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Forest Genetic Resources Working Papers

Preliminary review of

biotechnology in forestry,

including genetic modification

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Background

At its 13th Session, in November 2003, the FAO Panel of Experts on Forest Gene Resources reviewed the relevance and discussed the feasibility of a global review of biotechnology in forestry, as part of an FAO effort to assess the global status and trends of forest tree genetic diversity. This paper is the first attempt to provide statistical information on the extent and patterns of biotechnology research and applications in forest trees, worldwide. Other studies are anticipated, in particular analyses of economic and other forces driving biotechnology development in forestry.

Disclaimer

The Forest Genetic Resources Working Papers report on issues and activities related to the conservation, sustainable use and management of forest tree genetic diversity. The purpose of these papers is to provide early information on on-going developments in the field, and to stimulate discussion.

The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. As a Working Paper, this document does not necessarily reflect the views of FAO.

Quantitative information has been compiled according to sources, methodologies and protocols identified and selected by the authors. For standardized methodologies and assessments on forest resources, please refer to *FAO, 2005. State of the World's Forests 2005*; and to *FAO, 2001. Global Forest Resources Assessment 2000 (FRA 2000). FAO Forestry Paper No 140*.

Biotechnology techniques and products in use or in the pipeline in developing countries and countries with economies in transition described in this Working Paper have been added to the searchable database FAO-BioDeC where they can be consulted for more details (www.fao.org/biotech/inventory_admin/dep/default.asp). Information can also be found on the FAO website (www.fao.org), in particular regarding biotechnology (www.fao.org/biotech/sector5.asp) and forest genetic resources (www.fao.org/forestry/fgr).

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Executive summary

Nicholas Wheeler

The overall objective of the FAO work programme on forest biotechnology is to contribute to a global assessment of the discipline's status and trends. The purpose of this document is to provide a summary of four FAO-sponsored reports on global forest biotechnology research activities and their applications. Individual reports are provided in Appendixes 2.1–2.4. Information compiled in these reports came from questionnaires, systematic searches of CAB Abstracts¹ and other scientific databases, Internet searches of public and private websites, and personal communications. Though no doubt incomplete, the resulting data set of over 2 700 major biotechnology activities (from the last 10 years) and responses to questionnaires provides a comprehensive summary of worldwide activities, particularly those in the public domain.

The term **biotechnology**, as used here, is defined as “*any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use*”². As noted in FAO's 2004 report on the state of the world's food and agriculture (‘SOFA 2004’)³, “*biotechnology is more than genetic engineering*”. Indeed, a significant majority (81 percent) of all biotechnology activities in forestry over the last 10 years was not related to genetic modification. During that period, major forest biotechnology activities were reported for 142 genera in over 80 countries, with activities relatively evenly spread among major categories: genetic modification, 19 percent; characterizing genetic diversity, 26 percent; genomics, genetic maps and marker-assisted selection (MAS), 21 percent; and vegetative propagation or micropropagation, 34 percent. The majority of cited activities excluding genetic modification occurred in developed countries (71 percent of the total), though China (6 percent) and India (9 percent) were exceptionally well represented in developing countries and countries with economies in transition. Regionally, forest biotechnology activities excluding genetic modification were most numerous in Europe (39 percent), Asia (24 percent) and North America (23 percent), and least numerous in Oceania (6 percent), South America (5 percent), Africa (3 percent) and the Near East (less than one percent). The majority of major biotechnology activity (64 percent) has been focused on only six genera (*Pinus*, *Populus*, *Eucalyptus*, *Picea*, *Quercus* and *Acacia*).

Worldwide, more than 210 field trials of genetically modified (GM) trees exist in 16 countries, but the great majority occurs in the United States. Field trials of GM trees are restricted largely to four genera (*Populus*, 51 percent; *Pinus*, 23 percent; *Liquidambar*, 11 percent; and *Eucalyptus*, 7 percent). Approximately half of all reported tree genetic modification activities are related to methods development (e.g. gene stability, gene expression) or basic biological questions (e.g. functional genomics, tissue culture). Of the remaining activities, herbicide tolerance (13 percent), biotic resistance (12 percent), wood chemistry (9 percent) and fertility issues (6 percent) dominate the most studied groups of traits. Only China has reported the commercial release of GM trees (ca 1.4 million plants on 300–500 ha in 2002). These releases followed two stages of field trials and required government regulatory approval.

¹ www.cabi-publishing.org/

² FAO. 2001. *Glossary of biotechnology for food and agriculture: a revised and augmented edition of the glossary of biotechnology and genetic engineering*. FAO Research and Technology Paper No. 9. Rome.

³ FAO. 2004. *The state of food and agriculture (SOFA) 2003-2004. Agricultural biotechnology: meeting the needs of the poor?* FAO Agriculture Series No. 35. Rome.

Overall, genetic modification activities in forestry occur in at least 35 countries and *Populus* remains the most commonly studied tree genus (52 percent of activities).

Reported forestry biotechnology activities excluding genetic modification are still largely confined to the laboratory (>95 percent), though the application of micropropagation tools in field plantings is becoming more common. Notably, though significant effort has been made to characterize the genetic diversity of numerous tree species, few if any applications in forest tree genetic resource management or conservation have been cited in public-domain literature.

Large investments in tree genomics, MAS and genetic maps have been made over the last 5–10 years, particularly in North America (34 percent of activities) and Europe (43 percent). As noted in 'SOFA 2004', the most significant breakthroughs in biotechnology are coming from research into the structure of genomes and the genetic mechanisms underlying economically and adaptively important traits: "...genomics is providing information on the identity, location, impact and function of genes affecting such traits – knowledge that will increasingly drive the application of biotechnology in all agricultural sectors". The science of proteomics is gaining some attention as well. The most notable accomplishment in forest genomics is the recent completion of a genome sequence for *Populus*. Identification of and partial sequences for nearly all the genes in *Pinus* are anticipated by many within 5 to 10 years. The discovery of alleles within candidate genes with measurable effects on quantitative traits such as cold tolerance is setting the stage for the application of association genetics, possibly within a decade. In this approach, MAS is directed towards the selection of superior alleles in candidate genes directly controlling phenotypic variation in traits of interest. Nevertheless, while expectations for the application of biotechnologies excluding genetic modification for tree breeding and selection remain high, there are virtually no publicly cited cases of commercial use except for micropropagation (rooted cutting technology) which is developed in at least 63 countries, and for over 80 genera (*Pinus*, *Eucalyptus*, *Picea*, *Tectona*, *Acacia*, *Populus* and *Larix* predominate). The use of somatic embryogenesis, though promising for clonal forestry, and at the advanced field test stage for some species including *Pinus taeda*, has to date provided only limited commercial applications.

Trends in forest biotechnology can be partitioned into four categories:

- Biotechnology excluding genetic modification, research: Emphasis appears to be on discovering and identifying the function of all the genes in a few model tree species (in genera such as *Populus*, *Eucalyptus*, *Pinus*). Genes are fuelling association genetics studies. Investment largely derives from public sources in developed countries.
- Biotechnology excluding genetic modification, application: Micropropagation tools will likely dominate these biotechnology applications worldwide for many years, though simple use of markers for fingerprinting, paternity analysis and MAS will grow, rapidly for a few selected species, more slowly for others.
- Genetic modification, research: Genetic modification may be used to evaluate gene function in publicly funded programmes.
- Genetic modification, application: 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. Some authors anticipate commercial releases in countries besides China within a decade.

1. Synthesis: a snapshot of the global status and trends in forest biotechnology

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This chapter is an updated synthesis of Appendixes 2.1–2.4 of this Working Paper.

1.1 PURPOSE

The overall objective of the FAO work programme on forest biotechnology is to contribute to a global assessment of the discipline's status and trends. The purpose of this chapter is to summarize the findings and conclusions of four individual studies seeking to define the current state of forest biotechnology research and application. This 'statistical survey' of the types of biotechnologies being used globally will ultimately be coupled with analyses of economic and other forces driving biotechnology development in forestry to provide a comprehensive view of the current status of and trends in the discipline. This Working Paper is not intended to provide descriptive summaries of biotechnology tools, their precise classifications, areas of applications, or perceived contributions to forestry development. These topics have been extensively treated in other publications (see citations in references and annexes in this Working Paper).

1.2 BACKGROUND

Between 2002 and 2004, the FAO Forestry Department commissioned four studies to investigate the extent and patterns of biotechnology research and application on forest trees, worldwide. The studies are presented in this Working Paper (the Appendix numbers are indicated in brackets).

- Chaix, G and Monteuis, O. CIRAD Forestry Department. Biotechnology in the forestry sector. 2004. (Appendix 2.1)
- Walter, C. and Killerby, S. New Zealand Forest Research. A global study on the state of forest tree genetic modification. 2003. (Appendix 2.2)
- Wang, H. Chinese Academy of Forestry. The state of genetically modified forest trees in China. 2003. (Appendix 2.3)
- El-Kassaby, Y. A. University of British Columbia. Anticipated contribution to and scale of impact of biotechnology in forestry. 2004. (Appendix 2.4)

The authors of these reports have compiled comprehensive information sources, including databases from questionnaires, systematic searches in CAB Abstracts and associated global scientific databases, Internet searches of public and private websites, and personal communications with scientists active in the field.

All attempts to be thorough and accurate have been made but capturing a sense of the status and trends of forest biotechnology research and application is a daunting task given the global spread of practitioners and the extremely rapid rate of progress in some technological areas such as genomics. Despite the authors' best efforts, the FAO-sponsored studies reported here are sure to suffer from some largely unavoidable deficiencies such as:

- Inability to access, literally or in a timely manner, the vast literature and databases in Russian, Chinese and several other languages.
- Inability to access information on progress of and future plans for research and application in the private sector. This is particularly true for, but by no means exclusive to, R&D on genetic modification.
- Inability to stay abreast of rapid progress, the results of which may have immediate and large impacts on the science.
- Inability to report progress on work currently underway or completed, but not reported (often a lag of a few years).
- Inability to equate the number of citations of particular technologies with the actual resources invested in those technologies.

As an example of the last point, the number of citations and patents noted for somatic embryogenesis does not accurately reflect the several millions of dollars invested in the technology in North America alone over the last 15 years. In short, this Working Paper is likely to be a good indicator and possibly predictor of trends in forest biotechnology activity, but is equally likely to be lagging in defining the most current research activities and applied results. Future updates of the global status of forest biotechnologies will address many of the drawbacks pointed out here.

1.3 DEFINITIONS

The current working definition of **biotechnology** used by the FAO is “*any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use*” (Convention on Biological Diversity⁴). In the broadest sense, this definition would include historical or traditional tree improvement technologies. For this report, the interpretation is narrowed to consider only modern DNA, molecular biology and plant reproductive technologies. **Genetic modification** or genetic engineering is defined here as *the use of recombinant DNA and asexual gene transfer methods to alter the structure or expression of specific genes and traits*.

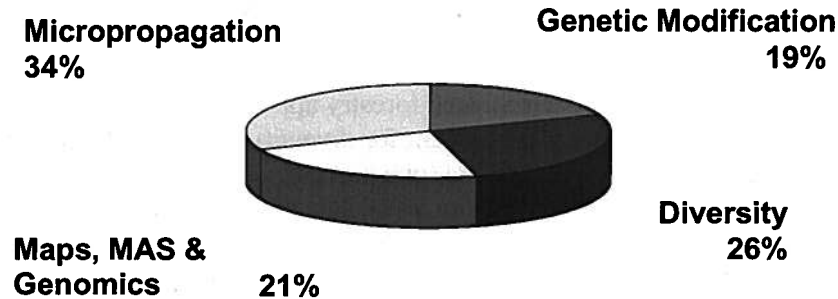
1.4 RESULTS

Over 2 700 references representing the main forest biotechnology activities (publications of major research findings, field plantings, propagation programmes, etc.) reported within the last decade were compiled and synthesized in project surveys. The majority of these references (60 percent) were reported within the last 5 years, suggesting the scope and scale of biotechnology research and application is accelerating. Approximately 81 percent of the cited activities refer to biotechnologies excluding genetic modification. Chaix and Monteuiis (see Appendix 2.1) differentiate three categories of these activities (Figure 1.1):

- marker development and estimates of diversity (referred to below as ‘diversity’);
- mapping, marker-assisted selection (MAS) and genomics (collectively referred to as MMG);
- micropropagation.

⁴ See: www.biodiv.org/.

Figure 1.1 Proportion of biotechnology activities, by major categories, indicated in the public domain (from Chaix and Monteuis, Appendix 2.1)



1.5 STATE OF BIOTECHNOLOGIES IN THE FORESTRY SECTOR, EXCLUDING GENETIC MODIFICATION

1.5.1 Overview

Forest biotechnology research and application is truly global in scope with activities identified in 76 countries. A significant majority of cited activities occurs in developed countries (68 percent), with the United States (14 percent), France (9 percent) and Canada (8 percent) the most active participants represented in the data set (percentages are of total citations of main biotechnology activities). India (9 percent) and China (6 percent)⁵ were far and away the most active of the developing countries and countries in transition. Regionally, forest biotechnology activities were most numerous in Europe (39 percent), Asia (24 percent) and North America (23 percent), and least numerous in Oceania (6 percent), South America (5 percent), Africa (3 percent) and the Near East (less than one percent).

While forest biotechnology research and application has spread to at least 140 genera, the great majority of activity (62 percent) has been focused on only six genera (*Pinus*, *Eucalyptus*, *Picea*, *Populus*, *Quercus* and *Acacia*, in descending order of activity).

Total activities in biotechnology excluding genetic modification were relatively evenly distributed between the categories identified by Chaix and Monteuis (see above and Appendix 2.1), diversity, MMG and micropropagation (32 percent, 26 percent and 42 percent, respectively), but became skewed when the number of genera studied was considered (diversity, 75 genera; MMG, 36 genera; micropropagation, 78 genera). Each category will be discussed at length in the following sections.

⁵ Figures should be taken with caution: for example, a significant amount of literature in Chinese is not covered by international databases.

Biochemical and molecular markers play a significant role in many forest biotechnology activities, and marker development for trees has closely followed that for humans and agricultural crops. Isozymes, randomly amplified polymorphic DNAs (RAPDs) and restriction fragment length polymorphisms (RFLPs) have been widely used for genetic diversity and mapping studies, though the current trend favours microsatellites (nuclear and cytoplasmic) and AFLPs (amplified fragment length polymorphisms). Currently, ESTs (expressed sequence tags) and SNPs (single nucleotide polymorphisms) represent the most active area of marker development, though the total number of citations for these markers remains small suggesting the literature does not yet reflect the current status of this area of research. Work with these markers is being driven by large genomic and association genetics projects.

The vast majority of biotechnology activities in forestry appears to be restricted to the laboratory (99 percent, 97 percent and 94 percent for diversity, MMG and micropropagation, respectively) with some supporting field trials (one percent, 2.5 percent and 5 percent, respectively). Reported commercial application of biotechnologies appears to be restricted to micropropagation (but forms less than one percent of reported activities for that category), though it is known from non-public sources that commercial applications exist to a modest degree for all categories.

1.5.2 Characterization of genetic diversity

In general, reported activities in this category relate to the use of molecular markers to characterize amounts and patterns of genetic diversity in tree populations (studies on population structure), and to estimate the influence of human activities on forest genetic resources. More specifically, these activities include descriptive studies of (i) measures of diversity (57 percent of total activities in this category) and tree conservation with logging assessment (8 percent); (ii) tree mating systems, estimation of effective population size and gene flow within and among populations (15 percent); (iii) paternity analysis and fingerprinting applications (14 percent); and (iv) taxonomic and phylogenetic applications (6 percent). Most studies targeting genetic diversity appear to be addressing and validating population genetic models or providing baseline data for the elaboration of conservation strategies. However, the application of these data for conservation purposes at the operational level is generally not obvious in the literature. Marker applications for fingerprinting and paternity analysis have found considerable utility in both basic science and commercial applications, though the commercial applications are not widely publicized (e.g. Lambeth *et al.* 2001).

Ninety-nine forest tree genera are represented in this category, though efforts are directed largely at the six noted in Section 1.5.1, Overview. In addition, *Fagus*, *Abies*, *Betula*, *Castanea*, *Pseudotsuga* and *Larix* have received considerable attention. While 66 percent of the studies on genetic diversity characterization occur in Europe or North America, studies of species originating from (native to) these regions represent only 37 percent of the total activities. The remaining distribution, by region, for the origin of species studied is evenly divided among Asia (19 percent), Oceania (New Zealand and Australia, 11 percent), South America (9 percent), Africa (4 percent) and the Near East (less than one percent), with 19 percent of species of non-ascertained origin, or hybrids.

Studies on genetic diversity characterization have been largely based on RAPD and AFLP markers in the past, with a gradually increasing use of nuclear and cytoplasmic microsatellites. Virtually all of these markers are considered neutral (not related to selection pressure) and may not be ideal for understanding genetic patterns influenced by evolutionary forces.

There is a small but growing trend to use SNP markers located in coding regions of candidate genes controlling phenotypic expression in adaptive traits to study the influence of evolutionary forces on allele frequencies.

1.5.3 Mapping, marker-assisted selection and genomics (MMG)

During the decade of the 1990s significant biotechnology activity centred on the development of molecular markers, test populations, genetic linkage maps, and statistical means of identifying quantitative trait loci (QTLs). QTLs represent statistical associations between markers and genes that control some proportion of the genetic and phenotypic variation of a quantitative trait (generally less than 10 percent per QTL). QTLs have several potential applications including (i) genetic dissection of complex quantitative traits, (ii) providing the basis for MAS, and (iii) providing guidance for selection and prioritization of candidate genes (discussed later). Linkage and QTL maps have been created for over two dozen tree species⁶ and though more maps are likely to appear, most current efforts appear to focus on increasing the density and type of markers located on these maps. The current trend in MAS is towards the selection of superior alleles in candidate genes directly controlling phenotypic variation in traits of interest. This approach, termed association genetics, differs in application from traditional QTL studies primarily in the form of the test population being studied. Traditional methods use pedigreed populations for within-family selection while association studies rely on populations of unrelated individuals. Though MAS using QTLs has found utility for specialized populations of commercial species in a few developed and developing countries, association genetics holds promise for application across many populations, species and countries following appropriate development.

Over the last half-dozen years tremendous resources have been invested in genomics sciences, though this may not yet be reflected in the activities compiled here. Genomics encompasses a wide range of activities including gene discovery (ESTs), gene space and genome sequencing, gene function determination (database blast searches, expression profiling using arrays and slides, etc.), comparative studies among species, genera and families, physical mapping and the burgeoning field of bioinformatics. The ultimate goal of genomics is to identify every gene and its related function in an organism.

The recent completion of a whole-genome sequence for *Populus* (a project led by the United States Department of Energy) has laid the foundation for reaching this goal for a model species, and efforts follow to replicate this deed in *Eucalyptus*, though progress is slower. Public and private EST libraries for conifers now probably exceed one million entries. The immediate applications of genomics include identification of candidate genes for association studies and targets for genetic modification studies. Also, comparative studies of ESTs from different trees have revealed the tremendous similarity among taxa throughout the conifers, and raise hope that what is learned from one species will benefit many others.

Finally, this category includes activities in the areas of proteomics and metabolomics, disciplines that currently enjoy very modest efforts in Europe and North America, but which are likely to expand in level of effort and geographic area in the near future.

Data set analyses, though confounded by the many technologies noted here, remain useful for tracking trends. MMG activities are conducted globally, but are concentrated in Europe (43 percent) and North America (34 percent). About 40 genera have been studied, though four genera dominate the MMG landscape (*Pinus*, *Populus*, *Eucalyptus*, *Picea*).

⁶ See: <http://dendrome.ucdavis.edu/>.

Methodological approaches to MMG studies heavily favour gene discovery, functional genomics and candidate gene identification (65 percent of total studies), while linkage and QTL mapping account for 31 percent of activities. Proteomics activities are modest (2 percent). Where identified, MMG studies have targeted primarily genes controlling wood property traits (57 percent) and resistance to abiotic (20 percent) and biotic (4 percent) stresses. Growth rate (6 percent), genetic diversity (8 percent) and flowering (5 percent) make up the remaining major categories.

1.5.4 Micropropagation

Micropropagation is a term used here to describe methods of *in vitro* vegetative multiplication including rooted cuttings, organogenesis and somatic embryogenesis. Micropropagation is used to create large numbers of individual clones or genotypes. Because vegetative propagation bypasses the genetic mixing associated with sexual reproduction, it represents an ideal way to deliver genetic gain: select individuals are replicated precisely. The majority of biotechnology activities excluding genetic modification compiled by Chaix and Monteuis (Appendix 2.1) (42 percent) relate to micropropagation.

Micropropagation by rooted cutting is commonly used in more than 20 species of commercial importance, the majority of which are angiosperms. Many of the activities noted suggest the technology is advanced and commercially viable. Conifers are less easily rooted than angiosperms, though modest programmes for several genera exist. Somatic embryogenesis is defined by an array of steps that result in the creation of embryos from somatic tissues (as opposed to zygotic embryos from germinal cell lines). Though technically difficult, the technology has the potential to produce literally millions of genetically identical individual plants. It has received considerable R&D attention for highly valued conifer species, primarily in developed countries, for many years. Though large-scale commercial plantings of somatic embryos do not yet exist, progress in the technology appears promising and small-scale field testing is increasing (for example, on *Pinus taeda* in the United States). The delivery of somatic embryos to the field remains a significant hurdle to reducing plantlet costs and, therefore, large-scale use. Excellent progress in the creation of manufactured seed⁷ appears to provide a solution to this problem, though further research is likely to be needed.

Organogenesis, or the creation of plantlets from tissues such as cotyledons, has largely fallen out of favour in forestry operations and is used infrequently. In the future, the use of vegetatively propagated trees for intensively managed, high-yielding plantations is anticipated to increase significantly in all regions of the world. It is the author's view that rooted cuttings are likely to dominate in angiosperm propagation in developing countries while somatic embryos will dominate in conifer propagation in developed countries.

Micropropagation activities occur in at least 64 countries in all regions of the world, though efforts are concentrated in Asia (38 percent of total), Europe (33 percent) and North America (16 percent). These activities include more than 80 genera of forest trees, the most commonly used being *Pinus*, *Picea*, *Eucalyptus*, *Acacia*, *Quercus*, *Tectona*, *Populus* and *Larix*. A breakdown of activities within micropropagation suggests most reported work is within somatic embryogenesis (65 percent), along with cell and tissue culture (17 percent) and microcuttings (13 percent).

⁷ Source: Weyerhaeuser Company, Federal Way, WA, USA.

1.6 STATE OF BIOTECHNOLOGIES IN THE FORESTRY SECTOR: GENETIC MODIFICATION

1.6.1 Overview

Few if any technological advancements in crop improvement methods have attracted as much attention from the scientific and lay communities as genetic modification. This is as true for forestry as it is for agriculture. In fact, genetic modification is so embedded in the public consciousness, that it is often considered synonymous with the term biotechnology. As detailed earlier, biotechnology encompasses much more than genetic modification, and publicly cited work on biotechnology activities excluding genetic modification significantly outnumbers genetic modification activities in the forestry sector (see Figure 1.1). However, in view of the intense scrutiny of and interest in genetic modification today, and its potential for significant influence on the ecological and economic landscapes, it is given disproportionate attention in this review.

The FAO-commissioned studies reviewed in this Working Paper provide detailed accounts of and insights into the state of genetic modification activities in world forestry as of early 2004. Walter and Killerby (Appendix 2.2) have summarized results of a detailed questionnaire sent to well over 500 institutions and scientists potentially engaged in or concerned with work on genetic modification in forestry. In addition, like Chaix and Monteuis (Appendix 2.1), Walter and Killerby have searched public Internet databases, such as the APHIS (US Department of Agriculture – Animal and Plant Health Inspection Service) website for field trial permit applications in the United States, for any reference to work on genetic modification not typically reported in the literature. Collectively, these reports, taken together with information collated by Wang (Appendix 2.3), provide a reasonably consistent picture of the status of and trends in forestry research on genetic modification.

Forestry genetic modification activities are taking place in at least 35 countries, 16 of which host some form of experimental field trials (Figure 1.2). These field trials are generally very small (12 to 2 850 plants in reported studies) and typically of short duration. In many countries such trials must be destroyed before seed bearing occurs. In the remaining 15 countries, experimentation is restricted to laboratories or greenhouses. To date, only China (see Wang, Appendix 2.3) has reported the establishment of approved, commercial plantations of GM trees (discussed in subsequent sections).

Nearly two-thirds of the 520 reported genetic modification activities in forestry (see Chaix and Monteuis, Appendix 2.1) occur in North America (48 percent) and Europe (32 percent). Asia follows with 14 percent of reported activities, Oceania with 5 percent, South America with one percent and Africa with less than one percent. The year 2004 has seen a sharp increase in field test applications for GM forest trees in the United States (Figure 1.3).

Populus was the first tree to be genetically modified (1986) and is by far the most commonly studied tree genus for genetic modification purposes today (47 percent of activities). This is no doubt a function of the ease with which some genotypes of the genus can be transformed and vegetatively propagated for experimental purposes. *Pinus* (19 percent), *Eucalyptus* (7 percent), *Liquidambar* (5 percent) and *Picea* (5 percent) make up the majority of the remaining experimental studies. Field trials of GM trees are restricted largely to four top genera (*Populus*, 51 percent; *Pinus*, 25 percent; *Liquidambar*, 11 percent; and *Eucalyptus*, 7 percent).

Figure 1.2. Forest genetic modification activities worldwide

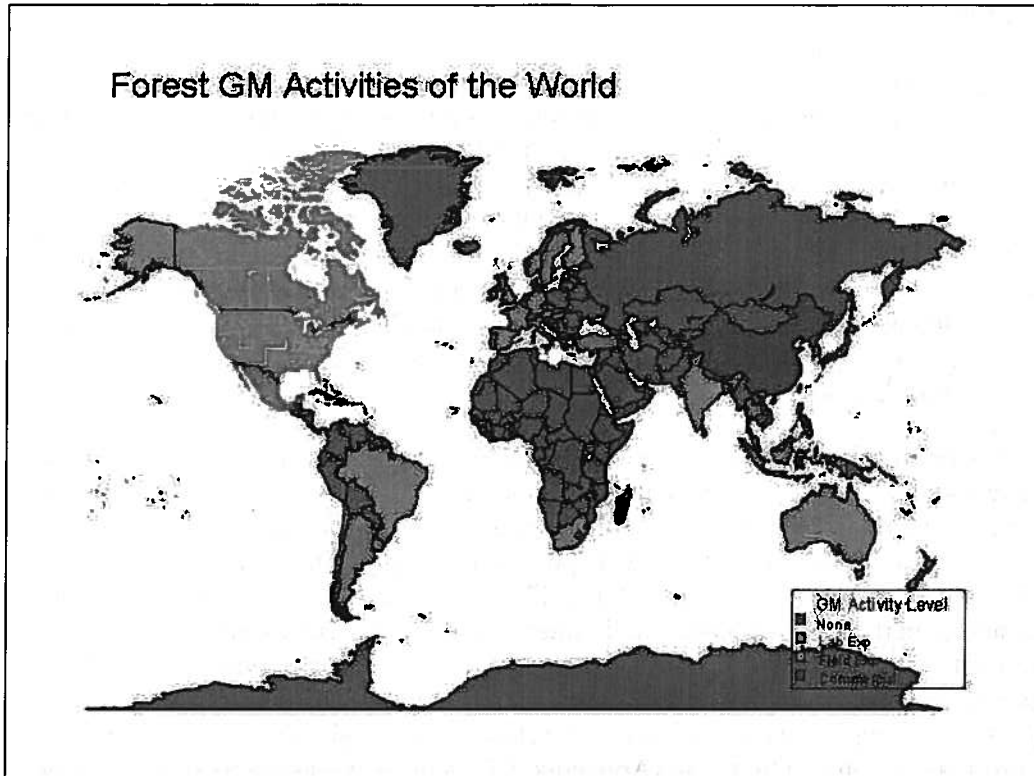
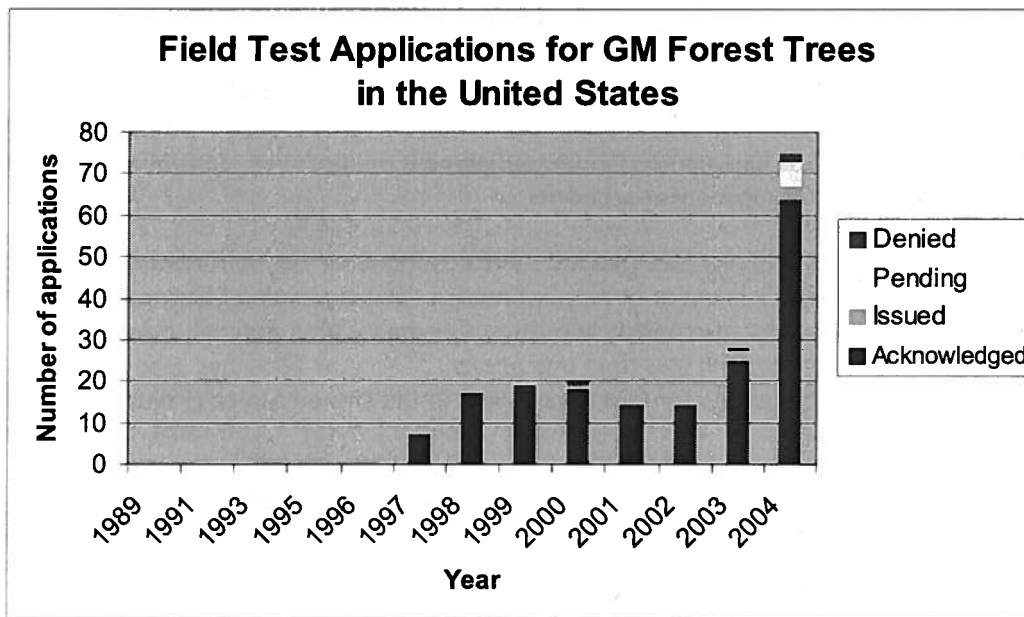


Figure 1.3. Field test applications for GM forest trees in the United States



Source: US Animal and Plant Health Inspection Service (APHIS) GM field test release permits database, according to APHIS notification categories.

Approximately half of all reported genetic modification activities with trees are related to methods development (e.g. gene stability, gene expression) or basic biological questions (e.g. functional genomics, tissue culture). Of the remaining activities, ostensibly guided by commercial deployment objectives, herbicide tolerance (13 percent), biotic resistance (12 percent), wood chemistry (9 percent) and fertility issues (6 percent) dominate the most studied groups of traits. This area will be discussed in greater detail later.

1.6.2 Responses to the genetic modification questionnaire

The questionnaire was designed not only to assess the current status of genetic modification in trees worldwide, but also to capture the views of practitioners on future developments and means to keep the public informed of their activities. A copy of the questionnaire is available from the FAO.

Of the 418 questionnaires sent out by Walter and Killerby⁸, 49 were returned (11.7 percent) and of these, 23 respondents were conducting research on genetic modification in forest trees⁹. For simplicity, these 23 respondents will be referred to as the 'core' respondents henceforth. Respondents did not include some private companies known to be conducting genetic modification activities in trees, most notably in North America where the majority of activity is reported. Regardless, the comments and trends noted, while not comprehensive, are likely to reflect those of the global research community. Of the core respondents, ten represented research institutions, nine universities, two private industries, and two were categorized as 'other'. Two of the total respondents represented non-profit organizations concerned with appropriate use of biotechnology. Fifty-seven percent of the core respondents currently publish information about their work on the Internet.

1.6.3 Project scope

The first three questions sought to determine the scope of genetic modification activities in contained laboratory experiments (68 reported), field-based experiments (26 reported) or commercial plantings (none reported), primarily with respect to traits and species evaluated. Of the 68 laboratory projects, all but two were identified as research targeted with the remaining two related to commercial applications. Four projects serviced both functions. Seventeen different gene/trait groups were identified in the laboratory, the majority of which were related to methods development or stability (markers, reporters, antibiotic resistance; 26 projects), reproductive development (19) or herbicide resistance (11). Other notable traits included wood properties (eight), insect resistance (seven) and wood chemistry (eight). The species studied were largely as noted in Section 1.6.1, Overview, though the percentages of each varied slightly from the list generated by the complete data set. Most projects were reported to be working with more than one species and on more than one trait or gene. Eleven respondents reported a total of 26 projects with field trials, 20 of which have been established (1–3 per year) over 13 of the last 15 years. Of these 26, 20 (77 percent) involved *Populus*, two *Pinus*, and one each *Betula*, *Eucalyptus* and *Picea*. Markers (nine projects), herbicide resistance (six), lignin biosynthesis (five), insect resistance (three), and reproductive development (two) dominated the traits being investigated in field trials. Approximately half of the respondents with field trials identified the issues addressed by these trials to be related to plant growth/performance (five projects), gene expression stability (four), environmental risk assessment (three), horizontal gene transfer (two), herbicide applications (two) and four others (one each).

⁸ Other questionnaires were sent out by FAO or posted on mailing lists and biotechnology discussion fora.

⁹ Several respondents commented on genetic modification activities in ornamental and fruit trees. Data on these will be noted where appropriate.

To judge future trends better, respondents were asked whether they had pending plans for future trials of GM trees. A total of 12 laboratory and field trials were specified for four genera in five countries, with establishment anticipated over the next 3 years.

One respondent from China reported the commercial release of two transgenic poplar genotypes transformed for leaf-eating insect resistance. The largest release was with *Populus nigra* transformed with the *Bt* gene *cry1Ac*. Following a successful 1-ha pilot planting in 1994, 80 ha of field trials were established on eight sites in seven provinces in 1999. Subsequent authorization from state regulating agencies permitted the establishment of *ca* 1 million trees in 2002, on some 300 ha. A smaller release with a hybrid poplar clone transformed with both *cry1Ac* and API followed in 2003. No other respondents to the questionnaire reported plans for commercialization.

1.6.4 Links with breeding, propagation or other research

The majority of the 68 projects involving GM trees had no or very weak connections to traditional breeding programmes (60 percent) or vegetative propagation programmes (66 percent), though ongoing development of some links was noted. The remainder noted strong links to such efforts. Of the core respondents, 65 percent noted the use of genetic modification for underpinning other research goals such as testing gene function, evaluating environmental impact, QTL detection, MAS evaluation, microarray and EST mining for genes, etc.

1.6.5 Regulatory framework

Respondents were asked to identify whether, and from whom, regulatory oversight existed for their genetic modification projects. Core respondents representing 15 countries identified regulatory agencies (see Appendix 2.1) and categorized their involvement in (i) pre-risk assessment (13 countries), (ii) surveillance, monitoring and quarantine (12 countries), or (iii) management of failure, redress and control (12 countries). At least nine countries regulate all three levels of involvement, and no country is totally unregulated. When asked whether the regulatory framework in their country was adequate to assess the benefits and risks related to their experiments, 15 of the core respondents (65 percent) indicated it was, five thought it inadequate, and three did not answer. Comments on how the process could be improved were many, and seemed to focus on how the process could be streamlined and made less expensive. Others suggested more risk assessment research was required. None of the core respondents indicated they were testing GM trees abroad.

1.6.6 Intellectual property

Of the core respondents, eight (35 percent) indicated they owned intellectual property (IP) in relation to their production of GM trees, ten did not, and five respondents did not answer. Nine of the respondents confirmed they had IP agreements with other institutions. None of the respondents indicated they had IP arrangements with institutions in developing countries and countries in transition, though some indicated willingness to have these where appropriate and required. Eleven areas were noted as being the target for IP ownership including, but not restricted to, genes, gene expression, transgene development and testing, modulation of lignin biosynthesis, flowering control and embryogenesis.

1.6.7 Future of GM trees

Many of the 49 respondents contributed suggestions as to how GM trees might be used for environmental or health benefits in the future, but only a few respondents predicted further field releases, suggesting that high costs and regulatory burdens of conducting such trials are too great. Some suggestions for future plans included development of GM trees for tolerance to drought and extreme temperatures, candidate gene testing, production of secondary compounds (pharmaceuticals), site remediation, fibre quality, etc. The generally dour view of the future of genetic modification in forestry can be attributed to a number of factors including, but not restricted to: consumer rejection of or unease with GM products; public relations risks for companies engaged in research on genetic modification; unpredictable and costly government regulations, not only for conducting the research but for international trade; inadequate research support; intellectual property costs; and so forth. These factors are in contrast to the largely unregulated and less costly biotechnologies excluding genetic modification or traditional means of tree improvement.

1.6.8 Benefits from GM trees

Respondents were asked what commercial, environmental or human health benefits might be attributed to the use of genetic modification technology in forestry. Respondents often indicated more than one benefit per category. With respect to commercial benefits, increased wood production (15 responses) or improved wood quality (12) were most cited, followed by resistance to insects (nine) and disease (seven), reduced production and processing costs of wood or chips (five), and reduced chemical costs for pulping (four). Several other responses were given once each (a total of 60 possible benefits mentioned). Two notable environmental benefits were reduced pressure on natural forests (12) and reduced use of chemicals in forests and in processing (12). The use of GM trees for phytoremediation and carbon sequestration¹⁰ were also identified as environmental benefits (seven responses each), while increased productivity per managed hectare, adaptation to stresses, reduced erosion, and renewable energy largely completed the list. Human health benefits from GM forests were mentioned least often, though reduction in pollen and allergy problems was identified seven times. Reduced environmental pollution (five responses) and environmental protection and restoration (five) were also noted. Three respondents felt there were no likely health benefits. Benefits of other kinds were also noted. It was noted that genetic modification in forestry could lead to potential economic benefits for developing countries and countries in transition, and could significantly accelerate conventional breeding programmes. There were indications that genetic modification could also provide basic biological knowledge and employment, and contribute to reduce global warming.

