). This is seen as an opportunity by many authors, who advocate using advanced biotechnologies to accelerate domestication for the benefit of wood production (Robinson, 1999; Campbell *et al*., 2003; Boerjan, 2005; Tuskan, 2007).

In any case, forest biotechnology applications have historically been developed for the benefit of planted forests. But today forests are still planted from undomesticated reproductive material, as explained below. A few forest tree species are in the early stages of domestication (Nelson and Johnsen, 2008) but even so, they are semi-domesticated at best (Figure 1). Forest biotechnology applications are specific to each type of forest.

Figure 1. Types of planted forests sorted by degree of domestication. Planted forests compose 5% of the world’s forested areas and only a fraction of these are in the early stages of domestication (Nelson and Johnsen, 2008).



Here, we present a brief overview of some main applications of conventional technologies in the forestry sector in developing countries.

2.1 Forest tree improvement

Forest tree improvement spread as a concept in the twentieth century, well after the advent of quantitative genetics and World War II. The primary goal was to identify and select wild seed sources suitable for planted forests. Few recurrent breeding programmes developed from this. Breeding cycles were lengthy, in part because population-level improvement was essential. Forest tree improvement proceeds along a separate trajectory from agriculture.

Namkoong, Barnes and Burley (1980) wrote on their opening page: “Tree breeding is now an accepted activity in approximately half of the countries of the world…the breeding strategy has stopped at the first generation concepts of selection, progeny tests and clonal or seedling seed orchards.” Three decades later, this still holds true. The decision to settle for a known seed or propagule source can be traced to shortfalls in the long-range stability, funding and continuity of efforts required to sustain any forest planting programme: political instability, policy shifts, timber surplus, land sales, warfare, famine, drought, extreme weather events, lost manufacturing capacity and shift in global markets. Rarely has the decision to halt a tree improvement programme rested on the choice of forest tree species, but shortfalls here include forest disease and pest outbreaks, poor wood quality and even a surfeit of seed production.

Tree improvement for indigenous species gained momentum after World War II, mostly in Europe, Canada, Australia, New Zealand and the United States when reliable and well-adapted seed sources were needed for massive planting programmes. The next step, making selections in natural stands that served as seed parents, was viewed as a radical practice that contrasted with natural regeneration, dysgenic logging and the occasional haphazard seed collection. These early programmes were government-led.

A few tree-improvement programmes matured into recurrent forest tree-breeding programmes. Given large land and financial requirements, these became enduring public-private partnerships among governments, universities and timber companies. Vegetative propagation was used only in the early years, provided that the species could be propagated easily at a low cost. Whether seedlings or cuttings, the idea of a known/tested source of germplasm rapidly spread to Southern Hemisphere countries such as Brazil, Colombia, Chile, Argentina, India, China, Congo, Zimbabwe, South Africa and Malawi, where introduced and indigenous forest species alike grew much faster than in the Northern Hemisphere. Notable among these were some of the world’s most successful exotic species today: *Pinus radiata*, *Eucalyptus* spp. and *Acacia* spp.

Southern Hemisphere tree improvement was founded on naturalized introductions, imported exotic species and a few indigenous species. Its link to markets and manufacturing grew with global trade. Multinational timber corporations could grow timber more cheaply in some Southern Hemisphere countries, and this spurred closer connections between forest research initiatives in developed and developing countries. Planted forests and tree improvement programmes have reaped considerable benefits from globalization.

2.2 Recurrent tree breeding

Recurrent tree breeding here refers to the application of Mendelian genetics principles within a given silvicultural system for the purpose of improving the genetic quality of the forest. Its goal is to *improve the genetic value of the population while maintaining genetic diversity*. This advanced-generation or recurrent breeding programme refers to population-level improvement, not to the development of breeds or inbred lines. Few of the many forest tree species planted today have been subjected to even a single generation of population-level improvement. This is a subtle but important point when comparing advances in forest biotechnology with advances in crop or livestock biotechnology. Forest tree breeders weigh the importance of genetic gain against the importance of sufficient genetic diversity, the avoidance of inbreeding depression and long-term uncertainty.

As such, the breeding programme requires highly skilled experts, considerable investment funds and continuity of effort, because it continuously provides the best individuals for planting with each new breeding-testing-selecting cycle (White and Carson, 2004; Balocchi, 1998). Selections are placed in a production population which can be a small indoor or outdoor orchard. For some programmes, only a few seeds are needed for multiplying via vegetative propagation. Either seed or propagules may be sold or planted as “varietals”, even though each is highly heterozygous. To date, forest-tree breeding programmes do not develop inbred lines or hybrid crosses as applied to crop breeding.

In any breeding programme, the selection goal needs to be well defined. One important trait to consider in breeding programmes, be they conventional or biotechnology-based, is wood formation (Plomion *et al*., 2000; Plomion, Pionneau and Baillères, 2003), which drives profit margin through age of harvest and product recovery. For conifers, annual rings within a single tree generate differences in market value, and so much attention has centred on how to alter this aspect of wood formation. For example, the early corewood rings for *Pinus* spp. are less valued than the outer rings owing to their different warping and pulping qualities. Annual rings laid down at older ages compose so-called mature wood. Finding the genetic controls for wood quality at early and later stages of development is a critical step for conifer plantations, because higher quality wood in the early rings would lower the age of harvest or the "rotation age". The rotation age is the earliest age in the tree's lifespan at which harvest becomes profitable. Most forest trees can live decades or even centuries beyond the rotation age.

Even in the most intensively managed forest tree programmes, tree improvement has not followed the same path as agriculture. Genetic gain is carefully balanced against genetic diversity. Forest tree breeders, unlike their agricultural counterparts, attach great importance to maintaining genetic diversity for population-level improvement. Genetic diversity is seen as an insurance policy against catastrophic loss beyond a single generation. Forest tree breeding programmes, so integral to molecular applications of forest biotechnology, work on long timelines as a biological necessity.

This biological imperative to balance genetic gain against genetic diversity has not only given rise to forest tree programmes that do not resemble those for crops or livestock, but also to novel solutions. One common approach in tree improvement programmes is to safeguard genetic diversity (Krutovskii and Neale, 2001; Tanaka, Tsumura and Nakamura, 1999). Grafted archives are often established at multiple locations. Unlike agricultural crops, these are needed because there are no repositories to insure against the loss of indigenous forest tree species. The payoff for these backup collections often comes when these archives provide germplasm for disease resistance, catastrophic weather events or a change in market demands. Another and more cost-efficient method has been the multiple-population breeding strategy which uses divergent selection and multiple populations for a 2-for-1 programme conserving genetic diversity at the same time as making genetic gain (Eriksson, Namkoon and Roberds, 1995; Williams, Hamrick and Lewis, 1995). In this respect too, the forest biotechnology portfolio follows a separate path from crops and livestock.

3. Current status of application of forest biotechnologies in developing countries

Forest biotechnology can contribute to improving productivity and reducing vulnerability of forest ecosystems to disease, degradation and human disturbance. The challenge continues to be to ensure sufficient genetic gains while maintaining genetic diversity at the ecosystem and landscape level. To date, forest biotechnology has provided knowledge on how to mitigate the effects of forest fragmentation on genetic diversity, and on how to promote gene flow by managing tropical forest ecosystems for pollination, seed dispersal and soil symbionts.

Below, we provide an overview of application of biotechnologies in naturally regenerated tropical forests (Section 3.1) and in planted forests (Section 3.2). Some of the biotechnologies overlap, although the forest systems differ considerably.

3.1. Naturally regenerated tropical forests

Today, most molecular marker systems are DNA-based systems, such as microsatellites (Yazdani *et al*., 2003; Brondani *et al*., 1998) or amplified fragment length polymorphism, AFLP, (Cervera *et al*., 2000), although biochemical markers such as isozymes continue to provide important insights into tropical forest ecosystems (e.g. Brown and Moran, 1981; Hamrick, 2004). Molecular markers have been used for decades and are extensively reviewed in FAO (2007).

Molecular marker methods are available for a growing number of tropical hardwood species such as *Aucoumea klaineana*, *Bagassa guianensis*, *Entandrophragma cylindicum*, *Hopea odorata*, *Hymenea courbaril*, *Dryobalanops aromatica*, *Neobalanocarpus heimeii*, *Koompasia malaccense* and the endangered *Shorea lumutensi* (Born *et al*., 2006; Born *et al*., 2007; Garcia *et al*., 2004; Hamrick and Murawski, 1990; Lacerda, Kanashiro and Sebbenn, 2008; Lee *et al*., 2000; Lee *et al*., 2002; Lee *et al*., 2003; Lee and Krishnapillay, 2004; Lee *et al*., 2004a, 2004b; Lee *et al*., 2006, Lee *et al*., 2007, Lee, Tnah and Ng, 2008; Lim *et al*., 2002; Naito *et al*., 2005; Ng, Lee and Koh, 2004; Ng *et al*., 2006; Sebben *et al*., 2008; Silva *et al*., 2008). Today, findings are available to guide operational forest management plans in developing countries, but only for a very limited number of the hundreds of tree species that are managed in naturally regenerated tropical forests. This area of forest biotechnology continues to expand, moving from tools development into more hypothesis-driven knowledge acquisition (Table 3). Such research inquiry is a powerful source of pertinent knowledge for protecting tropical forests.

Table 3. Hypothesis-driven molecular marker applications for indigenous tropical forests which are naturally regenerated. The hypotheses were tested using DNA-based forest biotechnology tools in combination with other information sources such as meteorology, ecology and/or taxonomy.

Topics	**Region, biota or taxa**	**Reference**
Life history and potential for resilience to climate change	Tropical forests	Hamrick, 2004
Phenology	Neotropics	Clark, 2004
Silvicultural diversity	Tropical forests	Finkeldey and Ziehe, 2004
Selective logging	*Shorea megistophylla*	Murawski, Dayanandan and Bawa, 1994
Organellar DNA diversity	*Cedrela odorata*	Cavers, Navarro and Lowe, 2003
Forest fragmentation	Many tree species	Nason and Hamrick, 1997; Young and Clarke, 2000
Genetic bottlenecks	*Pinus maximartinezii*	Ledig *et al*., 1999
Reproductive biology	*Dunalia arborescens*	Cruz, 1981
Fitness by life cycle stage	*Platypodium elegans*	Hufford and Hamrick, 2003
Outcrossing rates	*Cordia alliodora*	Boshier *et al*., 1995
Genetics of invasiveness	*Pinus* spp.	Richardson and Petit, 2006

This research community is also moving from molecular markers into genomics. Genomics refers to sequencing DNA either from the nuclear genome or from plastid and mitochondrial organelles. Unlike other areas of forest biotechnology, genomics data are often found in the public domain, usually internet databases (see review in Dean, 2006) and this affords the opportunity for DNA-based computational biology research. This availability of DNA sequencing data brings a distinct advantage to worldwide research on tropical forests.

To date, genomics data are yielding new insights into comparative biology for tropical forests (Table 4). Perhaps the application of most immediate use is an international plant barcoding project under the Consortium for the Barcode of Life (CBOL) to identify genes that can be used to distinguish between plant species (http://barcoding.si.edu/plant_working_group.html).

Table 4. More recent applications from DNA sequencing are emerging for the study of naturally regenerated tropical forests.

Research areas	**Region, biota or taxa**	**Reference**
Phylogeny, phylogeography, nuclear DNA diversity	Agroforestry *Leucaena* spp.	Hughes, Eastwood and Bailey, 2006
Speciation and the study of mechanisms which generate biodiversity	Neotropical forest genus *Inga*	Richardson *et al.*, 2001
Rapid species identification via DNA Barcoding	Worldwide	CBOL Plant Working Group, 2009; Waugh 2007
DNA-based Phylogeny Tree of Life Project	Worldwide	Burleigh and Matthews, 2004

This emerging use of genomics has been applied to several areas of inquiry, including phylogeny, which refers to comparing two or more DNA sequences from related forest trees with their near relatives to infer past divergence and speciation events. DNA sequences can be assumed to diverge in a steady-state, linear way such that they serve as a molecular clock (Table 3). A closely related area of inquiry is phylogeography, which refers to using DNA-based sequence data to infer the history and formation of one or more taxa (Table 4).

Genomics has yet to provide its full benefit: it is a growth area for the forest biotechnology portfolio. DNA sequencing can encompass well-characterized genes, entire chromosomes or even entire genomes. Not only are related taxa being compared but interrelationships among components of entire forest ecosystems can be studied. Taxonomy, complemented by phylogeny, has now given way to phylogeography and phylogenomics, where functional genes are compared across taxa (Eisen and Fraser, 2003; Burleigh and Matthews, 2004). DNA sequence data are available for comparative analyses via Internet databases (Table 5).

Table 5. Some examples of relevant DNA sequence databases (adapted from Dean, 2006). Analyses require specialized software, also available at some of these sites.

Database	URL
NCBI Taxonomy Browser Entrez Site Map	www.ncbi.nlm.nih.gov/ www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/ www.ncbi.nlm.nih.gov/Entrez/ www.ncbi.nlm.nih.gov/Sitemap/
EMBL-EBI UniProt Site Map EMBL-Heidelberg Bioinformatics Tools	www.ebi.ac.uk/ www.ebi.ac.uk/uniprot/index.html www.ebi.ac.uk/services/index.html www.embl.de/services/bioinformatics/index.php
DENDROME Treegenes	http://dendrome.ucdavis.edu/ http://dendrome.ucdavis.edu/treegenes/

Biotechnology tools such as molecular markers and genomics can therefore provide important knowledge about naturally regenerated tropical forests and important insights into the nature of the entire tropical forest ecosystems, including the relationship between the forest trees and the microbial communities with which they interact, which can influence the strategies employed for managing tropical forests.

Mycorrhizae are symbiotic associations that form between the roots of plant species and fungi. The hyphae (thread-like structures that are part of the body of the fungi) spread through the soil, taking up nutrients such as phosphorus and absorbing water, and transporting them to the plant root. In return, the fungi receive sugars from the plant (FAO, 2008a). Trees colonized with fungal symbionts are therefore likely to be more resistant to microbial pathogens and less stressed by drought. These benefits hold particular relevance for tropical forest ecosystems, given that drought and pathogen increases are predicted under climate change.

Genomics-based research is elucidating how this symbiotic complex functions. First, not all fungal symbionts have the same mechanisms, as genomics knowledge is confirming. The two major types of associations are ectomycorrhizae (EM) and vesicular-arbuscular mycorrhizae (VAM). While both buffer the tree host against diseases and abiotic stress, EM is more desirable for slowing forest degradation (Connell and Lowman, 1989) and for hastening re-colonization of abandoned land (Viera, Holl and Peneireiro, 2009). To this end, Connell and Lowman (1989) hypothesized that EM would confer a greater advantage to their host species than VAM.

The symbiont complex is more than the forest tree’s roots and the fungal symbiont. Symbiont EM fungi also have specific bacterial associates which together form complexes with the host tree’s roots (Bonfante and Anca, 2009). Together, this fungal-bacterial complex with the tree’s roots confers benefits within the tree’s roots and the surrounding area. Genomic sequencing of some fungal symbionts has been completed (Kuhn *et al*., 2008) and this is leading the way towards an emerging field known as community genomics, which uses DNA sequencing tools to unravel these and other complex interactions within an entire forest ecosystem (Whitham *et al*., 2006). This plethora of DNA sequencing methods not only applies to a single species or its near relatives, but also can provide insights into a tropical forest ecosystem. Its potential is already emerging for testing ideas about paleoecology and community ecology.

3.2 Planted Forests

Although there is some overlap, the biotechnologies used for planted forests are generally quite different from those used for naturally regenerated forests. It is also important to emphasize that there are different kinds of planted forests. Plantations can have different types of management systems (e.g. intensive, semi-intensive) and use different types of genetic material (e.g. wild material, genetically improved trees). Depending on the level of management intensity and the genetic material used in the planted forest, different groups of biotechnologies can be used. For simplicity, three different groups of biotechnologies can be identified according to the type of planted forests, ranging from the least sophisticated to the most advanced.

a) Basic forest biotechnologies

This group of biotechnologies is suitable for the least intensively managed planted forests, and includes a range of vegetative propagation methods such as tissue culture, biofertilizers and genetic fingerprinting using molecular markers. It could also be complemented by conventional technologies, such as early-stage tree improvement programmes. For these least intensively managed planted forests, the tropical forest restoration staircase (Chazdon, 2008) is the example that illustrates this type. This starts with planting reliable and well-adapted seed or propagule sources for reforestation. Poorly adapted, dysgenic plantings cannot hope to achieve such outcomes as restoring soil fertility for crop or forestry use, payment for ecosystem services, timber production or biodiversity recovery (Quesada *et al*., 2009). Forest biotechnologies, in this first stage of planted forests, contribute to the health and quality of indigenous tropical forests and of exotic species.