1.6.9 Risks of genetic modification in forestry

Respondents were next asked to identify anticipated commercial, environmental or human health risks associated with the use of genetic modification in forestry. Commercial risks ranged from public resistance (nine) and large financial investment risks (five), to biological risks such as transgene instability (three), plantation failure (two), wood quality issues (two) and use of monocultures (two). Ecoterrorism and development of tolerance by insects to GM resistance were each mentioned once in a total of 31 suggested risks. Of 16 responses to a question about human health risks, 11 suggested these were negligible or non-existent. Two votes each were given for potentially new allergens or toxic metabolites being created.

¹⁰ Questionnaires were filled in before the ninth session of the Conference of the Parties to the UN Framework Convention on Climate Change in December 2003.

Potential environmental risks noted included gene (eight) or plant (seven) escape into natural ecosystems, impact of resistance genes on non-target species (two), and clonal failure in plantations (two). Four other responses were given once each and eight respondents indicated there were no or limited environmental risks. A broad array of general responses was given about other potential risks, for example: (i) GM forests may render natural forests valueless; (ii) means exist to ameliorate environmental risks such as gene escape; (iii) regulatory/legal/commercial constraints will always slow commercialization of best technologies; and (iv) genetic modification will encourage clonal plantation forestry, which is undesirable.

When asked how risks involved with GM trees can be best addressed respondents noted most frequently the need for increased risk assessments in field and laboratory studies (13 total responses), introduction of sterility genes in GM crops (five), and research and education (two). Several other comments were given once each.

Respondents were specifically asked how their own R&D programmes addressed risk. Responses were many, varied and constructive. They included anticipated use of flowering control in GM products, inclusion and promotion of biodiversity conservation and ecological restoration of native/natural forests in association with GM plantations, studies of pollen dispersal and ecological interactions between GM trees and herbivores, and use of harmless selectable markers (to detect the presence of transgenic cells) rather than antibiotics.

Finally, respondents were asked about the risks of not using genetic modification technology. Seven respondents felt that this would compromise the ability to reduce pressure on the world's natural forests, while four felt there was little or no risk at all. Several other answers were recorded, including (i) non-users could be at a competitive disadvantage in intensive plantation production; (ii) loss of one approach for saving endangered species (e.g. the American chestnut, *Castanea dentata*); (iii) loss of the best, long-term opportunity for increasing forest productivity; and (iv) disproportionate loss of the technology could lead to a decrease of the timber industry in some countries relative to others.

1.6.10 Obstacles to genetic modification applications in trees

The questions of what obstacles to the application of genetic modification technologies in trees exist, and what means exist to address those obstacles, were asked. The overwhelming response to the first question was that public perception, regulatory issues and adversity toward science constituted the major set of obstacles (22 of 49 responses). The remaining nine obstacles, which drew 2–4 responses each, included such items as technical hurdles, lack of interest and support from the timber industry and growers, lack of funding, environmental risks, lack of integration with traditional breeding programmes and scientists, forest certification schemes and IP issues. Sixty-five percent of respondents (13 of 20) listed better education of and communication with the public as ways to overcome obstacles. Several other excellent suggestions dealt with both technical and strategic use issues, including: (i) target traits with obvious benefit to consumers; (ii) provide technology to ensure gene flow does not occur between GM and natural trees; (iii) seek means to certify products from GM plantations; (iv) increase R&D funds, particularly from public sources; and (v) eliminate the need for GUS (β -glucuronidase) and antibiotic resistant genes in the genetic modification process. There was also support for increased field testing to validate benefits and risks, possibly even to the exclusion of seeking commercial release until data are solidly supportive.

1.6.11 Perception and communication

The final series of questions in the questionnaire addressed issues of public perception of specific programmes and how organizations using genetic modification communicated their findings to the public. Twenty-five respondents (22 core, three additional) contributed an array of answers summarized by Walter and Killerby (see Appendix 2.2). These covered the gamut from very positive to very negative (vandalism of GM trials), and virtually all recognized the importance of public perception to their ultimate success. Some respondents perceived resistance from local governments while sensing support at the national level, while in some countries resistance was experienced from all aspects of society and government. Of core respondents, 61 percent reported they have some form of direct public communication, either as part of a corporate strategy or as individual communication strategies. Three respondents had no form of communication and two relied on collaboration with outside agencies for their communication about GM trees.

1.6.12 Ornamental and fruit tree genetic modification

Over 70 field trials of GM fruit and ornamental trees, conducted in 13 countries, were noted by respondents and from database searches. A significant majority (43 of 73) of the trials are located in the United States. Taxa most studied include *Carica* (papaya, 18), *Malus* (33), and *Prunus* (13). These trials target fundamentally different suites of genes from the forestry sector, concentrating on viral (15), fungal (17) and insect (11) resistance, rooting (five) and fruit ripening (11).

1.6.13 Publicly available information on field trials of GM trees

While the information provided by respondents to the questionnaire was immensely useful in gauging the status of and trends in genetic modification in forestry, it was clear from surveys of public databases and regulatory sites that all activities were not accounted for in the formal survey. This was particularly true for field trials of GM trees in the United States¹¹.

In general, getting an accurate estimate of the total number of field trials with GM trees is difficult. Best estimates place the number at greater than 225 trials, with as many as 150 or more in the United States. While the general world trend appears to be a gradual decline in the number of field trials being established, the current trend in the United States appears to be that field trial establishment is stable or increasing. This is largely a function of one or more private organizations focusing on the delivery of GM products to the forestry community.

1.7 CONCLUSIONS: STATUS AND TRENDS OF BIOTECHNOLOGY IN FORESTRY

Biotechnology in forestry, as in agriculture in general, encompasses a wide range of research tools used to understand and manipulate the genetic make-up of select organisms. Forest biotechnology is much broader than genetic modification, and includes micropropagation, molecular marker applications, and the rapidly expanding area of genomics. Indeed, a significant majority (81 percent) of all the biotechnology activities in forestry compiled from the last 10 years was not related to genetic modification.

Though most biotechnology tools were originally developed for use with human beings and agricultural crops, the forestry research community has been fast to adapt them.

¹¹ See: www.isb.vt.edu/cfdocs/fieldtests1.cfm or www.aphis.usda.gov/.

In many respects, forest trees, traditionally considered difficult organisms to study genetically, may constitute model organisms for technologies seeking to understand and use natural sources of variation. This is due in part to the unparalleled genetic and phenotypic diversity common to forest trees, relative to most crop species.

The global forestry biotechnology community, though small in comparison to agriculture, is fairly robust and widely divergent in research, development and application targets. Cited activities in forest biotechnology have increased nearly three-fold in the most recent 5-year period, compared to the previous 5-year period. Thus, there is an upward trend in the development of biotechnologies focused on characterization and utilization of naturally occurring genetic variation within species, while publicly funded and reported programmes on genetic modification technologies in trees, particularly those targeting field testing and deployment, seem flat or diminishing. Private sector genetic modification activities, though narrowly based, appear dynamic and the world's first commercial GM forest plantations have been established on a small scale in China.

Overall understanding of tree molecular biology has advanced dramatically in recent years. The completion of genome sequences for model plants such as *Arabidopsis* and especially *Populus*, along with development of high throughput genomics tools for gene discovery and functional assignment have set the stage for continued rapid progress. This includes our understanding of the genetic characterization of complex traits such as growth, cold and drought tolerance, insect and disease resistance, and so forth. Knowledge of, and access to, genes, will, in the author's view, cease to be a significant hindrance to progress in genetic modification or other biotechnology applications, over the next 10 years. To capture fully the value of this expanding resource, significant investment in bioinformatics infrastructure is needed. Finally, though small steps are appearing in some countries, globally the coordination and complementarity of biotechnology tools and conventional applied tree breeding programmes seem poorly developed.

Perceived trends in the development and application of biotechnology tools are highly varied.

- *Molecular markers*: The creation of new types of markers has recently declined (in the last 5 years), but the development and optimization of specific markers such as microsatellites and SNPs for tree species is currently very active. Markers will probably continue to enjoy increased application in forest genetic studies (diversity and conservation, phylogeography, mating systems) and tree improvement (fingerprinting, paternity analysis, breeding and testing, QTL mapping, MAS, association genetics), though most effort is likely to be concentrated on a few highly valued species. These applications are becoming increasingly commercial in scope.
- *Genomics/proteomics*: This is the most rapidly expanding area of publicly funded research in forest biotechnology, and centres on gene discovery and function elucidation. Whole genome or expressed gene sequences are expected to be largely known for at least one conifer (*Pinus*) and possibly two angiosperms (*Populus* and *Eucalyptus*) within 5 years. Thereafter, functional genomics tools (microarrays, model species comparisons, genetic modification) will probably receive most support. Within 10–15 years, virtually complete physical and genetic maps are likely to identify and locate most of the genes in model conifer and angiosperm tree species and large unigene sets may be available for expression studies. Comparative genomics studies suggest this information will be applicable across species, genera, and even family boundaries. Applications for this information will gradually increase, including technologies complementing traditional tree improvement such as association genetics (identification of superior alleles at known genes) and identification of genes for studies on genetic modification.

- *Micropropagation*: Vegetative propagation will probably expand concomitantly with intensively managed, clonal plantations throughout the world. The development of more intensive plantations seems a driving force in micropropagation biotechnology and genetic modification. Application will certainly increase if and when genetic modification finds acceptance due to the desire to deliver gain through clones in most cases. Research in rooted cutting technology is mature and declining, but use of rooted cuttings will continue to grow. Somatic embryogenesis (SE) research still enjoys significant support, particularly for application in conifers, but some technical hurdles remain to commercializing the technology on a large scale. The use of manufactured seed to deliver embryos may make SE more widely affordable and open the door to more extensive clonal forests for select species, markets and applications. Advances in automation will also be necessary in all steps of the SE process.
- *Genetic modification*: Globally, publicly funded research in genetic modification targeting forest deployment seems to be flat or diminishing, and some scientists have expressed mixed feelings about future applications of the technology. A notable exception is China, which appears to sponsor a robust R&D programme in several institutes and has sanctioned small commercial releases of GM poplar. A relatively few but apparently quite active privately funded organizations in the United States, New Zealand, and possibly elsewhere, are pursuing R&D with commercial deployment of GM trees in their business plans before 2010. Many private forestry products companies are reluctant to engage in research on genetic modification, probably as a function of several factors: consumer unease, public relations risks for companies engaged in the research, unpredictable and costly government regulations, government bans against genetic modification, limitations due to fragmented patent estates and stringent intellectual property rights, etc. Despite these uncertainties, some authors anticipate an increasing use of GM forest trees in the near future (10 years). This will probably occur largely in short-rotation clonal forests, where investment can be rapidly recouped, for specialty applications such as saving species endangered by pests or, more commonly, as a genomics tool for studying gene function. Many of the challenges to securing the benefits of GM crops for the poor, noted for agricultural crops (FAO 2004), will be equally or more difficult to overcome in forestry.

With the exception of some micropropagation tools, research and application of forest biotechnology tools will continue to be primarily sponsored by and used in developed countries. With the shifting of industrial, high-yield forestry to semi-tropical climates, application of biotechnologies in developing countries is likely to follow relatively quickly. The biggest challenge to the forest biotechnology community may be to find ways to enhance growth and yield of non-industrial forests for use in developing countries and countries with economies in transition.

1.8 REFERENCES

- FAO. 2004. *The state of food and agriculture (SOFA) 2003–2004. Agricultural biotechnology: meeting the needs of the poor?* FAO Agriculture Series No. 35. Rome.
- Lambeth, C., Lee, B.-C., O'Malley, D.M. & Wheeler, N.C. 2001. Polymix breeding combined with paternal analysis (PMX/WPA) of progeny: An alternative to full-sib breeding and testing systems. *Theor. Appl. Genet.*, 103: 930–943.

2. Appendixes

2.1 Biotechnology in the forestry sector

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With assistance and inputs from Jean Marc Bouvet, Jean Marc Gion, Yves Prin, Daniel Verhaegen and Philippe Vigneron and Antoine Galiana (for the genetic modification section)

2.1.1 INTRODUCTION

“Biotechnology provides important tools for the sustainable development of agriculture, fisheries and forestry and can be of significant help in meeting the food needs of a growing and increasingly urbanized population,” reads an FAO press release dated 15 March 2000¹². The field of modern biotechnology is indeed often considered as one of the fields of scientific research in which the most rapid advances have been made in recent years.

Several elements can explain the growing interest of forest scientists, conservationists and tree growers in modern biotechnologies. They include 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. Special features of interest to scientists and geneticists include the low level of domestication of forest trees and their rich genetic diversity; their long life cycles, long generation times and late sexual maturity; their spatial requirements; the multiplicity of species and the low degree of heritability of traits of interest, linked to weak juvenile–adult correlations and the importance of genotype–environment interactions. Application of biotechnologies in forests has been seen as a unique opportunity for obtaining new information on the extent, patterns and functioning of tree genetic diversity; and for providing new tree varieties and reproductive materials adapted to changing environmental, social and economic environments (Fenning and Gershenson 2002).

2.1.1.1 Background and methodology

Specific developments in biotechnology in the forestry sector have been addressed in a large number of conferences, meetings, publications, electronic fora and Internet web pages¹³. Owing to this abundant literature, this report, commissioned from CIRAD-Forêt by FAO in December 2003, will not describe the types and classifications of forest biotechnology in detail. It aims instead to fill a gap in global data and statistics on research in and applications of biotechnology for forest trees. Given the scientific and technical potential created by an increasingly accurate knowledge of forest tree species genomic structure, it is important to have an overall picture of the current status of forest biotechnology developments, together with trends and future prospects. The objective of this document is thus to review and summarize research, and the suitability and practical use of biotechnology in the forestry sector, and to provide tentative global analyses.

For the purpose of the study, a simple data set has been developed. The data set gathers major biotechnology activity (i.e. a given technology developed or used in a given country, on a given tree species or variety, by a given laboratory team, for a given purpose [Appendix 2.7.1]).

¹² See: www.fao.org/WAICENT/OIS/PRESS_NE/PRESSENG/2000/pren0017.htm or www.fao.org/biotech.

¹³ See, in particular, Appendix 2.7.2 for Internet sites.

Data originated from (i) systematic searches in CAB Abstracts and associated global scientific databases, (ii) searches on the Internet (including sites of private companies, governmental and non-governmental institutions and linked references, and (iii) personal enquiries, observations and communications. Most significant publications, including those produced by major laboratories and teams, have been included in the data set. The study was mainly conducted between February and September 2004.

The data set included basic fields such as country, type of biotechnology, information source, reference or Internet site, stage of development, species or genera involved and, whenever available, year when the activity was conducted. These fields were considered to be a minimum set of requirements for entering a biotechnology activity in the database. The reference period covers approximately the last 10 years, although more than 75 percent of the data were from between 2000 and 2004. Internet references, however, could not always be dated.

The data set is in no way comprehensive, and some of its limitations reflect the difficulties of such information gathering. The study revealed language limitations (international databases cover only a fraction of the literature in Chinese and Russian, for example). International databases also tend to reflect past research activities, and only a small share of on-going research work. An additional flaw of the data set is related to private (corporate) research and commercial applications, for which public-domain information is generally scarce. Despite its drawbacks, the data set provides a sample (of unknown global representativeness) of materials available in the public domain. No attempt has been made to gather classified information. In total, data on 2 716 activities were collected, and their analysis supports the present report. Data, statistics and conclusions presented in this report should therefore be considered with caution and as general indicators.

2.1.1.2 Outline of the report

A quick review of the literature shows that various classification systems have been used to categorize modern biotechnologies used in forestry¹⁴. In some countries, the term biotechnology refers to genetic modification exclusively. Yanchuk (2001) proposed three main categories: (i) biotechnology employing molecular markers, (ii) biotechnology aimed at enhancing plant propagation and large-scale production of uniform plant material, and (iii) biotechnology for modifying the genome of forest tree species. In the present report, the broad categories include:

1. The characterization of genetic diversity of forestry species, including diversity structure studies, gene flow and human impacts on forest stands: characterization, population genetics and diversity studies.
2. The functional and applied aspects of genetic investigation: genetic mapping, marker-assisted selection (MAS) and genomics (collectively referred to as MMG).
3. Research and applications in vegetative propagation: micropropagation (in the broad sense).
4. The alteration of genomes by insertion of genes: genetic modification.

Individual data (biotechnology entries) were categorized according to the above classification once they had been entered into the data set.

¹⁴ See: www.fao.org/biotech/sector5.asp.

The report presents detailed analyses of the data sets for the categories 1–4 above, and provides separate summaries for biotechnologies excluding genetic modification, and genetic modification technologies.

2.1.2 SUMMARY OF THE STATE OF BIOTECHNOLOGY IN THE FORESTRY SECTOR, EXCLUDING GENETIC MODIFICATION.

The information compiled in the data set of biotechnology activities excluding genetic modification represents 2 196 references (or 81 percent of all activities reported). Activities were reported in 76 countries, broken down by regions as follows: 39 percent of the activities were reported in Europe, 24 percent in Asia, 23 percent in North America, 6 percent in Oceania, 5 percent in South America, 3 percent in Africa and less than one percent in the Near East (Figure 2.1.1A). Both developed countries (24 countries, representing 68 percent of biotechnology activities) and developing countries and countries in transition (52 countries, or 32 percent of activities) were represented (Figure 2.1.1B). Developing countries and countries in transition were mainly represented by India (27 percent of these countries' activities), China (17 percent), Brazil (7 percent), South Africa (5 percent) and Malaysia (4 percent). Three countries (India, China and Brazil) accounted for 52 percent of all biotechnology work reported in developing countries and countries in transition.

Species surveyed belonged to 142 botanical genera. Sixty-two percent of the information collected in the database regarded research carried out on less than six genera including *Pinus* (20 percent of biotechnology activities excluding genetic modification), *Eucalyptus* (11 percent), *Picea* (9 percent), *Populus* (9 percent), *Quercus* (7 percent) and *Acacia* (6 percent) (Figure 2.1.2). Just four genera (*Pinus*, *Eucalyptus*, *Picea* and *Populus*) account for almost half of the compiled biotechnology activities excluding genetic modification.

Work was found to be relatively evenly spread between the three main categories of biotechnology categories apart from genetic modification: characterization of tree species genetic diversity represented 32 percent of biotechnology activities, MMG 26 percent, and micropropagation 42 percent (Figure 2.1.3A). Differences were more marked when tree genera were considered (Figure 2.1.3B). The forestry sector appears to have rapidly adopted markers developed for agricultural crops (Figure 2.1.4). Isozymes and random amplified polymorphic DNAs (RAPDs) have been widely used for genetic diversity description although the present trend seems to favour microsatellites (nuclear and chloroplast) and amplified fragment length polymorphisms (AFLPs). Driven by research on genomics, expressed genome banks (ESTs [expressed sequence tags]) are being widely developed.

The majority of the work reported is still mainly at the experimental stage in the laboratory. Genetic diversity characterization has less than one percent of its reported activities in the field, MMG 2.5 percent and micropropagation 5 percent (Figure 2.1.5). Field tests are still mainly geared to supporting laboratory research. These results possibly reflect the origin of the information in the data set. While research activities in the public sector are relatively easy to collect, especially through international research storage databases, information on commercial applications is generally restricted and incomplete.

Commercial applications of micropropagation are, however, generating increasing interest. The potential is huge although, up to now, only several thousand hectares seem to have been established globally using micropropagated materials.

In South America, particularly in Brazil, some companies are reported to be integrating micropropagation into the clonal propagation process: micropropagation is used to 'store' clones in mother blocks (gene banks) in the laboratory as potential sources of responsive nursery stock plants for large-scale mass propagation. The use of rooted cuttings has allowed the propagation costs to be lowered significantly.

Great expectations have been raised about the possible contribution of biotechnology to tree selection and breeding, and its commercial applications. Genomics and proteomics should greatly help breeders in tree selection, in particular in the identification of traits of interests. However, it remains difficult to predict when new forest tree varieties selected with biotechnology tools will become available on the market. Although genetic diversity characterization started some 30 years ago, very limited large-scale commercial application has yet been reported in forest tree genetic resources conservation and management.

Figure 2.1.1A. Distribution of reported forest biotechnology activities (excluding genetic modification) by world region

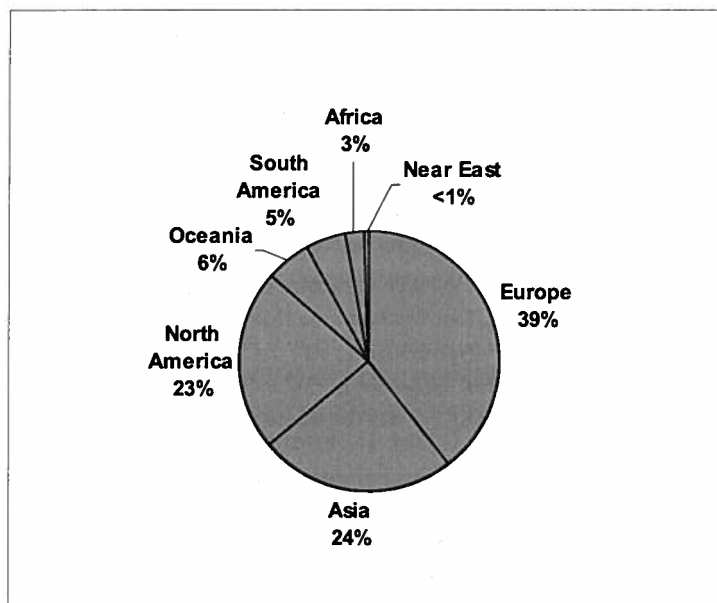


Figure 2.1.1B. Distribution of reported forest biotechnology activities (percent of activities in the data set, excluding genetic modification) by country (for the 15 countries most represented, making up 77 percent of the data set of entries excluding genetic modification)

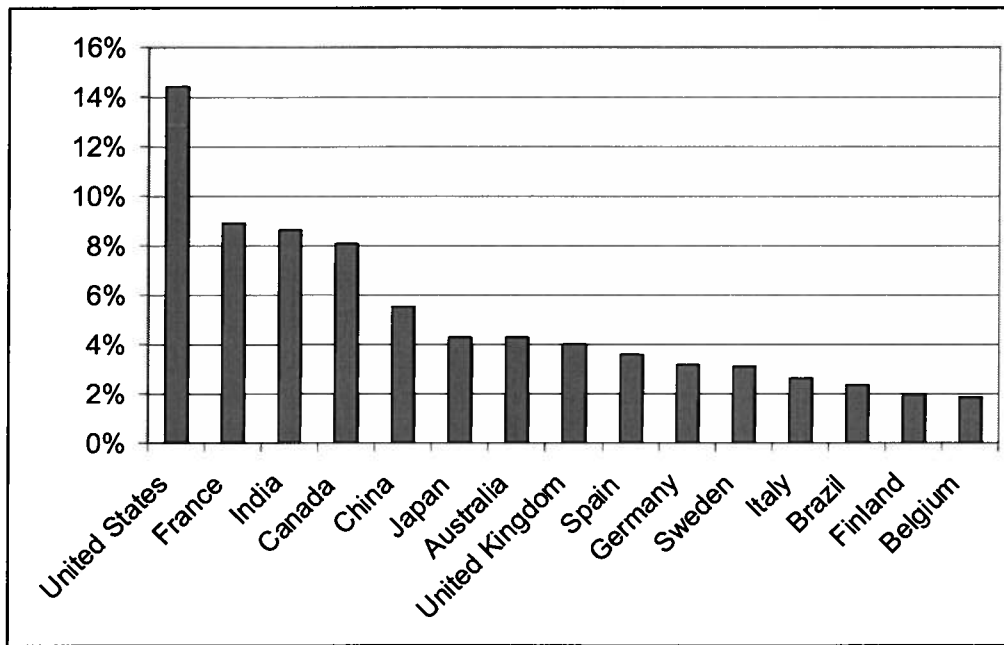


Figure 2.1.2. Distribution of reported forestry biotechnology activities, excluding genetic modification, by genus

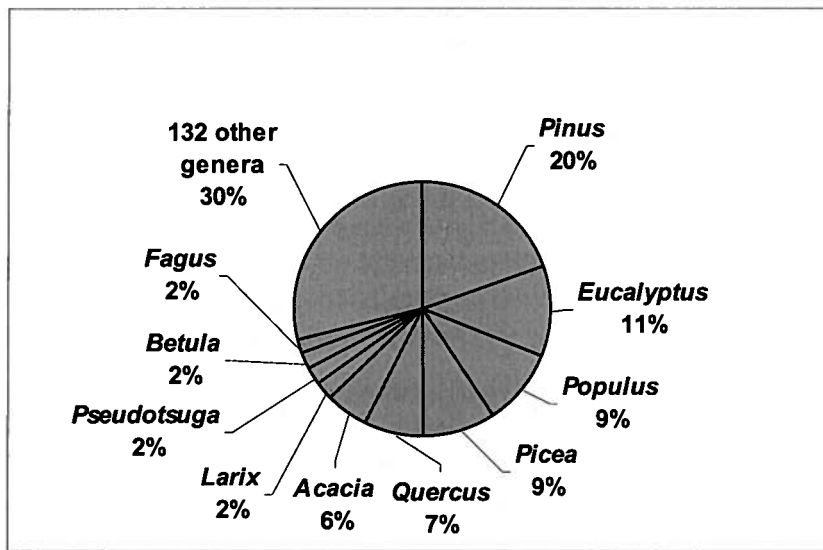


Figure 2.1.3A. Distribution of biotechnology activities, excluding genetic modification, by broad category (genetic diversity characterization; mapping, marker-assisted selection and genomics [MMG]; and micropropagation)

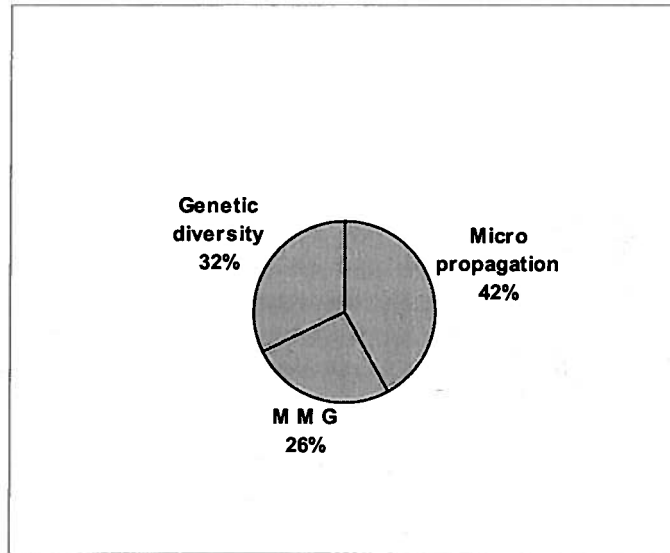


Figure 2.1.3B. Distribution of biotechnology activities, excluding genetic modification, (genetic diversity characterization; mapping, marker-assisted selection and genomics [MMG]; and micropropagation) by number of tree genera

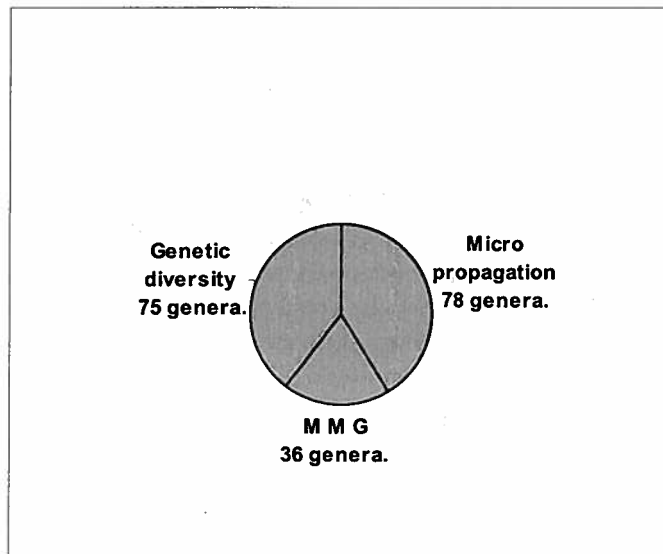


Figure 2.1.4. Distribution of molecular markers used in forest biotechnology activities, excluding genetic modification

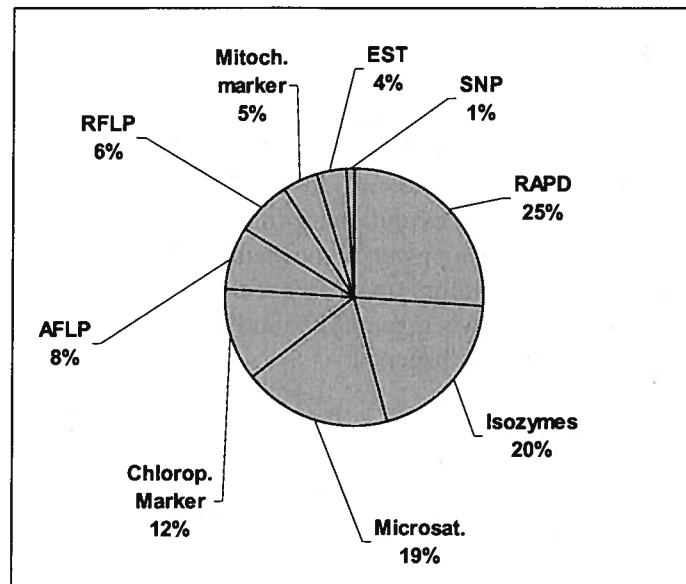
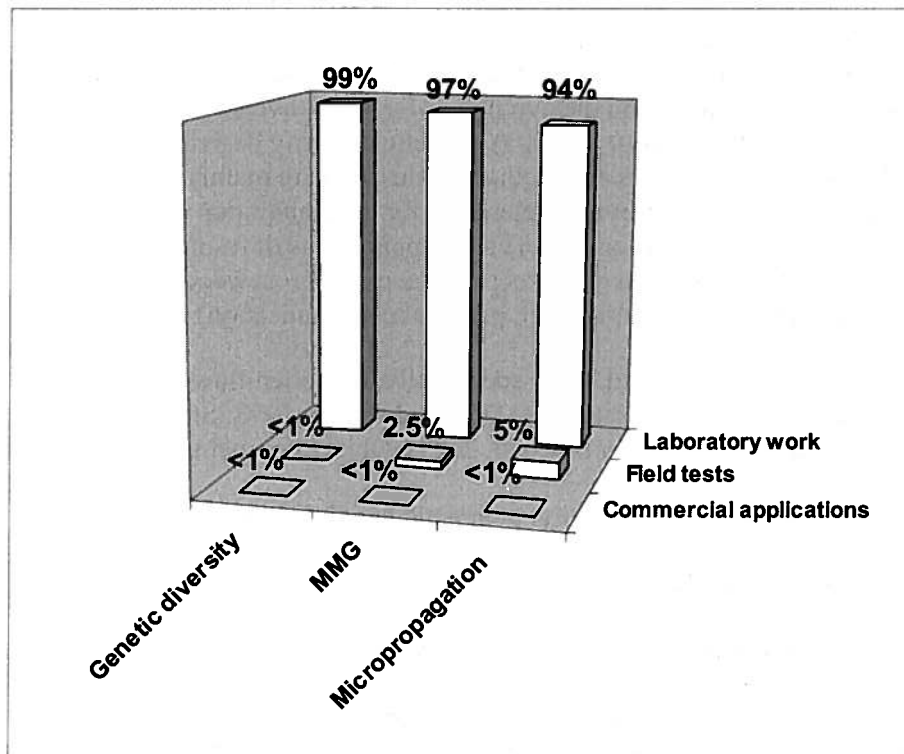


Figure 2.1.5. Distribution of reported forest biotechnology activities, excluding genetic modification, by category and applications (laboratory studies, field trials and commercial deployments)¹



¹MMG: mapping, marker-assisted selection and genomics.

2.1.3 CHARACTERIZATION OF GENETIC DIVERSITY: POPULATION GENETICS AND DIVERSITY STUDIES

2.1.3.1 Introduction

Genetic diversity is sometimes considered to be the invisible dimension of biological diversity. The present structure of genetic diversity is the result of the evolutionary history of the species exposed to natural selection pressures in variable environmental conditions. Natural selection at the local level is an evolutionary force opposed to gene flow. The combination of the two forces creates a powerful mechanism for maintaining within-species diversity. The use of molecular descriptors (markers) of the genome has allowed the measurement of genetic variation between genotypes and within/between populations, as well as the effectiveness of seed and pollen dispersal.

Global review

Studies carried out on forest tree genetic structure and on reproductive regimes use RAPD and AFLP markers, as well as nuclear markers and microsatellites, to increase understanding of the relationship between genome variations and genetic diversity. Molecular markers, especially neutral markers, provide an important tool for studying the structure of populations. However, experience shows that the resulting information on diversity, for example in ecology or taxonomic studies, is not always a good indicator of the patterns of variation in traits subject to selection pressures. A complementary approach is often required, consisting of studying the genetic diversity in specific regions of the genome. For example, SNPs (single nucleotide polymorphisms) are used to study the impact of evolutionary forces on the variability of allelic forms of a given gene, and their phenotypic expression.

Work reported on tropical trees often involves studies of the impacts of tree harvesting and fragmentation on species' reproductive patterns and changes in genetic diversity at the population level (Chase *et al.* 1996; Dawson *et al.* 1997; Dick 2001; Dutech *et al.* 2002; White *et al.* 2002). In temperate species, several studies focused on gene flow in natural populations (*Quercus*, *Cedrus*) and the evolution of genetic diversity under forest management (Streiff 1998; Fady *et al.* 2003). Genetic diversity descriptions are numerous and diversified in terms of species and methodologies, and are mainly aimed at verifying some theoretical aspects, or at providing elements for the elaboration of conservation strategies (Petit *et al.* 1998; Newton *et al.* 1999). Applications of results at operational level have been infrequently reported as yet, except in the case of rosewood (*Aniba rosaeodora*) and sandalwood (*Santalum*) (J. M. Bouvet, personal communication).

On-going studies on gene flow and, more specifically, on pollen flow, are based on the use of molecular markers, mainly microsatellites (Dow and Ashley 1998; Streiff 1998; Chaix 2003) and on paternity analysis (Gerber *et al.* 2000, 2003). Objectives include:

- estimating and studying the evolution of genetic variability over time;
- assessing effective population sizes;
- studying biological mechanisms of reproduction, either in natural populations or in improved populations (in selection programmes);
- studying pollen pollution in tree seed production areas (seed stands or seed orchards) or in the context of GM trees.

Data set analysis

Most of the activities compiled in the data set have the overall objective of conserving and managing forest genetic resources in a sustainable way, and providing a better understanding of genetic diversity and evolution through the study of gene flow. This includes analyses of the consequences of human practices on the evolution of forest tree genetic diversity, as well as development and validation of theoretical models of population genetics.

Work has been reported from all regions of the world (Figure 2.1.6A) including Europe (44 percent), followed by North America (22 percent), Asia (19 percent), South America (6 percent), Oceania (6 percent) Africa (3 percent) and the Near East (less than one percent). When one considers the origin of the species being studied, the situation shows broadly similar patterns (Figure 2.1.6B).

At least 99 genera are being worked on: *Pinus* (19 percent), *Quercus* (12 percent), *Eucalyptus* (8 percent), *Acacia* (7 percent) and *Picea* and *Populus* (6 percent) are the most frequently cited genera (Figure 2.1.7). Reported objectives of genetic diversity characterization studies (Figure 2.1.8) include: evaluation of genetic diversity (57 percent), gene flow studies (15 percent), fingerprinting (12 percent), and conservation (6 percent).