Vegetative propagation of forest tree species:

This covers a wide range of techniques which are useful for the rapid multiplication of genotypes. This has been useful for species which produce few or recalcitrant seeds or seedlings and for multiplying selected genotypes in a short period of time. It is also among the most ancient of forest applications, dating back eight centuries in China (Minghe and Ritchie, 1999). In India, there are about 8.9 million hectares of teak forest, much of which is propagated by tissue culture (Tiwari, Tiwari and Siril, 2002). The National Chemical Laboratory in Pune and the Tata Research Institute in Delhi produce up to a few million teak plantlets annually. Phytosanitary measures also require tissue culture when moving germplasm from one country to another. This reduces the spread of plant viruses. Some of the disadvantages are the high costs of maintaining a tissue culture laboratory and quality control. Without quality control, one often sees the occurrence of somaclonal variations and deformed plantlets.

Micropropagation is the development of clonal lines from small tissue samples such as buds, roots or embryos extracted from seeds (Yanchuk, 2001) and some examples are provided in Table 6. The principles and achievements relating to plant tissue culture and micropropagation have been well reviewed by Haines (1994), Yanchuk (2001) and FAO (2004). Thorpe, Harry and Kumar (1991) listed over 70 angiosperm and 30 gymnosperm tree species for which successful methods for the production of plantlets have been reported. Almost two decades ago, Le Roux and van Staden (1991) listed over 25 species of *Eucalyptus* alone. This, therefore, is a maturing part of the forest biotechnology portfolio.

Table 6. A few of the many forest tree plantation species which have been multiplied through tissue culture on a commercial scale in developing countries.

Countries	**Species**
India	*Tectona grandis,* *Anogeissus latifoglia* *Bamboo spp*
Malaysia, Indonesia, Vietnam	*Acacia mangium* and *Acacia mangium* x *Acacia auriculiformis* hybrids
Vietnam, India and South America	*Eucalyptus* spp.
Chile	*Pinus radiata*
Indonesia, Brazil, Thailand and Malaysia	*Tectona grandis*

Between 2002 and 2004, FAO commissioned four studies to investigate the extent and patterns of research and application in biotechnologies in forest trees worldwide. Results from the studies indicate that Asia accounts for 38% of the activities in forest tree micropropagation, followed by 7% in South America, 3% in Africa and 2% in Oceania (FAO, 2004). As expected, micropropagation of tree species is active mostly in countries with significant tree planting programmes (Galiana *et al*., 2003; Watt *et al*. 2003; Goh and Monteuuis, 2005; Goh *et al*. 2007). While a large number of tree species (78 to 80) have been used for vegetative propagation research, little of this effort continues. Most halt in the laboratory stages (94%), so few even get as far as the field-testing stage (5%). Less than just 1% of the species developed clonally and tested have reached the commercial application stage (FAO, 2004).

Biofertilizers:

Soils are dynamic living systems that contain a variety of micro-organisms such as bacteria, fungi and algae. Maintaining a favourable population of useful microflora is important from a fertility standpoint. The most commonly exploited micro-organisms are those that help in fixing atmospheric nitrogen for plant uptake or in solubilizing/mobilizing soil nutrients such as unavailable phosphorus into plant-available forms, in addition to secreting growth-promoting substances for enhancing crop yield. As a group, such microbes are called biofertilizers or microbial inoculants. The use of biofertilizers has yielded positive results for indigenous forest species in the eastern Madagascar littoral forests as well as for exotic forest species including eucalypts, acacia and cypress (Kisa *et al*., 2007; Duponnois *et al*., 2007; Duponnois *et al*., 2008; Ouahmane *et al*., 2007; Remigi *et al*., 2008). Other symbionts that are being considered include nitrogen-fixing bacteria such as *Rhizobium*, and *Azolla*, blue-green algae and mycorrhizal fungi (Caesar, 2009). In addition to the least intensively managed planted forests in developing countries, biofertilizers have also proved useful in forests under more intensive management.

Genetic fingerprinting with molecular markers:

All types of molecular and biochemical markers have been used for decades in these early-stage tree improvement programmes. A few examples are as follows:

- Measuring genetic diversity of breeding population accessions between indigenous provenances and naturalized landrace origins;
- Testing paternity contributions to offspring grown in field tests;
- Verifying genetic identity during vegetative propagation.

b) Intermediate forest biotechnologies

The second group of biotechnologies can be used for planted forests that provide industrial raw materials on a large planting scale. The single species used for plantations might be indigenous or they might be exotic, but the plantations are intensively managed.

Somatic embryogenesis:

Somatic embryogenesis (SE) is a tissue-culture technique that can also be used for the micropropagation of forest trees, where a small group of vegetative cells, which are stem-cell like, are induced on culture media to undergo tissue differentiation to form a somatic embryo. The somatic embryo goes through a maturing process before being “germinated” for planting (Tartorius, Fowke and Dunstan, 1991).

Regeneration through SE has been reported for over 50 woody species, encompassing over 20 angiosperm families, and at least a dozen conifer species (Park, Barret and Bonga, 1998; Wann 1988; Tartorius, Fowke and Dunstan, 1991; Attree and Fowke, 1991; Watt *et al*., 1991). Potential multiplication rates, particularly from cell suspension cultures, are very high. Additional advantages include the amenability of the process to handling in automated bioreactors and the possibility for mechanized delivery of the emblings (plants propagated from SE) through synthetic seed technology. SE is also ideally suited for efficient genetic transformation procedures because of the single-cell origin of embryos.

The advantages of SE in comparison to micropropagation by *in vitro* cuttings, especially with regards to multiplication rate and genetic modification, explain why large research investments have been made towards developing this technique. Although successes have been reported using this technique in some of the commercial species, there are still major obstacles to the large-scale operational application of the technique to forest trees. Most of the reports (Table 7) demonstrate that the results obtained are still in the experimental stages and are yet to reach the commercialization phase.

Table 7. Species in which SE is at the experimental stage.

Country	**Species reported**	**References cited**
Chile	*P. radiata, P.taeda*	Park, 2002; Jones, 2002;Lelu-Walter and Harvengt, 2004
Brazil and India	*Eucalyptus globulus; E. grandis*; and *E. dunnii*	Pinto *et al.* 2002; Watt *et al.*, 1999
India	*Tectona grandis* *Bamboo* Sandal	Krishnadas and Muralidharan, 2008; Godbole *et al.*, 2004; Shali and Muralidharan, 2008; Rathore *et al.*, 2008
Bangladesh	*Gmelina arborea, Artocarpus chaplasha, A. heterophyllus, Azadirachta indica* and *Elaeocarpus robustus*	Sarker, Islam Rafiqul and Hoque, 1997; Roy, Islam and Hadiuzzaman, 1998.

Moreover, SE is a costly high-precision operation, usually funded through multinational timber companies which own or lease land. Only the most elite selections are propagated using SE, where the genetic gains from recurrent breeding are maximized through the high-volume propagation of a single genotype.

Molecular markers, maps and QTL analyses:

As part of this high-precision operation, molecular markers also take on new functions. Breeding and selection in the recurrent breeding programme can be optimized by localizing chromosomal regions which influence the trait of interest.

No longer used only for genetic fingerprinting, markers are now used to find associations between traits and chromosomal regions. Forest trees, as perennial plants, have an added temporal dimension which can be challenging (Gwaze *et al*., 2003) even for a single pedigree. If a marker interval is found to change the trait value then it becomes known as quantitative trait locus (QTL) or a QTL haplotype, delineated by the relative position of two molecular markers. A QTL haplotype is not a gene but a single chromosomal segment inherited from either the maternal or paternal parent. It can include one or many genes exerting some degree of influence over a phenotypic trait.

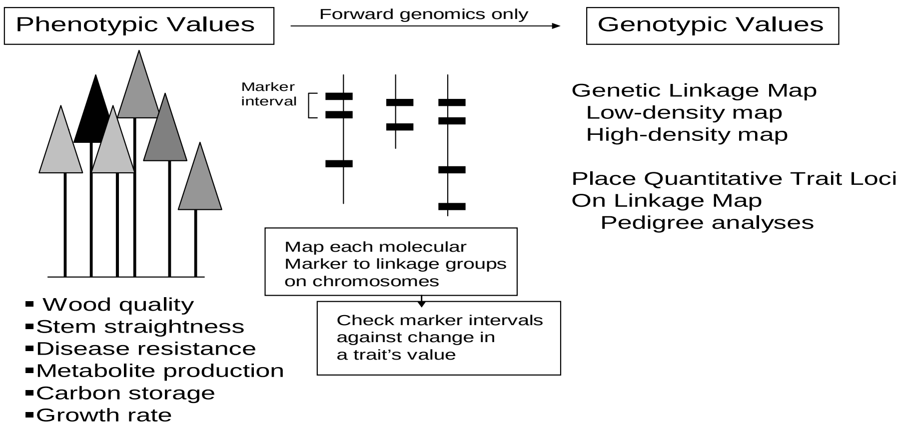
Finding QTL for forest trees is more costly and more computationally demanding than for most crop and livestock species, because forest tree pedigrees are outcrossing and highly heterozygous (Devey *et al*., 2003; Williams and Reyes-Valdes, 2007; Williams, Reyes-Valdés and Huber, 2007). Large pedigrees are rare with few generations and the populations have no breed structure or strong degrees of differentiation. However, numerous reports have cited the identification of QTL for major traits ranging from growth to wood quality and disease resistance in both Northern and Southern Hemisphere countries for a cadre of forest tree species. Moving from the genetic map to the physical map is more feasible with small hardwood genomes such as poplar and eucalypts. For species with large genomes, molecular cytogenetics technology, or placement of dye-tagged DNA segments from known genes on a fixed chromosome squash, is proving to be a useful bridge technology (Doudrick *et al*., 1995).