Figure 2.1.6A. Distribution of forest genetic diversity characterization activities by region where the work is/was carried out

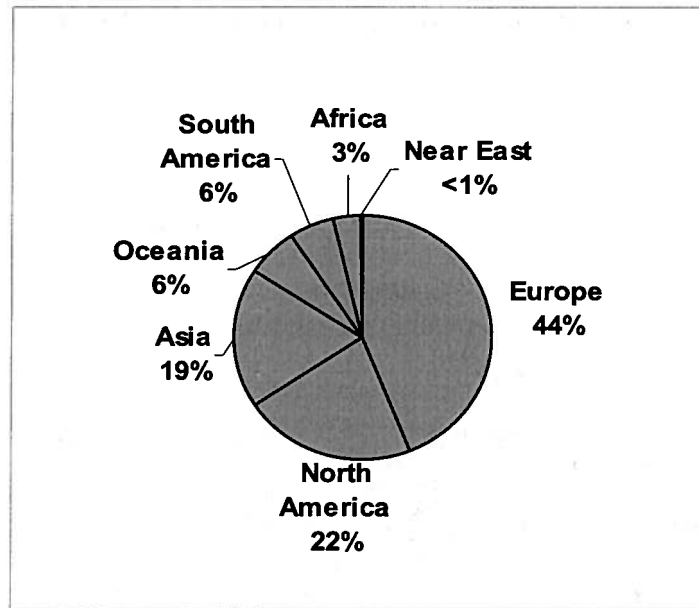


Figure 2.1.6B. Distribution of forest genetic diversity characterization activities by region of origin of the species studied; 'unknown' relates to undocumented sources, or to hybrid species, varieties and clones

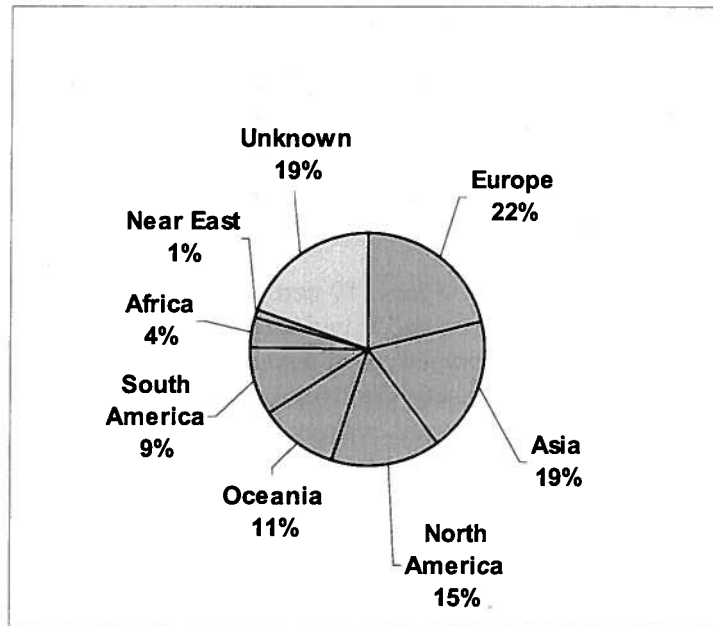


Figure 2.1.7. Distribution of genetic diversity studies by forest tree genus

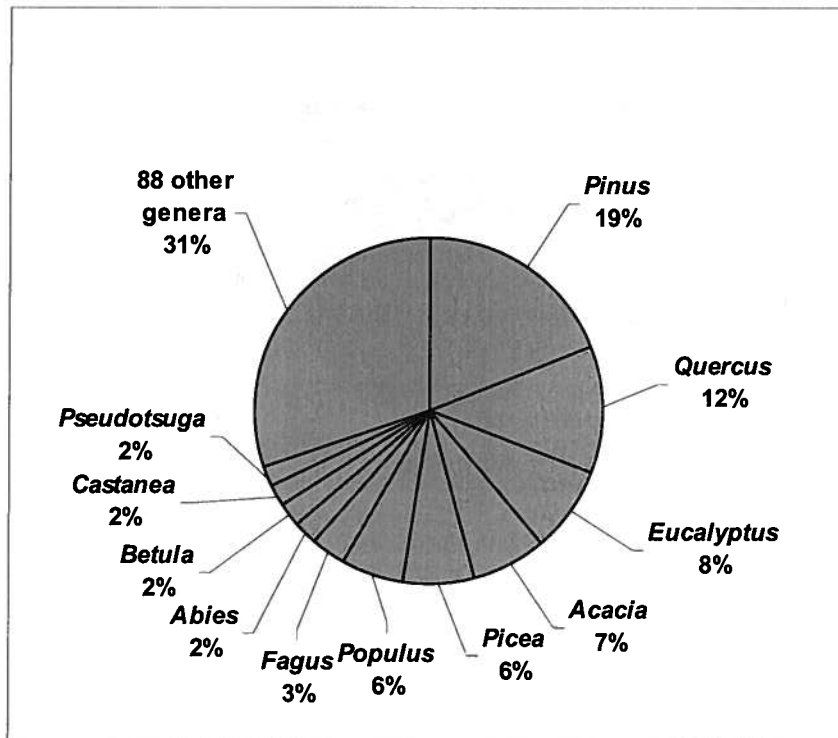
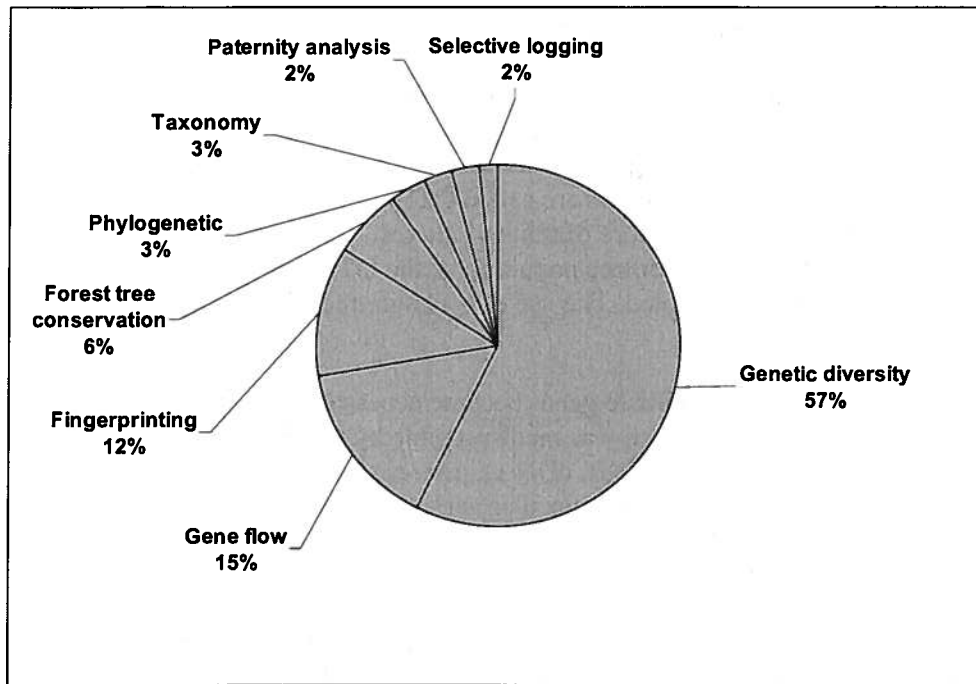


Figure 2.1.8. Main objectives of reported studies on forest tree genetic diversity

2.1.3.2 Genetic maps, marker-assisted selection and genomics

Introduction

For a long time, forest tree seed has been collected from natural populations and, less frequently and only recently, from artificial stands (including planted stands or seed orchards). Since genetic information was lacking, most collections were based on phenotypic selection. At the beginning of the twentieth century, more attention started to be given to seed origin; numerous arboreta were established around the world and international seed exchange, documented to a greater or lesser extent, increased significantly.

Later, growing awareness of the importance of intraspecific variation led to a more systematic exploration of the natural distribution of species of proven or perceived interest. With the assistance of national and international organizations, comprehensive explorations were made in the 1960s and 1970s for several genera and species including *Tectona grandis*, *Gmelina arborea*, *Eucalyptus*, *Pinus* and *Acacia*. Seeds collected were used in provenance tests, which in turn provided basic information and new materials for seed production areas or tree seed orchards. Nowadays, biotechnology tools allow more in-depth exploration of within-species genetic diversity, through the study of genomic variability.

Use of biotechnology in tree breeding and selection

Marker-assisted selection (MAS) has given further impetus to tree breeding and selection. Molecular markers are genetically linked to a given allele on a given locus and can therefore be used to predict the presence of the allele with great accuracy. The first markers developed (RAPDs, RFLPs [restriction fragment length polymorphisms] AFLPs and microsatellites) were neutral in the sense that their function, if they had any, was unknown.

They have provided a way of estimating genetic diversity and allowed comparison between individuals in different growth and development conditions. They have also been used in profiling to tag and then single out individual trees.

Co-segregation of RAPD and AFLP type markers has allowed the construction of genetic maps. Genetic maps can be used to assess the degree of evolutionary relatedness of a number of species and identify the zones where gene variations are statistically linked to the variability of quantitative traits. These zones are called QTLs (quantitative trait loci). QTL research focuses on the genetic architecture of traits of interest. However, QTL research results are not easily transferred to other tree populations: the QTLs identified so far are closely linked to the populations sampled. The use of QTLs in tree selection is thus increasingly questioned.

For the above reasons, the use of candidate genes became necessary for applying MAS to forest trees. The candidate gene approach was made possible by the development of gene search methods (AFLP, EST banks and SSR, cDNA), transcriptomics and proteomics. In order to use those genes in selection, their molecular variability must be linked to the variation of the targeted trait of interest. This requires associating appropriate field experiments with genetic mapping.

Proteomic analysis offers an important field of basic research that many research teams are willing to develop. Proteomic studies can find applications in such fields as biotic and abiotic stress response, effects of genetic mutations, gene expression regulation, QTL validation and genetic variability (Plomion 2000; Pilate *et al.* 2002).

Information technology is an important asset since work on biotechnology requires the handling of massive catalogues of genes, transcripts and proteins. The use of bioinformatics, in particular, to transfer results from one given (model) species to other species, in variability studies, fingerprinting or tree breeding, is particularly valuable.

Database analysis

Mapping, MAS and genomics (MMG) studies are found in all regions of the world except the Near East (Figure 2.1.9). Europe and North America represent 43 percent and 34 percent, respectively, of the total MMG activities, followed by Asia (11 percent), Oceania (8 percent), South America (2 percent) and Africa (2 percent). The work documented in Africa (South Africa and Congo) and South America (Brazil) relates to *Eucalyptus* almost exclusively.

MMG activities are being currently applied to approximately 40 genera (Figure 2.1.10) among which *Pinus* (32 percent), *Populus* (18 percent), *Eucalyptus* (12 percent) and *Picea* (9 percent) are the most studied.

Objectives of the research work (Figure 2.1.11) include wood properties improvement in relation to lignin composition (57 percent), abiotic resistance for cold or drought stress (20 percent), genetic diversity (8 percent), growth rate (6 percent), flowering (5 percent) and phytosanitary aspects of biotic resistance (4 percent). The work is being pursued mainly on expressed gene products (53 percent), genetic map construction (27 percent), gene candidate research (12 percent) and QTLs (6 percent). More recent proteomics research constitutes 2 percent of the activities documented (Figure 2.1.12).

Figure 2.1.9A. Distribution of research related to mapping, marker-assisted selection and genomics (MMG) in forest tree species by region where the work is being carried out

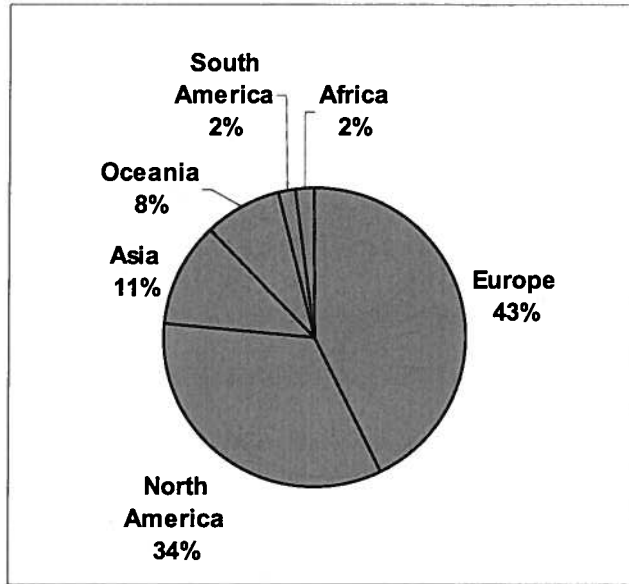


Figure 2.1.9B. Distribution of research related to mapping, marker-assisted selection and genomics (MMG) in forest tree species by region of origin of the species studied

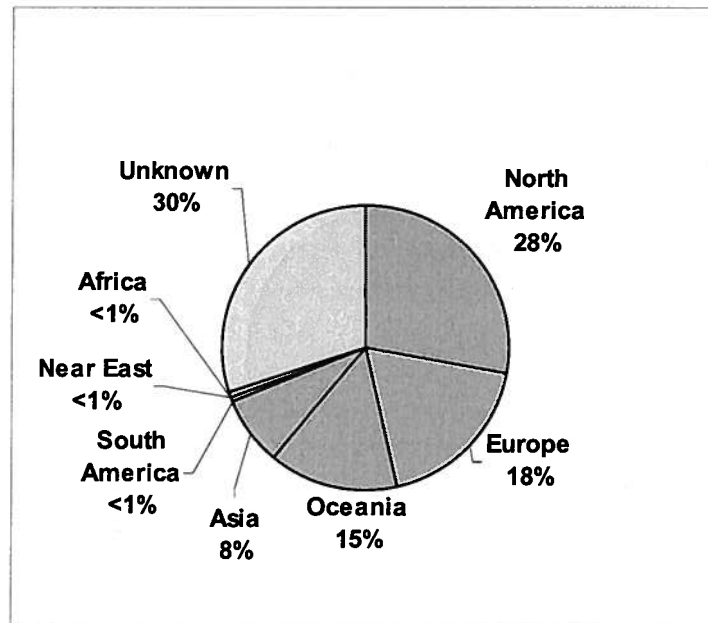


Figure 2.1.10. Distribution of mapping, marker-assisted selection and genomics (MMG) activities, by genus

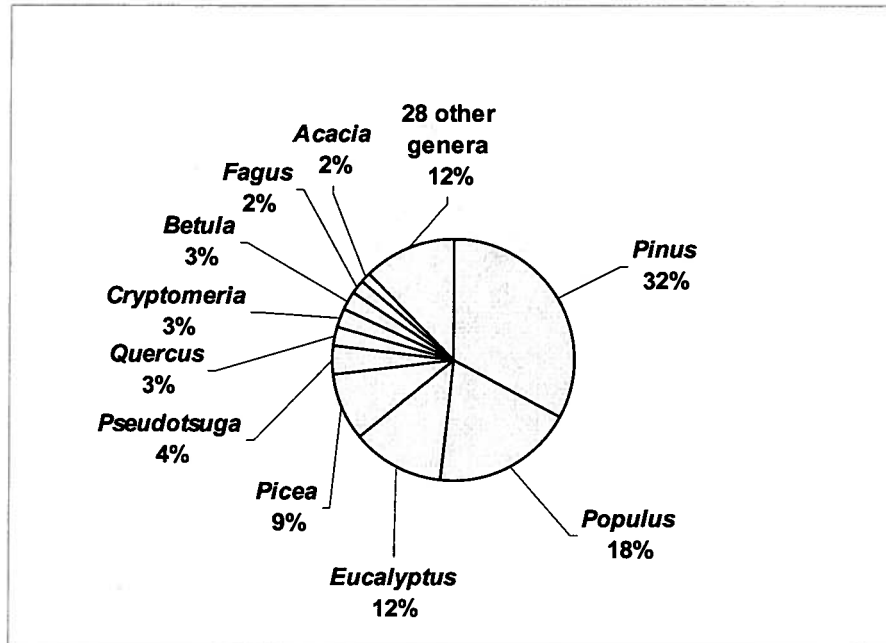


Figure 2.1.11. Distribution of the main traits targeted in marker-assisted selection studies

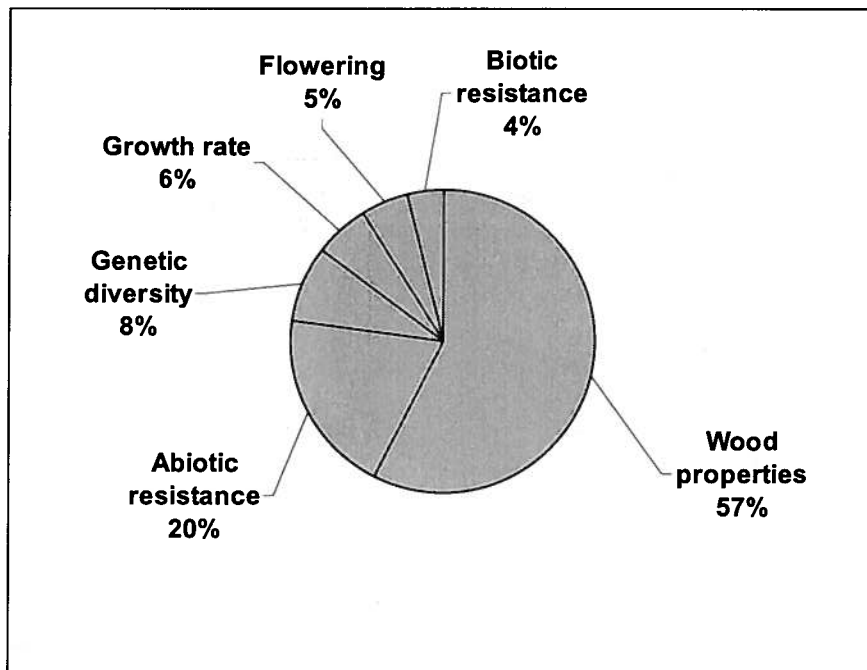
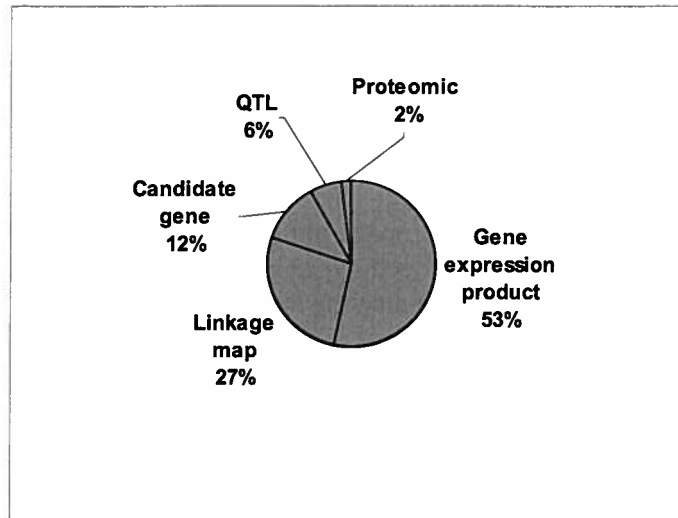


Figure 2.1.12. Methodological approaches associated with mapping, marker-assisted selection and genomics (MMG) in forestry



Genetic mapping

The construction of genetic maps is a prerequisite for MAS. Genetic mapping is currently being developed for more than 25 forest species with the main objective of making maps denser by positioning a larger number of markers. Genera and species include:

- *Eucalyptus* (Grattapaglia & Sederoff 1994; Verhaegen & Plomion 1996; Byrne *et al.* 1995; Marques *et al.* 1998; Brondani & Grattapaglia 1999; Gion *et al.* 2000; Myburg *et al.* 2003), including *E. camaldulensis*, *E. globulus*, *E. grandis*, *E. nitens*, *E. tereticornis* and *E. urophylla*;
- *Populus* (Cervera *et al.* 2001; Yin *et al.* 2002) in particular *P. deltoides*, *P. nigra* and *P. trichocarpa*;
- *Picea* (Plomion 2000) including: *P. abies*, *P. glauca* and *P. mariana*;
- *Pinus* (Kubisiak *et al.* 1995; Plomion 2000) including *P. brutia*, *P. edulis*, *P. elliottii*, *P. palustris*, *P. pinaster*, *P. radiata*, *P. strobus*, *P. sylvestris* and *P. thunbergii*;
- other species (Plomion 2000; Butcher *et al.* 2002; www.pierroton.inra.fr/genetics/Quercus): *Acacia mangium*, *Cryptomeria japonica*, *Larix leptolepis* [= *L. kaempferi*], *Pseudotsuga menziesii*, *Quercus robur* and *Taxus brevifolia*.

Several plant functional genomics programmes have undertaken linkage map construction, and significant developments in this area have been made with some forest trees. The search for linkages between coding regions and traits linked to wood quality has allowed some gene candidates to be identified (especially in lignin synthesis pathways) although they are not yet used in routine selection programmes. There are noteworthy EST bank development programmes underway for several species in several genera, including *Pinus*, *Populus*, *Eucalyptus* and *Betula*. The gene candidate approach has been used in a dozen species of *Pinus* (Plomion *et al.* 2000; Whetten *et al.* 2001; Brown *et al.* 2003; Garnier-Géré *et al.* 2003; Le Dantec *et al.* 2003; McMillan 2004), at least five *Eucalyptus* spp. including hybrids (Gion *et al.* 2000; Thamarus *et al.* 2002; De Melis *et al.* 1998; Plomion *et al.* 2003; Kirst *et al.* 2004; Ranik *et al.* 2004) and in three *Picea* spp. (McDougall 2000).

More recently, research work has been undertaken on other traits such as water stress tolerance (Dubos and Plomion 2003; Dubos *et al.* 2003). Proteomic studies have been initiated on wood properties for a limited number of species: *Pinus pinaster*, *Eucalyptus gunnii*, *Populus* spp. and *Picea sitchensis*.

2.1.3.3 Micropropagation

Introduction

In vitro vegetative propagation or micropropagation is aimed at cloning superior individuals or at 'bulk' (in mixture) propagating new genotypes with high genetic potential but available in limited quantities (such as materials obtained by controlled pollination).

Tree species can be micropropagated by microcuttings or by somatic embryogenesis.

Micropropagation methods

Micropropagation by microcuttings consists of mass producing vegetative copies of desired genotypes by either axillary or adventitious budding. In the latter case, differentiated cells, usually from superficial tissues, must undergo a de-differentiating process before new shoot formation is initiated. Ultimately, production of independent and self-sufficient individuals is completely dependent on the *de novo* formation of an adventitious root system.

Somatic embryogenesis, or production of embryos from somatic cells, is in fact a cloning technique, as opposed to zygotic embryogenesis in which germinal cells give rise to seedlings that are all genetically different. The process of somatic embryogenesis derives usually from callus formation induced by applying cytokinetic or auxinic exogenous growth regulators to very juvenile plant tissues. In the most favourable situations, some undifferentiated cells of these calli can evolve into somatic embryos characterized, similarly to zygotic embryos, by a shoot-root bipolar structure. This basically distinguishes somatic embryogenesis from microcuttings consisting first of a shoot from which an adventitious root must subsequently develop.

The majority of GM trees is likely to be used in the form of clonal materials and will need to be vegetatively propagated. In the case of conifers, somatic embryogenesis, especially when derived from a single cell, seems the most suitable regeneration and propagation technique. In broad-leaved species, vegetative propagation of GM materials is likely to use a combination of micropropagation and rooted stem cuttings, at least in the beginning.

Database analysis

Asia accounts for 38 percent of documented activities in forest tree micropropagation, followed by Europe (33 percent), North America (16 percent), South America (7 percent), Africa (3 percent), Oceania (2 percent) and the Near East (one percent) (Figure 2.1.13A). Sixty-four countries active in this field have been identified. A closer look shows that species from Asia (27 percent) and Europe (21 percent) predominate (Figure 2.1.13B), which may suggest that the activities in Asia are concentrated on indigenous species, while *Eucalyptus* spp. are micropropagated in all continents. Species originating from South America and Africa are almost absent from the data set. Micropropagation activities seem to take place mainly in countries with significant tree planting programmes.

Most micropropagation research activities are at a very advanced stage. Germplasm and protocols are likely to be available for large-scale deployment soon, if not already operational, for species and genera including:

- *Anogeissus* in India (Saxena and Dhawan 2001);
- *Acacia mangium* and *A. mangium* × *A. auriculiformis* in Malaysia (Galiana *et al.* 2003), Indonesia and Vietnam (O. Monteuis, personal observation);
- *Eucalyptus* in Vietnam, India (O. Monteuis, personal observation), Australia (Watt *et al.* 2003) and South America (Levis W. Handley, personal communication);
- *Pinus* in Canada and New Zealand (Lelu and Thompson 2000), and *Pinus taeda* in the USA (Levis W. Handley, personal communication);
- *Tectona grandis* in Brazil, Thailand and Indonesia (O. Monteuis personal observation) and Malaysia (Goh *et al.* in press).

Micropropagation techniques have been tested on at least 82 forest tree genera (many of them of proven or potential interest in forest plantations). Research work has been devoted to *Pinus* (13 percent), *Picea* (13 percent), *Eucalyptus* (11 percent), *Acacia* (7 percent), *Quercus* (6 percent), and *Populus*, *Larix* and *Tectona grandis* (4 percent each) (Figure 2.1.14). The first five genera account for 50 percent of all activities carried out in forest tree micropropagation. Somatic embryogenesis accounts for 65 percent of activities in micropropagation followed by cell/tissue culture (17 percent), micropropagation by microcuttings in *in vitro* conditions (13 percent), cryopreservation/conservation (4 percent) and embryo rescue (one percent) (Figure 2.1.15).

Figure 2.1.13A. Distribution of micropropagation activities by region of activity

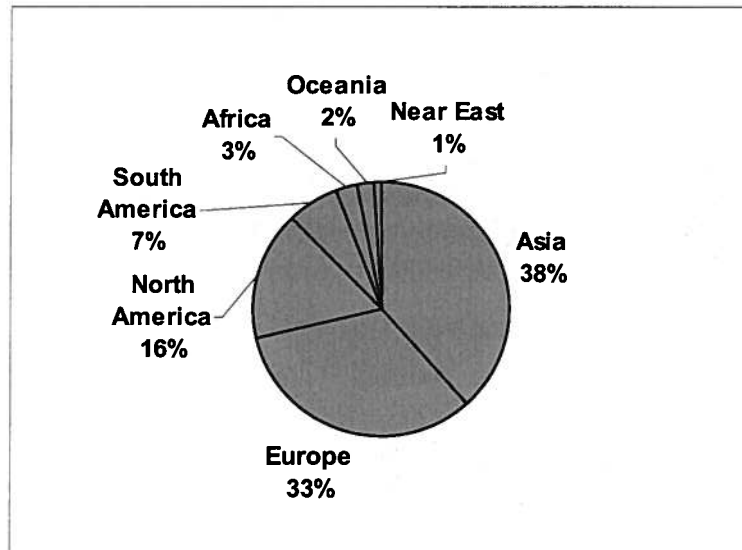


Figure 2.1.13B. Distribution of micropropagation activities by region of origin of the species studied

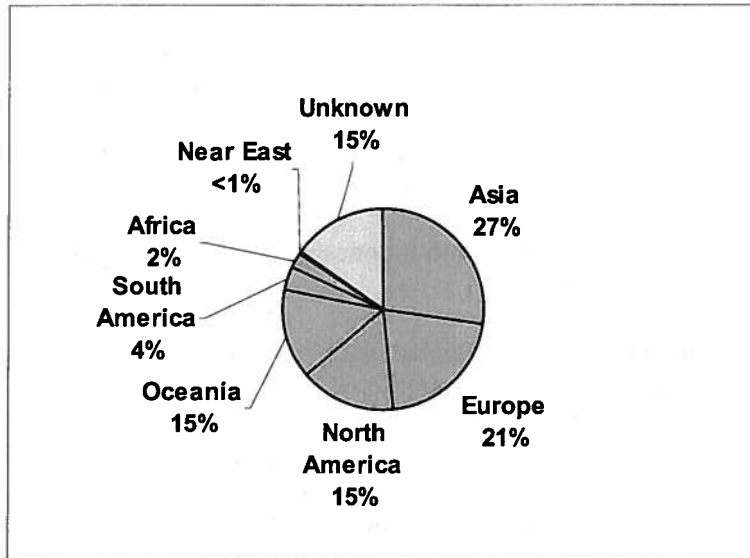


Figure 2.1.14. Distribution of micropropagation activities by genus

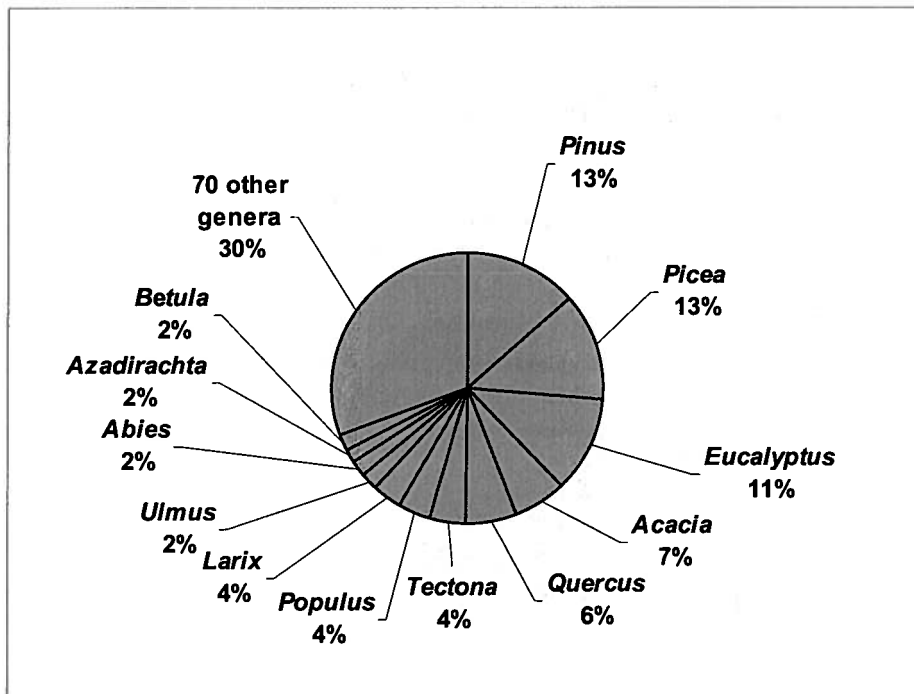
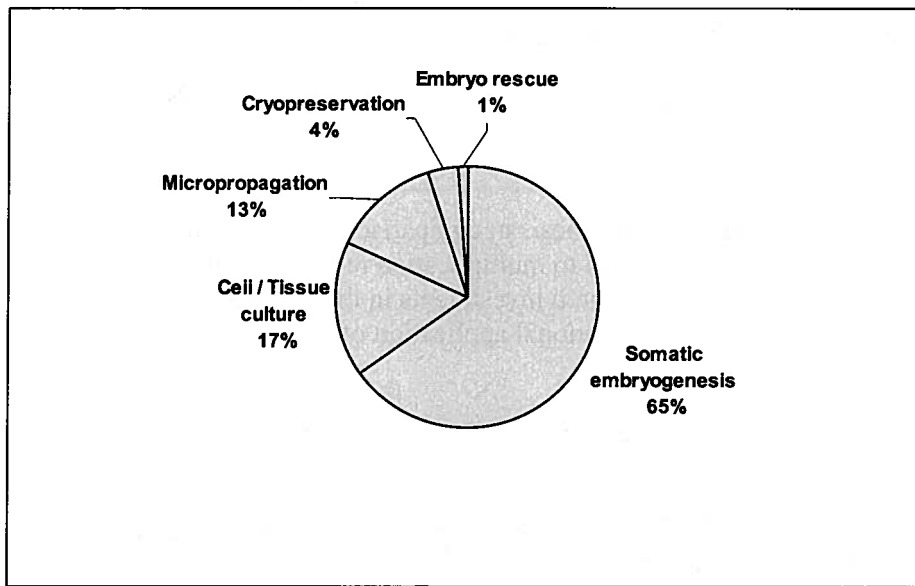


Figure 2.1.15. Categories of biotechnologies used in forest tree micropropagation

Micropropagation by microcuttings is carried out on more than twenty species including:

- *Populus alba*, *P. deltoides*, *P. tremula* and *Populus* hybrids in Germany and India (Cornu 1994), Spain (Bueno *et al.* 2003) and Lithuania (Kuusiene 2002);
- *Eucalyptus camaldulensis*, *E. globulus*, *E. grandis*, *E. nitens*, *E. tereticornis* and *E. urophylla* in South Africa, Spain and Portugal (Watt *et al.* 2003), India (Watt *et al.* 2003; Nadgauda in press), Vietnam and Thailand (O. Monteuis personal observation), and Australia (Bandyopadhyay *et al.* 1999);
- *Acacia mangium*, *A. melanoxylon*, *A. mangium* × *A. auriculiformis* in Malaysia and South Africa (Galiana *et al.* 2003; Monteuis *et al.* 2003; Quoirin 2003);
- *Tectona grandis* in India (Bonga and Von Aderkas 1992; Nicodemus *et al.* 2001; Nadgauda in press), Vietnam, Brazil and Indonesia (O. Monteuis personal observation), Thailand (Kjaer *et al.* 2000), Costa Rica (Schmincke 2000), Malaysia (Monteuis & Goh 1999; Goh & Monteuis 2001; Goh *et al.* in press) and Australia (Monteuis, personal observation) .;
- *Larix* in Canada and France (Charest 1996; Lelu and Thompson 2000);
- *Pinus radiata*, *P. taeda* and *P. pinaster* in France (Dumas and Monteuis 1991; Monteuis and Dumas 1992), New Zealand¹⁵ and the United States (Rahman *et al.* 2003);
- *Pseudotsuga menziesii* in the United States (Ritchie *et al.* 1994);
- *Sequoia sempervirens* and *Sequoiadendron giganteum* in France (Bonga and Von Aderkas 1992; O. Monteuis personal observation) and Germany (www.cnr.berkeley.edu/~jleblanc/www/Redwood/rdwd-Micropro.html);
- *Anogeissus latifolia* and *A. pendula* in India (Saxena and Dhawan 2001);
- *Betula pendula* in Norway (Saebo *et al.* 1995) and Finland (Cornu 1994);

¹⁵ See: www.forestresearch.co.nz.

- *Paulownia fortunei* in Australia (O. Monteuis personal observation);
- *Platanus acerifolia* in China (Liu and Bao 2003);
- other species include *Gmelina arborea*, *Artocarpus chaplasha*, *A. heterophyllus*, *Azadirachta indica* and *Elaeocarpus robustus* in Bangladesh (Sarker *et al.* 1997; Roy *et al.* 1998).

The advantages of somatic embryogenesis in comparison with micropropagation by microcuttings, especially with regard to multiplication rate and genetic modification applications, explain the major research investments in the technique. However, there are still serious obstacles to large-scale operational application of somatic embryogenesis to forest trees, for example:

- Only some species and, within these species, only some genotypes can produce somatic embryos.
- Success has been obtained, with few exceptions, mainly with juvenile tissues coming for instance from immature zygotic embryos.
- There are risks that somaclonal variation may decrease the value of the genotypes produced by somatic embryogenesis, resulting in a considerable waste of time, material and money. True-to-typeness, particularly, may remain a problem for certain genotypes, and efforts are still needed for optimizing this technique to make it more reliable, especially when using mature selected genotypes.

Although activities on somatic embryogenesis have been reported in all regions of the world, it seems that countries with conifer planting programmes show a stronger interest in this micropropagation technique:

- *Larix* in Canada (and France) (Charest 1996; Lelu and Thompson 2000);
- *Picea abies*, *P. glauca*, *P. mariana* and *P. sitchensis* in Canada, France and Ireland (Thorpe 1995; Charest 1996; Park *et al.* 1998; Lelu and Thompson 2000; Park 2002; Sutton 2002; Lelu-Walter and Harvengt 2004);
- *Pinus banksiana*, *P. patula*, *P. radiata*, *P. strobus* and *P. taeda* in Canada (Park 2002), South Africa (Jones 2002), New Zealand (Lelu and Thompson 2000), the United States (Jones 2002; Sutton 2002) and Chile (Lelu-Walter and Harvengt 2004);
- *Pseudotsuga menziesii* in the United States and Canada (Lelu and Thompson 2000, Sutton 2002);
- *Eucalyptus globulus* (Pinto *et al.* 2002), *E. grandis* and *E. dunnii* (Watt *et al.* 1999);
- *Gmelina arborea*, *Artocarpus chaplasha*, *A. heterophyllus*, *Azadirachta indica* and *Elaeocarpus robustus* in Bangladesh (Sarker *et al.* 1997; Roy *et al.* 1998).

Investments in vegetatively micropropagated species of commercial interest have resulted in successful large-scale applications in several countries in Europe, North America, Asia and Oceania. Unfortunately, there is a lack of basic information on areas planted with forest vitroplants.

2.1.4 GENETIC MODIFICATION IN THE FORESTRY SECTOR

2.1.4.1 Introduction

The first regeneration of a genetically modified (GM) forest tree was achieved in 1986 in *Populus*. Since then, the genus has become a model for genetic modification and related tree biotechnology studies. The first attempt to genetically modify a conifer (*Larix*) was reported in 1991 (Huang *et al.* 1991). Introducing targeted genes into the genome of a forest tree is a way to obtain GM plants. It is also a basic research tool for a better understanding of gene functioning in woody plants.

Review of objectives and methodologies

Transformation methods consist of inserting into the genome of the host plant a mutated gene or a gene from another organism either by microinjection/projection (direct transformation), or through a vector such as *Agrobacterium tumefaciens* (indirect transformation).

Most successful work on genetic modification of forest trees species genomes so far has been obtained by using juvenile material, often from explants produced from early germinations tissues which have much higher regeneration capacities than older material. Successful genome modification reports of adult selected plant material are very rare, except in poplars precisely because of its greater capacity to regenerate.

Traits subject to genetic modification are discussed below.

Herbicide resistance

A first method has consisted in introducing a mutated version of the gene encoding the enzyme target for various herbicides: glyphosate for *Populus alba* × *P. grandidentata*, *P. trichocarpa* × *P. deltoides*, *Eucalyptus grandis*, *Larix decidua* and *Pinus radiata* (the herbicide blocks the synthesis of tryptophan, tyrosine and phenylalanine), or chlorosulfuron for *Populus tremula* and *Pinus radiata* (the herbicide blocks the synthesis of leucine, isoleucine and valine).

A second and more frequently used strategy consists of introducing a microbial gene encoding an enzyme for the detoxification of the herbicide, and has been applied to *Populus alba*, *P. alba* × *P. tremula*, *P. tremula* × *P. alba*, *P. trichocarpa* × *P. deltoides* and *Eucalyptus camaldulensis* (Ho *et al.* 1998; Moralejo *et al.* 1998) and *Pinus radiata* and *Picea abies* (Bishop-Hurley *et al.* 2001).

Insect resistance

The first strategy consists of using microprojectiles to insert a gene encoding an endotoxin which binds to the receptors in the intestine of Lepidoptera, Coleoptera and Diptera, lysing the organ and killing the insect. This was done in *Populus alba* × *P. grandidentata*, *P. tremula* × *P. tremuloides* and *Picea glauca*. GM *Populus* trees and *Pinus radiata* expressing the *Bacillus thuringiensis* endotoxin 'Bt' have been obtained.

A second transformation method is based on the introduction of a gene coding for a protease inhibitor that modifies insect digestion, causing the death of the pest. Studies used potato gene *pin2*, a protease inhibitor introduced into *P. alba* × *P. grandidentata* through *A. tumefaciens* (Klopfenstein *et al.* 1991), and the gene of a rice protease inhibitor introduced into *P. tremula* × *P. tremuloides* (Heuchelin *et al.* 1997).

A third approach focuses on simultaneous modification of two genes for enhanced resistance to insects. This was achieved in *Liquidambar styraciflua*, combining a peroxidase anionic enzyme gene involved in cell growth and wall development with a 'Bt' gene (Sullivan and Lagrimini 1993).

Flowering modification

In order to produce sterile trees and prevent possible dispersal of transgenic pollen in the environment, an approach based on genetic ablation has been tested on poplar (Skinner *et al.* 2000). This technique, which consists of expressing a cytotoxic gene under the control of a very specific poplar floral promoter, resulted in more than 90 percent of transformed lines lacking floral structures. Other approaches, based on the suppression of key flowering genes, are being tested.

Quantitative and qualitative modification of lignin

Modification of lignin composition or content is being actively pursued because of the expected financial gains from pulp processing improvements. Lignins, which enhance cell wall mechanical properties and hardness, are difficult to process and are a significant limitation in processing wood into paper pulp by chemical treatment. Genetic transformation to modify lignin characteristics is a key research feature on species used in the paper industry. The aim is to regulate the activity of key enzymes involved in the lignin biosynthesis pathway (Lapierre *et al.* 1999; Jouanin *et al.* 2000; Li *et al.* 2003). Active on-going research targets the effects of lignin biosynthesis in *Populus* on soil carbon transformation and storage¹⁶.

Other traits

Other traits that have been considered for genetic modification include: resistance to diseases (Mayer 2004; www.genewatch.org/) in *Populus*, *Betula pendula*, *Alnus* and *Juglans*.; bioremediation in *Populus*; enhanced growth through higher efficiency of nitrogen assimilation in *Picea* and *Populus* (Gallardo *et al.* 1999), and modification of gibberellin synthesis in *Populus*.

2.1.4.2 Data set analysis

Genetic modification activities represent 520 entries in the data set, or 19 percent of all biotechnology activities recorded worldwide. Research is reported in 35 countries and on 29 tree genera. North America is the lead region, accounting for 48 percent of genetic modification activities on forest tree species, followed by Europe (32 percent), Asia (14 percent), Oceania (5 percent) and Africa and South America (less than one percent each) (Figure 2.1.16), while no activity is reported in the Near East. Genetic modification research at the country level is led by the United States (219 entries in the data set, or 42 percent of the world's recorded activities on GM forest trees) (Figure 2.1.17). Studies carried out by, or in cooperation with, agencies or companies from developed countries have been identified in Brazil, Chile, China, Indonesia, South Africa and Uruguay.

Populus accounts for 47 percent (Figure 2.1.18) of data set entries, followed by *Pinus* (19 percent), *Eucalyptus* (7 percent) and *Liquidambar* and *Picea* (5 percent each). The pedigree of a number of hybrid poplars used in China has not been ascertained. Several countries have already established GM tree field trials.

¹⁶ See: www2.ncsu.edu/unity/lockers/project/forestbiotech/news.html

Interestingly, while the first GM forest trees were developed some fifteen years ago, about three-quarters of the total number of species recorded in the dataset have been genetically transformed during the past five years.

The main studies underpinning genetic modification (Figure 2.1.19) target gene expression (21 percent), *in vitro* regeneration tissue culture (18 percent), herbicide resistance (13 percent), biotic resistance (12 percent) and lignin properties (9 percent).

Figure 2.1.16. Distribution of data set entries on genetic modification by region of the world

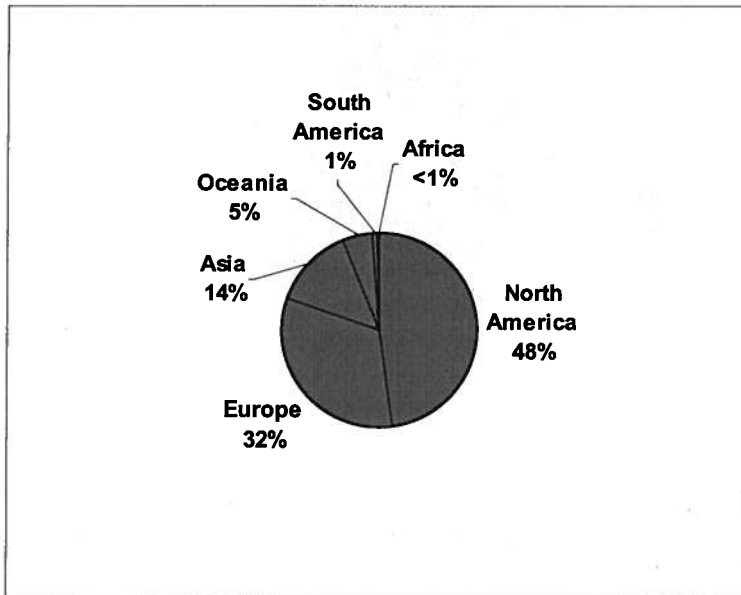


Figure 2.1.17. Distribution of reported forest tree genetic modification research activities by country (only those with more than five such activities in the data set are included)

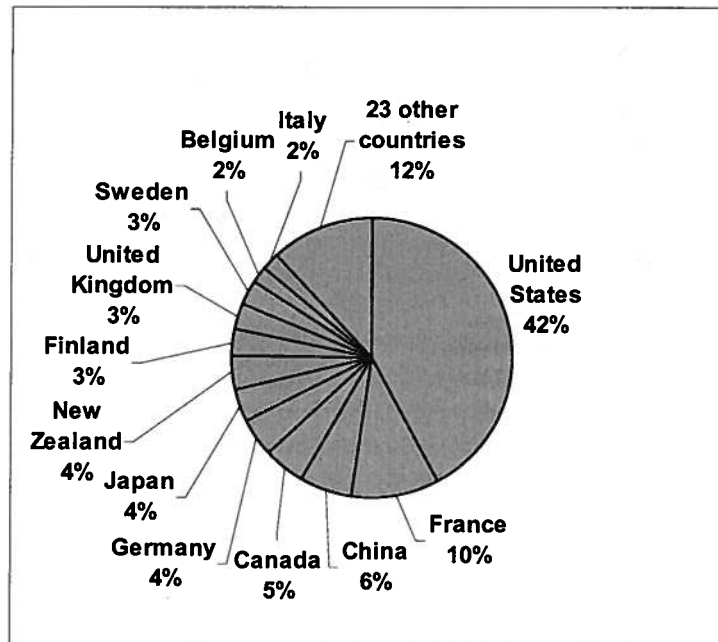


Figure 2.1.18. Distribution of reported forest tree genetic modification research activities by genus

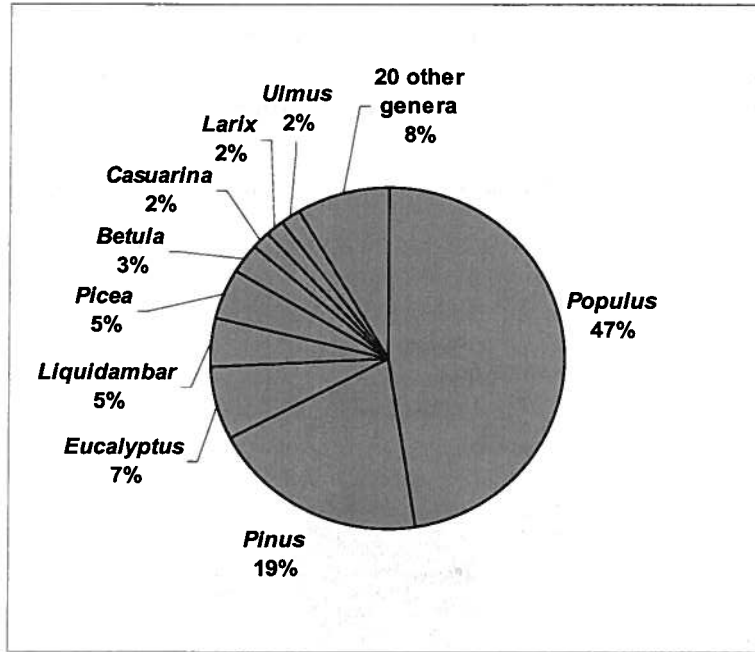
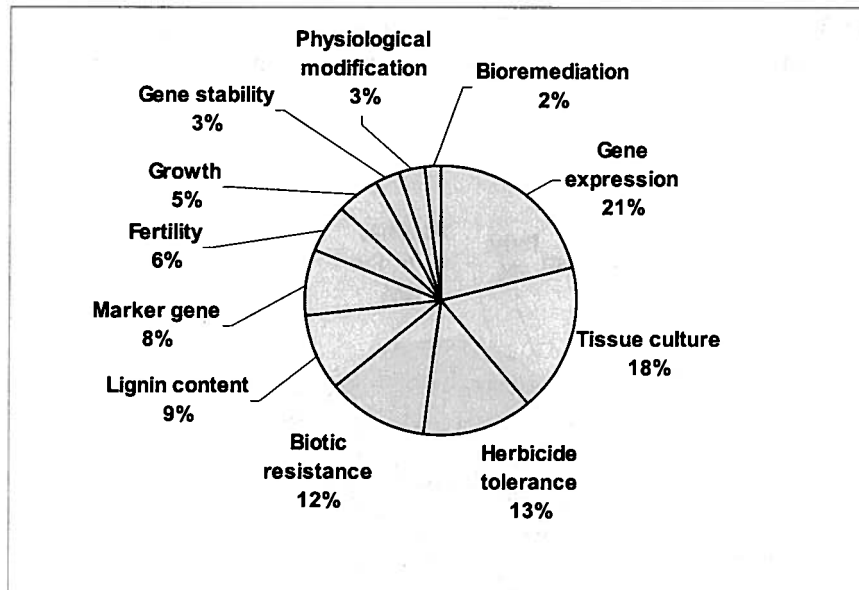


Figure 2.1.19. Main reported objectives of research activities in forest tree genetic modification



2.1.4.3 Summary of the state of genetic modification in the forestry sector

In vitro regeneration of transformed plants is still a technical limitation for many species and genotypes. Genetically transformed lines have been regenerated for *Populus*, *Eucalyptus*, *Pinus* (including *P. taeda*¹⁷) and *Picea*. Technical processes involved in regeneration are not always well controlled or even studied; especially in mature tissues where the situation is the most critical. No GM tree is reported to be commercialized in international databases to date (Mayer 2004; www.agbios.com).

Insertion of genes controlling traits of interest in a given forest species is limited by insufficient knowledge of the molecular control of these traits. Most of the traits important in wood production, such as growth rate, adaptability, and stem and wood quality, are under polygenic control, and therefore depend on more than a single gene. Biosafety regulations governing GM trees are often a concern due to the long period of time necessary to monitor environmental effects (including gene stability, pollen and seed dispersal, and impact on other elements of the ecosystem). Most existing regulations are both stringent and involve high development costs compared to annual crops. Collaborative genomics programmes such as those carried out on *Populus*, *Pinus* and *Eucalyptus*, with the on-going characterization of genes of interest (including those coding for flowering, and lignin and cellulose biosynthesis), and the identification of specific promoters of transgenic expression, are expected to boost advances in genetic modification of forest trees. Studies to assess the stability of GM tree growth under abiotic stress, and their environmental impact, have been initiated.

2.1.5 CONCLUSIONS: STATUS AND TRENDS OF BIOTECHNOLOGY RESEARCH AND APPLICATIONS IN FORESTRY

Unlike work carried out on crop or farm animal species, the domestication of forest trees is, with few exceptions, very recent. However, within a few decades, knowledge transfer from agriculture to forestry has been so fast that genetic knowledge of a few tree model genera, such as poplar (*Populus*), pine (*Pinus*) and *Eucalyptus*, is about to become as important, in terms of complexity and quality, as the genetic knowledge of the main crop species. Modern biotechnologies have potentially outstanding applications in the forestry sector, mainly because of the potential genetic gains they could confer. Expected gains for tree breeders include new genetic pools and significant reduction in tree selection time.

Owing to technical difficulties, high costs, and time needed to evaluate forest species, tree breeding programmes have long been limited to the mass selection of the best individuals. Between the 1970s and 1990s, development of horticultural techniques such as grafting, propagation by cuttings and controlled pollination, as well as information technology development, contributed to the implementation of sophisticated and efficient tree improvement activities. Long-term conventional tree breeding programmes and large-scale clonal propagation techniques have resulted in, and will continue to provide, significant productivity gains, especially for fast-growing species such as *Eucalyptus* and *Populus*.

While the objectives of tree breeding are clearly defined and address end-users' (tree growers') requirements (in terms of yield, wood quality, and biotic and abiotic resistance), the situation in biodiversity conservation is less clear. Several issues (including knowledge of species biology, ecosystem conservation, restoration of endangered species, and decision-making tools for ecosystem or forest management) are common to biological diversity and forestry.

¹⁷ Source: APHIS; www.aphis.usda.gov/

However, in spite of a considerable number of studies carried out on hundreds of forest tree species, there are still very few reported biotechnology applications. Many countries are currently engaged in biotechnology-supported forest biodiversity conservation programmes. Nonetheless, there are only a few reported direct impacts at policy or technical levels.

The impact of biotechnology on genetic studies has allowed the use of cutting-edge methodologies with forest tree species in spite of the late domestication process compared with crop species (Sedjo 2003). The sequencing of the *Populus* genome has significant implications for forestry (Campbell *et al.* 2003). *Eucalyptus* gene sequencing, which was reported during a IUFRO (International Union of Forest Research Organizations) Tree Biotechnology meeting in 2003, has since been pursued by the Eucalypt Genome Initiative¹⁸, the Eucalyptus Genome Sequencing Project Consortium¹⁹ and the Brazilian project Genolyptus. The Dendrome Project²⁰ is a collection of forest tree genome databases and other forest genetic information resources, with a special emphasis on conifers and *Pinus*. It is in genome sequencing that research seems currently most active.

The development of large-scale vegetative propagation techniques, essentially based on micropropagation or somatic embryogenesis, makes it theoretically possible to deploy superior planting materials rapidly and effectively. This is already the case for some coniferous and broad-leaved tree species including *Eucalyptus* and teak (*Tectona grandis*). Micropropagation techniques are also necessary to regenerate GM plants.

Genetic modification has been applied to forest trees mainly during the last two decades, at the experimental stage in the laboratory, to alter various traits such as herbicide, metal, salt, or insect resistance or cold tolerance. The rationale for producing trees with reduced lignin content for the pulp and paper industry is generally based on the expected environmental and economic benefits (Christensen *et al.* 2000). Equally if not more important, genetic modification is often considered as a tool to improve knowledge of tree biology and functioning, with significant potential applications; for example, for studying cell wall properties and wood formation. Since genes regulating secondary tissue and lignin formation in the annual plant *Arabidopsis thaliana* were identified (Goujon and Jouanin 2003), research on the genetic basis of wood quality in forest trees has significantly increased (mainly in *Eucalyptus*, *Pinus* and *Populus*).

With the exception of micropropagation, for which some applications are reported, most public domain information relates to research activities. Field applications appear extremely limited, according to the literature. Nevertheless, and in spite of unclear economic rationale, the use of biotechnology in forestry research has increased and provided new knowledge on tree biology and functioning. It also appears that forest biotechnology is increasingly funded by the public sector in academic research, while the private sector seems to focus investments more on specific traits of commercial interest such as wood quality.

Commercial deployment of new biotechnology-based forest tree varieties of a limited number of taxa (*Eucalyptus*, *Pinus taeda*, *P. radiata*, *P. pinaster*, *Populus*) can be expected in the near future. The impact of biotechnology applications on global wood supply may, however, take longer to materialize (not before 2020, according to Seppälä 2003). In this context, genetic modification appears to be the most significant and controversial sector of forest biotechnology. It is noteworthy that in some countries the terms 'biotechnology' and 'genetic modification' are used as synonymously, adding to the confusion.

¹⁸ See: www.up.ac.za/academic/fabi/eucgenomics/EGI/.

¹⁹ See: <http://forests.esalq.usp.br/>.

²⁰ See: http://dendrome.ucdavis.edu/Gen_res.htm.

During the last decade, several collaborative groups or consortia have been created by public or private partners for applying specific forest biotechnologies in a number of countries, including:

- ArborGen, created in 1999, with the aim of enabling research on genetic modification, has been working on herbicide resistance, growth and fibre quality for paper pulp in *Eucalyptus*, *Pinus radiata*, *Populus*, *Pinus taeda* and *Liquidambar* sp.
- GenForSA, created in 1999 with the main objective of modifying and testing disease resistance of *Pinus radiata*, while improving wood quality and formation (Owusu 1999).
- Monfori Nusantra Indonesia, created in 1996, has been involved in mass production of tissue cultures of *Tectona grandis*, *Acacia* and *Eucalyptus* for field trial establishment and commercialization.
- The Oregon State University's Tree Genetic Engineering Research Cooperative (TGERC) has carried out research on the use of GM poplar in plantations.
- The Poplar Molecular Genetics Cooperative (PMGC) has been established with the aim of improving knowledge of genetic and molecular mechanisms responsible for productivity and quality trait variations in hybrid poplars.
- The North Carolina State University Forest Biotechnology Industrial Research Consortium (FORBIRC) mission is to integrate genome technology, metabolic engineering, traditional tree breeding and wood and paper science into a research organization directed to the creation of superior wood as a raw material and as a product.

The interest induced by the first genetic modifications of tree species at the end of the 1990s has since declined. Regulatory frameworks have been established in many countries for GM organism (GMO) field experiment or commercial deployment, with increased testing costs. In a number of countries, hostile reaction from the public or/and environmental groups has also limited deployment of GM trees. Some authors have questioned the profitability or return on investment of MAS techniques, at least in the short term and the medium term (Robinson 1999).

In Europe, forestry research priorities now focus on stronger synergies between biotechnology and conventional selection and breeding programmes mainly for wood improvement. The aim is to fill the gap between forest trees and agricultural crops in terms of the level of domestication. As a result, tree improvement tends to use genomic and proteomic approaches for achieving trait selection more rapidly. This is done by taking advantage of the advances obtained in model plants such as *Arabidopsis* (Kirst *et al.* 2003), and of the considerable increase of information exchange resulting from the latest bioinformatics progress. More and more effort is devoted to research aimed at better understanding biotic and abiotic stress responses. Significant investments have been made in genomics research on *Populus* and *Pinus* within the framework of the Poplar Genomics Initiative and the Pine Mapping Project.

In the tropics, wood production in volume per hectare is significantly higher than in the temperate zone. Several large-scale companies have recently invested in tree planting in tropical and subtropical areas. Some paper companies tend to fund their own research activities and keep them confidential. More emphasis seems to be put in genomics and proteomics, and particularly sequencing, thanks to developments on *Arabidopsis thaliana*, *Oryza* and *Populus*.

At the same time, there is increasing information coming from genome banks on wood quality, growth and stress resistance. Good examples of EST development include *Eucalyptus* in the framework of the Genolyptus project in Brazil, *Populus*²¹, *Pinus taeda*²², and *P. pinaster* and *Quercus*²³, although applications for practical field selection are very unlikely to be operational for at least a decade.

Some predict that the next few years are more likely to see significant field applications of forest genomics research than of genetic transformation research, whose application is seriously hindered by strong environmental opposition and stringent regulations. Developments in agricultural crops are very meaningful in this respect. Genomics and proteomics outcomes are expected to boost conventional tree breeding and improve the efficiency of existing programmes.

The production costs of a new forest tree variety using conventional selection (well-known species, with a short juvenile phase) is roughly estimated at about US\$400 000 and requires 15 to 20 years to be developed (Fenning and Gershenzon 2002). The calculation does not include the investment required for the selection of the suitable species. On the other hand, genomics, supported by bioinformatics in the framework of MAS, requires substantial equipment and running budget (as an indication, a supportive biotechnological platform costs several million dollars). In turn, such biotechnologies are expected to enable breeders to access more accurate and numerous traits of interest for greater improvements, while reducing the new variety production costs thanks to bioinformatics.

As regards developing countries and countries with economies in transition, there are few references available on their involvement in forestry biotechnology. The limited literature mainly refers to micropropagation in Vietnam, Malaysia, Indonesia and India. Malaysia has a reported strong oil palm molecular biology programme, including genetic modification.

However, some emerging countries with advanced financial, institutional and human capacities (including Brazil, India²⁴, and China) have made significant breakthroughs in advanced forest biotechnology. Brazil has been actively involved in research on *Eucalyptus* through the Genolyptus project that constitutes at the moment the cutting edge in research on the genus worldwide. China is very active in poplar genetic modification. It can be reasonably assumed that after a period of time, results from research in technically advanced countries will benefit an increasing number of developing countries and countries in transition.

African countries, with the exception of Kenya and South Africa, are far less advanced in forest biotechnology. However, notable development of agricultural crop biotechnology in countries like Nigeria, Uganda, Côte d'Ivoire, Morocco and Algeria (particularly through AAB²⁵) and even in the forestry sector (ISAAA in Kenya²⁶), constitute positive indicators suggesting that the situation is improving. Adoption of well-targeted forest biotechnology, possibly for drought-resistance, seems a crucial issue for the future of such activities.

Micropropagation has for the past several years been the most striking example of forest biotechnology applications, at least in terms of numbers of plants produced and acclimatized. Applications have been reported mainly in Asian countries, although there is still a lack of reliable information as far as operational field planting is concerned.

²¹ See: <http://Poppel.fysbot.umu.se>, <http://mycor.nancy.inra.fr/PoplarDB.html>.

²² See: <http://cbc.umn.edu>, <http://dendrome.ucdavis.edu>.

²³ See: www.pierroton.inra.fr.

²⁴ See: <http://dbtindia.nic.in/ebc/ptc.htm>.

²⁵ See: www.aab.org.dz.

²⁶ See: www.isaaa.org/Projects/Africa/trees.htm.

2.1.6 REFERENCES

- Bandyopadhyay, S., Cane, K., Rasmussen, G. & Hamill, J.D.** 1999. Efficient regeneration from seedling explants of two commercially important temperate eucalypt species – *Eucalyptus nitens* and *E. globulus*. *Plant Sci.*, 140: 189–198.
- Bishop-Hurley, S.L., Zabkiewicz, R.J., Grace, L., Gardner, R.C., Wagner, R.C. & Walter, C.** 2001. Conifer genetic engineering: transgenic *Pinus radiata* (D. Don) and *Picea abies* (Karst) plants are resistant to the herbicide Buster. *Plant Cell Rep.*, 20: 235–243.
- Bonga, J.M. & Von Aderkas, P.** 1992. *In vitro culture of trees*. Dordrecht, Netherlands, Kluwer, 236 pp.
- Brondani, R.P.V. & Grattapaglia, D.** 1999. *Development of a genus wide reference linkage map for Eucalyptus based on microsatellite markers*. Plant & Animal Genome VII Conference, Town & Country Hotel, San Diego, CA, 17–21 January 1999, P166.
- Brown, G.R., Bassoni, D.L., Gill, G.P., Fontana, J.R., Wheeler, N.C., Megraw, R.A., Davis, M.F., Sewell, M.M., Tuskane, G.A. & Nealea, D.B.** 2003. Identification of quantitative trait loci influencing wood property traits in loblolly pine (*Pinus taeda* L.). III. QTL verification and candidate gene mapping. *Genetics*, 164: 1537–1546.
- Bueno, A., Gomez, A. & Manzanera, J.A.** 2003. Propagation and DNA markers characterization of *Populus tremula* L. and *Populus alba* L. In S.M. Jain & K. Ishii, eds. *Micropropagation of woody trees and fruits, forestry sciences*, pp. 37–74. Dordrecht, Netherlands, Kluwer.
- Butcher, P., Williams, E., Whitaker, D., Ling, S., Speed, T. & Moran, G.** 2002. Improving linkage analysis in outcrossed forest trees - an example from *Acacia mangium*. *Theor. Appl. Genet.*, 104: 1185–1191.
- Byrne, M., Murrell, J.C., Allen, B. & Moran, G.F.** 1995. An integrated genetic linkage map for eucalypts using RFLP, RAPD and isozyme markers. *Theor. Appl. Genet.*, 91: 689–875.
- Campbell, M., Brunner, A.M., Jones, H.M. & Strauss, S.T.** 2003. Forestry's fertile crescent: the application of biotechnology to forest trees. *Plant Biotechnol. J.*, 1: 141–154.
- Cervera, M.T., Storme1, V., Ivens, B., Gusmão, J., Liu, B.H., Hostyn, V., Van Slyckenc, J., Van Montagu, M. & Boerjan, W.** 2001. Dense genetic linkage maps of three *Populus* species (*Populus deltoides*, *P. nigra* and *P. trichocarpa*) based on AFLP and microsatellite markers. *Genetics*, 158: 787–809.
- Chaix, G., Gerber, S., Razafimaharo, V., Vigneron, P., Verhaegen, D. & Hamon, S.** (2003). Gene flow estimation with microsatellites in a Malagasy seed orchard of *Eucalyptus grandis*. *Theor. Appl. Genet.*, 107: 705–713.
- Charest, P.J.** 1996. Biotechnology in Forestry: Examples from the Canadian Forest Service. *For. Chron.*, 72(1): 37–42
- Chase, M.R., Moller, C., Kessel, R. & Bawa, K.** 1996. Distant gene flow in tropical tree. *Nature (London)*, 383: 398–399.

- Christensen, J.H., Baucher, M., O'Connell, A., van Montagu, M. & Boerjan, W.** 2000. Control of lignin biosynthesis. In S.M. Jain & S.C. Minocha, eds. *Molecular biology of woody plants*, Vol. 1, pp. 227–267. Dordrecht, Netherlands, Kluwer.
- Cornu, D.** 1994. Forêt, de la gélose à la terre. *Biofutur*, Février 1994: 25–31.
- Dawson, I.K., Waugh, R., Simons, A.J. & Powell, W.** 1997. Simple sequence repeats provide a direct estimate of pollen-mediated gene dispersal in the tropical tree *Gliricidia sepium*. *Mol. Ecol.*, 6: 179–183.
- De Melis, L.E., Brugliera, P., Pongracic, S. & Stevenson, T.W.** 1998. Nucleotide sequence of a *Eucalyptus globulus* cDNA clone encoding cinnamyl alcohol dehydrogenase. *Plant Physiol.*, 116: 1191.
- Dick, C.W.** 2001. Genetic rescue of remnant tropical trees by an alien pollinator. *Proc. R. Soc. Lond., Ser. B, Biol. Sci.*, 268: 2391–2396.
- Dow, B.D. & Ashley, M.V.** 1998. High levels of gene flow in bur oak revealed by paternity analysis using microsatellites. *Heredity*, 89: 62–70.
- Dubos, C. & Plomion, C.** 2003. Identification of water-deficit responsive genes in maritime pine (*Pinus pinaster* Ait.) roots. *Plant. Mol. Biol.*, 51: 249–262.
- Dubos, C., Le Provost, G., Pot, D., Salin, F., Lalane, C., Madur, D., Frigerio, J.-M., Plomion, C.** 2003. Identification and characterization of water-deficit responsive genes in hydroponically grown maritime pine (*Pinus pinaster* Ait.) seedlings. *Tree Physiol.*, 23:169–179.
- Dumas, E. & Monteuis, O.** 1991. Régénération in vitro de pins maritimes âgés par bourgeonnement adventif sur euphylls. *Ann. AFOCEL*, 1989–1990: 43–58.
- Dutech, C., Seiter, J., Petronelli, P., Joly, H. & Jarne, P.** 2002. Evidence of low gene flow in a neotropical clustered tree species in two rainforest stands French Guiana. *Mol. Ecol.*, 11: 725–738.
- Ellis, D.D., McCabe, D., McInnis, S., Ramacandran, R., Russel, D.R., Wallace, K., Martinell, B.J., Roberts, D.R., Raffar, K.F. & McCown B.H.**, 1993. Stable transformation of *Picea glauca* by particle acceleration. *Bio/Technol.*, 11: 84–89.
- Fady, B., Lefèvre, F., Reynaud, M., Vendramin, G.G., Bou Dagher-Kharrat, M., Anzidei, M., Pastorelli, R., Savouré, A. & Bariteau, M.** 2003. Gene flow among different taxonomic units: evidence from nuclear and cytoplasmic markers in *Cedrus* plantation forests. *Theor. Appl. Genet.*, 107: 1132–1138.
- FAO.** 2000. How appropriate are currently available biotechnologies for the forestry sector in developing countries?. Background Document to Conference 2 (25 April to 30 June 2000) of the FAO Biotechnology Forum. (www.fao.org/biotech/C2doc.htm)
- Fenning, M. & Gershenzon, J.** 2002. Where will the wood come from? Plantation forests and the role of biotechnology. *Trends Biotechnol.*, 20: 291–295.
- Galiana, A., Goh, D., Chevallier, M.H., Gidiman, J., Moo, H., Hattah, M. & Japarudin, Y.** 2003. Micropropagation of *Acacia mangium* × *A. auriculiformis* hybrids in Sabah. *Bois For. Trop.*, 275: 77–82.
- Gallardo, F., Fu, J., Cantón, F.R., García-Gutiérrez, A., Cánovas, F.M. & Kirby, E.G.** 1999. Expression of a conifer glutamine synthetase gene in transgenic poplar. *Planta*, 210: 19–26.

Garnier-Géré, P., Bedon, F., Pot, D., Austerlitz, F., Léger, P., Kremer, A. & Plomion, C. 2003. *DNA sequence polymorphism, linkage disequilibrium and haplotype structure in candidate genes of wood quality traits in maritime pine (Pinus pinaster)*. In *Treebiotechnologies*, Umea, Sweden, 7–12 June 2003, s6.19.

Gerber, S., Mariette, D., Streiff, R., Bodénès, C. & Kremer, A. 2000. Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. *Mol. Ecol.*, 9: 1037–1048.

Gerber, S., Chabrier, P. & Kremer, A. 2003. FaMoz: a software for parentage analysis using dominant, codominant and uniparentally inherited markers. *Mol. Ecol. Notes*, 3: 479–481.

Gion, J.M., Rech, P. & Grima-Pettenati, J. 2000. Mapping candidate genes in *Eucalyptus* with emphasis on lignification genes. *Mol. Breed.*, 6: 441–450.

Goh, D. & Monteuuis, O. 2001. *Production of tissue-cultured teak: the plant biotechnology laboratory experience*. In *Proceedings, 3rd Regional Seminar on Teak: 'Potential and opportunities in marketing and trade of plantation teak: challenge for the new millennium'*, pp. 237–247. Yogyakarta, Indonesia, 31 July – 4 August, 2000.

Goh, D.K.S., Alloysius, D., Gidiman, J., Chan, H.H., Mallet, B. & Monteuuis, O. 2005. Selection and propagation of superior teak for quality improvement in plantations: case study of the ICSB/Cirad-Forêt joint project. In Bhat, K.M., Nair, K.N., Bhat, K.V., Muralidharan, E.M. & Sharma, J.K., eds. *Quality timber products of teak from sustainable forest management*, pp. 390–399. Kerala Forest Research Institute, India & International Tropical Timber Organization, Japan.

Goujon, T. & Jouanin, L. 2003. Genes involved in the biosynthesis of lignin precursors in *Arabidopsis*. *Plant Physiol. Biochem.*, 41: 677–687.

Govindaraju, D.R. 1989. Estimates of gene flow in forest trees. *Biol. J. Linn. Soc.*, 37: 345–357.

Grattapaglia, D. & Sederoff, R. 1994. Genetic linkage maps of *Eucalyptus grandis* and *E. urophylla* using a pseudo-testcross mapping strategy and RAPD markers. *Genetics*, 137: 1121–1137.

Heuchelin, S.A., Jouanin, L., Klopfenstein, N.B. & McNabb, H.S. 1997. Potential of proteinase inhibitors for enhanced resistance to *Populus* arthropod and pathogen pests. In Klopfenstein, N.B., Chun, Y.W., Kim, M.S. & Ahuja, M.R., eds. *Micropropagation, genetic engineering, and molecular biology of Populus*. Gen. Tech. Rep. RM-GTR-297, pp. 173–177. Fort Collins, CO, US Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Ho, C.K., Chang, S.H., Tsay, J.Y., Tsai, C.J., Chiang, V.L. & Chen, Z.Z. 1998. *Agrobacterium tumefaciens*-mediated transformation of *Eucalyptus camaldulensis* and production of transgenic plants. *Plant. Cell. Rep.*, 17: 675–680.

Huang, Y., Diner, A.M., & Karnosky, D.F. 1991. *Agrobacterium rhizogenes* mediated genetic transformation and regeneration of a conifer: *Larix decidua*. *In Vitro Cell Dev. Biol.*, 27: 201–207.

Jones, N. 2002. Somatic embryogenesis as a tool to capture genetic gain from tree breeding strategies: risks and benefits. *South. Afr. For. J.*, 195: 93–101.

- Jouanin, L., Goujon, T., de Nadaï, V., Martin, M.T., Mila, I., Vallet, C., Pollet, B., Yoshinaga, A., Chabbert, B., Petit-Conil, M. & Lapierre, C. 2000. Lignification in transgenic poplars with extremely reduced caffeic acid-*O*-methyltransferase activity. *Plant Physiol.*, 123: 1363–1373.
- Kirst, M., Johnson, A.F., Baucom, C., Ulrich, E., Hubbard, K., Staggs, R., Paule, C., Retzel, E., Whetten, R. & Sederoff, R. 2003. Apparent homology of expressed genes from wood-forming tissues of loblolly pine (*Pinus taeda* L.) with *Arabidopsis thaliana*. *Plant Biol.*, 100: 7383–7388.
- Kirst, M., Myburg, A. & Sederoff, R.R. 2004. *Microarrays and transcript level Qtls identify candidate genes for the quantitative traits of wood quality and growth in Eucalyptus*. In Plant & Animal Genomes XII Conference, San Diego, CA, USA, 10–14 January 2004, P611.
- Kjaer, E.D., Kaosa-Ard, A. & Suangtho, V. 2000. Domestication of teak through tree improvement: Options, potential gains and critical factors. In *Site, technology and productivity of teak plantations*, FORSPA Publication No. 24/2000, Teaknet Publication No. 3, pp. 161-189.
- Klopfenstein, N.B., Shi, N.Q., Kernan, A., McNabb, H.S., Hall, R.B., Hart, E.R. & Thornburg, R.W. 1991. Transgenic *Populus* hybrid expresses a wound-inducible potato proteinase inhibitor II-CAT gene fusion. *Can. J. For. Res.*, 21: 1321–1328.
- Kubisiak, T.L., Nelson, C.D., Nance, W.L. & Stine, M. 1995. RAPD linkage mapping in a longleaf pine × slash pine F1 family. *Theor. Appl. Genet.*, 90: 1110–1127.
- Kuusiene, S. 2002. Sexual and non sexual plant reproduction in the laboratory (available at www.genfys.slu.se/staff/dagl/nova02/Abstracts_Nova02.doc)
- Lapierre, C., Pollet, B., Petit-Conil, M., Toval, G., Romero, J., Pilate, G., Leplé, J.C., Boerjan, W., Ferret, V., de Nadaï, V. & Jouanin, L. 1999. Structural alterations of lignins in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acid *O*-methyltransferase activity has an opposite impact on the efficiency of industrial kraft pulping. *Plant Physiol.*, 119: 153–163.
- Le Dantec, L., Chagné, D., Pot, D., Bedon, F., Géré-Garnier, P., De Daruvar, A. & Plomion, C. 2003. *Data mining in pine ESTs: II. Development of SNP markers*. In *Trebiotechnologies*, Umea, Sweden, 7–12 June 2003, s6.22.
- Lelu, M.A. & Thompson, D. 2000. L'embryogenèse somatique des conifères: une filière pour produire du matériel forestier de reboisement. *C. R. Colloq. Sainte Catherine*, 2000: 167–174.
- Lelu-Walter, M.A. & Harvengt, L. 2004. L'emeryogenèse somatique des conifères, état et perspectives. *Afocel Inf.-For.*, 694: 6.
- Li, L., Zhou, Y., Cheng, X., Sun, J., Marita, J.M., Ralph, J. & Chiang, V.L. 2003. Combinatorial modification of multiple lignin traits in trees through multigene co-transformation. *PNAS*, 100(18): 4939–4944.
- Liu, G. & Bao, M. 2003. Adventitious shoot regeneration from *in vitro* cultured leaves of London plane tree (*Platanus acerifolia* Willd.). *Plant Cell Rep.*, 21: 640–644.
- Lourmas, M. 2001. Analyse des flux de gènes efficaces dans la cédraie du petit lubéron. Mémoire de DEA, Université Montpellier II, France, 33 pp.