Translating QTL identification into marker-assisted selection (MAS) moves into the realm of commercial applications. Choosing MAS requires a cost-benefit analysis, as described for *Pinus radiata* (Wilcox *et al*., 2001) and this is used for poplars, *Eucalyptus* spp., *Pinus radiata* and a few cases of temperate-zone *Pinus* spp. especially those planted as an exotic. MAS has led to some novel breeding strategies when applied to forest trees (e.g. El-Kassaby and Lstiburek, 2009).

The selection of QTL haplotypes is not straightforward, because a given pedigree can be segregating for more than one or even two QTL haplotypes of interest, which can result in ambiguous genetic models for QTL inheritance. But MAS is operational, lending yet another level of forest biotechnology precision to plantation forestry. Figure 2 shows how large numbers of molecular markers are assayed on gels for segregation patterns, then placed on a genetic map. Each individual now has a known genetic fingerprint, a collection of marker intervals or haplotypes – and some trait measurements. From these elements, QTL haplotypes can be identified.

But as mentioned earlier, finding QTL in single pedigrees is an arduous process for forest trees. Other methods for identifying QTL have since been developed or borrowed from other biological systems. These include association genomics, which was developed for humans and other mammals (see review by Darvasi and Shifman, 2005) but these methods are well-suited for forest trees (Brown *et al*., 2004). Association genomics is a population-level QTL detection methods that is only effective if enough gametic disequilibrium is present in the population. This has indeed been the case for several intensively managed forest trees in both Northern and Southern Hemisphere regions.

Figure 2. Forward genomic methods.



Trait measurements constitute the phenotypic value of an individual tree. Trait-based genomics approaches such as QTL mapping are known as forward genomics. The reverse genomics approach (Figure 3) is gene-based. More intensive methods use both forward and reverse approaches.

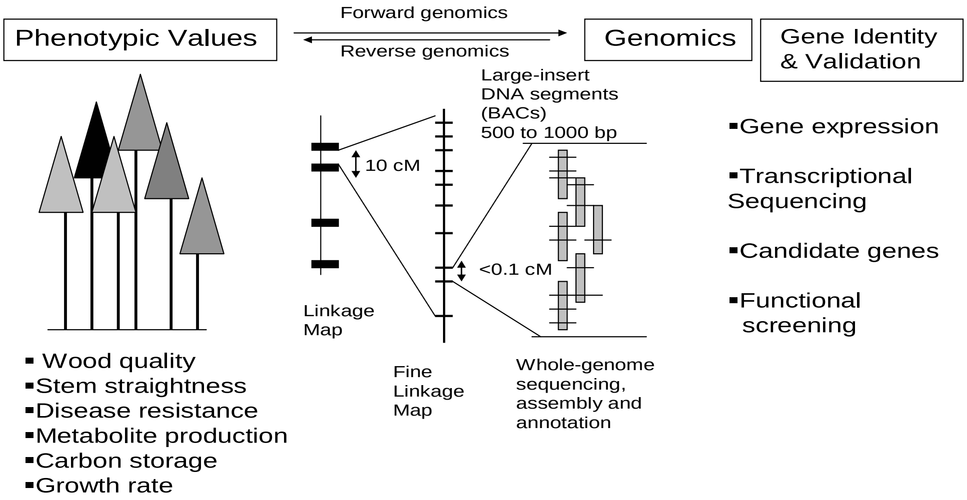
The trait measurements for each individual can be compared with its marker haplotypes (Figure 2). This is known as co-segregation between linked molecular markers and a putative QTL haplotype.

***Whole-genome sequencing projects for forest tree species*.**

To reap the full benefits of genomics, one must use both forward and reverse genomics methods (Figure 3). Forward genomics moves from the traits to the genotype. But gene-based or reverse genomics is also needed to identify and characterize the underlying genes. For hardwood species such as *Eucalyptus* spp. or *Populus* spp. adding the reverse genomics is rapid and feasible because genome sizes are in the same range as those for rice, tomato and Arabidopsis (Wakamiya *et al*., 1993). Conifer genomes, by contrast, are larger than any commodity species in agriculture. The poplar genome was the first forest tree species to be sequenced in its entirety (Tuskan, 2007). The *Eucalyptus* genome initiative for whole-genome sequencing is an even larger effort that is being coordinated between 130 scientists in 18 countries including Brazil and South Africa. Sequencing the pine genome has less momentum given its enormous size, almost seven times larger than the human genome. A number of groups are completing part of the pine genome at present. A total of 100 large chromosomal segments (also known as bacterial artificial chromosomes or BACs) from *Pinus taeda* are presently being sequenced at the Joint Genome Institute in the United States.

The impetus for sequencing these forest tree genomes tends to comes from large-scale wood production in intensively managed plantations worldwide, but forest health has also provided an equally compelling case. One of the side-benefits of a large-scale DNA sequencing project is a rich store of new molecular markers such as single nucleotide polymorphisms (SNPs).

Figure 3. Forward and reverse genomic methods.



Trait measurements constitute the phenotypic value of an individual tree. Reverse genomics is gene-based while forward genomics is trait-based. Reverse genomics identifies, tests and validates specific genes controlling the trait of interest. Together, they provide an integrated picture of which genes or chromosomal segments are influencing the trait of interest and the degree of independence among these genes. The most sophisticated forest biotechnology portfolios at this time use both forward and reverse approaches but these are limited to *Eucalyptus* spp, *Populus* spp. and *Pinus* spp.

Functional genomics:

In recent years, sequencing entire genomes has shifted emphasis from analyzing sequence data to the elucidation of gene function, also referred to as functional genomics. Gene function is inferred by using sequence alignment-based comparisons, identifying homologues between and within organisms, transcript profiling to determine gene expression patterns for small numbers of transcripts and yeast two-hybrid interaction analysis for identifying metabolic pathways, gene networks and protein complexes.

It is often conducted using microarrays, which refers to the parallel assessment of gene expression for tens of thousands of genes. It works on the principle of competitive hybridization between complementary DNA (cDNA) strands. This approach can identify candidate genes for quantitative traits in forestry, a form of reverse genomics. As an example, cDNA microarray technology generated a transcript-level profile of wood forming tissues (differentiating xylem) for a pedigree composed of individuals from a *Eucalyptus grandis* x *E. globulus* F1 hybrid x *E. grandis* backcross population (Kirst *et al.*2004). Microarrays are information-rich sources of information about genes controlling the trait of interest.

Proteomics:

Just as a genome describes the genetic content of an organism, a proteome defines the protein complement of the genome. Proteomics includes the identification of proteins in cells or tissues, the characterization of their physio-chemical properties such as post-translational modifications, function and expression level. Proteomics is a powerful tool for studying proteins and their modifications under different developmental stages and/or in response to various environmental stimuli.

In the cell, proteins form transitory or stable complexes as part of pathways, and act within protein networks. These protein-protein interactions can be used to unravel the various interactions. After processing and modifications, a single gene may express between one and a few dozen different protein products. A combination of methods is required to characterize expressed proteins (or proteome) fully.

A standard procedure is two-dimensional gel electrophoresis (2-DE) as the separation method, followed by mass spectrometry (MS) analysis of the separated and enzymatically digested proteins. The peptide mass fingerprints typically obtained by MS are matched against sequence databases using dedicated bioinformatics tools. The whole procedure can be automated and robotized for high-throughput purposes. The aim of this technique is to evaluate the modifications of protein expression with respect to genetic, environmental and developmental factors. The question is which quantitative variation of proteins is responsible for which quantitative phenotypic variation. The application of two-dimensional gel electrophoresis coupled with mass-spectrometry (2DE-MS) in forest tree genomics to map the expressed genome has been well reviewed by Plomion, Pionneau and Baillères (2003).

c) Advanced forest biotechnologies

This uses the most sophisticated forest biotechnology portfolio yet. It includes recurrent tree breeding, backward and reverse genomics approaches, whole-genome sequencing, low-cost vegetative propagation and the genetic modification of forest trees. The latter will be the focus of this section.