- McDougall, G.J.** 2000. A comparison of proteins from the developing xylem of compression and non-compression wood of branches of Sitka spruce (*Picea sitchensis*) reveals a differentially expressed laccase. *J. Exp. Bot.*, 51: 1395–1401.
- McMillan, L.K., Cato, S.A., Wilcox, P.L. & Echt, C.S.** 2004. *Candidate gene selection in Pinus radiata finding the needles in the haystack.* In Plant & Animal Genomes XII Conference San Diego, CA, 10–14 January 2004, W84.
- Marques, C.M., Araujo, J.A., Ferreira, J.G., Whetten, R., O'Malley, D.M., Liu, B.H. & Sederoff, R.** 1998. AFLP genetic maps of *Eucalyptus globulus* and *E. tereticornis*. *Theor. Appl. Genet.*, 96: 727–737.
- Mayer, S.** 2004. *Non-food GM crops: new dawn or false hope.* GeneWatch UK report, Part 2. Grasses, flowers, trees, fibre crops and industrial uses. 48 pp. (available at www.genewatch.org)
- Monteuuis, O. & Dumas, E.** 1992. Morphological features as indicators of maturity in acclimatized *Pinus pinaster* from different in vitro origins. *Can. J. For. Res.*, 22: 1417–1421.
- Monteuuis, O. & Goh, D.** 1999. About the use of clones in teak. *Bois For. Trop.*, 261: 28–38.
- Monteuuis, O., Alloysius, D., Garcia, C., Goh, D. & Bacilieri, R.** 2003. Field behavior of an in vitro-issued *Acacia mangium* mature selected clone compared to its seed-derived progeny. *Aust. For.*, 66: 87–89.
- Moralejo, M., Rochange, F., Boudet, A.M. & Teulières, C.** 1998. Generation of transgenic *Eucalyptus globulus* plantlets through *Agrobacterium tumefaciens*-mediated transformation. *Aust. J. Plant Physiol.*, 25: 207–212.
- Myburg, A., Griffin, A.R., Sederoff, R.R. & Whetten, R.W.** 2003. Comparative genetic linkage maps of *Eucalyptus grandis*, *Eucalyptus globulus* and their F1 hybrid based on a double pseudo-backcross mapping approach. *Theor. Appl. Genet.*, 107: 1028–1042.
- Nadgauda, R.S., Kendurkar, S.V. & Kularni, V.M.** In press. *Tissue culture for improved productivity of teak.* In International conference on quality timber products of teak from sustainable forest management. Peechi, Kerala, India, 2–5 December 2003.
- Newton, A.C., Allnut, T., Gillies, A.C.M., Lowe, A.J. & Ennos, R.A.** 1999. Molecular phylogeography, intraspecific variation and the conservation of tree species. *Tr. Ecol. Evol.*, 14: 140–145.
- Nicodemus, A., Nagarajan, B., Mandal, A.K. & Suberbramanian, K.** 2001. *Genetic improvement of teak in India.* In Proceedings, 3rd Regional Seminar on Teak: 'Potential and opportunities in marketing and trade of plantation teak: challenge for the new millennium', pp. 277–294. Yogyakarta, Indonesia, 31 July – 4 August, 2000.
- Owusu, R.A.** 1999. *Summary : GM technology in the forest sector : a scopy study for WWF*, 8pp. (available at www.wwf-uk.org)
- Park, Y.S.** 2002. Implementation of conifer somatic embryogenesis in clonal forestry: technical requirements and deployment considerations. *Ann. For. Sci.*, 59: 651–656.
- Park, Y.S., Barret, J.D. & Bonga, J.M.** 1998. Application of somatic embryogenesis in high-value clonal forestry: deployment, genetic control, and stability of cryopreserved clones. *In Vitro Cell. Dev. Biol.-Plant*, 34: 231–239.
- Petit, R., El Mousadik, A. & Pons O.** 1998. Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.*, 12(4): 844–855.

- Pilate, G., Pâques, M., Leplé, J.C. & Plomion, C.** 2002. Les biotechnologies chez les arbres forestiers. *Rev. For. Fr.*, 54: 161–180.
- Pinto, G., Santos, C., Neves, L. & Araùjo, C.** 2002. Somatic embryogenesis and plant regeneration in *Eucalyptus globulus* Labill. *Plant Cell Rep.*, 21: 208–213.
- Plomion, C.** 2000. Développement de la génomique pour l'étude de la variation des caractères d'intérêts chez le pin maritime. Mem. HDR Université Paris Sud UFR Scientifique Orsay, 125 pp.
- Plomion, C., Pionneau, C., Brach, J., Costa, P. & Baillères, H.** 2000. Compression wood-responsive proteins in developing xylem of maritime pine (*Pinus pinaster* Ait.). *Plant Physiol.*, 123: 959–969.
- Plomion, C., Pionneau, C. & Baillères, H.** 2003. Identification of tension-wood responsive proteins in the developing xylem of *Eucalyptus*. *Holzforschung*, 57: 353–358.
- Quoirin, M.** 2003. Micropropagation of *Acacia* species. In S.M. Jain & K. Ishii, eds. *Micropropagation of woody trees and fruits*, pp. 245–268. Dordrecht, Netherlands, Kluwer.
- Rahman M.S., Messinamg, M.G. & Newton, R.J.** 2003. Performance of loblolly pine (*Pinus taeda* L.) seedlings and micropropagated plantlets on an east Texas site. I. Above- and belowground growth. *For. Ecol. Manage.*, 178: 245–255.
- Ranik, M., Creux, N. & Myburg, A.A.** 2004. Transcriptome analysis of xylogenesis in *eucalyptus* using cDNA-AFLP and Li-cor automated DNA analysers. In Plant & Animal Genomes XII Conference San Diego, CA, USA, 10–14 January 2004, P610.
- Ritchie, G.A., Steven, D.D. & Timmis, R.** 1994. Maturation in Douglas-fir: II. Maturation characteristics of genetically matched Douglas-fir seedlings, rooted cuttings and tissue culture plantlets during and after 5 years of field growth. *Tree Physiol.*, 14: 1261–1275.
- Robinson, C.** 1999. Making forest biotechnology a commercial reality. *Nature Biotechnol.*, 17: 27–30.
- Roy, S., Islam, M. & Hadiuzzaman, S.** 1998. Micropropagation of *Elaeocarpus robustus* Roxb. *Plant Cell Rep.*, 17(10): 810–813.
- Saebo, A., Skjeseth, G. & Appelgren, M.** 1995. Light quality of the *in vitro* stage affects the subsequent rooting and field performance of *Betula pendula* (Roth). *Scand. J. For. Res.*, 10: 155–160.
- Sarker, R.H., Rafiqul Islam, M. & Hoque, M.I.** 1997. In vitro propagation of neem (*Azadirachta indica* A. Juss) plants from seedlings explants. *Plant Tissue Cult.*, 7(2): 125–133.
- Saxena, S. & Dhawan, V.** 2001. Large-scale production of *Anogeissus pendula* and *A.latifolia* by micropropagation. *In Vitro Cell. Dev. Biol.-Plant*, 37: 586–591.
- Schmincke, K.H.** 2000. Teak plantations in Costa Rica: precious woods experience. *Unasylva*, 201: 29–35.
- Sedjo, R.A.** 2003. Biotech and planted trees: some economic and regulatory issues. *Agbioforum* 6: 112–119.
- Seppälä, R.** 2003. Forest biotechnology and the global forest sector. In McCord, S. & Gartland, K., eds. *Forest biotechnology in Europe: The challenge, the promise, the future*, pp. 39–43. Institute of Forest Biotechnology.

- Skinner, J.S., Meilan, R., Brunner, A.M. & Strauss, S.H.** 2000. Options for genetic engineering of floral sterility in forest trees. In S.M. Jain & S.C. Minocha, eds. *Molecular biology of woody plants*, Vol. 1, pp. 135–153. Dordrecht, Netherlands, Kluwer.
- Streiff, R.** 1998. *Structure génétique spatiale et flux polliniques dans une chênaie mixte: Quercus petraea (Matt.) Liebl., Quercus robur L.* Université de Montpellier II Sciences et Techniques du Languedoc, 90 pp. (thesis)
- Sullivan, J. & Lagrimini, L.M.** 1993. Transformation of *Liquidambar styraciflua* using *Agrobacterium tumefaciens*. *Plant Cell Rep.*, 12: 303–306.
- Sutton, B.** 2002. Commercial delivery of genetic improvement to conifer plantations using somatic embryogenesis. *Ann. For. Sci.*, 59: 657–661.
- Thamarus, K.A., Groom, K., Murrell, J., Byrne, M. & Moran, G.F.** 2002. A genetic linkage map for *Eucalyptus globulus* with candidate loci for wood, fibre, and floral traits. *Theor. Appl. Genet.*, 104: 379–387.
- Thorpe, T.A.** 1995. *In vitro embryogenesis in plants*. Dordrecht, Netherlands, Kluwer, 558 pp.
- Verhaegen, D. & Plomion, C.** 1996. Genetic mapping in *Eucalyptus urophylla* and *E. grandis* using RAPD markers. *Genome* 39: 1051–1061.
- Watt, M.P., Blakeway, F.C., Termignoni, R. & Jain, S.M.** 1999. Somatic embryogenesis in *Eucalyptus grandis* and *E. dunnii*. In: S.M. Jain, P.K. Gupta & R.J. Newton, eds. *Somatic embryogenesis in woody plants*, Vol 5, pp. 63–78. UK, Kluwer.
- Watt, M.P., Blakeway, F.C., Mokotedi, Meo, Jain, S.M.** 2003. Micropropagation of *Eucalyptus*. In S.M. Jain & K. Ishii, eds. *Micropropagation of woody trees and fruits*, pp. 217–244. Dordrecht, Netherlands, Kluwer.
- Whetten, R., Sun, Y.H., Zhang, Y. & Sederoff, R.** 2001. Functional genomics and cell wall biosynthesis in loblolly pine. *Plant Mol. Biol.*, 47(1–2): 275–91.
- White, G., Boshier, D.H. & Powell, W.** 2002. Increased pollen flow counteracts fragmentation in a tropical dry forest: an example from *Swietenia humilis* Zuccarini. *Ecology*, 99: 2038–2042.
- Yanchuk, A.D.** 2001. The role and implications of biotechnological tools in forestry. *Unasylva*, 204(52): 53–61.
- Yin, T., Zhang, X., Huang, M., Wang, M., Zhuge, O., Tu, S., Zhu, L.H. & Wu, R.** 2002. Molecular linkage maps of the *Populus* genome. *Genome*, 45: 541–555.

2.1.7 ANNEXES

2.1.7.1 Methodology of data collection

Data analysed in this study were stored in a simple Excel data set. The data set gathers major biotechnology entries (i.e. a given technology developed or used in a given country, by a given laboratory team, on a given species or variety, for a given purpose). Since a single activity can be reported and published many times in different journals, a significant amount of time has been devoted to singling out the activities and selecting the latest (or most comprehensive) reference (= source of information, or activity, or entry, or record).

Collection dates: February to April 2004 with revisions in September 2004.

Methodology: *Systematic searches on CAB Abstracts from 1994 to 2004 using combinations of key words (including forest trees, MAS, micropropagation, proteomics, genomic, EST, lignin, transformation, GMO). Examples: forest trees × genetic diversity, forest trees × micropropagation, proteomics × eucalyptus.

*Research on Google using the key words indicated above. A search in Google provided 632 000 references to 'forest biotechnology' in November 2004 (all languages).

*Team research by topic, on the Internet, through personal contacts, and through reviews of literature. Topics included:

- Micropropagation
- Genetic transformation
- Genetic improvement, genomics, etc.
- Structuration of genetic diversity
- Mycorrhizae, *Rhizobium*

*Review of the proceedings of the latest international conferences

Confidentiality: *Only public information was gathered, including personal communications and observations.

The following databases and international data sets have been used through CABI: AGRICOLA, AGRIS, ASFA, CAB Abstracts, Econlit, FSTA, TROPAG & RURAL, and ULRICH International Periodicals.

Data structure: In total, 2 716 records have been collected. Of these, 2 644 records were directly related to forest trees, while 72 records addressed forest trees indirectly: studies on mycorrhizae (*Frankia* and *Rhizobium*) accounted for 36 records, fruit trees and ornamental trees 30 records, and bamboos and rattans six records.

Information on crop trees has not been searched systematically, and the data set is not representative of activities taking place on domesticated trees. However, a number of genera that comprise wild populations and cultivated varieties (including *Ficus*, *Malus*, *Morus*, *Pyrus*, *Prunus*) have been included, when insufficient information was available from the source of information to ascertain the degree of domestication of the germplasm on which activities were carried out. These classification uncertainties represent approximately one percent of the data set.

A number of forest tree species cannot be categorized in specific regions of provenance. This is the case for most hybrid *Populus* clones, including euramerican poplars (*Populus* × *P. euramericana*) and many Asian varieties.

Eurasian species (including *Pinus sylvestris*, *Populus nigra* ...) have been categorized as of European origin.

2.1.7.2 Main Internet sites used

<http://biotech.jrc.it>
<http://dendrome.ucdavis.edu>
<http://elonmerkki.net>
<http://fabinet.up.ac.za>
<http://mediaforest.net>
<http://ogm.agriculture.gouv.fr/>
<http://webdoc.sub.gwdg.de>
<http://web.ahc.umn.edu/biodata/>
www.agbios.com
www.agnet.org
www.americanlands.org
www.aphis.usda.gov/
www.arborea.ulaval.ca
www.arborgen.com/
www.beckerunderwood.com
www.biorize.com
www.biospace.com
www.cellfor.com
www.checkbiotech.org
www.cirad.fr
www.cnr.berkeley.edu
www.csiro.au
www.ctnbio.gov.br
www.edinburgh.ceh.ac.uk
www.efi.fi
www.ermanz.govt.nz
www.etfrn.org
www.fao.org
www.forestresearch.co.nz
www.fsc.org
www.fungi.com
www.genet-info.org
www.genewatch.org
www.greenpeace.org
www.grolife.com
www.ipgri.cgiar.org
www.isb.vt.edu
www.itto.or.jp
www.iufro.org
www.ncl-india.org
www.nf-2000.org
www.nrcan-rcan.gc.ca
www.phcmexico.com.mx
www.philombios.com
www.pierroton.inra.fr

www.premiertech.com
www.rbge.org.uk
www.rti.org
www.strategis.ic.gc.ca
www.ucl.ac.be
www.wrm.org.uy

2.1.7.3 Distribution of countries and territories by geographic region as used in this study²⁷

Africa: Cameroon, Congo, Côte d'Ivoire, Ethiopia, Ghana, Kenya, Madagascar, Senegal, South Africa, United Republic of Tanzania, Uganda

South America: Argentina, Brazil, Chile, Costa Rica, Cuba, Guyana, Mexico, Uruguay, Venezuela (Bolivarian Republic of).

North America: Canada, United States of America

Asia: Bangladesh, China (including the province of Taiwan), India, Indonesia, Japan, Republic of Korea, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Singapore, Thailand, Viet Nam

Near East: Egypt, Iran (Islamic Republic of), Kuwait, Saudi Arabia, Tunisia, United Arab Emirates

Europe: Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Israel, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, Serbia and Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, United Kingdom.

Oceania: Australia, New Zealand.

²⁷ Note: the distribution of countries and territories by regions of the world is in line with the FAO regional classification. See:
www.fao.org/countryprofiles/physical_presence.asp?lang=en.

2.2 A global study on the state of forest tree genetic modification

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2.2.1 SUMMARY

The information provided in this report was resourced from practitioners in the area through a detailed questionnaire. Particular emphasis was given to the views of practitioners on how they see genetic modification contributing to forestry, and how they view future development. To complement information from the questionnaire, other publicly available information, such as that available on Internet sites, was included in the assessment.

2.2.2 INTRODUCTION

Genetic modification in forest trees is widely reported and results from field testing are beginning to appear (Pilate *et al.* 2002, C. Walter, unpublished). In 1999, Owusu reported that, despite efforts by national regulatory agencies to keep track of research and applications of genetic modification in forest trees, as well as attempts to set up and maintain global information systems on GMOs (genetically modified organisms) and genetically modified (GM) trees, there was no comprehensive data set recording research and applications of genetic modification in forestry (Owusu 1999). The lack of global compilation of forest genetic modification makes it difficult to gauge the current status of such a controversial issue and to predict future trends. The present report is an attempt to quantify the existing situation with regard to developments on genetic modification in forest trees, using information and data provided by technology developers. It is, however, acknowledged that this approach can provide a snapshot of the situation only, and regular updating is recommended.

2.2.3 GLOBAL STATUS OF TREE GENETIC MODIFICATION. THE QUESTIONNAIRE

A questionnaire was developed to assess the current status of tree genetic modification worldwide, and to scope the views of scientists and organizations involved in tree biotechnology on the current and future issues related to genetic modification in trees. The questionnaire was also designed to capture the views of practitioners on future developments, and on strategies required to provide scientific information to the public. A copy of the original questionnaire is available from the FAO. Responses were collected by FAO and New Zealand Forest Research Institute Ltd (NZFRI) and analysed by the authors. The following provides a detailed analysis together with an interpretation of the results. Where appropriate, tables or graphics summarize results.

Some respondents included ornamentals/fruit trees in their responses. These were included in the analysis, because it was deemed important to capture the views of individuals and organizations practising genetic modification, with tree genetic modification as understood in the widest possible sense.

2.2.3.1 Response rate

Based on the NZFRI address list, 418 questionnaires were sent out by the authors to institutions possibly involved in genetic modification of woody plants and another 105 institutions and individuals were contacted by FAO. Further, Internet lists such as ETFRN²⁸ and METLA²⁹ were used to distribute information, as were the IUFRO (International Union of Forestry Research Organizations) Task Force on Biotechnology, the FAO Panel of Experts on Forest Gene Resources, the EUFORGEN³⁰ national co-ordinators and colleagues at various organizations. It can be assumed that the majority of forest geneticists, breeders and molecular biologists worldwide were informed about the study and were asked to contribute. Questionnaires were available in English, French and Spanish.

A deadline of 24 May 2003 was given for receipt of questionnaires, but this was extended, mainly owing to the fact that many key organizations had not responded by that date. The latter were contacted again directly and a few more questionnaires were returned. As of 30 September 2003, a total of 49 responses was received and entered onto a spreadsheet. This represents a response rate of 11.7 percent (based on 418 questionnaires sent originally). No more responses were received as of the end of 2003.

When data from the responding individuals and organizations were compared with publicly available data, it was noticed that not all organizations conducting research or field evaluations have made their data available, although much is publicly available from websites³¹.

2.2.3.2 Respondents (Q 1.1–1.3³²: Name, address, institutional framework, Internet)

The respondents were located in 26 different countries (Table 2.2.1).

Twenty-three of the 49 respondents (47 percent) were conducting research on genetic modification in forest trees, with one other being a non-profit organization advocating appropriate use of biotechnology.

Of the 49 respondents, 24 (49 percent) were from research institutes, 14 (29 percent) from universities, six (12 percent) from private commercial companies, and two (4 percent) from non-profit organizations. Another three (6 percent) did not state what type of organization they belonged to, or else wanted to keep the nature of their organization confidential (one respondent).

Of the 23 respondents conducting research on genetic modification in forest trees, ten (44 percent) were in research institutes, nine (39 percent) in universities, two (8.5 percent) in a private industry organization, and two (8.5 percent) in 'other organization' including one confidential response. The other 'other organization' was CHH (Carter Holt Harvey) in New Zealand, who stated that they are currently only completing work already 'in the pipeline'.

Fifteen of the 49 respondents (31 percent) are currently publishing information relating to genetic modification on the Internet; 21 (43 percent) do not publish.

²⁸ Contact: etfrn@iac.agro.nl.

²⁹ Contact: forestgen@metla.fi.

³⁰ IPGRI's European Forest Genetic Resources Programme.

³¹ For examples, see the websites of regulating authorities in Appendix 3.8.1.

³² Numbering refers to the numbers of the questions in the questionnaire.

Thirteen respondents (27 percent) did not answer these questions. However, when only those organizations that work with genetic modification were analysed, the picture is different: 13 of the 23 respondents (57 percent) working with genetic modification of trees publish information about genetic modification on the Internet. Ten (43 percent) of them do not use this medium for publishing information.

Internet addresses were submitted (see Appendix 3.8.1) by all respondents answering 'yes', but one did not want the Internet address to be published, for reasons of confidentiality.

Table 2.2.1. Responses by country. Numbers of respondents (total) and respondents using genetic modification are listed by country

Country	Number of respondents	Number of respondents using genetic modification
Algeria	1	0
Argentina	1	1
Australia	1	1
Austria	1	0
Belgium	1	1
Canada	4	2
China	1	1
Costa Rica	1	0
Ireland	2	0
Finland	1	1
France	3	2
Germany	5	2
Italy	2	0
Japan	2	2
Mexico	1	1
Morocco	1	0
New Zealand	3	2
Norway	1	0
Portugal	2	1
Romania	1	0
South Africa	1	0
Spain	1	0
Sweden	4	3
USA	6	2
Israel	1	1
Uruguay	1	0
TOTAL	49	23

2.2.3.3 Contained research (Q 2.1: The scope of genetic modification in contained laboratory research)

Of the 23 respondents who reported work on genetic modification of forest trees, 14 (61 percent) were conducting research on *Populus*, nine (39 percent) on *Pinus*, eight (35 percent) on *Picea* and four (17 percent) on *Eucalyptus*. Three respondents (13 percent) were working on *Betula*, two (9 percent) with *Larix*, two (9 percent) with *Chamaecyparis* and two (9 percent) with *Ulmus*. Other species being worked on, by one respondent each, were in the genera *Pseudotsuga*, *Robinia*, *Paulownia*, *Quercus*, *Juglans* and *Tectona*³³.

When asked whether the genetic modification work was related to a research or commercial project, all but two experiments were described as research. The two commercial projects were by one organization and the taxa involved were *Populus nigra* and a hybrid poplar. However, three respondents classified a total of four experiments as both research and commercial. The species included were in the genera *Populus* and *Eucalyptus* (twice each), perhaps indicating that there are plans to use those trees for a commercial project in the future.

Many different traits and associated genes were mentioned by respondents as targets. Evaluation of these responses is somewhat difficult since different terms were obviously used for the same trait. Also, it can be assumed that most if not all transformation events include markers, and most probably antibiotic resistance genes, but these were not always mentioned. Table 2.2.2 (Figure 2.2.1) lists the traits by the frequency with which they were cited. Names of genes (as given in the responses) were used in this evaluation to arrive at a clearer picture of what traits were actually meant, and to group some traits.

The projects associated with commercial goals were in wood properties (three), insect resistance (two) and detoxification of pollutants (one).

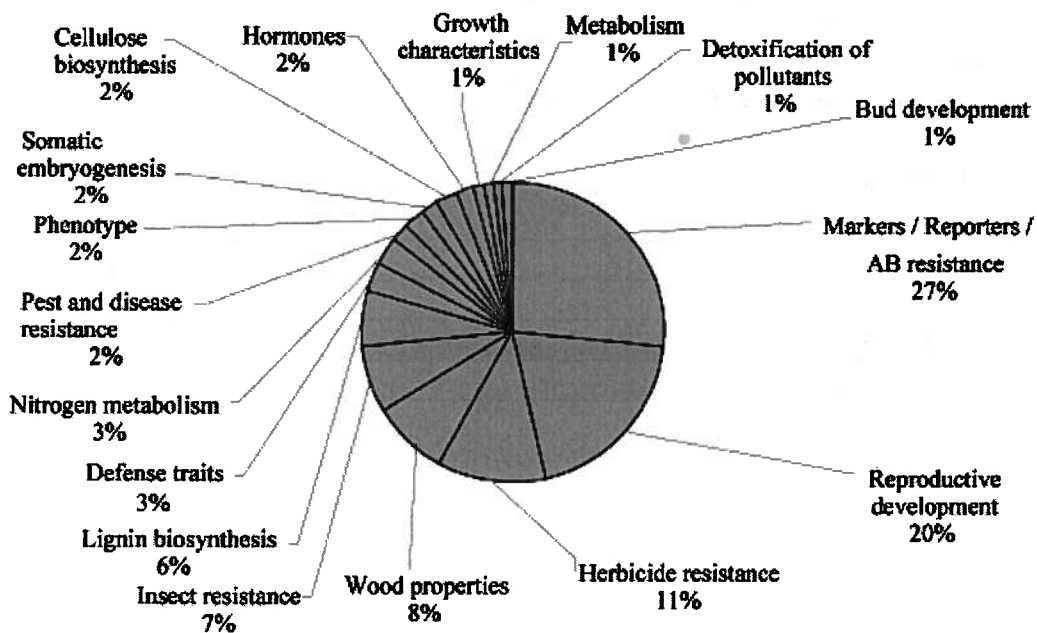
A total of 68 projects was referred to and four (6 percent) of them were classified as applied for (regulatory approval), 50 (74 percent) as ongoing, and four (6 percent) as both. One project each (1.5 percent) was in development and had been stopped. However, no comments were made on eight projects (12 percent).

³³ Note that percentages add to more than 100 because most respondents work on more than one species.

Table 2.2.2. Traits targeted in genetic modification projects, in laboratory based experiments

Trait/gene	Number of times cited
Markers/reporters/antibiotic resistance	26
Reproductive development	19
Herbicide resistance	11
Wood properties	8
Insect resistance	7
Lignin biosynthesis	6
Defence traits	3
Nitrogen metabolism	3
Pest and disease resistance	2
Phenotype	2
Somatic embryogenesis	2
Cellulose biosynthesis	2
Hormones	2
Growth characteristics	1
Metabolism	1
Detoxification of pollutants	1
Bud development	1

Figure 2.2.1. Traits targeted in genetic modification projects, in laboratory based experiments



2.2.3.4 Field trials (Q 2.2: The scope of genetic modification in field tests)

Eleven respondents of 49 (22.5 percent) reported projects with GM trees involving field analysis and a total of 26 projects were mentioned. Of those, 20 (77 percent) were with *Populus* and two (8 percent) with *Pinus*. One project each (4 percent each) was reported for *Betula*, *Eucalyptus*, *Picea* and other species in other genera.

Traits/genes targeted in these projects are listed in Table 2.2.3 (Figure 2.2.2). There are significant differences in the relative ranking of traits targeted in laboratory research (Table 2.2.2) and traits studied in field trials, in particular with regards to reproductive development.

For 22 projects, respondents provided information on the establishment of the trials. Table 2.2.4 lists the numbers of GM tree field trials established by year. Two of the 22 projects were described as 'ongoing', with no commencement date given. Information on size of trial and numbers of trees, where available, is given³⁴.

When asked about the issues addressed in field trials, all but five respondents who had trials in place provided answers (Table 2.2.5 and Figure 2.2.3).

Table 2.2.3. Traits/genes targeted in projects with GM trees in field trials

Trait/gene	Number of field trials cited
Marker	9
Herbicide resistance	6
Lignin biosynthesis	5
Insect resistance	3
Reproductive development	2
Wood properties	1
Metabolism	1
Detoxification	1
Sterility	1
Transgene stability	1
Metabolism	1
Activation tagging	1

³⁴ These data are probably a vast underestimate of the actual number of trees installed in field trials.

Figure 2.2.2. Traits/genes targeted in projects with GM trees in field trials

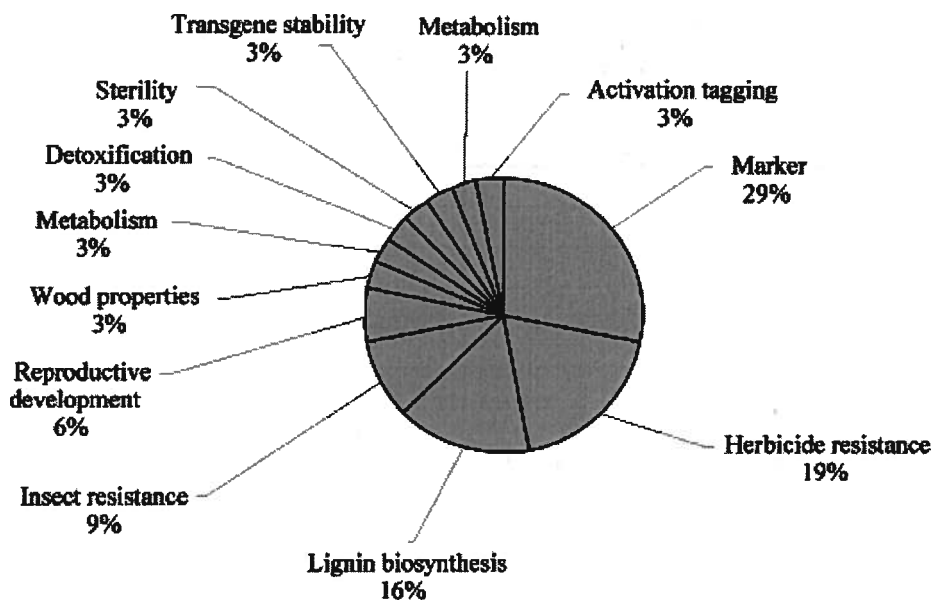


Table 2.2.4. Established field trials of GM trees¹

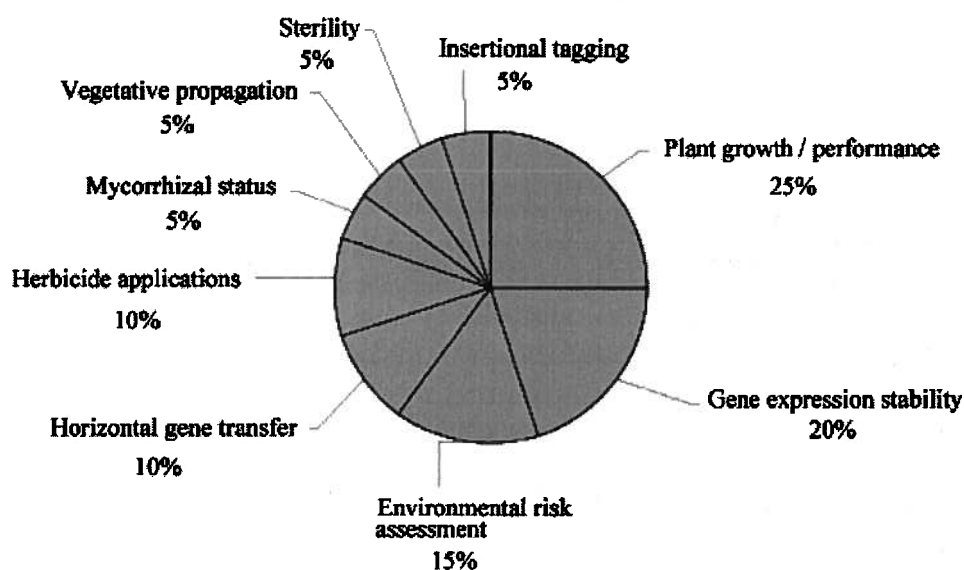
Year	Number of trials	Size of trial ²	Number of trees
1989	1	0.012 ha	100
1990	–	–	–
1991	1	–	–
1992	2	0.015 ha each	90 each
1993	–	–	–
1994	1	1.13 ha	1080
1995	3	<1 acre/–/–	477/–/–
1996	1	0.5 ha	337
1997	1	–	–
1998	1	1 acre	904
1999	2	total 80 ha	–/–
2000	3	pots/0.1 ha/0.2 ha	120/190/–
2001	1	–	–
2002	1	1.5 acres	2852
2003	2	total 1 ha	57/16
‘Ongoing’	2	0.004 ha/–	12/–

¹–: No data provided.

²1 acre ~ 0.4 ha.

Table 2.2.5. Issues addressed in field trials with GM trees (number of projects addressing a particular issue)

Issue addressed	Number of projects
Plant growth/performance	5
Gene expression stability	4
Environmental risk assessment	3
Horizontal gene transfer	2
Herbicide applications	2
Mycorrhizal status	1
Vegetative propagation	1
Sterility	1
Insertional tagging	1

Figure 2.2.3. Issues addressed in field trials with GM trees (percentage of projects addressing a particular issue)

2.2.3.5 Commercial plantations (Q 2.3: Scope of genetic modification in commercial plantations)

Most respondents reported that they had no immediate plans for commercialization, one of them commenting that it would take decades of research to test whether desired traits were realized and were stable, to assess risks, and to gain public and regulatory backing for commercial release.

One respondent felt that if there were any commercial plantations of GM forest trees in the world, those responsible were very successful in keeping it secret.

However, it has been reported (Su *et al.* 2003) that there are such plantations and one respondent described the commercial release of two GM tree species in China:

- *Populus nigra*, modified with the *Bt* gene³⁵ for resistance to leaf-eating insects, with 80 ha of field trials on eight sites in seven provinces since 1999 and 200–300 ha of commercial planting since 2002 (over one million cuttings have been propagated).
- Hybrid poplar 741 (*P. alba* × [*P. davidiana* + *P. simonii*] × *P. tomentosa*), modified with *cry1Ac*³⁶ and *API*³⁷ for resistance to leaf-eating insects, with field trials since 2001 and commercial planting since 2003 (currently only indicative data available on area planted).

The respondent further reported that there are significant problems keeping track of the area planted, mainly because poplar is vegetatively propagated and there is confusion in nurseries as to what is genetically modified and what is not.

2.2.3.6 Traditional breeding programmes (Q 2.4: On the support for genetic modification programmes by traditional breeding programmes)

Respondents commented on the integration of their tree genetic modification projects with traditional breeding programmes. Of a total of 68 projects involving GM trees (in 23 organizations), 27 (40 percent) reported full integration with traditional breeding projects. Nine respondents (13 percent) reported that either connections or the development of an integrated approach were weak, 29 (43 percent) indicated that their tree genetic modification projects were not integrated at all with traditional breeding projects. A further respondent who represented three projects (4 percent) made the statement that there are no links seen, and no links are anticipated.

2.2.3.7 Vegetative multiplication (Q 2.5: On the support for genetic modification programmes by vegetative propagation programmes)

Comments were requested on the integration of tree genetic modification projects with vegetative propagation projects. Of a total of 68 projects involving GM trees (in 23 organizations), 23 (34 percent) reported full integration with clonal multiplication projects. Seventeen (25 percent) respondents reported weak connections or connections in development, while 21 (31 percent) had no integration with such programmes. Seven (10 percent) of the respondents did not offer any information under this heading.

2.2.3.8 Other research (Q 2.6: The scope of genetic modification projects underpinning other research goals)

Under this question, respondents were asked about research projects that use genetic modification technology but do not anticipate deploying GM trees in commercial forestry. Of the 23 organizations using tree genetic modification, 15 (65 percent) reported underpinning research, seven (30 percent) answered 'no' and one (5 percent) did not respond. Of those organizations that did not use genetic modification for trees (27 out of 49 respondents, or 55 percent), 17 (63 percent) did not respond to this question. Eight of them (30 percent) reported no non-tree research in genetic modification, while two (7 percent) reported such research.

A total of 17 out of 49 respondents (35 percent) reported underpinning research and 12 gave more detailed descriptions of such research. The key areas were:

³⁵ The *cry1Ac* gene.

³⁶ *Cry1Ac* codes for a protein that is toxic to a specific class of insects.

³⁷ Another gene of which the product is toxic to insects.

- testing gene function
- environmental impact
- MAS, QTL detection, genetic maps
- microarray and EST mining for genes
- flowering-related genes/cone production
- evaluation of safety, risk assessment, consumer protection
- fibre-development genes/wood formation
- biotic and abiotic stresses
- genomics and proteomics
- physiological and hormonal studies

2.2.3.9 The regulatory framework (Q 3.1: What is the regulatory framework covering genetic modification work in the respective country?)

Respondents provided information on which agency in their country was responsible for regulating genetic modification work in contained laboratories, field trials and commercial release. This information is summarized in Appendix 3.8.1. Respondents were from 15 countries and indicated whether the respective authority was involved in pre-risk assessment (13 countries), surveillance, monitoring and quarantine (12 countries), and management of failure, redress and control (12 countries). For our analysis (Table 2.2.6), only respondents involved in some type of research in genetic modification were included. Note that there are some discrepancies in responses from the same countries, probably due to misunderstandings with the questions, or different viewpoints. The data provided in Table 2.2.6 should therefore be taken with caution.

2.2.3.10 Pending applications (Q 3.2)

The aim of this question was to understand the immediate future of GM tree testing. Respondents listed a total of 12 projects pending. This includes development in containment, field testing of GM trees and release of GM trees. Note that some countries distinguish between field tests and release, whereas others do not and consider both as a release. Table 2.2.7 provides details of 12 projects.

Table 2.2.6. Involvement of regulatory agencies in pre-risk assessments of genetic modification projects, surveillance, monitoring and quarantine (S/M/Q) and management of failure, redress, control (M/R/C), by country¹.

Country	Pre-risk assessment	S/M/Q	M/R/C
Argentina	Y	Y	Y
Australia	Y	Y	Y
Belgium	Y	N	Y
Canada	–	–	–
	Y	Y	Y
China	Y	Y	Y
Finland	Y	N	–
France	Y	N	Y
	–	Y	Y
Germany	–	–	–
	N	Y	Y
Israel	Y	Y	–
Japan	Y	Y	Y
	Y	Y	Y
Mexico	–	–	–
New Zealand	Y	Y	Y
	Y	Y	Y
Portugal	Y	Y	Y
Sweden	Y	Y	Y
	Y	Y	?
	Y	Y	Y
USA	Y	–	–
	Y	Y	Y

¹–: indicates no answer.

Table 2.2.7. GM tree projects for which regulatory approval is currently sought

Country	Type ¹	Species	Numbers	Size (area) ²	Date
Belgium	D	<i>Populus tremula</i> × <i>P. alba</i>	75 transclones	–	2004
China	T	<i>Populus tomentosa</i>	–	–	–
France	D	<i>Picea abies</i>	1000	–	2004
	D	<i>Pseudotsuga menziesii</i>	1000	–	2004
	D	<i>Pinus pinaster</i>	1000	–	2000
Germany	D	<i>Populus tremula</i> hybrids	–	–	2004
	D/T	<i>Populus tremula</i> hybrids	–	–	2005
	D/T	<i>Populus tremula</i> hybrids	–	–	2006
USA	–	<i>Populus</i> sp.	–	–	2003/04
	R	<i>Populus tremula</i> × <i>P. alba</i>	588	< 1 acre	2003
	R	<i>Populus tremula</i> × <i>P. alba</i>	300	< 1 acre	2003
	R	<i>Populus tremula</i> × <i>P. alba</i>	3000	3.5 acres	2003

¹D: Development in contained laboratories; R: Release; T: Field testing; –: no information available.

²1 acre ~ 0.4 ha.

2.2.3.11 Adequacy of the existing framework (Q 3.3)

Respondents were asked about the regulatory framework in their country and whether the framework was adequate, from their point of view, to assess the benefits and risks related to their experiments. The evaluation of the response includes all respondents that are involved in genetic modification projects with trees (a total of 23 respondents). Three respondents (13 percent) made no comments, five (22 percent) felt the framework was not adequate and 15 (65 percent) felt it was. Additional comments were offered on perceived shortcomings of the regulatory process and how improvements could be made. These included:

- process too slow, bureaucratic and costly
- exceptionally robust process, but too costly
- overcautious approach by regulatory agencies
- too many approvals required, often with the same assessment requirements, same information requested several times
- risk assessment is based on process – should be on product
- no consideration given to comparison with conventional methodology
- site safety concerns lead to requirement to keep location confidential
- policies need to be better publicized by regulatory agencies
- procedures need to be defined more clearly and made more efficient
- more risk assessment research required
- rules for contained research need to be relaxed
- applications need to be simplified for standard genes and promoters that have been assessed previously
- need to ensure that the process cannot be abused by opponents

2.2.3.12 Testing abroad (Q 3.4: Does the respondent have laboratories, field tests or commercial plantations abroad?)

None of the 23 respondents involved in genetic modification projects answered ‘yes’ to the question about whether they had research facilities abroad. Eighteen (78 percent) responded with ‘no’ and five (22 percent) did not respond at all. The responses were similar for field trials and commercial plantations. However, one respondent from Belgium reported that they have a field test in France. This respondent also confirmed that the regulatory process abroad was appropriate to assess the risk and benefit of the project.

2.2.3.13 Intellectual property regime (Q 4.1–4.3)

It is always difficult to obtain information related to intellectual property (IP). Much of this is confidential business information; however, respondents were encouraged to share information that they consider public. The responses from 23 respondents who use genetic modification in their projects were analysed. When asked whether they owned IP in relation to their production of GM trees, eight (35 percent) confirmed that they did, ten (44 percent) did not and five (22 percent) did not respond. The following list illustrates the broad areas in which IP is owned by these respondents:

- modulation of lignin biosynthesis
- transgene development
- vegetative multiplication
- testing of transgenes
- flowering control
- gene expression
- fibre development
- embryogenesis
- new varieties
- cell wall control

Nine of the 23 respondents (39 percent) confirmed that they have IP agreements with other institutions, eight (35 percent) did not and six (26 percent) did not respond. One respondent commented that since patent protection lasts for only 20 years and most GM trees grow slowly (many years to maturity), patent protection does not offer an advantage.

Eighteen respondents (78 percent) confirmed that they did not currently have IP arrangements with institutions in developing countries, five (22 percent) did not respond to this question. However, when additional comments were made, there appeared to be a preparedness to enter into IP arrangements where appropriate and required.

2.2.3.14 Future plans on use of genetic modification in trees (Q 5.1)

Here the organizations were asked for their plans on future use of genetic modification in trees. The analysis of responses included all respondents, since many organizations not using genetic modification at present made comments under this heading. The following lists represent the responses.

Prospects:

- 10-year timing horizon is envisaged to commercialization
- long term views (10–20 years) (20–30 years)
- “we have a restriction on research in British Columbia, a 10-year moratorium”
- research only, possibly field trials
- GM trees as research tools only, no commercialization anticipated
- concern that government regulation and public perception make tree genetic modification uneconomic
- GM trees as research tools only, no commercialization anticipated
- concerns related to unpredictability of expression

Developments:

- sterility to combat allergies
- assessment of environmental impacts

Potential products/uses:

- trees producing useful substances (for example pharmaceuticals)
- GM trees for site remediation (phytoremediation or bioremediation)
- use of genes for lignin reduction, increased yield and quality
- saving species (American chestnut, *Castanea dentata*, for example)
- assessing gene expression
- pest and disease resistance
- adaptive and fitness traits
- fibre quality
- resistance to dryness and high temperature
- candidate gene testing
- herbicide tolerance

It is difficult to quantify the responses in this category, but it is still interesting to note that many responses indicate an environmental or health benefit is expected from future use of GM trees.

2.2.3.15 Expected field release (Q 5.2: If and when a GM plantation is expected)

Only a few respondents predicted further field releases. One release is expected for 2004 and two further projects (potentially including many species) in around 10 years time. One respondent did not want to disclose information for reasons of confidentiality. Another respondent mentioned that no further tests would be conducted in the near future because of extremely high costs associated with field test applications (New Zealand). A respondent from Sweden felt that less than one percent of the Swedish forests would contain GM trees by 2050. However, this respondent also commented that in the long term GM trees will be planted more, because their characteristics can be manipulated more effectively.

Note that the terms 'field test' and 'field release' are not clearly defined. Some respondents regarded field tests as releases, others appeared to regard releases as full commercial plantations.

2.2.3.16 Benefits from GM trees (Q 5.3)

Respondents were asked about commercial, environmental, human health and other benefits of genetic modification of trees.

Several anticipated commercial benefits were mentioned by respondents using or not using genetic modification in tree projects. These are listed below in Table 2.2.8 (Figure 2.2.4) together with the number of times each expected benefit was cited.

Environmental benefits mentioned by respondents are listed in Table 2.2.9 (Figure 2.2.5). One respondent commented that 35 percent of the value of GM trees will lie in environmental benefits.

Human health benefits are probably more difficult to identify than commercial and environmental benefits. However, respondents made the comments listed in Table 2.2.10 (Figure 2.2.6).

Table 2.2.8. Commercial benefits anticipated from GM trees

Benefit	Number of times cited
Increased wood production	15
Improved quality	12
Resistance to pests	9
Resistance to diseases	7
Reduced production and processing costs	5
Reduced chemicals for pulping	4
New products	3
Adaptability	1
Remediation of toxic sites	1
Sterility	1
Company shares increasing in value	1
Traits not available in current breeding population	1

Figure 2.2.4. Commercial benefits anticipated from GM trees (percentage of respondents citing benefit)

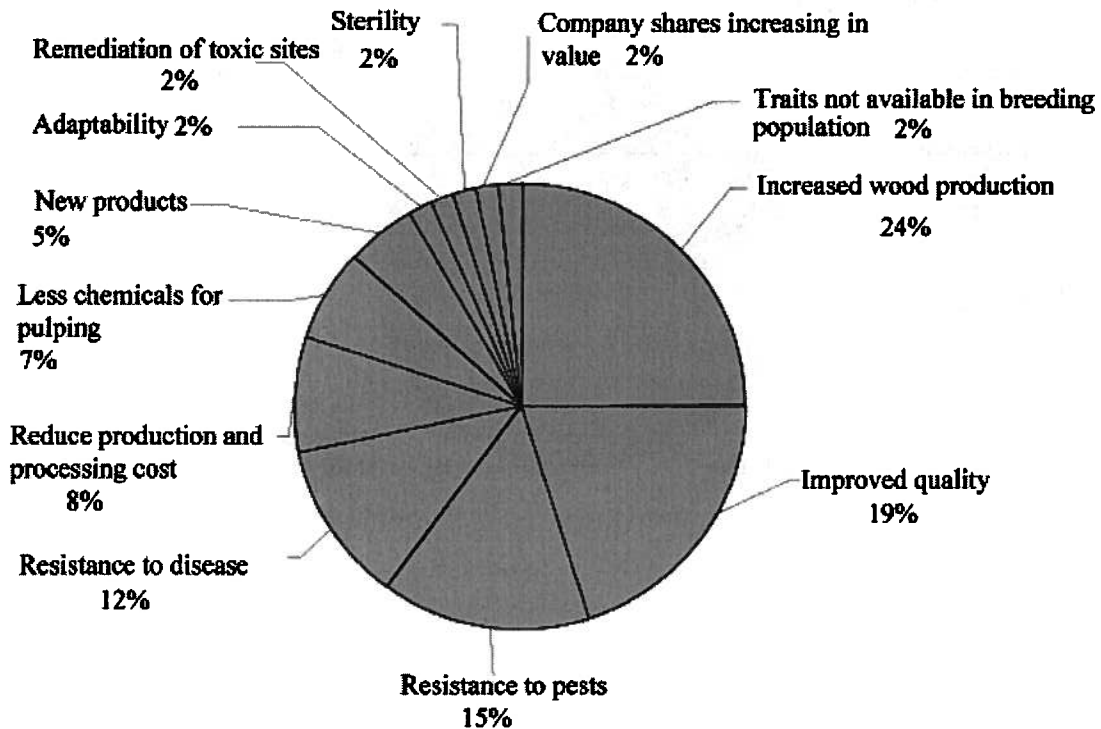


Table 2.2.9. Environmental benefits expected from GM trees

Benefit	Number of times cited
Take pressure off natural forests	12
Reduce chemical use in forests and processing	12
Bioremediation/phytoremediation	7
Carbon sequestering	7
Higher productivity per hectare	3
Adaptation to stress	3
Reduced erosion	2
Renewable energy	1
Renewable raw material	1

Figure 2.2.5. Environmental benefits expected from GM trees (percentage of respondents citing benefit)

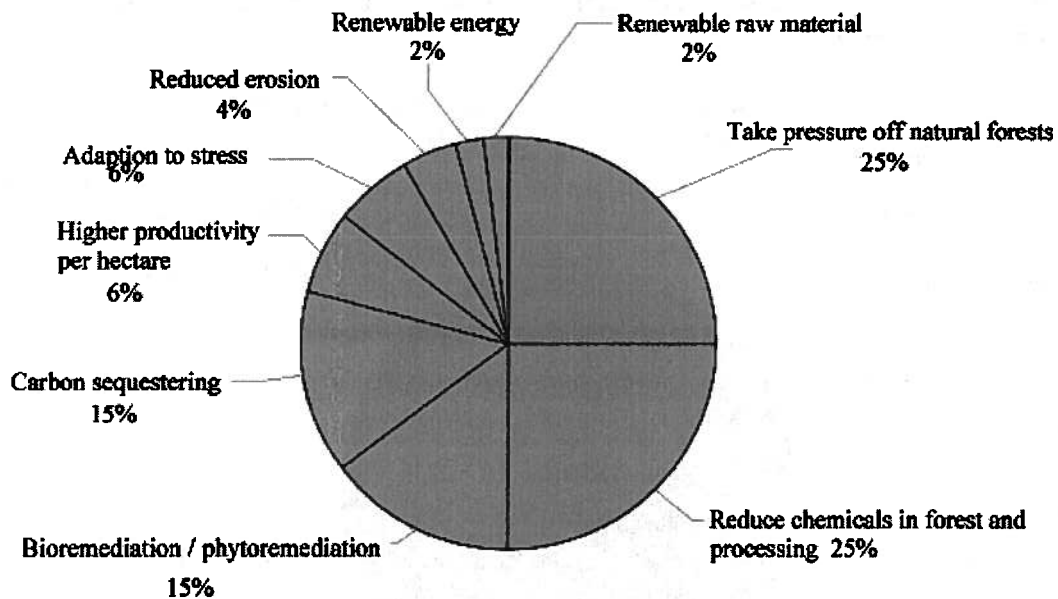
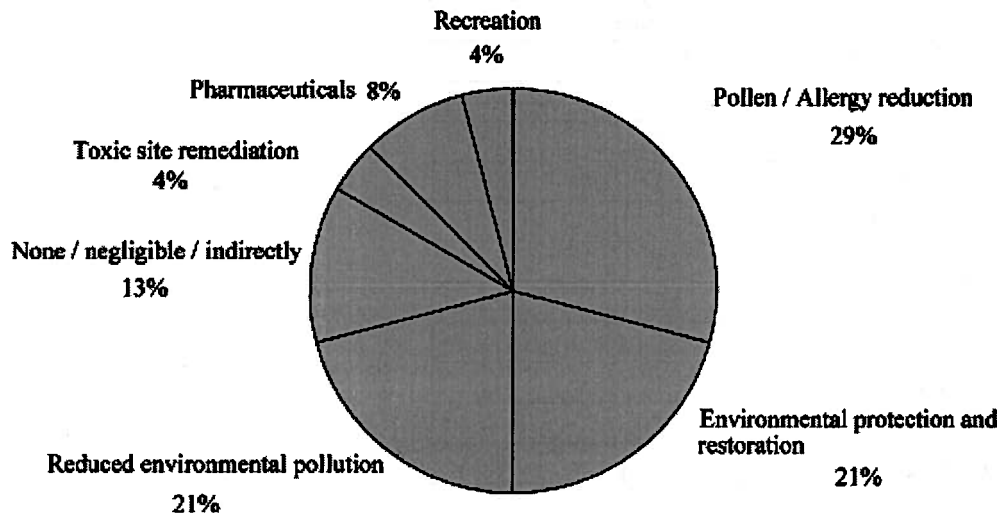


Table 2.2.10. Human health benefits expected from GM trees

Benefit	Number of times cited
Pollen/allergy reduction	7
Environmental protection and restoration	5
Reduced environmental pollution	5
None/negligible/indirectly	3
Toxic site remediation	1
Pharmaceuticals	2
Recreation	1

Figure 2.2.6. Human health benefits expected from GM trees (percentage of respondents citing benefit)

Respondents were also asked to make any other comments with regard to benefits they would expect from GM trees in forestry. The following list reflects the responses:

- potential economic benefit to developing countries
- protection against insects and fungi
- significant acceleration of conventional breeding
- shortened breeding cycles
- focus by environmental groups on genetic modification has reduced their focus on clonal and conventional techniques
- knowledge and employment
- better forests, general improvements in forestry
- potentially decreasing global warming

2.2.3.17 Risks in GM tree use (Q 5.4)

Respondents were also asked to comment on potential risks in the use of GM trees in forestry.

Anticipated commercial risks are identified in Table 2.2.11 (Figure 2.2.7), human health risks in Table 2.2.12 (Figure 2.2.8), and environmental risks in Table 2.2.13 (Figure 2.2.9). Comments by respondents on other risks are also listed.

It is notable that the biggest threat to the commercial use of GM trees is seen to be associated with social rather than technical issues.

Table 2.2.11. Anticipated commercial risks of GM trees

Commercial risk	Number of times cited
Public resistance	9
High financial risk	5
Monopoly positions	3
Transgene instability	3
Lack of operational data	2
Plantation failure	2
Quality issues	2
Monocultures/biodiversity	2
Resistance development (insects)	1
Ecoterrorism	1
Low risk if managed properly	1

Figure 2.2.7. Anticipated commercial risks of GM trees (percentage of respondents citing risk)

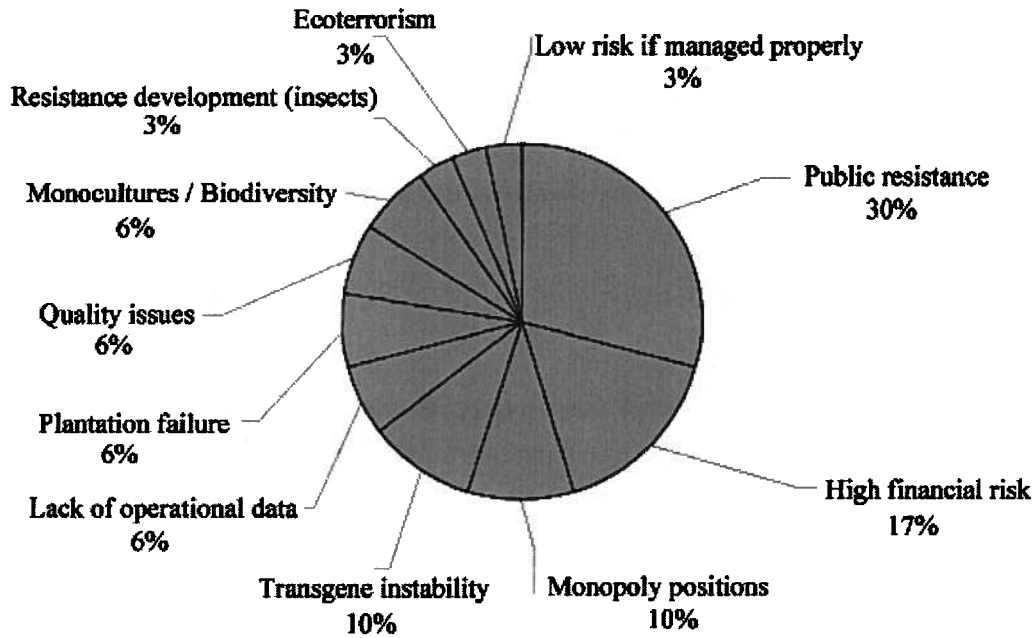


Table 2.2.12. Anticipated human health risks of GM trees

Health risk	Number of times cited
Limited/none/negligible	11
Emerging new allergies	2
Emerging new toxic metabolites	2
Not exceeding that in conventional technology	1

Figure 2.2.8. Anticipated human health risks of GM trees (percentage of respondents citing risk)

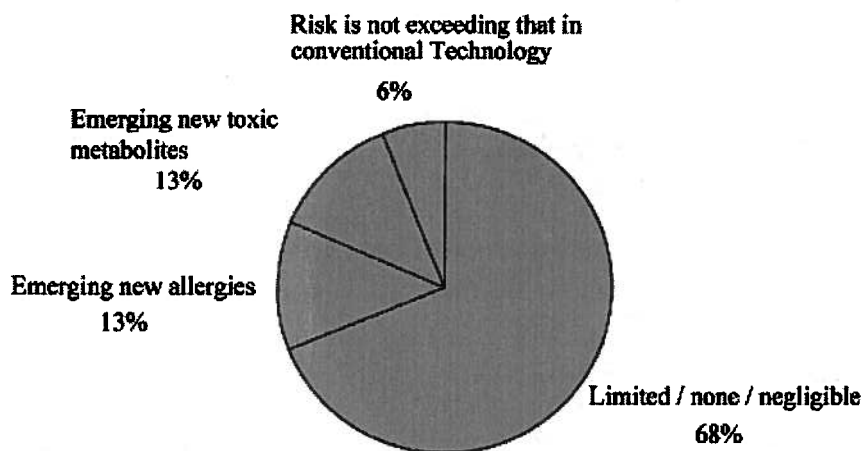
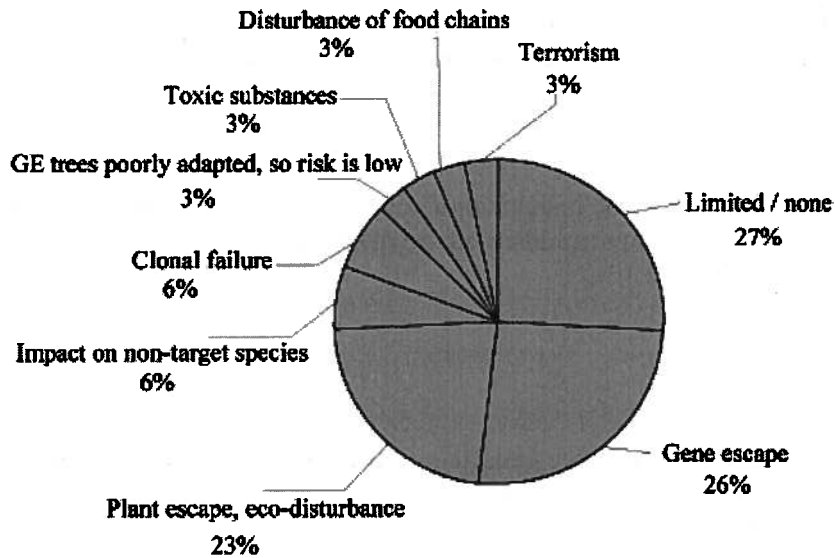


Table 2.2.13. Anticipated environmental risks of GM trees

Environmental risk	Number of times cited
Limited/none	8
Gene escape	8
Plant escape, eco-disturbance	7
Impact on non-target species	2
Clonal failure	2
GM trees poorly adapted, so risk low	1
Toxic substances	1
Disturbance of food chains	1
Terrorism	1

Figure 2.2.9. Anticipated environmental risks of GM trees (percentage of respondents citing risk)



Further comments by respondents on 'other risks' (and in some cases their mitigation, which is dealt with in more detail in Table 2.2.14 [Figure 2.2.10]) are represented in the following list:

- GM forests may render natural forests valueless.
- Sterile trees may be developed to prevent or inhibit transgene spread.
- Government research priorities take funding and priorities and attention from other matters.
- Spread of competence in genetic modification increases risks connected with 'bad will' applications.
- Genetic modification will redirect resources from biological to legal matters.
- Legal and commercial matters reduce the potential for combining the best genes into one variety. This will delay release of varieties and promote the use of the same variety for a longer time, thus increasing the distance between technical progress and what is planted in the field.
- Genetic modification will encourage clonal forestry with a few clones that are not locally adapted (and used over a larger area than clones are currently).
- Radical actions against biotechnology as happened in the United Kingdom (in 1999), when extremists removed several crops of GM maize and a GM tree plantation.

- Given the invasive traits of some trees planted in commercial forests, there may be difficulties in controlling them if they are made genetically resistant to herbicides.
- Economic consequences are potentially high if resistance is broken by a new generation of parasites (whether resistance is given through genetic modification technology or not). This risk seems to be higher when (i) resistance is provided by a single gene, or a limited number of genes, (ii) for trees, compared with other plants, given the difference in growth times between a pest and its tree host, and (iii) when planted areas are significant (e.g. poplar and poplar leaf rust).
- Perhaps ecoterrorism.

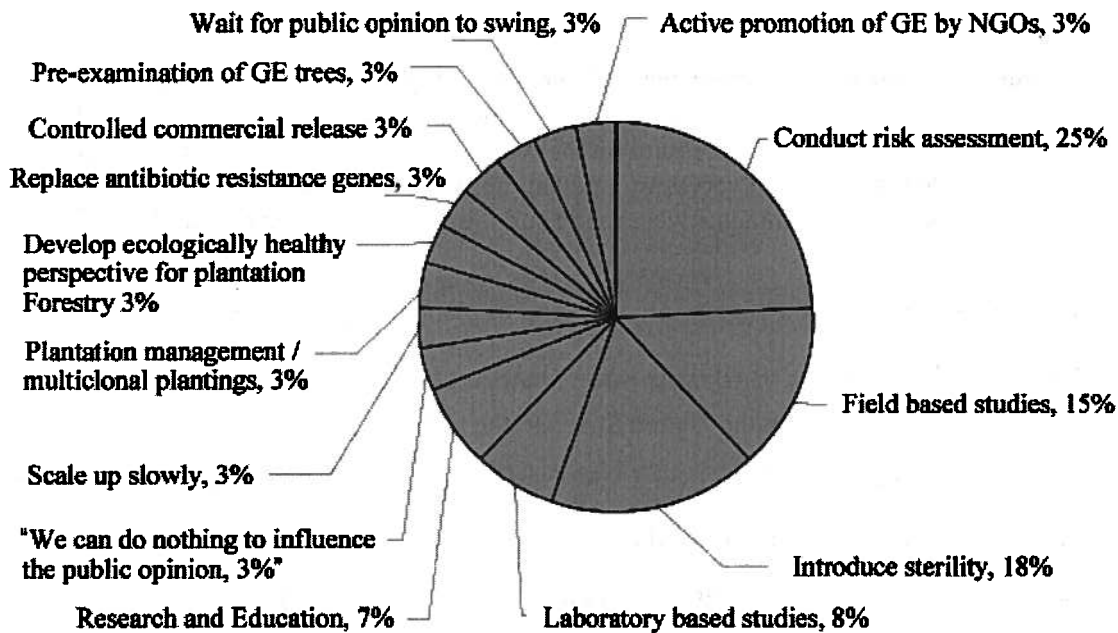
The questionnaire also tried to gain some insights into how respondents felt risks could be addressed and how their R&D programme addressed the risks in particular. The results are compiled in Table 2.2.14 (Figure 2.2.10).

Table 2.2.14. How risks involved with tree genetic modification can be addressed

Response	Number of times given
Conduct risk assessment	7
Field-based studies	4
Introduce sterility	5
Laboratory-based studies	2
Research and education	2
“We can do nothing to influence the public opinion”	1
Scale up slowly	1
Plantation management/multiclinal plantings	1
Develop ecologically healthy perspective for plantation forestry	1
Replace antibiotic resistance genes	1
Controlled commercial releases	1
Pre-examination of GM trees	1
Wait for public opinion to swing	1
Active promotion of genetic modification by NGOs ³⁸	1

³⁸ Non-governmental organizations.

Figure 2.2.10. How risks involved with tree genetic modification can be addressed (percentage of respondents giving reply)



In a further question, respondents were asked to describe how their R&D programme addressed risk. The following list summarizes the responses:

- Research on flowering control.
- Study of pollen dispersal.
- Commercial trees will contain sterility genes when required.
- Use of D-serine resistance as a harmless selectable marker.
- By promoting biodiversity conservation as an issue to be dealt with within plantation forestry.
- Promoting plantation forestry as a tool for ecological restoration of native/natural forest types at risk.
- Study ecological interactions between GM tree material and herbivores (several insect species) and mycorrhizae.
- Collaboration with researchers working with societal and ethical questions related to GM plants.
- Cautious, step-by-step approach.
- "Significant R&D issue of the research programme of the Ministry of Consumer Protection; five research projects funded by the Ministry of Education and Research."
- Safety assessment.
- Promoting forest tree genetic modification as an environmentally sustainable way to provide the world with timber, thereby creating the option to leave natural forests alone.

- “Even though we do not have an R&D programme, our objective is to serve as a facilitator for diverse groups to conduct appropriate research and to put the results into practice in an appropriate way.”
- No risk of genetic modification addressed since trees solely used for fundamental research.

At this point, the question was asked “what do you regard as the risks of not using genetic modification technology in commercial forestry?” Seven respondents felt that this would compromise the ability to reduce pressure on the world’s forests, three felt there were no risks at all, one regarded the risks as “very few” and another respondent did not foresee any economical risks. Further comments were made and they are summarized in the following list:

- Inability to compete at the highly intensive end of plantation production.
- Shortage of industrial wood during next century.
- Loss of a major industry in the United States to the tropics.
- Inability to save a species from extinction if it meets a serious threat (pest) to its survival.
- Depending on traits that are affected/modified, some environmental benefits are lost.
- Increases in pollution originating from traditional intensive forestry.
- Increased degradation of soil quality.
- “Intensive agriculture including forest plantation and the pulp and paper industries will always have a bad effect on the environment due to their intensive use of land resources and chemicals. Genetic engineering is the only way we can address these problems and reduce the damage.”
- Increased global warming and non-sustainable forestry will continue.
- Slowing down of traditional breeding programmes, while the demand for delivery of standardized products is always more pressing and urgent.
- “In a perspective of a few decades there is no risk, but in a longer time perspective we would abstain from the most powerful way of manipulating trees genetically.”
- “Genetic modification technology is just an extension of traditional tree breeding. To jettison the science would be a disservice to Mankind.”

2.2.3.18 The obstacles in the application of genetic modification to trees, and ways to overcome those obstacles (Q 5.5)

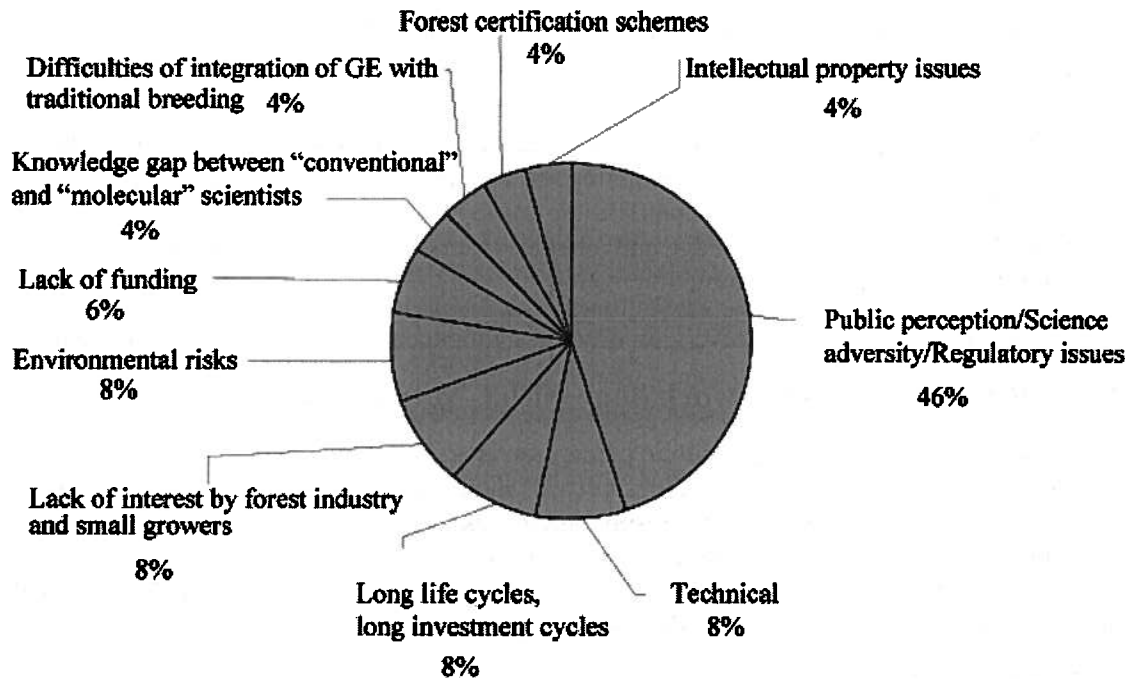
Respondents were asked about the obstacles they can see to applying genetic modification to forest trees.

Twenty-nine out of 49 (59 percent) of the respondents explained what they see as the major obstacles in using genetic modification in tree plantations, summarized in Table 2.2.15 (Figure 2.2.11).

Table 2.2.15. Obstacles to the use of genetic modification in trees. Most respondents identified more than one obstacle

Obstacle	Number of times cited
Public perception/science averseness/regulatory issues	22
Technical	4
Long life cycles, long investment cycles	4
Lack of interest by forest industry and small growers	4
Environmental risks	4
Lack of funding	3
Knowledge gap between 'conventional' and 'molecular' scientists	2
Difficulties of integrating genetic modification with traditional breeding	2
Forest certification schemes	2
Intellectual property issues	2

Figure 2.2.11. Obstacles to the use of genetic modification in trees. Most respondents identified more than one obstacle (percentage of respondents citing obstacle)



When asked for suggestions on how to overcome these obstacles, respondents gave very complex answers. A total of 20 responses (41 percent of total) were received and 13 of them (65 percent) suggested better education of the public and communication with the public as a way to overcome obstacles. However, a number of other comments were made and they are listed below (in slightly edited form, to increase clarity):

- In the forestry sector, it would be wiser to tackle important traits, such as resistance/tolerance to drought/water stress, and to avoid gene transfer from distantly related organisms.
- Sterile/late flowering trees need to be developed.
- It might help if genetic modification were used with sterility genes, to limit genetic pollution.
- Implement a strategy for using an ‘antifitness-gene’ in combination with the ‘gene-of-interest’ to prevent the establishment of the transgenic line in natural ecosystems.
- Improve genetic modification technology in order to determine genetic transformation devoid of GUS (β -glucuronidase) and antibiotic resistant genes.
- Promote use of genetic modification technology only where there is a clear threat to the survival of a species (e.g. chestnut blight, *Cryphonectria parasitica*).
- “Can we increase the amount of conserved natural/old/wild forests/their biodiversity by producing the wood we need more effectively?”
- Avoid release of ‘useless’ GMOs, without obvious benefits to the final consumer/user (for example: resistance to herbicide).
- Close collaboration between pulp/paper industry and scientific research.
- Promote the possibility of certifying GM plantation products.
- Provide more R&D funds.
- Do not actively promote ‘commercial’ applications for GM forest trees over the next few decades (e.g. from FAO). Let the experiences from agriculture accumulate a little before looking for commercial applications. But do some field experiments to accumulate needed experience and knowledge and a better understanding of the consequences when commercial applications become interesting.
- “We need much more field-testing in order to gain experience, both of risks and benefits.”

2.2.3.19 Perception of the work done with GM trees (Q 6.1)

Respondents were asked whether public perception is an issue and whether they had experienced positive or negative attitudes towards their work. Out of the 23 working with tree genetic modification, 22 (96 percent) responded to this question. Also, three further respondents who did not work with tree genetic modification made comments. The following list of comments summarizes the responses. Note that the list does not contain all individual responses but it captures the main points made.

- NGOs are opposed to GMOs.
- “We have not had any negative comments directed at us because of our research, but then we are not carrying out field trials.”
- “Public perception is very important. Our institution is very active in science communication and has many different means of communication (website, leaflets, brochures, presentations, exhibition, school projects, etc.). GM trees are not a specific topic in this communication, but much information is provided on GM plants in general. With a transgenic poplar field trial, we experienced negative attitude from green activists. The trial was destroyed by these activists in July 1999.”

- Government agencies at local level strongly discourage non-research use of GM trees. At the national level, there is more support, but national agencies (Canadian Forest Service) have little power in provincial resource base decisions.
- “Public perception is important; forests are seen as our dear national property! Government agencies, I feel, do not understand our research work and its goals. The general public is interested and mostly relies on scientific risk assessments that regard ongoing research safe (we don't have commercial applications!). Then there is one small and very active activist group against GM trees, being also very skilful in getting a lot of publicity compared to their actual critical mass.”
- Positive, responsible attitude expressed by local authorities.
- “In Sweden the attitudes vary. Research was allowed. However, there was not enough evidence provided that GM trees are safe for the environment and human health. GM plants could not be planted in the field, only in greenhouses and incubators. Also there were newspapers reports of genetic modification of Norway spruce with a gene that confers freezing resistance. The gene was found in a deep-sea fish, so public opinion was that the trees would produce fish instead of cones and seeds. Outside the academic field, the perception of GM organisms is the same, as something terrible and evil, not for utilizing and even less for eating. This is the overriding opinion in my country.”
- Even though such activities are not under way in Romania, both forest authorities and the public have negative attitudes towards work on GM trees.
- “Yes, I do experience a negative attitude from the department, university and other parts of the establishment, because I say that I do not believe in fast application of genetic modification or the blessing of genetic patents as far as long-rotation forestry is concerned. As a result I get fewer research resources. But I feel that I should give my honest opinions on matters within forest genetics, when I feel there is a need to do so. From the ‘public’ (neighbours and so on) I meet a slightly negative attitude because they believe I am part of an ongoing transformation of the Swedish forest to GM forest, in spite of the fact that this initiative is a long way off.”
- The need for certification from the Forest Stewardship Council (FSC) has led Swedish forest companies to disassociate themselves from transgenic conifers even for research purposes (e.g. by refusing to allow trials of transgenic species on land supervised by the research organization).
- “We have experienced positive and negative attitudes towards our work. Mostly positive, except for members of Greenpeace.”
- “We are very concerned about public perception. A couple of years ago nearly 1 000 of our transgenic trees were vandalized by ‘eco’terrorists.”

2.2.3.20 Communication strategy (Q 6.2)

Respondents were asked whether their organization had a communication strategy to inform the public about developments in genetic modification. Nineteen out of 23 (83 percent) respondents working with GM trees made comments under this heading. Fourteen (74 percent) of them reported some form of direct communication, either as part of a corporate strategy or as individual communication strategies. Three (16 percent) respondents did not have any form of communication in the area of GM trees and two (11 percent) said that they relied on collaboration with outside agencies for their communication about GM trees.

2.2.3.21 Concluding comments (Q 7)

The opportunity was provided to make any additional comments that respondents wished to be considered in this study. The comments made here were mostly clarifications of what has been said earlier, and requests for copies of the study when available, plus requests for confidentiality.

2.2.4 FURTHER INFORMATION COLLECTED FROM PUBLIC SOURCES

To complement responses received to the questionnaire, and assess its overall representativeness, further information on field tests was collected from public sources in January 2004. They mainly included databases available on the Internet, often maintained by regulatory agencies (for detailed information and Internet addresses, see Appendix 3.8.1). Unfortunately, there is no database that covers all field tests worldwide and the individual databases have their own formats. The amount of data related to a specific trial varies to a great extent between the databases. Also, most often it cannot be determined from the database whether a specific trial has actually been established or whether its status is approved, but the trial not yet planted. However, in the following analysis all field tests listed in the databases are included and it is assumed that they have been planted or will be planted in the near future. Some databases did not appear to be up to date (they show field trial information up to a specific date which is sometimes years ago).

Both data sets referred to in this study (resulting from (i) the questionnaire and (ii) additional searches) were compared in detail to evaluate whether all trials specified in the questionnaire were actually covered in the additional analysis as well. Only two trials were not covered in the publicly available databases

All other trials that were reported in the questionnaire were also represented in the data set obtained independently from other resources. This indicates that the data in the questionnaire are very reliable. However, many (over 100) field trials found in other resources are not represented in the data set retrieved from the questionnaire.

2.2.4.1 Analysis of publicly available information

Numbers of field tests by country

In this analysis, a distinction was made between forest trees and a second category including both ornamentals and fruit trees (Table 2.2.16). Focusing on forest trees, the United States appear to have the majority of field trials active (103 trials reported in January 2004). Next is China with nine trials; two of them, a world-first, are commercial plantations. The countries in Europe, combined, have a total of 23 trials. For fruit trees and ornamentals, the United States is again the frontrunner with 47 trials, followed by Italy with eight trials.

It becomes evident from this analysis that the United States is under-represented in the responses to the questionnaire. It is of concern that the views of the major player(s) in forest biotechnology are not represented in this study, owing to a poor response from these organizations/companies. The views of researchers and organizations in the United States are poorly represented in the analysis of data from the questionnaire (especially from the private sector).