To date, the only report of commercial plantings with genetically modified (GM) trees is for poplar, in China (Xhiao-hu *et al*., 2003; FAO, 2004). However, most tree species used in planted forests have been successfully transformed at the experimental level, and results have demonstrated the correct expression of new genes in these plants (Fillatti *et al*., 1987; Walter *et al*., 1998 and 1999; Bishop-Hurley *et al*., 2002).

Benefits from genetic modification can arise in particular from the transfer of traits from species as wide apart as bacteria or other plants that are not readily available either in the breeding population or in the forest tree species as a whole. Traits that have been the subject of extensive research for genetic modification include stem shape (taper and “roundness”), herbicide resistance, flowering characteristics, lignin content, insect and fungal resistance (Li *et al*., 2003; Grace *et al*., 2005; Punja, 2001, Shin *et al*., 1994, Tang and Tian, 2003).

The potential environmental benefits of such technology (Gianessi *et al*., 2002) include new means to combat pathogen and pest outbreaks. Some intensively managed tree improvement programmes have been investigating the use of gene transfer methods for many years. An example is the introduction of the *Cry1Ac* gene from the bacteria *Bacillus thuriengensis* (Bt) into radiata pine, where the ultimate goal is to enhance resistance to the pine shoot tip moth (Grace *et al*., 2005). Less attention has been given to other applications of GM forest trees such as environmental remediation, land reclamation and mercury sequestration. Forest trees (conifers and hardwoods) are useful for land reclamation purposes, even without genetic modification, so the potential value is considerable.

As mentioned earlier, a small area of GM poplar is also planted on a commercial scale in China. China has a highly productive forestry plantation programme with six national forest planting programmes. To date, at least seven million hectares have been planted with fast growing poplars (FAO, 2008b).

The first successful transformation was done on *Populus nigra* with the *Bt* gene *Cry1Ac* in 1993. This tree was used in field testing as early as 1994 and was subsequently deployed in further pilot plantings. In 2000, the Chinese regulatory authority permitted the establishment of about one million trees on 300 hectares (Hu *et al*., 2001). This was followed by a smaller release with a hybrid poplar clone transformed with both *Cry1Ac* and *PI* genes (Xiao-hua *et al*., 2003). The toxicity of this transformed clone was greatly enhanced as the GM plants contained two insect resistance genes. Subsequently, the transformation of poplars for disease resistance and tolerance to environmental stresses has been achieved, though these are still at the laboratory stage (Xiao-hua *et al*., 2003).

Genetic modification is part of the reverse genomics approach that is used to evaluate gene function but its commercialization is shifting investment from the public domain into proprietary areas. As noted by FAO (2004), a notable trend is that the “numbers of publicly funded projects appear to be waning, while privately funded projects appear to be increasing, judging by field trials established in recent years”. This is a capital-intensive effort requiring long-term continuity of funding, scientists and infrastructure. Developing a GM genotype on a commercial scale first requires a well-established gene transfer technology (Walter *et al*., 1998). Each GM genotype must then be vegetatively propagated on a large scale before shareholders can expect a return on the steep initial investment.

The issue of GMOs has received considerable attention over the last decade in scientific and non-scientific circles and from policy-makers worldwide. The focus of attention has been on the crop sector, which is where most GMOs have been commercialized. In 2008, an estimated 125 million hectares were cultivated with GM crops compared to just 400 hectares of Bt poplars in China, with 20 000 seedlings prepared for planting in 2009 (James, 2008). If or when further GM forest trees are released commercially this situation may change. A regulatory framework to govern research and the applications of GM forest trees is essential. The issue goes beyond the country level, because pollen flow and seed dispersal do not respect national boundaries. National and international regulatory systems should contain provisions for preliminary risk assessments, monitoring and control and for liability and redress.

Many countries currently have regulations for agricultural crops, including fruit-trees, although many developing countries lack such frameworks and the capacity to implement them. There are, however, no regulations specific to the use of GM in forestry. Although policies and regulations adopted for agricultural crops are also likely to be used for forest trees, forest trees present special challenges (they have long time frames and life spans, they form a wild resource and are major constituents of an ecosystem). Forests are not only trees, and forest ecosystems are more fragile, longer-lived and less closely controlled than crop fields. Decision-making is complicated by the fact that while agriculture is primarily viewed as a production system, forests are generally viewed as a natural system, important not only for the conservation of biodiversity but also for social and cultural values. Thus the use of GM forest trees is viewed more as a political and environmental than as a technical or trade issue (El-Lakany, 2004).

3.3. Summary of applicability and anticipated contribution

Based on the current analysis, Table 8 attempts to summarize and compare the current status, key issues and future perspectives for a number of conventional approaches and biotechnologies in developing countries. The different technologies differ in respect of public acceptance, the technical capacity and infrastructure/materials required for their use and costs. For the near future, it is predicted that the potential impact is high for tree improvement, genomics, DNA bar coding and fertilizers. To complement this, Table 9 summarizes the anticipated contribution of forest biotechnology applications to natural and planted forests for developing and developed countries, based on the worldwide survey commissioned by FAO (2004).

Table 8. Current status of some conventional approaches and biotechnologies and factors influencing their applicability in the forestry sector in developing countries.

Emerging forest biotechnology applications	Extent of use	Public and government acceptance	Current technical capability for using technology	Current technical capability for adapting/developing new technology	Infrastructure and/or materials and tools available for using	Relative cost	Skills required for application	Potential for generating impact (time frame < 10 years)
Tree improvement	High	High	High	Low	Medium	Medium	Medium	High
Recurrent tree breeding	Low	High	Low	High	Medium	High	High	Medium
Molecular markers	Medium	High	Medium	Medium	Low	Medium	Medium	Medium
Genomics	Low	High	Low	Low	Low	Medium	High	High
Bioinformatics	Medium	High	Medium	Medium	Medium	Medium	High	Low
Genetic modification	Low	Low to Medium	Low	Low	Low	High	High	Low
Biofertilizers	High	High	High	High	Low	Low	Low	High
Comparative phylogeny	Low	High	Medium	Medium	Low	Medium	High	Low
DNA Bar coding	Low	High	Medium	Medium	Medium	Medium	Low	High

Table 9. Anticipated contribution of forest biotechnology applications to natural and planted forests worldwide (adapted from Table 2.4.2 in FAO, 2004).

Applicable forestry component	Spatial scale	Development elements relevant to biotechnology	Broad technologies						
			Molecular applications						Regeneration
			Bioinformatics	Diversity measurement	Gene discovery	Genetic modification	Biosensors	Product verification	
Natural populations	Tree–population	Genetic resources characterization	X	X	X				
	Population	Mating system/gene flow		X					
	Population–landscape	Conserving diversity		X					
	Population–landscape	Silvicultural impact assessment		X					
Breeding populations	Tree	Selection	X	X	X				
	Tree–population	Mating designs		X					X
	Tree	Testing	X	X	X			X	X
	Population	Diversity management		X	X				
Production populations	Population	Mating system	X	X					X
	Population	Gene flow	X	X				X	X
	Population–landscape	Silvicultural impact		X			X		X
Regeneration	Stand	Natural		X	X			X	X
	Stand	Planted		X	X	X		X	X
Domestication	Population	Native species diversity	X	X	X		X	X	
	Population	Exotic species suitability	X	X	X		X	X	
Gene conservation	Population	Diversity assessment	X	X					
	Population	Gene flow/contamination		X			X		X
	Tree	Reproduction		X	X		X		X
Forest health	Tree–stand	Risk/hazard assessment	X	X			X		
	Tree	Resistance screening	X	X	X	X	X		X
Processing/ Value added	Logs	Pulp processing	X		X	X	X	X	
	Logs	Wood treatment	X		X	X	X	X	

4. An analysis of successes and /or failures of forest biotechnologies in developing countries

To date, the use of biotechnologies has been beneficial only at very advanced stages of selection and improvement programmes. Unlike with crops, where the number of species to choose from is relatively limited, an immense diversity at both the interspecific and intraspecific level is used in forestry. Thanks to this important diversity, the early stages of classical selection (exploration, collection, testing of genetic resources) provide important gains. By way of example at the species level, *Acacia crassipcarpa,* which is currently the main plantation species in swamp areas, was "unknown" as a plantation species only 20 years ago. At intraspecific level, coordinated, multilocational provenance trials have shown sometimes 200% variation in adaptive traits among populations across the natural range of distribution of the same species. Individual variation within populations is also very important, and selection at this level also yields important initial gains, in particular through clonal development (the traditional rooting of cuttings).

For most species and forest tree management systems, advances registered in developing countries until now have been made without any incorporation of biotechnologies. There are very good examples of advanced tree breeding programmes using biotechnology tools in developing countries, too, but they refer to a small part only of the forest area (although their share of timber production is relatively high).

One main reason for failure is an inadequate assessment of the real costs and benefits of using biotechnology tools in given conditions (the level of improvement and the intensity of management), often under pressure from offering sides. As a result, expectations are not met and unjustified costs are high. This is a common risk in the early stages of development of new technologies. The same problem during the development of clonal forestry occurred a few decades ago. The development of protocols for the mass vegetative propagation of eucalyptus (rooting cuttings) was a real breakthrough in the 1970s, making it possible to take advantage of outstanding individuals from highly heterogenous interspecific hybrid progenies (the genetic gain could not be captured otherwise). The first large scale plantations and gains in Congo and Brazil were very impressive. But a perverse side-effect was that insufficiently informed programmes (or projects that were under pressure from active clonal forestry promoters) overestimated the benefit from vegetative propagation and neglected all the necessary but time-consuming and demanding basic work (systematic species and provenance exploration and testing, individual selection and breeding, etc). This resulted in disappointments and misconceived strategies in some cases.

Much still needs to be done along the lines of upgrading the skills of researchers by ensuring that they receive higher education or appropriate higher level training to be able to plan, develop and execute proper tree improvement programmes. Sufficient financial resources also need to be committed at the national level to ensure that such programmes are carried out successfully with the final aim of producing improved and bred reproductive material.

In developed countries, the applications of advanced forest biotechnologies have developed faster than predicted by Robinson (1999) a decade ago. A shift in technology transfer models has contributed to this success. In particular, this has come from the engagement and contributions of many Southern Hemisphere governments and universities. These institutions have contributed funding, talent and impetus to virtual forest research consortia in areas such as whole-genome sequencing and other genomic applications. This is a contrast to older technology transfer models characteristic of early tree improvement programmes. Unlike tree improvements, the consortia are less formally structured within a government. These grassroots scientific exchanges often include one or more government partners, and they are hastening forest biotechnology in interesting ways that bear little resemblance to traditional models of technology transfer. The advances being made in developed countries are also relevant to the progress in the application of forest biotechnologies in developing countries.

An analysis of forest biotechnology successes and/or failures leads to the following observations:

Observation 1. Forest biotechnology applications are developing along a separate path from crop biotechnology. Policy-makers tend to put forest biotechnology and crop biotechnology on the same plane, but the benefits, goals, risks and deliverables are distinctly different. This points to an important knowledge deficit about forestry biotechnology that needs to be addressed.

Observation 2. Forest biotechnology is now expanding to a wide range of forest types. The forest biotechnology portfolio *sensu lato* appears to be growing beyond its utility to forest plantations. Tropical forest complexity, health and recovery are also benefiting from forest biotechnologies in the form of genomics and its panoply of related methods. This has new relevance for tropical forests given the major current focus on slowing climate change.

That forest biotechnology applications have rapidly expanded in the past 5–10 years is also apparent from FAO (2004) which indicates that 64 percent of research and application activities in forest biotechnologies worldwide were focused on only six genera (*Pinus*, *Populus*, *Eucalyptus*, *Picea*, *Quercus* and *Acaci*a). As discussed earlier, this is no longer the case. In this respect, genomics can be seen as a technology spillover, no longer restricted to planted forests, but being used also for the management of naturally regenerated tropical forests.

Forest biotechnology has advanced over the past decade during favorable economic conditions. This is expected to change. The next decade may see slower progress because the forestry industry itself has some inherent problems, which may be accentuated by the global financial crisis. Like other research and development (R&D) areas, this downturn could reduce forest biotechnology investment at a critical time, and shape how developing countries choose to invest in forest biotechnology. These problems are unique to the forestry context, as summarized by Robinson (1999).

Observation 3. Plantation forestry has less flexibility in tailoring its raw materials delivery due to long lead times. This means that tailoring raw materials for markets that are years or even decades into the future is a high-risk proposition. Historically, this is a point which has not been well understood by biotechnology leaders in the agricultural biotechnology or pharmaceutical industries (Robinson, 1999).

This suggests that it might be timely to re-examine the role of biotechnology within the wood manufacturing processes rather than modifying the raw material supply years in advance of market demand. This emerging field of science is known as molecular wood biotechnology. Biotechnology benefits to date have included energy savings, waste reduction, remediation of toxic chemicals (see reviews by Breen and Singleton, 1999; Mansfield and Esteghlalian, 2003; Ahuja, Gisela and Moreira, 2004). Perhaps the best-known example is the use of microbial (fungal) enzymes that degrade lignin, a component of the plant cell wall. The use of microbial enzymes is a time-honoured method that has been applied in pulping processing since 1975. Economic feasibility studies have shown that recent microbial biotechnology applications can raise mill productivity by 30% (Mansfield and Esteghlalian, 2003). The genetic improvement of fungi, bacteria and other microbes is a faster way of improving pulping processes efficacy and degraded mill waste than attempting to modify the raw materials of forest trees.

Observation 4. The private forestry sector is cautious about investing in forests and forest biotechnologies on lands which are not wholly owned. This holds true both for forest biotechnology and for intensive plantation management. As noted above, most forests are not privately owned. A related issue is that for-profit licensing for genetically enhanced forest trees tends to have been a tricky business model in the past due to long timelines, low investment rates and public ownership of forests as a worldwide norm (Robinson, 1999). Thus forestry and its research, including tree breeding, are now more vulnerable to funding reductions and loss of continuity than before the financial crisis.

In most developing countries, the industrial sector is dominated by foreign firms that do not often solicit or require research input from local research establishments, as they rely on research conducted in their countries of origin. The situation, however, is different for the agriculture, forestry and horticultural sectors, where research is heavily supported by public research institutions. This scenario does not bode well for the development of biotechnology because commercial biotechnology has its roots in academia. Researchers in universities and research institutes carry out nearly all basic research from which biotechnologies and biotechnology processes are developed. Thus, private venture-capital companies, which could help supply equity capital in support for development of local biotechnology is lacking. Besides, local firms are very unlikely to invest their already limited financial resources in long-gestation projects when interest rates are often higher if the funds are kept in banks.

However, there are interesting experiences of genetic improvement cooperatives that have pooled the resources of various private companies and universities to establish a single genetic improvement programme to benefit all participants. This model was applied successfully in Chile, where the state forest service, the main forest companies and a university created a cooperative. Most of the advances in biotechnologies both in Pines and Eucalypts were made by this consortium.

Observation 5. Costs and consequences of expanding the range of forest biotechnologies. To these stresses and strains, one must add the cost of expanding the forest biotechnology portfolio itself. Such an expansion itself can generate financial strain, too. Burdon (1992) considered how molecular-based forest biotechnology would fit with classic breeding programmes. He foresaw severe institutional strain "…without skilful and sensitive management various competitive forces can subvert the safe and successful application [of molecular-based methods]." He was also concerned that this internal tension could imperil the collaboration within and between organizations which had already brought so much success to tree breeding. That "proprietary technology is very tempting…" added yet another source of strain on forest tree breeding. Managing forest biotechnology well requires strategic oversight.

Observation 6. Roller-coaster R&D funding. Another point is that forestry – and its R&D budget – has always had a "roller-coaster ride", as Robinson (1999) mentions. Managing against the vagaries of a roller-coaster budget makes the success of forest biotechnology to date even more impressive. This is true for government-sponsored research too. Unstable raw material costs, fixed labor and overhead costs coupled with small, uneven profit margins all mean that the scale of forestry operation tends to become ever larger to clear net profits. The roller-coaster ride is an external force which threatens the stability and continuity required for long-term research on long-lived forest species.

Observation 7. Infrastructure and capacity constraints. In most developing countries, the use of biotechnology has been mostly limited to using tissue culture technology for the multiplication of selected clones. While some form of a master plan for the development of biotechnology is present, there have been no real efforts to popularize this technology in the countries, mainly because of socio-economic factors. The introduction of biotechnology to developing countries has been by means of multilateral or bilateral collaboration. Experts from the collaborating (often developed) countries have visited and worked in the developing countries as short- or long-term experts and counterparts. They have helped establish laboratories and equip them with the relevant facilities to carry out research. While work continues during the period of collaboration, it slows down considerably once the collaboration phase is over. There are several reasons for this:

- The local counterparts are not adequately trained to continue the work independently once the collaboration ends.
- Once the collaboration period is over and the experts have returned home, the work in the laboratories slows down considerably as a result of financial constraints or the lack of technical knowledge.
- When equipment breaks down, it takes a long time to be repaired or purchased due to lack of funds.
- The purchase of chemicals needed for the work can be delayed as a result of a shortage of funds or the need to wait for them to be imported into the country.