Table 2.2.16. Field trials of GM forest trees and GM ornamental/fruit trees recorded in publicly available databases, by country

Country	Number of trials with forest trees cited	Number of trials with ornamentals/fruit trees cited
Argentina	0	0
Australia	1	2
Belgium	0	2
Brazil	2	1
Canada	7	0
Chile	3	0
China	9, partly commercial evaluation	0
Finland	5	0
France	4	0
Germany	4	1
India	1	0
Indonesia	1	0
Ireland	1	0
Israel	1	0
Italy	0	8
Japan	0	1
Mexico	0	1
Netherlands	0	3
New Zealand	3	3
Norway	1	0
Portugal	1	0
South Africa	1	0
Spain	1	0
Sweden	0	1
Thailand	0	2
United Kingdom	6	2
United States	103	47
Uruguay	2	0

GM genera/species involved

In the following analysis, forest trees were again separated from ornamental/fruit trees. It is noted that only a few forest trees are currently of significant interest for inclusion in forest biotechnology projects (Table 2.2.17). *Populus* is most often used in trials (82 trials), followed by *Eucalyptus* and *Pinus* (34 and 31 trials, respectively). In the fruit tree/ornamentals category, *Malus* is most often used in trials (33 trials), followed by *Carica papaya* and *Prunus* (18 and 13 trials, respectively).

Table 2.2.17. Field trials with GM forest trees and fruit trees/ornamentals

Category	Taxon	Number of trials cited
Forest tree	<i>Eucalyptus</i>	34
	<i>Populus</i>	82
	<i>Picea</i>	6
	<i>Pinus</i>	31
	<i>Betula</i>	3
	Total	156
	Fruit species/ornamental	<i>Carica papaya</i>
<i>Malus</i>		33
<i>Olea</i>		2
<i>Prunus</i>		13
<i>Cyphomandra</i>		1
<i>Juglans</i>		2
<i>Belladonna</i>		1
<i>Citrus</i>		1
<i>Persea</i>		1
<i>Castanea</i>		1
Total		73

Genes/attributes involved

It is very difficult to extract comprehensive information on genes and traits from field-test databases (Table 2.2.18). It appears that those databases often contain some voluntary information that was provided by the organizations conducting tests, but this is not standardized and often one can only guess at the genes involved from the trait information given. Also, in many cases the genes are not specified (confidential business information). Further, it can be assumed that, at least at the time of the survey, many GMOs in field trials have an antibiotic resistance gene integrated into their genome ('Reporter and marker genes' in Table 2.2.18). This is important for selection of the transformed event and most researchers still use the *nptII* gene for this purpose. This gene confers resistance to the antibiotics kanamycin or geneticin. However, only 43 trials refer to this or a similar gene/trait. When assessing the additional traits of commercial importance to forestry, it is evident that herbicide resistance is the most important trait in forest trees (41 trials), followed by insect resistance (21 trials) and lignin modification (15 trials). In fruit trees/ornamentals the picture is significantly different with resistance against microorganisms (viruses, fungi, bacteria) appearing most important (35 trials), followed by insect resistance (11 trials).

Table 2.2.18. Genes and traits related to field tests

Trait involved	Trials ornamentals and fruit trees	Trials forest trees	Genes
Reporter and marker genes	2	43	<i>nptII</i> ; <i>uidA</i> ; <i>aphIV</i>
Fruit ripening	7	0	
Viral resistance	15	0	coat protein; PRSV replicase; <i>TaMV</i> coat protein
Fungal resistance	17	2	cecropin; chitinase; defensin; <i>npr1</i> ; STS; oxalate oxidase; osmotin; attacin
Herbicide resistance	1	41	
Lignin modification	0	15	4coumarate CoA ligase; CAD; OMT
Nitrate reductase synthesis	0	4	
Metabolites	0	3	
Heavy metal phytoremediation	0	5	mercuric ion reductase
Bacterial resistance	3	3	antimicrobial peptide; lysozyme
Salt resistance	0	1	
Rooting	5	0	<i>rol</i>
Altered ethylene production	4	0	ACC synthase
Plant development	2	9	
Altered sugar alcohol levels	4	0	sorbitol dehydrogenase
Metabolism of halogenated hydrocarbons	0	1	P450
Sterility	0	3	diphtheria toxin A
Altered fruit ripening	7	0	<i>S</i> -adenosylmethionine transferase
Altered gene expression	0	1	
Altered polyphenol oxidase levels	3	0	polyphenoloxidase
Changes in reproduction (not sterility)	0	5	
Insect resistance	11	21	<i>cry1Ac</i> ; <i>cry3A</i> ; hyoscamine 6 β -hydroxylase; agglutinin; chitinase; chitobiosydase
Sugar content	1	0	

2.2.5 WHERE TO FROM HERE? THE FUTURE OF GENETIC MODIFICATION IN FORESTRY

The future of genetic modification in forestry appears difficult to predict. It is instructive, however, to assess the current situation in agricultural crops, where data from a decade of commercial application of genetic modification technology are now available (James 2004), along with ample reports on the environmental and economic sustainability of this technology. Most importantly, during this time the world has witnessed a continued and significant increase in GM crop acreage worldwide, with approximately 81 million hectares planted in 2004. Once plantations of GM crops were approved for a specified area or country, uptake by farmers has been swift, and more studies are forthcoming that substantiate the economic and ecological benefit of these crop plantations (FAO 2004).

Forestry is different in that rotation times are usually in the order of several decades (with the exception of short-rotation tree crops such as eucalypts). Nevertheless, where intensive plantation forestry can be practised, there are prospects of genetic modification eventually leading to field applications, provided objectives are well targeted and operational risks are managed appropriately. Studies have suggested high economic gain (Li *et al.* 2003), and comprehensive field trials with GM poplar with altered lignin composition have indicated that economic and environmental gains can be made, at least for producing pulpwood, with this technology (Pilate *et al.* 2002). As another example, field trials with GM *Pinus radiata* are beginning to yield data that demonstrate the continued correct expression of transgenes over 4–6 years and without selection for this expression (C. Walter, personal observation). The first commercial GM plantation, although relatively small in a plantation forestry sense (up to one million trees), is currently growing in China. GM *Populus nigra* trees have a *Bacillus thuringiensis* toxin gene integrated and the resistance of GM trees to insect attack has been demonstrated. More plantation projects using GM forest trees have been reported in China.

A number of issues that need special attention in the near future are detailed below, representing the authors' personal analyses.

Biotechnologies including genetic modification have shown huge potential in the agricultural sector, to produce more food on less land, avert or mitigate certain undesirable environmental effects, and provide benefit to farmers, producers, consumers and the environment.

The Gene Revolution on-going in agriculture is fundamentally distinct from the Green Revolution of the 1960s and 1970s, in the sense that it is mainly driven by the private multinational sector, focuses on a very small number of crops and traits, and is protecting intellectual property in plant innovations (FAO 2004). Four countries (Argentina, Canada, China and the United States) and two traits (insect resistance and herbicide resistance) accounted for 99 percent of the global area planted with GM crops in 2003.

Molecular biology in forestry is now well advanced. Tree genomes and transcriptomes are being analysed and genetic transformation technology is available for many tree species, particularly those that are significant or potentially significant species for clonal plantation forestry.

Initial testing of GM trees in the laboratory, glasshouse and field has indicated that tree genetic modification can provide some tremendous advantages to growers and processors, while at the same time providing environmentally sustainable solutions for wood production.

Wood demand worldwide is on the increase, a trend that is likely to continue. The share of wood harvesting from natural forests will probably decrease, and be increasingly regulated. Forest biotechnology, in combination with plantation forestry, can provide options to protect natural forests from detrimental harvest while meeting the world's increasing demand for wood.

New biotechnologies, in particular genetic modification, raise concerns. Admittedly, many questions remain unanswered for both agricultural crops and trees, and in particular those related to the impact of GM crops on the environment. Given that genetic modification in trees is already entering the commercial phase with GM *Populus* in China, it is very important that environmental risk assessment studies are conducted with protocols and methodologies agreed upon at a national level and an international level. It is also important that the results of such studies are made widely available.

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2.2.7 REFERENCES AND FURTHER READING

- Beals, T.P. & Goldberg, R.B.** 1997. A novel cell ablation strategy blocks tobacco anther dehiscence. *Plant Cell*, 9: 1527–1545.
- Binns, A.N. & Thomashow, M.F.** 1988. Cell biology of *Agrobacterium* infection and transformation of plants. *Ann. Rev. Microbiol.*, 42: 575–606.
- Bishop-Hurley, S.L., Zabkiewicz, R.J., Grace, L., Gardner, R.C., Wagner, A. & Walter, C.** 2001. Conifer genetic engineering: transgenic *Pinus radiata* (D Don) and *Picea abies* (Karst) plants are resistant to the herbicide Buster. *Plant Cell Rep.*, 20: 235–243.
- Burdon, R.D.** 2001. Genetic aspects of risk – species diversification, genetic management and genetic engineering. *N.Z. J. For.*, 44: 20–25
- Burdon, R.D. & Walter, C.** 2004. Exotic pines and eucalypts: perspectives on risks of transgenic plantations. In: S.H. Strauss & H.D. Bradshaw, eds. *The bioengineered forest: challenges for science and society*. Washington, DC, RFF (Resources for the Future) Press.
- Carson, M., Carson, S. & Walter, C.** 2004: *The future of forest biotechnology – challenge paper*. Proceedings, Forest Biotechnology Workshop, Global Biotechnology Forum, Concepcion, Chile, 2–5 March 2004.
- Conner, A.J., Glare, T.R. & Nap, J.-P.** 2003. The release of genetically modified crops into the environment. *Plant J.*, 33: 19–46.
- Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D. & Rees, M.** 2001. Transgenic crops in natural habitats. *Nature (London)*, 409: 682–683.

- Dale, P.D.** 1999. Public concerns over transgenic crops. *Genome Res.*, 9: 1159–1162.
- Dale, P.J., Clarke, B. & Fontes, M.G.** 2002. Potential for the environmental impact of transgenic crops. *Nature Biotechnol.*, 20: 567–574.
- De la Cruz, I. & Davies, I.** 2000. Horizontal gene transfer and the origin of species: lessons from bacteria. *Trends Microbiol.*, 8: 128–133.
- De Vries, J., Meier, P. & Wackernagel, W.** 2001. The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microb. Lett.*, 195: 211–215.
- Droege, W., Puehler, A. & Selbitschka, W.** 1999. Horizontal gene transfer among bacteria in terrestrial and aquatic habitats as assessed by microcosm and field studies. *Biol. Fertil. Soils*, 29: 221–245.
- Ellstrand, N.C.** 2001. When transgenes wander, should we worry? *Plant Physiol.*, 125: 1543–1545.
- FAO.** 2004. *The state of food and agriculture 2003-2004. Agricultural biotechnology: meeting the needs of the poor?* FAO Agriculture Series No. 35. Rome.
- FAO.** FAOSTAT interactive database (available at <http://apps.fao.org/>)
- Fenning, T.M. & Gershenzon, J.** 2002. Where will the wood come from? Plantation forests and the role of biotechnology. *Trends Biotechnol.*, 20: 291–296.
- Fischer, R., Buddle, I. & Hain, R.** 1997. Stilbene synthase gene expression causes changes in flower colour and male sterility in tobacco. *Plant J.*, 11: 489–498.
- Fox, J.** 2003. Resistance to *Bt* toxin surprisingly absent from pests. *Nature Biotechnol.*, 21: 258–259.
- Gianessi, L., Sivers, C.S., Sankula, S. & Carpenter, J.E.** 2002. *Plant biotechnology: Current and potential impact for improving pest management in US agriculture. An analysis of 40 case studies.* Washington, DC, National Center for Food and Agricultural Policy. (available at www.ncfap.org/40CaseStudies.htm)
- Gianessi, L., Sankula, S. & Reigner, N.** 2003. *Plant biotechnology: potential impact for improving pest management in European agriculture, a summary of nine case studies.* , Washington DC, National Center for Food and Agricultural Policy, 15 pp. (available at www.ncfap.org/Europe.htm)
- Grace, L.J., Charity, J.A., Gresham, B., Kay, N. & Walter, C.** 2005. Insect-resistant transgenic *Pinus radiata*. *Plant Cell Rep.*, 2005. (published online first)
- Ho, M.-W., Ryan, A. & Cummins, J.** 1999. Cauliflower mosaic viral promoter – a recipe for disaster? *Microbiol. Ecol. Health Dis.*, 11: 194–197.
- Hoefig, K.P., Moyle, R.L., Putterill, J. & Walter, C.** 2003. Expression analysis of four *Pinus radiata* male cone promoters in the heterologous host *Arabidopsis*. *Planta*, 217: 858–867.
- Hull, R., Covey, S.N. & Dale, P.** 2000. Genetically modified plants and the 35S promoter: assessing the risk and enhancing the debate. *Microbiol. Ecol. Health Dis.*, 12: 1–5.

- Jain, R., Rivera, M.C. & Lake, J.A.** 1999. Horizontal gene transfer among genomes: the complexity hypothesis. *Proc. Natl. Acad. Sci. USA.*, 96: 3801–3806.
- James, C.** 2004. *Preview: Global status of commercialized biotech/GM crops: 2004*. ISAAA Briefs No. 32. ISAAA. Ithaca, NY, USA.
- Krattiger, A.F.** 1997. *Insect resistance in crops: a case study of Bacillus thuringiensis (Bt) and its transfer to developing countries*. ISAAA Briefs No. 2, 42 pp. Ithaca, NY, USA, International Institute for Applied Systems Analysis (IIASA).
- Kube, P. & Carson, M.** 2004. A review of risk factors associated with clonal forestry of conifers. In: C. Walter & M.J. Carson, eds. *Plantation forest biotechnology for the 21st century*. Kerala, India, Research Signpost.
- Kumar, S. & Fladung, M.** 2001. Gene stability in transgenic aspen (*Populus*). II. Molecular characterisation of variable expression of transgene in wild and hybrid aspen. *Planta*, 213: 731–740.
- Levin, S.A. & Andreasen, V.** 1999. Disease transmission dynamics and the evolution of antibiotic resistance in hospitals and communal settings. *Proc. Natl. Acad. Sci. USA*, 96: 800–801.
- Levy, S.B., Marshall, B., Schluederberg, S. & Rouse, D.** 1988. High frequency of antimicrobial resistance in human fecal flora. *Antimicrob. Agents Chemother.*, 32: 1801–1806.
- Li, L., Zhou, Y., Cheng, X., Sun, J., Marita, J.M., Ralph, J. & Chiang, V.L.** 2003. Combinatorial modification of multiple lignin traits in trees through multigene co-transformation. *Proc. Natl. Acad. Sci. USA*, 100: 4939–4944.
- Lindgren, K. & Lindgren, D.** 1996. Germinability of Norway spruce and Scots pine pollen exposed to open air. *Silva Fenn.*, 30: 3–9.
- Lorenz, M.G. & Wackernagel, W.** 1994. TBacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev.*, 58: 563–602.
- Losey, J.E., Raynor, L.S. & Carter, M.E.** 1999. Transgenic pollen harms monarch larvae. *Nature (London)*, 399: 214.
- Matzke, M.A. & Matzke, A.J.M.** 1995. How and why do plants inactivate transgenes? *Plant Physiol.*, 107: 679–685.
- Meyer, P.** 1995. Variation of transgene expression in plants. *Euphytica*, 85: 359–366.
- Morel, J.-B. & Tepfer, M.** 2000. Pour une evaluation scientifique des risques: le cas du promoteur 35S. Are there potential risks associated with use of the cauliflower mosaic virus 35S promoter in transgenic plants? *Biofutur*, 201: 32–35.
- Nester, E.W., Thomashow, L.S., Metz, M. & Gordon, M.** 2002. *100 years of Bacillus thuringiensis: a critical scientific assessment*. American Academy of Microbiology, 18 pp. (also available at www.asm.org/Academy/index.asp?bid=2129)
- Nielsen, K.M., Bones, A.M., Smalla, K. & van Elsas, J.D.** 1998. Horizontal gene transfer from transgenic plants to terrestrial bacteria – a rare event? *FEMS Microb. Rev.*, 22: 79–103.

- Ochmann, H., Lawrence, J.G. & Groisman, E.A.** 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature (London)*, 405: 299–304.
- Owusu, R.A.** 1999. *Summary: GM technology in the forest sector, a scoping study for the WWF*. 8 pp. (available at www.wwf-uk.org/filelibrary/pdf/gmsummary.pdf)
- Phipps, R.H. & Park, J.R.** 2002. Environmental benefits of genetically modified crops: Global and European perspectives on their ability to reduce pesticide use. *J. Anim. Feed Sci.*, 11: 1–18.
- Pilate, G., Guiney, E., Holt, K., Petit-Conil, M., Lapierre, C., Leple, J.C., Pollet, B., Mila, I., Webster, E.A., Marstorp, H.G., Hopkins, D.W., Jouanin, L., Boerjan, W., Schuch, W., Cornu, D. & Halpin, C.** 2002. Field and pulping performances of transgenic trees with altered lignification. *Nature Biotechnol.*, 20(6): 558–560.
- Pray, C.E., Huang, J., Hu, R. & Rozelle, S.** 2002). Five years of *Bt* cotton in China the benefits continue. *Plant J.*, 31: 423–430.
- Punja, Z.K.** 2001. GE of plants to enhance resistance to fungal pathogens – a review of progress and future prospects. *Can. J. Plant Pathol.*, 23: 211–215.
- Quist, D. & Chapela, I.H.** 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature (London)*, 414: 541–543.
- Rhymer, J.M. & Simberloff, D.** 1996. Extinction by hybridization and introgression. *Ann. Rev. Ecol. Syst.*, 27: 87–109.
- Sedjo, R.** 2004. Potential for biotechnology applications in plantation forestry. In: C. Walter & M.J. Carson, eds. *Plantation forest biotechnology for the 21st century*. Kerala, India, Research Signpost.
- Shin, D.I., Podila, G.K., Hunag, Y. & Karnosky, D.F.** 1994. Transgenic larch expressing genes for herbicide and insect resistance. *Can. J. For. Res.*, 24: 2059–2067.
- Snow, A.** 2002. Transgenic crops – why gene flow matters. *Nature Biotechnol.*, 20: 542.
- South, D.B.** 1999. How can we feign sustainability with an increasing population? *New For.*, 17: 193–212.
- Stewart, C.N., Richards, H.A. & Halfhill, M.D.** 2000. Transgenic plants and biosafety: Science, misconceptions and public perceptions. *BioTechniques*, 29: 832–843.
- Strauss, S.H., Rottmann, W. H., Brunner, A.M. & Sheppard, L.A.** 1995. GE of reproductive sterility in forest trees. *Mol. Breed.*, 1: 5–26.
- Tang, W. & Tian, Y.** 2003. Transgenic loblolly pine (*Pinus taeda* L.) plants expressing a modified delta-endotoxin gene from *Bacillus thuringiensis* with enhanced resistance to *Dendrolimus punctatus* Walker and *Crypythothelea formosicola* Staud. *J. Exp. Bot.*, 54: 835–844.
- Tepfer, D., Garcia-Gonzales, R., Mansouri, H., Seruga, M., Message, B., Leach, F. & Mirna Curkovic, P.** 2003. Homology-dependent DNA transfer from plants to a soil bacterium under laboratory conditions: implications in evolution and horizontal gene transfer. *Transgen. Res.*, 12: 425–437.

- Thompson Campbell, F.T.** 2000. Genetically engineered trees: questions without answers. Washington, DC, American Lands Alliance (available at www.americanlands.org/forestweb/getrees.htm)
- Van den Belt, H.** 2003. Debating the precautionary principle: “Guilty until proven innocent” or “Innocent until proven guilty”? *Plant Physiol.*, 132: 1122–1126.
- Walter, C.** 2004. Review: genetic engineering in conifer forestry: Technical and social considerations. *In Vitro Cell Dev. Biol.-Plant.*, 40: 434–441.
- Walter, C. & Fenning, T.** 2004. Deployment of genetically-engineered trees in plantation forestry – an issue of concern? The science and politics of genetically modified tree plantations. In: Walter, C. & Carson, M.J., eds. *Plantation forest biotechnology for the 21st century*. Kerala, India, Research Signpost.
- Whiteman, A. & Brown, C.** 1999. The potential role of forest plantations in meeting future demands for industrial wood products. *Int. For. Rev.*, 1(3): 143–152.
- Su, X.H., Zhang, B.Y., Huang, Q.J., Huang, L.J. & Zhang, X.H.** 2003. *Advances in tree genetic engineering in China*. Paper submitted to the XII World Forestry Congress, Quebec, Canada. (www.fao.org/DOCREP/ARTICLE/WFC/XII/0280-B2.HTM)
- Yasueda H., Yui, Y., Shimizu, T. & Shida, T.** 1983. Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J. Allergy Clin. Immunol.*, 71: 777–786.
- Zhu, S., Tomberlin, D. & Buongiorno, J.** 1999. *Global forest products consumption, production, trade and prices: global forest products model projections to 2010*. FAO Global Forest Product Outlook Study Working Paper No: GFPOS/WP/01. Rome

2.2.8 ANNEXES

2.2.8.1 Resources

Field test approval authorities (as advised by questionnaire responses, plus additional resources).

Country	Authority	Website
Argentina	CONABIA	http://siiap.sagyp.mecon.ar/http-hsi/english/conabia/frameing.htm
Australia	Office of the Gene Technology Regulator	www.health.gov.au/ogtr/
Belgium	The Minister of Health	www.health.fgov.be/
Canada	Canadian Food Inspection Agency	www.inspection.gc.ca/english/toce.shtml
	Agriculture Canada	www.agr.gc.ca/index_e.phtml
China	National Committee for Biosafety of Agricultural GMOs, National Forestry Administration	www.fao.org/forestry/FOR/FORFORM/FOGENRES/regions/Update/updNEa.stm
Finland	Finland National Board for Gene Technology Ministry of Social Affairs and Health	www.vn.fi/stm/english/
France	Commission on Biomolecular Modification Ministry of Country Planning and Environment	www.environnement.gouv.fr/english/default.htm
	Commission on Genetic Modification	www.recherche.gouv.fr/commis/genetique/default.htm
Germany	Robert Koch Institut	www.rki.de/
Israel	Plant Protection and Inspection Services, Ministry of Agriculture	www.moag.gov.il/english/
Japan	Ministry of Education, Culture, Sports, Science and Technology	www.mext.go.jp/english/
	Ministry of Agriculture, Forestry and Fishing	www.maff.go.jp/eindex.html
Mexico	SEMARNAT (National Ministry for the Environment)	www.semarnat.gob.mx/
	CONAFOR (Comision Nacional Forestal)	www.conafor.gob.mx/
New Zealand	Environmental Risk Management Authority (ERMA)	www.ermanz.govt.nz
Portugal	Ministry of the Environment	
Sweden	Skogsstyrelsen (The National Board of Forestry)	www.svo.se/eng/default.htm
USA	USDA – Animal and Plant Health Inspection Service (APHIS)	www.aphis.usda.gov/

Internet addresses of organizations publishing information on the Internet (compiled from responses to questionnaires).

Country	Organization	Internet address
Australia	CSIRO Forestry and Forest Products, PO Box Kingston, ACT 2604, Australia	www.ffp.csiro.au/tigr/molecular/molbiology.html
Belgium	Department of Plant Systems Biology, Vlaams Instituut voor Biotechnologie (VIB), Rijvisschestraat 120, 9052 Zwijnaarde, Belgium	www.psb.rug.ac.be/ ; Follow - Research: Research divisions: Molecular Genetics: Tree Biotechnology to find research details
Canada	Atlantic Forestry Centre, PO Box 4000, Fredericton, NB, Canada E3B5P7	www.nrcan.gc.ca
Finland	Punkaharju Research Station, Finlandiantie 18, FIN-58750, Punkaharju, Finland	www.metla.fi/tutumus/index-en.htm ; search for "biotechnology" or Project 3198 under the topic 'Forest Genetics and Tree Breeding'
France	Unité Amélioration Génétique et Physiologie Forestières, INRA-Orléans, Avenue de la Pomme de Pin, BP20619 Ardon, 45166, Olivet Cedex, France	www.inra.fr/
	AFOCEL Biotech, Domaine de l'Etancon, F-77370 Nangis, France	www.afocel.fr/
Germany	Institute for Forest Genetics and Forest Tree Breeding, Federal Research Centre for Forestry and Forest Products, Sieker Landstr. 2, D-22927 Grossgansdorf, Germany	www.rrz.uni-hamburg.de/GeneTree/
Japan	Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki-ken, Japan 305-8687	http://ss.ffpri.go.jp/e_version/majorprograms/mjprograms_j
New Zealand	The Horticulture and Food Research Institute of New Zealand Ltd, Private Bag 92 169, Auckland, New Zealand	www.hortresearch.co.nz
	New Zealand Forest Research Institute Ltd, Sala Street, Rotorua, New Zealand	www.forestresearch.com
Sweden	Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Box 7080, S-750 07 Uppsala, Sweden	www.genfys.slu.se
USA	Institute of Forest Biotechnology, PO Box 13399, Research Triangle Park, NC 27709-339, USA	www.forestbiotech.org
	Tree Genetic Engineering Research Cooperative, 348 Richardson Hall, Oregon State University, Corvallis, OR 97331-5752, USA	www.fsl.orst.edu/tgerc/index.htm

2.3 The state of genetically modified forest trees in China

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2.3.1 SUMMARY

The purpose of this paper is to review thoroughly the current status of research on and applications of genetically modified (GM) forest trees in China. All research into GM forest trees has been carried out with species or hybrids of broadleaved genera, *Populus*, *Eucalyptus* and *Betula* using genes conferring resistance to insect pests and disease pathogens and tolerance to environmental stress. European black poplar (*Populus nigra*) and the hybrid white poplar clone GM 741, transformed with genes for insect resistance, were approved for commercialization by regulatory authorities, and GM plantations were established in China in 2002. Research on genetic modification of larch (*Larix*), targeting reduced lignin content, is still in its infancy. China has a regulatory framework and government policies on genetically modified organisms (GMOs) in place for agricultural crops, and special regulations for forest trees are in the pipeline.

2.3.2 INTRODUCTION

A GMO is an organism that has been transformed by the insertion of one or more genes (called transgenes) (FAO 2001). Accordingly, GM forest trees can be regarded as trees that have been modified by gene technology or have inherited particular traits from the organism initially modified by gene technology for the traits (Office of the Gene Technology Regulator 2002). Genetic modification does not include traditional breeding or natural hybridization, i.e. GM trees cannot be obtained through conventional tree breeding methods. Because of this, the formulation and use of GM trees in applied forestry has increasingly drawn attention from the scientific and non-scientific communities as there is concern about the potential impacts on human health, the environment and the international trade (FAO 1999, 2002).

The ability to transfer new traits rapidly from one species to another has the potential to enhance traditional tree improvement and breeding since generation times for forest tree species are rather prolonged. Therefore, this technique may be of greater significance in forestry than in agriculture. At present, the traits of interest for research on genetic modification in forestry in China mainly include insect pest resistance, drought and salt tolerance for environmental rehabilitation and soil and water restoration, in particular for northwestern China, and wood property improvement to lower amounts of lignin for the paper-making industry.

The process of creating and using GM trees in China can be outlined as follows:

- Phase I, GM plants are created through genetic engineering in the laboratory;
- Phase II, the GM plants are vegetatively propagated in a multiplication area (nursery) to form GM lines;
- Phase III, the GM lines are tested in the field in pilot trials to select clones;
- Phase IV, the selected clones are deployed to establish commercial plantations.

Phases II and III are generally called environmental releases since the GM trees are planted out in the field. Assessments of GM trees must be made at phase III in terms of biology, including biosafety, and silviculture before they are commercially used in plantations.

In China, GMOs are regulated by the 'Biosafety Act for GMOs in Agriculture', which was adopted at the 38th Session of the State Council in May 2001. In the act, GMOs are defined as organisms that have been modified by gene technology, including all gene-transferred plants, animals and micro-organisms and their derived products. In 2002, China had the world's fourth largest area of GM crops, over 2.1 million hectares (Yan 2003; FAO 2004). According to the Bio-Dec database, six GM commercial crops are cultivated in China, including two cotton varieties, two tomato varieties, a green pepper and petunia.

Research into GM forest trees in China started in the late 1980s and has developed very quickly in the last few years. The State Council approved a national programme on research and commercialization of GM plants in agriculture for a period of 5 years. The programme was launched jointly in 1999 by the Ministries of Finance and Science and Technology, with support for 116 projects, three of which were related to forest trees. Most of the research activities in forest trees are undertaken in the Chinese Academy of Forestry (CAF) and forestry universities as well as at institutes of the Chinese Academy of Sciences (CAS).

2.3.3 STATUS OF RESEARCH

2.3.3.1 Insect resistance

Research into GM forest trees was initiated in late 1980s by the Research Institute of Forestry, CAF, in cooperation with the Institute of Microbiology, CAS, with *Populus nigra* for insect resistance. It was first reported (Tian *et al.* 1993) that the toxin gene of *Bacillus thuringiensis* (*Bt*) was successfully inserted into the genome of *Populus nigra* through *Agrobacterium tumefaciens*. A total of 54 GM trees was created. An international field project (CPR/88/041, funded by UNDP [United Nations Development Programme] and implemented by FAO from 1990 to 1995) assisted CAF in high-yield forestry capacity building, technology transfer and laboratory support. The project was instrumental in the first confirmation of tree transformation by genetic modification in the country (Gabriel 1992, 1993, 1994). The 54 regenerated plants were tested by allowing *Apochemia cinerarius* and *Lymantria dispar*, which were major insect pests attacking poplar in China, to feed on them. The results indicated that the mortality of the insect larvae ranged from 80 percent to 96 percent and 100 percent, respectively, 5 and 9 days after feeding. Survival of field transplanted regenerated plants was fairly low. Three GM plants were selected on the bases of growth and toxic performance in the experiment. Subsequently, another study on insect resistance was carried out with *P. nigra* into which *Bt* and *Pi* genes had been simultaneously inserted. The toxicity of this transformed clone was greatly enhanced as the GM explants contained two insect resistance genes (Li *et al.* 2000a).

Chen *et al.* (1995) reported another experiment on insect resistance in which *NPTII* and *Bt*, carried by *Agrobacterium tumefaciens* strain LBA 4404, were integrated into the chromosomes of *Populus deltoides*. It was found that there was significant variation in the number of adventitious buds and rooting rates between GM plants and the control treatment. Rooting rates of just 8–9 percent were achieved with GM plants, which placed a considerable constraint on propagation.

There has been one study reported on resistance to wood-eating insects through genetic modification in China. Li *et al.* (1996) reported that *P. deltoides*, *P. nigra* and *P. × euramericana* were genetically modified through the insertion of the antibacterial gene *LcI*. Results showed that the mortality of the wood borer, *Anoplophora glabripennis*, reached over 75 percent on the GM plants. It was also found that there was significant variation between species in the percentage rooting of adventitious buds cultured on a medium of 40 mg/litre kanamycin for 15–22 days (94 percent, 66 percent and less than 1 percent for *P. deltoides*, *P. × euramericana* and *P. nigra*, respectively).

Tian *et al.* (2000) and Zheng *et al.* (2000), respectively, reported a study on GM trees that was jointly carried out by the Agricultural University of Hebei Province, China and the Institute of Microbiology, CAS with explant materials of hybrid clone 741. The hybrid was developed in 1974 from a rather sophisticated combination of (*Populus alba* × [*P. davidiana* × *P. simonii*]) × *P. tomentosa*. However, foliage of the hybrid appears to resemble that of *P. tomentosa*. The hybrid clone 741 was a sterile female plant, which provided the advantage that there was no gene flow from GM to non-GM populations. In this study a binary expression vector, *pBTiA*, was first constructed with partially modified *Bt Cry1Ac* gene and *API* gene, then the vector containing the two genes was integrated into the genome of hybrid clone 741 through *Agrobacterium*-mediated transformation. In this way GM 741 was produced. The larvae of *Clostera anachoreta* and *Lymantria dispar* were fed on the GM 741 plants. Three plants were selected and propagated by cuttings because they exhibited greatest insect resistance, with mortality of over 70 percent of the total number of larvae tested. Finally, PCR (polymerase chain reaction) and Southern blotting analyses were conducted and indicated that both the *Bt Cry1Ac* and *API* genes had been integrated into the genome of hybrid clone 741. Follow-up research on the technique of vegetative propagation for the GM 741 poplar was also described (Zheng *et al.* 2001).

N-106, a hybrid poplar clone (*P. deltoides* × *P. simonii*; Wang *et al.* 1991), is widely planted in the transition from warm temperate to subtropical zones in China. It is tolerant of hardy and salty conditions but has been subjected to serious insect damage in recent years. A scorpion neurotoxin gene, *AaIT*, separated from *Androctonus australis* which is native to northern Africa, was inserted into the genome of N-106 poplar through a binary vector and 62 GM plants were regenerated. Insect feeding tests indicated that plant A5 was significantly resistant to *Lymantria dispar*. ELISA testing revealed that the *AaIT* gene was expressed in the GM poplar (Wu *et al.* 2000).

Lin *et al.* (2002) reported that the *CpTI* gene was inserted into the genome of a triploid poplar hybrid, (*Populus tomentosa* × *P. bolleana*) × *P. tomentosa*, and the total soluble protein level expressed in the GM plants was increased. The same research group also reported that three major insect pests, *Malacosoma disstria*, *Lymantria dispar* and *Leucoma candida* [= *Stilpnolia candida*], which attack poplars in northwestern China, were tested on plants of the GM lines, TG04, TG07 and TG71, and larval mortality was perceptibly increased (Zhang *et al.* 2002).

Transformation of an insect resistance gene into birch was first reported by Zhan *et al.* (2001). GUS (β -glucuronidase) and Southern blotting analyses revealed that the spider insecticidal peptide gene was integrated into the genome of *Betula platyphylla*, though no further reports have been published on the propagation or field testing of GM plants.

2.3.3.2 Disease resistance

Compared with research on genetic modification of trees for resistance to insects, little research has been undertaken for resistance to disease. Zhao *et al.* (1999) reported that the rabbit alexin gene *NP-1* had been inserted into the genome of *Populus tomentosa*. Results demonstrated that infection by many micro-organisms was inhibited in GM plants but further information on follow-up research has yet to be reported.

Eucalyptus urophylla is one of the major eucalypt plantation species in southern China. It is found, however, that *E. urophylla*, along with a few other species, is significantly damaged by *Pseudomonas solanacearum*. To develop clones more resistant to the pathogen, the cecropin D gene was inserted into the genome of U6, which is a clone locally selected from the introduced populations of *E. urophylla* in southern China. All experimental materials, including the gene, were provided by the Forestry College, South China Agricultural University in Guangzhou. The GM plants were inoculated with *P. solanacearum* and results showed that, 30 days after inoculation, 56.7 percent of GM plants were damaged relative to 86.7 percent of the control treatment (Shao *et al.* 2002).

Another study in GM forest trees, undertaken in Taiwan Forestry Research Institute, is more interesting. The gene coding for C4H (cinnamate 4-hydroxylase) isolated from *Populus tremuloides* was transferred into a selected clone of *Eucalyptus camaldulensis* that had been introduced from Australia. Two GM lines, one with sense and the other with anti-sense C4H (FAO 2001), were obtained with over 100 cuttings regenerated and planted out for further characterization. The objective of this research was to regulate lignin content in the wood. Assessment of the field trials will be made 2 years after planting (Chen *et al.* 2001). Similar research is being undertaken with larch in the Research Institute of Forestry, CAF, although no breakthrough has been made yet.

2.3.3.3 Tolerance to environmental stress

Liu *et al.* (2000) reported that the *MtID* gene had been successfully inserted and expressed in the genome of a poplar (*Populus* × *Xiao Zhannica* cv. 'Balizhuangyang'). The plant material was provided by Shandong Agricultural University and the *MtID* gene was cloned by the Institute of Genetics, CAS. In a subsequent paper it was stated that the GM plants had shown strong tolerance to a concentration of 0.4 percent NaCl in the growth medium (Sun *et al.* 2002a). The methodology of micropropagation for the GM plants was described by the same authors in another paper (Sun *et al.* 2002b). Unfortunately, the exact identity of the plant material used in the study was not clear since various Latinized names were used, perhaps inappropriately. In May 2003, the GM poplar was officially registered for the protection of breeder's right under a Chinese common name, Taiqing No.1 poplar (PVP Office 2003; www.cnvpv.net/announcement.htm). In June 2003, the environmental release was officially approved by the Ministry of Agriculture to establish 100 mu (equal to about 6.6 ha) of plantations with 'the transgenic poplar' (without any specific name) in Tianjin City and Shandong Province. In December of 2003, the research project was thoroughly reviewed, the GM poplar was given another Chinese common name, Zhongtianyang, and assigned a cultivar name as (*Populus* × *xiaozhuanica* W.Y. Hsu and Liang cv. 'Balizhuangyang-zhongtian'). The GM poplar, whatever it is, has now been planted in coastal areas in Shandong Province, where the soil has been so seriously saline that there is a limited choice of tree species for planting.

Few species are adapted to the severe physical environments of northwestern China. An attempt has been made to genetically modify tree species to be more tolerant to environmental stresses (e.g. drought, saline soil and cold).

Yang *et al.* (2001) reported that a salt tolerant gene, *Bet-A*, was inserted into the genome of poplar hybrid, *P. simonii* × *P. nigra*, which is widely planted in northern China. However, no further information is available about how many GM plants were obtained and or whether tolerance was enhanced.

Another study of gene transformation for tolerance to saline soil was carried out with another poplar hybrid (*P. deltoides* × *P. cathayana*). *Mt1D/gutD* as fusion genes were integrated into the genome of the hybrid and four GM explants were obtained in the experiment. Physiological tests indicated that the GM plants showed, to a certain extent, improved tolerance to saline medium at a concentration of 0.4 percent NaCl (Fan *et al.* 2002).