In spite of these shortcomings, countries such as Vietnam have successfully developed elite clonal hybrids of *Acacia mangium* x *Acacia auriculiformis* for planting programmes. There are currently 127 000 hectares under clonal acacia hybrids cultivation (van Bueren, 2005) and the planted area continues to increase each year. With the recent rapid growth of the economy in the country, the situation looks poised to change as the government commits more funds towards education, training, research, skilled manpower development and infrastructure development. The biotechnology agenda is also being given priority in the National Development Plan in the respective countries.

5. Case studies of applications of forest biotechnologies in developing countries

5.1 *Eucalyptus* plantations in Brazil

More than 700 species of eucalyptus are found in Australia but their performance as a plantation species is far greater elsewhere (Borralho, 2001) and especially in Brazil. The Aracruz Cellulose's plantations in Brazil are perhaps the most widely-cited success story. The company won the coveted Wallenberg Prize for its intensification efforts. This operation comprises a total of 300 000 hectares of eucalypts of which half are produced by low-cost vegetative propagation (Aracruz Cellulose, 2008).

In support of this intensive plantation effort, Brazil has a large-scale eucalyptus genomic research initiative that is known as the Genolyptus Project or the Brazilian Network of *Eucalyptus* Genome Research (Grattapaglia, 2004). The Genolyptus Project builds on the international whole-genome sequencing effort. It aims for a genome-wide understanding of the molecular basis for wood formation in *Eucalyptus* and is coupled with ongoing tree breeding programmes. The project and its talented scientist team is generating a suite of biological and computational resources to discover, sequence, map, validate and understand the underlying variation of genes and genomic regions of economic importance in *Eucalyptus,* with a focus on wood formation and disease resistance (Grattapaglia, 2008). The project is based on a partnership among agencies within the Brazilian federal government through the MCT–Fundo Verde Amarelo. The academic/research sector is represented by seven universities and the Brazilian Agricultural Research Corporation (EMBRAPA). Industry is represented by twelve forestry companies. The Genolyptus Project could be considered a good example of how genomics can be successfully integrated with traditional breeding programmes to return value in a reasonably short time. This project also represents a good model of how universities, government agencies and private enterprises can work together to benefit different categories of stakeholders.

5.2 Clonal propagation of teak in Malaysia

Teak, *Tectona grandis*, is widely planted in many countries in Asia, South and Central America and Africa. A fifteen-year collaboration between the Sabah Foundation Group (Malaysia) and the Centre de Coopération Internationale en Recherche Agronomique pour le Developpement (CIRAD, France), exploiting molecular markers and micropropagation, has led to the availability of superior quality planting material, both for the local market as well as for export (Goh *et al*. 2007; Muralidharan, 2009a). The candidate trees for producing the superior clones were selected with reference to intrinsic wood qualities (e.g. natural durability, shrinkage, sapwood percentage, etc.). Simple sequence repeat (SSR) markers were developed to determine the genetic background and diversity in order to reduce inbreeding and ensure the genetic fidelity of the clones mass produced by tissue culture. There is now widespread demand for these clones globally. In addition, a clone identification form provides detailed information on each clone, including the DNA fingerprinting profile.

5.3 Micropropagation applied to tree breeding of fast-growing forest tree species in Latin America

Biotechnology was introduced in Latin America in the 1980s. Networking has been very important for the research community there. By December 2008, there were 5 467 researchers in 738 agricultural biotechnology laboratories in 32 countries in the Technical Co-operation Network on Plant Biotechnology in Latin America and the Caribbean (REDBIO), based at the FAO Regional Office in Chile. The network has been in operation since 1991 to develop biotechnology for the sustainable use of regional genetic resources, promote the safe and responsible application of the technologies – especially in fragile environments, and enhance the regional development of new strategic technologies, such as molecular genomics. It also encourages the application, whenever feasible, of advanced biotechnology tools in integrated crop management and sustainable production systems.

In terms of planted forests, the largest areas are in Brazil (7 million hectares, 4.1 million of which are industrial man-made forests). Chile has 2.25 million hectares of planted forest areas, practically all for industrial purposes; Argentina has 0.7 million hectares; Venezuela, 0.5 million hectares; Cuba, 0.4 million hectares; Peru, 0.3 million hectares; Colombia and Mexico, about 0.2 million hectares each; and Uruguay about 0.75 million hectares. In the other countries of Latin America and the Caribbean the reforested area is less than 100 000 hectares per country. The estimates of the current yearly forestation rate vary from 386 000 to 520 000 hectares (FAO, 2006). Practically all the planted forests have been established on abandoned agricultural lands where erosion is prevalent. The overwhelming majority has been established with fast-growing exotic species in the *Eucalyptus* and *Pinus* genera. Many of these planted forests have been established by clonally propagated elite plants in the case of *Eucalyptus* or through somatic embryogenesis in the case of the *Pinus* species.

5.4 Bioprotection in Kerala, India

At the Forest Protection Division of the Kerala Forest Research Institute, India, investigations into control of a serious insect pest of teak viz. the teak defoliator (*Hyblaea purea*), have been carried out for several years. A Hyblaea purea Nuclear Polyhedrosis Virus (HpNPV) isolated from the natural populations of the insect larvae resulted eventually in a very effective biological control method. A permanent preservation plot where the pest outbreak was kept under control over several years with regular spraying of the HpNPV formulation clearly demonstrated the benefits in terms of increased volume of timber compared with the control plots. Research then went into the rearing of the insect larvae in the laboratory on an artificial diet and the mass multiplication of the virus, followed by the formulation of the pesticide incorporating UV protectants and other adjuvants, and finally the spraying technique in the planted forests. Almost two decades of research finally culminated in a successful solution to a serious problem. Nevertheless, the technology has remained in the laboratory and there is no indication that it will make it to the standard package of practices of the teak plantings immediately. Since most teak in India today comes under control of the State Forest Departments, acceptability by the forestry professionals is important. Some farmers had shown interest and willingness to use the product in their plantations, and the initial response showed that the technology was effective. The case study demonstrates that research in forest biotechnology has a much better chance of producing results when conceived, developed and implemented in a broader framework that involves not only scientists and technologists but also, at every stage, the forestry professionals who work at the field level and, at some level, policy-makers who eventually have to give their approval (Muralidharan, 2009b).

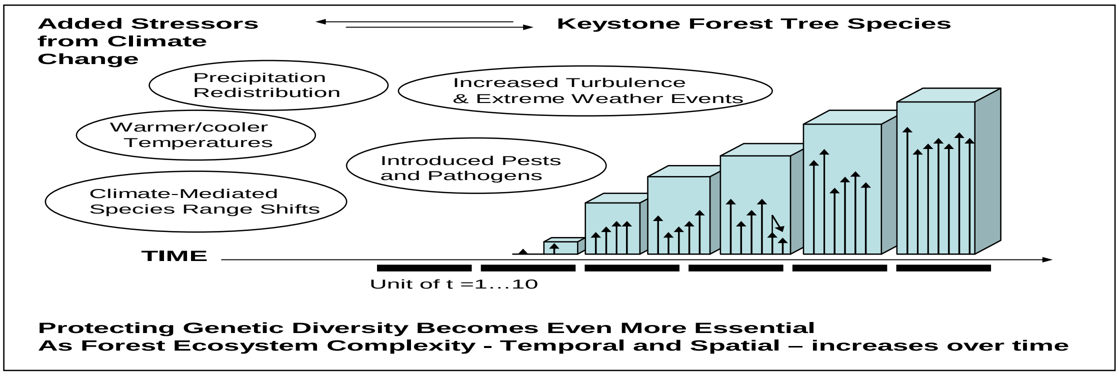
B. Looking Forward: Preparing for the Future

6. Key issues where forest biotechnologies could be useful

6.1. Adaptation to climate change

Forests, particularly tropical forests, play a central role in climate change and this is expected to shape the direction of forest biotechnology research in new ways. At the heart of the matter is how to ease forest adaptation. Forest adaptation is the foundation for all other forestry policy solutions aimed at slowing climate change (Hamrick, 2004; Millar, Stephenson and Stephens, 2007; Aitken *et al*., 2008). In addition, all forestry policy solutions, i.e. reduced emissions for deforestation and degradation (REDD), forestry offsets, biofuels and biomass depend on the health and resilience of forests while adapting to climate change (Clark, 2004, 2007). Thus forest adaptation deserves a closer look as predictive models for climate change effects become more regional. Already, predicted range shifts and assemblage mixing are being published to some degree, but no two regions will experience climate change in the same way. Tropical forests are especially vulnerable to climate change (see Figure 4).

Figure 4. A schematic diagram of a forested ecosystem which is adjusting to climate change. Climate change related problem-solving will dominate forest research and development, particularly for tropical regions.

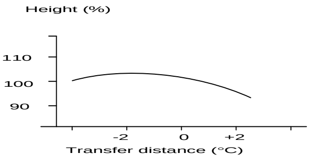


Assisted migration. Thinking about forest tree adaptation under climate change has led to the formulation of the concept of assisted migration (Aitken *et al*., 2009; Marris, 2009). Assisted migration refers to the practice of matching seed source to location, *assuming a different climate in the future*. This is an important concept given that forest trees are so narrowly adapted.

As climate-change models are becoming more regionally predictive, range shifts for indigenous forest trees are expected. Seed sources and provenances can be matched for optimum growth under these future site conditions (i.e. warmer, drier, colder). The conceptual example is shown in Figure 5. The issue is more critical at higher latitudes where forest rotation ages span 50 to 100 years, which is well within the realm of expected climate change by 2050.

With regard to biotic and abiotic stresses expected under climate-change scenarios, the development of biotechnology tools for resistance to pests, tolerance to climatic extremes, bioremediation and carbon sequestration will be more relevant in the near future both for naturally regenerating and for planted forest tree species.

Figure 5. Assisted migration. The concept refers to moving a local seed source to where its optimum growing conditions will be in the future. Optimum growth is defined here as height, and the distance refers to temperature change. Figure adapted from Aitken *et al.* (2008).



6.2. Sustainable management of forest genetic resources

Genetic diversity provides the fundamental basis for the evolution of forest tree species that has enabled forests and trees to adapt to changing conditions for thousands of years. Adaptation has resulted in a unique and irreplaceable portfolio of forest tree genetic resources. Fires, deforestation, new pests and diseases, and other factors are increasingly threatening forest genetic resources. The vast majority of forest genetic resources remain unknown and underutilized, although the sustainable use of forest genetic diversity has a great potential to contribute towards addressing new challenges and maintaining economic, social and cultural values, as well as providing environmental services and benefits. The field of forest genetic resources is undergoing significant changes. Traditionally, the sector has been concerned with technical issues of genetic conservation, tree improvement and seed supply for wood production. The scope of genetic management, however, is now expanding as the demand for products from forest species is increasing and diversifying (timber, fibre, fruits, resins and other non-wood products), which is contributing to food security and poverty reduction of rural populations. The emerging uses of forest genetic resources must be assessed to achieve sustainable use of these resources. Advances in biotechnology are rapidly enabling the improved use of genetic resources, and potentially greater economic and social contributions resulting from forest genetic resources. Biotechnology developments will also provide improved tools to enhance the effectiveness of conservation and development measures (knowledge about life-history traits and genetic diversity is lacking or inadequate for most tree species to define and implement conservation strategies).

7. Identifying options for developing countries

Based on the stocktaking exercise that has been carried out here, a number of specific options can be identified for developing countries to help them make informed decisions regarding the adoption of biotechnologies in the forestry sector in the future.

7.1 Biotechnologies should be integrated with conventional technologies

Forest biotechnology as a whole lacks strategic oversight to ensure the integration of its parts (Burdon, 1992). No policy exists that would ensure that tree breeding and molecular-based components of forest biotechnology work together in a complementary fashion. This is a problem, because biotechnologies such as molecular markers and mass propagation methods can be useful only if stable conventional forest breeding programmes are in place.

7.2 Promote public-private partnership at national level

Effective public-private partnerships are a key factor in most successful cases of the development and implementation of forest biotechnologies, especially as regards industrial wood production and processing. It is therefore an important option to be considered by developing countries.

7.3 Improve information and communication strategies for biotechnologies

Public access to good and updated information on forest biotechnologies is very important in developing countries. Benefits from their use can be optimized if the end-users know how to utilize them properly. Consolidated information and education mechanisms should be put in place to allow communication between the relevant sectors of society. Attention should be given to issues relating to the meaningful adoption of biotechnologies, including, socio-economic implications, efficiency, costs and benefits and environmental impacts.

8. Identifying priorities for action for the international community

The international community, including FAO and other UN organizations as well as NGOs, donors and development agencies, can play a key role in supporting developing countries by providing a framework for international cooperation as well as funding support for the generation, adaptation and adoption of appropriate biotechnologies. Here, we propose a set of Priorities for Action that will help the international community fulfill this role.

8.1 Improve access to peer-reviewed scientific information about forest biotechnologies in developing countries

Even with internet access, peer-reviewed journal and books continue to be central sources of information for scientists. Subscription costs for the best available scientific knowledge have risen exponentially, putting it out of the reach of many institutions, even in the most science-literate developed countries. While open-source journals are a step in the right direction, publications in traditional forestry outlets such as proceedings, conferences and government printing office publications are declining. Today, forest biotechnology is adversely affected by barriers to knowledge acquisition. These barriers, when coupled with publication bias (defined as the propensity to forego publishing negative results), can only slow scientific progress.

The international community is already acting to reduce these barriers. For example, FAO is coordinating the Access to the Global Online Research in Agriculture (AGORA, www.aginternetwork.org) programme, providing free or low-cost access to scientific journals in the fields of food, agriculture (including forestry), environmental science and related social sciences. A sister programme, called Online Access to Research in the Environment (OARE, www.oaresciences.org) also covers both forestry and biotechnology and its goal is to improve access to scientific research in developing countries by providing high quality, timely, relevant, environmental and related sciences journals and other scientific content for free or at nominal cost. OARE is an international public-private consortium coordinated by the United Nations Environment Programme, Yale University and leading science and technology publishers. Such initiatives from the international community should be encouraged and strengthened.

8.2 Build capacity for understanding forest biotechnology issues at all levels

For most policy-makers, scientists and even students, forest biotechnology is a form of agriculture. As discussed earlier, however, this is not the case. At best, it is a tribute to those who have developed capacity for agricultural biotechnology, but applying agricultural biotechnology to trees will not optimize the benefits to be obtained from forestry resources. Agricultural biotechnology does not constitute the best form of knowledge for forests. Forest biotechnology is an area that is separate from crop and livestock biotechnology and requires its own capacity-building. Capacity building initiatives in forest biotechnologies from the international community should be strengthened, in view of this important observation. The capacity-building initiatives should include training in emerging tools such as bioinformatics and computational biology for tropical forest studies. Intensive educational efforts for bioinformatics courses would benefit professionals and scientists in developing countries. This skill set provides capacity for testing hypotheses using available information from DNA sequences and related databases. In addition to educational workshops, this action will also require upgrades of computing infrastructure and perhaps bandwidth in some cases. A wealth of data is being produced by whole-genome DNA sequencing consortia, the Tree of Life project, the Consortium for the Barcode of Life and a host of other independent initiatives.

8.3 Review the status and potential of forest biotechnologies for developing countries

From this document, it is clear that the forestry sector in developing countries is in a very dynamic situation and facing a number of important challenges and opportunities for which biotechnologies can play a significant role. FAO commissioned a series of studies in 2002-2004 to investigate the extent and pattern of research and application of forest biotechnologies worldwide. Published in 2004, these studies have informed and influenced policy-making in developing countries, providing good indicators and possible predictions of trends in forest biotechnologies around the world. Such global surveys are important, and the international community should continue to provide period reviews of the status and potential of forestry biotechnologies in developing countries. The reviews should cover synergies with other biotechnology sectors, such as applications of biotechnology to micro-organisms to improve wood manufacturing processing, as well as with other fields of technology that may be useful, such as nanotechnology, information technology and synthetic genomics that may converge to the benefit of wood products manufacturing. Another potential area for convergence is the combination of genomics tools with Geographic Information Systems (GIS). Using GIS to track rare alleles, gene flow or expressed proteins is another area that deserves a closer look. Technology advances are delivering finer resolution at both the landscape and molecular ends of this molecule-to-landscape spectrum, and this will no doubt provide interesting ways to study all forest ecosystems.

8.4 Encourage North-South collaboration

As mentioned earlier in this document, the application of forest biotechnologies has advanced faster in developed countries than originally predicted (Robinson, 1999). As much of the research refers to processes and/or tree species that are relevant to developing countries, these advances are therefore of major potential relevance to developing countries as well. The international community should act to ensure that the results of research and application in forest biotechnologies in developed countries are made accessible to developing countries.

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