Some research is still ongoing at the Institute of *Populus tomentosa*, Beijing Forestry University, to improve resistance to low temperature. An attempt is being made to clone a cold resistance gene from *P. suaveolens* and transfer it into the genome of *P. tomentosa* (Lin *et al.* 2000; Lin 2001).

2.3.3.4 Research into sterility of GM plants

Genetic-use restriction ('Terminator') technology will be useful in relation to the deployment of non-sterile forest trees. This is, however, a difficult task. It is almost impossible to reduce the risk of gene flow from GM trees to non-GM trees through isolation distances because of the ease of natural hybridization between poplars of the same section, and poplar trees are so widely planted in northern China that pollen and seed dispersal cannot be prevented. An effort was made to develop a sterile mechanism in GM male *P. nigra* by inserting the *TA29-Barnase* gene (Li *et al.* 2000a). Another proposal is to breed sterile poplars for urban planting to prevent nuisance caused by the cotton-like seed flying around; however, there are no successful cases reported yet.

2.3.4 ENVIRONMENTAL RELEASE AND COMMERCIALIZATION

Most of the research on genetic modification in trees is still at the laboratory stage with no further results reported following gene transformation. Only two projects have gone as far as environmental release of GM plants.

In the spring of 1994, a pilot trial on about 1 ha was established with GM plants of *P. nigra*, as described above (Tian *et al.* 1993), in Manas Forest Farm, Xinjiang Uygur Autonomous Region, China. An assessment was carried out, with data collected in 1997 on the performance of the GM insect resistance trait and silvicultural parameters. The results showed that the average percentage of seriously damaged leaves on the GM trees was only 10 percent while that of the trees in control plots planted nearby reached 80–90 percent. The average number of pupae in the GM plantations was much reduced, down to 20 percent of that found in the non-GM control plots. The numbers of pupae and leaves damaged in the GM plantation were far below the threshold set for chemical control measures. It was also found that the non-GM trees mixed in the plantation benefited much owing to cross protection from insects through the presence of the GM trees (Hu *et al.* 2001). Similar results from the same research can be found in another paper by Wang *et al.* (1996).

Following this test, in 1998 the first environmental release of GM trees in China was approved by the Committee of Biosafety, Ministry of Agriculture, and in the following year 80 ha of pilot plantations were established with GM *P. nigra* on eight sites in Beijing, Jilin, Shandong, Jiangsu, Henan, Shanxi and Xinjiang provinces.

Approved by the Gene Security Committee, the State Forestry Administration, GM trees of this species were commercialized for planting in plantation forestry by 2002. It is estimated that one million GM *P. nigra* trees have so far been propagated and used in the establishment of plantations (i.e. about 300 ha of commercial plantations should have been established using GM materials; Su *et al.* 2003a). However, the accurate area of GM plantations cannot be assessed because of the ease of propagation and marketing of GM trees and the difficulty of morphologically distinguishing GM from non-GM trees. A number of individual nursery-men at markets declare that their planting materials are GM trees produced through high-tech, for a higher price. Consequently, a lot of materials are moved from one nursery to another and it is difficult to trace them.

Gao *et al.* (2003) reported the results of an investigation carried out in a 3-year old GM plantation which was established with GM materials of GM 741 in a forest farm close to Qinhuangdao, a coastal city 300 km north of Beijing. It was found that there were significant differences between the GM and non-GM plantation stands in terms of species composition, dominance and community structure of defoliating insects and their natural enemies (Tian *et al.* 2000; Zheng *et al.* 2000). It is estimated that some 0.4 million cuttings of GM 741 have been produced and planted in the field.

These are the only two cases of GM trees that have so far been reported as environmental releases in China. The size of the environmental releases of GM trees is mainly constrained by the quantity of selected GM plants which develop normally. Often, the number of GM plants regenerated from transformation events is too small to establish trials in the field. Another likely constraint is the time and effort required to get governmental approvals to release GM trees into the environment.

At present, only these two types of GM trees have gone through field testing and, following that, entered into commercialization. However, it is almost impossible to work out the exact area of commercial plantations that are established with GM materials in the country. A system should be established to monitor the status of the GM plantations and other associated components living in or impacted by the ecosystems containing GM trees.

2.3.5 REGULATORY FRAMEWORK

The Chinese government has made a large effort to establish a regulatory framework for GM plants, including GM forest trees. Regulations have been put in place or are currently being developed since there is concern about the potential impacts on human health and the environment as well as the trade of products derived from GM forest trees (Lu *et al.* 1999). The regulations are, with relevance to the existing international instruments, legally binding for GM trees from laboratory work to field testing, environmental release and seeking for approval for commercialization. The process of field release approval that must be gone through is a technical assessment which is made by an expert panel, organized by the State Forestry Administration and then a report to the National Committee for Biosafety of GMOs in Agriculture for approval.

Presently, China has committees and procedures in place to carry out evaluations involving the development, release or commercialization of GM forest trees in terms of benefits and biological safety. The regulations binding the R&D and commercial application of GM forest trees are as follows (See Yan 2003):

- Act of Biosafety for the Genetically Modified Organisms in Agriculture, adopted by the 38th Session of the State Council, China in May 2001 which came into force in June 2001.

- Regulation of Assessment on Biosafety of GMOs in Agriculture, issued by the Ministry of Agriculture, China in January 2001 which came into force in March 2002. The assessment of the biosafety of GM plants, animals and micro-organisms and the control measures of security for GMOs and their derived products in agriculture are all included in a separate annex to this regulation. The R&D of GM forest trees must, of course, follow this regulation.
- Regulation of Security for Imported GMOs in Agriculture, issued by the Ministry of Agriculture, China in January 2002 which came into force in March 2003.
- Regulation of Labelling GMOS in Agriculture, issued by the Ministry of Agriculture, China in January 2002 which came into force in March 2003.

At the current time, a specialized regulation for GM forest trees is being drafted by the State Forestry Administration as an addition to the regulations above that forestry has to follow.

Very recently, scientists of Hebei Agricultural University who produced the GM 741 trees have applied for breeders' rights to the new plant variety, and their application has been reviewed by a technical panel. It would be the second case to be granted intellectual property (IP) for GM forest trees in China, following 'Taiqing No.1', if their application were to be approved.

2.3.6 REMARKS AND PERSPECTIVES

In China all GM forest trees are fast growing, vegetatively propagated broadleaved species (poplars and eucalypts) managed in short-rotation plantations. There would be less risk of genetic contamination of non-GM populations if plantations were harvested before reproductive maturity; however, environmental risks must be closely monitored (as reported in an email conference by FAO (FAO 2002).

It will be a long time before GM conifer trees are produced even though scientists are working on larch in a few laboratories in research institutions and universities in China. Research into GM forest trees aimed at increasing tolerance to environmental stress, for instance drought and salt, and to lower the proportion of lignin in wood formation has attracted great support with huge government resources; however, very few results have yet been obtained since this study is still in its infancy in China. Currently, research projects on such subjects are being carried out by several research groups in the Research Institute of Forestry, CAF and other research organizations.

The methodology of gene transformation has almost become routine in the laboratory and many laboratories can currently carry out genetic modification of trees. Unfortunately, most of the research stops after the laboratory stage. Study of genetic modification of forest trees is bottlenecked by basic research in tree molecular genetics, which lags far behind that for agricultural crops, as pointed out by Su *et al.* (2003b). The regeneration of explants of most tree species is so low that materials for field testing and environmental release are limited. Additionally, a large proportion of explants are abnormal. A number of economic traits are changed by the insertion of pleiotropic effect genes or gene silencing. Furthermore, field testing and environmental release take quite a long time. General information on GM forest trees in China is summarized in Table 2.3.1.

There is a knowledge gap to be bridged between scientists doing research in traditional tree breeding and those in biotechnology. Scientists who work on biotechnology are mostly from the younger generation with less experience in practical forestry and little interest in tree

breeding and improvement programmes. On the other hand, people who are used to traditional tree breeding are lagging behind or not familiar with biotechnology. This is probably why GM forest trees have not been used effectively to develop a strategy for tree breeding programmes, or in combination with conventional breeding techniques.

Table 2.3.1. Summary of species and status of R&D on genetic modification in forest trees in China

Tree species	Status of project ¹	Traits targeted	Gene(s) inserted	Stage of development	References
<i>Betula platyphylla</i>	R	Insect resistance	Spider insecticidal peptide gene	On going	Zhan <i>et al.</i> 2001
<i>Eucalyptus camaldulensis</i>	R	Lowering lignin content	C4H	On going	Chen <i>et al.</i> 2001
<i>Eucalyptus urophylla</i>	R	Resistance to disease caused by <i>Pseudomonas solanaceanum</i>	cecropin D	On going	Shao <i>et al.</i> 2002
Poplar hybrid 741 (<i>P. alba</i> × [<i>P. davidiana</i> + <i>P. simonii</i>] × <i>P. tomentosa</i>)	E, C	Resistance to leaf-eating insects	<i>Bt Cry1</i> and API	Applied for, Commercial plantings in 2001	Zheng <i>et al.</i> 2000
<i>Populus</i> × <i>xiaozhuanica</i> W.Y. Hsu et Liang cv. 'Balizhuangyang-zhongtian'	E	Salt tolerance	<i>mtlD</i>	Approved for environment release in 2003. Granted breeder's right in 2003	Liu <i>et al.</i> 2000, Sun <i>et al.</i> 2002a, b, PVP Office 2003
<i>Populus deltoides</i>	R	Insect resistance	<i>Bt</i>	On going	Chen <i>et al.</i> 1995
<i>Populus deltoides</i> × <i>P. cathayana</i>	R	Resistance to leaf-eating insects	<i>mtlD/gutD</i>	On going	Fan <i>et al.</i> 2002
<i>Populus deltoides</i> × <i>P. simonii</i> (N-106)	R	Resistance to leaf-eating insects	<i>AaIT</i>	On going	Wu <i>et al.</i> 2000
<i>Populus nigra</i>	E, C	Resistance to leaf-eating insects	<i>Bt</i>	Applied for commercial plantings in 2002	Hu <i>et al.</i> 2001, Li <i>et al.</i> 2000a, b,
<i>Populus simonii</i> × <i>P. nigra</i>	R	Salt tolerance.	<i>Bet-A</i>	On going	Yang <i>et al.</i> 2001
<i>Populus tomentosa</i>	R	Resistance to disease and stress	<i>NP-1</i> (rabbit alexin)	On going	Lin <i>et al.</i> 2000, 2001

¹R: Research; E: Environmental release; C: Commercial planting.

Another gap lies in research planning. Research projects on GM trees are developed separately from those of applied tree breeding and improvement projects. Study on GM trees should be aimed at species of proven economic value for commercial forestry rather than species which are relatively technologically easier to work with for genetic modification. The superb genetic materials selected by traditional breeding programmes should be used as a priority in genetic engineering. This is the real reason, perhaps, that work on a number of GM trees stopped at the laboratory stage with no further activities as a follow up.

Resource allocation is not balanced for research programmes on traditional breeding and on biotechnology, with most of the governmental funds going to the development of biotechnology. Few research projects on traditional tree breeding can survive today, including seed orchards and provenance trials.

There is a theoretical debate among scientists in China on whether or not forest trees should be genetically modified for resistance to diseases and insects. The adaptation of insects and micro-organisms to changing environments and host trees is faster than the production cycle of GM trees. In other words, natural evolution never stops as the ecological balance is being broken up. Given the potential risks and impacts that GM trees would bring, it is argued by some that diseases and insects can be effectively controlled through the art of silviculture and forest management to keep them below outbreak levels.

On the other hand, GM forest trees are regarded differently from GM plants for food and agriculture. The latter raises issues of direct effects on human health or impacts on the environment, but many people think that 'wood is not food'. It can be foreseen that R&D of GM forest trees will develop even faster in the near future and there is no doubt that GM forest trees will be grown on a large scale in plantation forestry and for land reclamation in China as long as some technical obstacles, mentioned above, are overcome in the coming years.

The Chinese government has set a lofty target for forestry development: that forest coverage will reach 19 percent of the total land area by 2010 and 23 percent by 2020, which is definitely a great challenge facing forest science today and tomorrow. Forest genetics, genetic modification and domestication of forest trees will, beyond all doubt, be asked to make contributions to the goal.

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2.3.8 REFERENCES

- Chen, Y., Han, Y. & Tian, Y. 1995. Study on the plant regeneration from *Populus deltoides* explant transformed with Bt toxin gene. *Sci. Silv. Sin.*, 31(2): 97–103.
- Chen, Z.Z., Chang, S.H., Ho, C.K., Chen, Y.C., Tsai, J.B. & Chiang, V.L. 2001. Plant production of transgenic *Eucalyptus camaldulensis* carrying the *Populus tremuloides* cinnamate 4-hydroxylase gene. *Taiwan J. For. Sci.* 16(4): 249–58.
- Fan, J., Han, Y., Li, L., Peng, X. & Li, J. 2002. Studies on transformation of *mtiD/gutD* divalent genes to *Populus deltoides* × *P. cathayana*. *Sci. Silv. Sin.*, 38(6): 30–35.
- FAO. 1999. *Information note on biosafety*. Document C 99/INF/25. FAO Conference 30th Session. Rome, 12–23 November 1999. (available at www.fao.org/docrep/meeting/x3387e.htm)
- FAO. 2001 Glossary of biotechnology in food and agriculture. FAO Research and Technology Paper No. 9. Rome. (available at www.fao.org/biotech/index_glossary.asp)
- FAO. 2002. Gene flow from GM to non-GM populations in the crop, forestry, animal and fishery sectors. Summary document to Conference 7 (31 May to 5 July 2002) of the FAO Biotechnology Forum. (available at www.fao.org/biotech/logs/C7/summary.htm).

- FAO. 2003. Regulating GMOs in developing and transition countries. Background document to Conference 9 (28 April to 1 June 2003) of the FAO Biotechnology Forum. (available at www.fao.org/biotech/C9doc.htm).
- FAO. 2004. Agricultural biotechnology: meeting the needs of the poor? State of the world's food and agriculture 2003–2004. Rome.
- Gabriel, D.W. 1992. Report on the fourth and fifth missions of the forest genetics consultant. Project High Yield Poplar Plantations in Warm Temperate and Cold Semi-arid Areas of East China. FO:DP/CPR/88/041, Field Document 18. FAO, Rome (*unpublished*).
- Gabriel, D.W. 1993. Final report of the mission. Project High Yield Poplar Plantations in Warm Temperate and Cold Semi-arid Areas of East China. FO:DP/CPR/88/041, Field Document 25. FAO, Rome (*unpublished*).
- Gabriel, D.W. 1994. Follow-up mission report. Project High Yield Poplar Plantations in Warm Temperate and Cold Semi-arid Areas of East China. FO:DP/CPR/88/041, Field Document 26. FAO, Rome (*unpublished*).
- Gao, J., Zhang, F., Hou, D., Wu, B., Zhang, S. & Zhao, X. 2003. Structure of arthropod community in stands of transgenic hybrid poplar 741. *J. Beijing For. Univ.*, 25(1), 62–64.
- Hu, J.J., Tian, Y.C., Han, Y.F., Li, L. & Zhang, B.E. 2001. Field evaluation of insect-resistant transgenic *Populus nigra* trees. *Euphytica*, 121: 123–127.
- Li, Y., Chen, Y., Li, L. & Han, Y. 1996. Transformation of antibacterial gene Lcl into poplar species. *For. Res.* 9(6): 646–649.
- Li, L., Qi, L., Han, Y., Wang, Y. & Li, W. 2000a. A study on the introduction of male sterility of antiinsect transgenic *Populus nigra* by TA29-Barnase gene. *Sci. Silv. Sin.*, 36(1): 28–32.
- Li, M., Zhang, H., Hu, J., Han, Y. & Tian, Y. 2000b. Study on insect resistant transgenic poplar plants containing both *Bt* and *Pi* gene. *Sci. Silv. Sin.*, 36(2): 93–97.
- Lin, S. 2001. *Cold acclimation of freezing resistance of Populus tomentosa and identification of antifreeze protein in Populus suaveolens*. Beijing Forestry University, China, 110 pp. (Ph.D. thesis)
- Lin, S., Xiao, J. & Zhang, Z. 2000. Development in resistance gene engineering researches of poplars. *J. Beijing For. Univ.*, 22(6): 85–88
- Lin, Y., Zhang, Q. & Lin, S. 2002. Identification of expression of *CpTI* gene in transgenic poplars at protein level. Beijing Forestry University, *Forestry Studies in China*, Vol. 4, No. 2, pp. 33–37.
- Liu, F., Sun, Z., Cui, D., Du, B., Wang, C. & Chen, S. 2000. Cloning of *E. coli mtlD* gene and its expression in transgenic balizhuangyang (*Populus*). *Acta Genet. Sin.*, 27(5): 428–433.
- Zhao, S., Zu, G., Liu, G., Huang, M., Xu, J. & Sun, Y. 1999. Introduction of rabbit defensin NP-1 gene into poplar (*P. tomentosa*) by *Agrobacterium*-mediated transformation. *Acta Genet. Sin.*, 26(6): 715–720.
- Zheng, J., Liang, H., Gao, B., Wang, Y. & Tian, Y. 2000. Selection and insect resistance of transgenic hybrid poplar 741 carrying two insect-resistant genes. *Sci. Silv. Sin.*, 36(2): 13–20.
- Zheng, J., Pei, B., Liang, H., Wang, J., Wang, Y., Du, K., Yang, M., Gao, B., Tian, Y., Jia, Y., Yu, H., Myuhu & Ma, Y. 2001. The characteristic of two insect-resistant genes transformed hybrid poplar 741 and its propagation methods. *Hebei J. For. Orchard Res.* 16(2): 97–117.

2.4 Anticipated contribution to and scale of impact of biotechnology in forestry

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2.4.1 SUMMARY

Developments in and applications of forest biotechnology are rapidly expanding. They are becoming both more complex and more accessible throughout the world. There are fundamental differences in public perception and potential applications of biotechnology in forestry relative to agriculture. These differences stem from the multifaceted cultural and utilitarian role forests play, and their relatively recent history of domestication compared to that of agricultural crops. This report represents an attempt to describe these activities and anticipate their contribution in forestry.

2.4.2 INTRODUCTION

Many recent publications, including working groups and conferences of the FAO, have dealt with various aspects of biotechnology in the forestry context (e.g. Krutovskii and Neale 2001; Yanchuk 2001; FAO 2002). During the past 15 years, this field has seen rapid development of new technology and a large increase in the number of groups involved in research and applications around the world (Ellis *et al.* 2001; Campbell *et al.* 2003). Research and testing of modern biotechnology in the initial stages requires up-to-date laboratory facilities. While the technological tools for forest biotechnology are typically adapted from an agricultural background, the subsequent applications and impacts differ substantially when applied to trees (Owusu 1999; FAO 2002). Biotechnology in forestry, for example its use in intensively managed plantations, engenders a host of issues not addressed by agricultural models, and central to those is the relatively long rotation required for forest crops to reach economic maturity. The majority of these differences originate from the fundamental life history characteristics of trees as sessile, long-lived, outcrossing organisms which can disperse pollen and seed across very long distances, and would likely be planted in potential mating proximity to wild, compatible populations or related species (DiFazio *et al.* 1999). Other differences between agriculture and forestry which must be taken into account stem from public perception: people often regard trees as essential components of forest ecosystems which perform many functions and provide many tangible and metaphysical values simultaneously, unlike most crop plants (Owusu 1999; FAO 2002).

Although anthropological influences are traceable in most of the world's forests, and managed forests have been the norm in parts of the world for centuries, trees have been partly domesticated only in the past half century. In effect, very few of these trees are removed more than two or three generations from their wild congeners (Libby 1973). Since trees have fairly long generation times, breeding and deployed populations and even clonal lines produced through various vegetative propagation techniques, including biotechnological methods, are still very similar to their wild contemporaries, unlike many agricultural crop plants (Campbell *et al.* 2003). For example, teosinte has been transformed through millenia of traditional and intensive breeding into a narrow range of maize or corn lines, with a drastically different mating system, range, phenology, physiology and associated genetic characteristics.

2.4.3 CATEGORIES OF FOREST BIOTECHNOLOGY

Recent literature varies in how it categorizes forest biotechnology (e.g. Haines 1994; Yanchuk 2001; FAO 2001). Owing to the gradual evolution of our understanding of genetics, and the consequent development of new tools and technologies by refining and combining new knowledge with prior research, these categories overlap to some degree. The author used five major categories, each of which can be applied to a characteristic subset of applications (see Tables 2.4.1 and 2.4.2):

1. Markers (biochemical and molecular).
2. Propagation and multiplication.
3. Genomics (functional, structural, comparative, associative, statistical).
4. Marker-assisted selection (MAS) and breeding.
5. Genetic modification.

These tools and their utility are applicable on different scales, from the individual cell or plant to the landscape level (see Table 2.4.1). The only category which results in genetic manipulation of living trees is number 5, although development of some markers and DNA libraries for genomics may involve transformation of bacteria in the laboratory. The impact of these biotechnologies will therefore vary with the specific use, as will the associated benefits and risks (see Table 2.4.2; Arntzen *et al.* 2003). Certification of forest products, forest companies and lands is also potentially affected by the application of biotechnology. There are many certification agencies around the world, and some, such as the Forest Stewardship Council (FSC), have specifically excluded genetically modified (GM) trees from certifiability, or the land on which they grow, or other products obtained from stands containing these trees (FSC 1996). Some clonal forestry operations are certified, but most agencies have no explicit guidelines concerning genetic applications. Industrial processes which utilize genetic transformation of enzymes to chemically break down lignin in the harvested wood, e.g. pulping using enzyme digestion, have been certified on the grounds that this reduces toxic chemical use and discharge. Thus, certification standards vary among agencies, countries, products, processes and applications. Public opinion regarding genetic modification is more amenable to downstream (post-harvest) than upstream (pre-harvest) applications; the main cause for concern appears to be the potential for release of GM trees into the environment, which is an issue only for the latter (Pew Initiative on Food and Biotechnology 2001; Gartland *et al.* 2002).

Developed countries and developing countries or countries in transition have different priorities and applications for biotechnology (see Table 2.4.2; Anonymous 2003). DNA-based applications (some markers, genomics and genetic modification) require a large initial outlay of resources, may have continuing high costs depending on the project, and entail a highly trained work force (Ritland and Ritland 2000; Industry Canada – Life Sciences Branch 2001). Developed nations have so far been leaders in developing and applying these technologies for a variety of purposes (breeding, commerce and conservation; public and private uses), but it should be noted, based on the agriculture model, that barriers to the flow of capital and expertise associated with the introduction of biotechnology to new territories are virtually non-existent. In other words, the introduction of forest biotechnology tools to developing countries and countries in transition could be rapid as long as economic opportunities are present. Government agencies in developed countries typically provide much of the funding and infrastructure for the development of basic biotechnology research; however, it should be noted that applied research and development associated with any potential commercialization are mainly driven by the private sector.

The shift of funds supporting biotechnology from the public to the private sectors requires a new way of viewing the introduction of these tools into the forest sector. Thus, published information indicates that the majority of known forest biotechnology activities in developing countries and countries in transition have been more restricted in their scope, primarily to the use of markers and propagation, although other tools may have been introduced into the private sectors but, if so, information on the types of biotechnology application, their places of introduction and their frequency are unknown. The FAO Biotechnology in Developing Countries (FAO-BioDeC) database³⁹ could be a useful storage facility for such forestry biotechnology information.

The flow of some biotechnology through teams including partners both from developed countries and from developing countries and countries in transition, such as CAMCORE (International Cooperative for Tree Conservation and Domestication), have provided increased access to these resources and tools.

Risk assessment is a critical application of many of the new biotechnological tools, and must, in turn, be applied to innovations as they are developed (Government of Canada 1985; Owusu 1999). Targeted gene engineering is of key concern, both with respect to inserting and eliminating gene function. Gene flow assessments are being conducted for poplars (e.g. DiFazio *et al.* 1999), but much more information is required to allay public concerns and deal with ecological hazards surrounding the potential for gene escape (Pew Initiative on Food and Biotechnology 2001; FAO 2002; Giles 2003). This applies to pollen and seed dispersal, as well as the ability of species to sprout via roots or stumps, become vigorous weeds, or hybridize with wild sympatric congeners (Government of Canada 1985; Crawley *et al.* 2001; Dalton 2002; Pilate *et al.* 2002; Adam 2003). Escapes and introgression of agricultural genes have been documented, as has unauthorized planting of GM crop seeds, leading to assessment, monitoring and regulatory problems. The relative fitness effects conferred by each novel trait must be evaluated in the environmental and genetic context of the field in order to assess risks properly (Johnson and Kirby 2001; Dalton 2002; Pilate *et al.* 2002; Anonymous 2003). Promoter genes and other biosensors are being investigated as means of tracking GM material, a necessary precondition prior to approval for field testing or deployment. Experts have widely concurred that a case-by-case examination is necessary for approval of GM trees (Government of Canada 1985; Heron and Kough 2001; FAO 2002; Arntzen *et al.* 2003).

2.4.4 APPLICATIONS

The high cost of biotechnology will probably steer its commercial use towards short-rotation plantations as opposed to less intensively managed forests, but some applications are suitable for all forest types and can guide forest conservation activities (see Tables 2.4.1 and 2.4.2) (Sedjo 1999; Yanchuk 2001). An attempt to indicate the status of each tool and likely future trends in its use will be made in the following sections.

2.4.4.1 Markers (biochemical and molecular)

Biotechnology has progressed from phenotype to genotype, and has been trying to quantify relationships between the two ever since. Early work focused on biochemistry (e.g. terpene analysis), progressed to biosynthetic constitutive or induced protein expression (isoenzymes) and is now utilizing DNA-based tools (e.g. microsatellites, quantitative trait loci [QTLs]) since the advent of PCR (polymerase chain reaction) technology.

³⁹ See: www.fao.org/biotech/inventory_admin/dep/default.asp.

Molecular markers can be either dominant (only the dominant allele is expressed if both parents are genetically different at a given locus) or codominant (the genotypes of both parents are quantifiable at a locus). Dominant markers (amplified fragment length polymorphisms [AFLPs], randomly amplified polymorphic DNAs [RAPDs]) require larger sample sizes for statistical analysis than codominant markers (isoenzymes, microsatellites/simple sequence repeats [SSRs], restricted fragment length polymorphisms [RFLPs], sequence-tagged sites [STSs], expressed sequence tags [ESTs], single nucleotide polymorphisms [SNPs]).

The different markers all have varying benefits, drawbacks, costs and ease of development and all can be applied to a limited range of optimal applications, generally focused on neutral or non-adaptive genetic variation (see Table 2.4.1, Ritland and Ritland 2000, Krutovskii and Neale 2001, and others for reviews). Molecular tools have been widely used to measure gene flow and genetic diversity of natural and artificial populations of forest trees and associated species, and the impacts of anthropogenic disturbance on their evolutionary potential (Haines 1994; Ritland and Ritland 2000; Yanchuk 2001). The long-term viability of species and endemics subject to influences such as pollution or climate change can be modelled using genetic data. There are still no highly repeatable, easily assayed markers directly linked to quantitative traits. Although progress has been made in identifying QTLs for some adaptive traits in some species, a good genomic linkage map is essential, as are breeding and identification and sequencing of candidate genes for quantitative traits of interest. The application of marker technology in forestry is by far the most extensive use of other tools. It is not restricted to either developed countries or developing countries and countries in transition, but the type of markers, the speed of use and the frequency of utilization differs with the status of marker development and the biometrical methods needed for data analyses (see Tables 2.4.1 and 2.4.2).

2.4.4.2 Propagation and multiplication

Clonal forestry using both broadleaves and conifers is gaining in popularity due to the resulting uniformity and ease of silviculture, harvesting and processing. More importantly, it allows the capture of levels of genetic gain that cannot be attained through sexual reproduction. This technology is restricted to organogenesis and somatic embryogenesis, which require extremely detailed understanding of cell biology, multiplication, biochemical signalling, differentiation and the production of copies or clones in a laboratory setting. Both of the above methods capitalize on plant species' ability to regenerate an entire genetically identical individual from a single cell or group of differentiated or undifferentiated cells via *in vitro* tissue culture. Individuals with selected traits or the highest performers from testing trials or breeding programmes can be replicated on a large scale, capturing both additive and non-additive genetic variation from traditional breeding. The advantage of these methods is the ability to store regenerated tissues indefinitely in liquid nitrogen or under laboratory conditions (i.e. hold the genotype constant) while field testing is underway to identify elite lines. This allows selected lines with desirable attributes to be further multiplied for operational deployment. Currently some operational scale production is underway both in developed countries and in developing countries and countries in transition, but it should be noted that the scale of production in broadleaves is larger than that in conifers.

2.4.4.3 Genomics

Our understanding of fundamental biology and evolution is also being enriched through collaborative efforts using model species in genomics research around the world (Krutovskii and Neale 2001). Evolutionary synteny among taxa, even those as distantly related as algae and angiosperms, can be quantified and candidate genes or groups of proteins (microarrays) involved in biochemical pathways of interest can be identified easily: this process of identifying putative genes controlling certain traits is called gene discovery. Expression of key gene families or microarrays and their relative up- or down-regulation following stress or environmental changes can be gauged, possibly leading to identification of compounds of significance, and their interactions with genotype and the environment. Characterization of genetic components of disease or pest resistance is a rapidly expanding field (Ellis *et al.* 2001; Gartland *et al.* 2002). Genomics is a fairly new field with many subdisciplines (Krutovskii and Neale 2001), which requires substantial investment for start-up and maintenance: high-technology automata, costly supplies and chemicals, PCR and other machines, highly trained laboratory staff, marker (EST, SNP) development, as well as vast bioinformatics and associated statistical capacity are necessary. At present, the majority of activity in this area is at the research level.

2.4.4.4 Marker-assisted selection and breeding

While the phenotype is the desired end result of breeding, variation associated with differences in expression, genotype-by-environment interaction and non-additive or epistatic genetic variability necessitates large, costly and time-consuming field trials for trees with traits of interest (Haines 1994; Yanchuk 2001). Mendelian inheritance and our increasing ability to understand and target specific genes have enabled the development of MAS to complement traditional breeding (Haines 1994; Campbell *et al.* 2003). This technique has been used with tremendous success in agricultural crop breeding for various genes and traits over the past two decades, and is increasing in importance as more comprehensive genetic maps, and the locations of QTLs on those maps, are developed for each species. If MAS can be used to characterize and select tree genotypes, substantial cost savings may be realized by a much shorter breeding cycle (i.e. more rapid turnover between generations) (Haines 1994; Sedjo 1999). Although based on tools developed using markers, it requires the use of material of known pedigree and integrates the study of specific structural, functional and morphological attributes of species' genomes. Although this work is experimental at this stage, several studies both in developed countries and in developing countries and countries in transition on broadleaves and conifers are underway. Information on the progress of these studies is sketchy owing to their often mixed funding nature (i.e. public and private).

2.4.4.5 Genetic modification

Research on and the potential for deployment of GM trees have caused widespread public concern. Regulatory agencies have called for more research on gene flow, likelihood of horizontal and vertical gene transfer via escapes, hybridization and introgression, and a range of ecological impacts of GM material in the field (Owusu 1999; Johnson and Kirby 2001; Pew Initiative on Food and Biotechnology 2001; Gartland *et al.* 2002; Anonymous 2003). Concerns about GM trees share similarities with those about agricultural crops, but while the latter are grown and used for research and consumer products throughout many developed and developing nations, transforming trees has only recently begun, and is currently mainly at the experimental stage. In the laboratory, transformation has been achieved using biolistics and *Agrobacterium* species for conifer and broad-leaved genera of commercial importance.

Specific genes and regions, primarily conferring insect or disease resistance, sterility, and wood quality attributes, have been the focus of nearly all of the research (Pilate *et al.* 2002; Campbell *et al.* 2003). Some promoter and marker genes have also been tested. In some regions, pulp and paper processing has achieved dramatic reductions in the use of highly toxic chlorine and other chemicals by employing enzymes genetically modified to digest lignin (Ellis *et al.* 2001; Pilate *et al.* 2002; Campbell *et al.* 2003). The use of genetic modification during industrial processing does not currently appear to cause public concern, especially when the outcome includes substantial environmental benefits. It is difficult to give an accurate account of this type of work since the majority of it is being conducted by the private sector.

2.4.5 SUMMARY TABLES

Table 2.4.1 summarizes the anticipated contribution to and scale of impact of each broad area of biotechnology on elements of forest populations. For instance, the table shows the various forestry practices in which these tools will play a significant role as well as the spatial scale of their use (from the individual tree to population to landscape). Additionally, the specific component activities that these tools will contribute to are broken down into individual activities that are associated with knowledge accumulation in terms of the forest resource, selection, breeding, testing, forest management, IPM (integrated pest management), and downstream activities such as pulping. This table highlights biotechnology categories that are in frequent use, and the development and/or research status of the remaining categories (El-Kassaby and Krakowski 2005).

A summary of the various broad technologies where biotechnology is expected to play a significant role is presented in Table 2.4.2. It shows the broad application and overlapping functions of these categories in relation to various activities. For instance, each broad biotechnology category encompasses its use and a description of its specific tools (markers, tools, traits, areas of use), their frequency of use (from no use to very common), and a brief description of how they are used.

2.4.6 CONCLUSION

There is, to date, no single system systematically collating the different forest biotechnology activities around the world. This would be necessary to gauge current concerns and predict future trends, impacts and needs in this rapidly expanding field. International regulation of biotechnology is inconsistent, and even varies between jurisdictions. Research and development being done in the private sector subject to confidentiality or intellectual property agreements, patent protection and corporate competition preclude or prevent dissemination of some types of information. Other forestry biotechnology applications occur on private lands, or in areas where there is no requirement or framework to report their implementation or extent.

Technical development of forestry, in most cases, mirrors that of agriculture. The use of biotechnology tools in forestry has already caused confusion. As stated above, only one out of five possible biotechnology categories in forestry involves genetic modification and its state-of-the-art is mainly experimental (however, see Section 2.4.4.5, above). Nonetheless, public perception may preclude the use or affect the usefulness of the other biotechnology categories in forestry. The diversity of topics and the variable levels of competence in the field of forestry biotechnology require cross-functional team effort at national and international levels. Wide-ranging contributions that give species and geographic representation are required to ensure accuracy and balance.

2.4.7 REFERENCES

- Adam, D.** 2003. Transgenic crop trial's gene flow turns weeds into wimps. *Nature (London)*, 421: 462.
- Anonymous.** 2003. Missing the big picture. *Nature (London)*, 421: 675.
- Arntzen, C.J., Coghlan, A., Johnson, B., Peacock, J. & Rodemeyer, M.** 2003. GM crops: science, politics and communication. *Nat. Rev. Genet.*, 4: 839–843.
- Campbell, M.M., Brunner, A.M., Jones, H.M. & Strauss, S.H.** 2003. Forestry's fertile crescent: the application of biotechnology to forest trees. *For. Biotechnol.*, 1: 141–154.
- Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D. & Rees, M.** 2001. Transgenic crops in natural habitats. *Nature (London)*, 409: 682–683.
- Dalton, R.** 2002 Superweed study falters as seed firms deny access to transgene. *Nature (London)*, 419: 655.
- DiFazio, S.P., Leonardi, S., Cheng, S. & Strauss, S.H.** 1999. *Assessing potential risks of transgene escape from fiber plantations*. In P. Lutman, ed. Proceedings, Symposium on Gene Flow and Agriculture: Relevance for Transgenic Crops. No. 72. Farnham, UK, British Crop Protection Council.
- Ellis, D., Mellan, R., Pilate, G. & Skinner, J.S.** 2001. *Transgenic trees: where are we now?* In S.H. Strauss & H.D. Bradshaw, eds. Proceedings, 1st International Symposium on Ecological and Societal Aspects of Transgenic Plantations, pp. 113–123. College of Forestry, Oregon State University, Corvallis, OR, USA.
- El-Kassaby, Y.A. & J. Krakowski.** 2005. *Forest biotechnology deliverables*. In: Proceedings, North New Century, New Trees: Biotechnology as a Tool for Forestry in North America, Research Triangle Park, NC, USA, 16–17 November 2004. (in press)
- FAO.** 2001. *Biotechnology in the forest sector* (available at www.fao.org/biotech/sector5.asp)
- FAO.** 2002. Gene flow from GM to non-GM populations in the crop, forestry, animal and fishery sectors. Summary document to Conference 7 (31 May to 5 July 2002) of the FAO Biotechnology Forum. (available at www.fao.org/biotech/logs/C7/summary.htm)
- FSC.** 1996, revised 2000. *FSC Principles and criteria*. Forest Stewardship Council Document 1.2, Principle 6.
- Gartland, K.M.A., Kellison, R.C. & Fenning, T.M.** 2002. *Forest biotechnology and Europe's forests of the future*. Proceedings, Forest biotechnology in Europe: impending barriers, policy and implications, Edinburgh, UK.
- Government of Canada.** 1985, revised 2003. Seeds Act. c. S-8. (available at: <http://laws.justice.gc.ca/en/S-8/98571.html>)
- Haines, R.J.** 1994. Biotechnology in forest tree improvement: research directions and priorities. Forestry Paper No. 118. Rome.

Heron, D.S. & Kough, J.L. 2001. *Regulation of transgenic plants in the United States*. In S.H. Strauss & H.D. Bradshaw, eds. *Proceedings, 1st International Symposium on Ecological and Societal Aspects of Transgenic Plantations*, pp. 101–104. College of Forestry, Oregon State University, Corvallis, OR, USA.

Industry Canada – Life Sciences Branch. 2001. *Biotechnology in the forestry sector*. Natural Resources Canada (available at <http://strategis.ic.gc.ca/bo01532e.html>)

Johnson, B. & Kirby, K. 2001. *Potential impacts of genetically modified trees on biodiversity of forestry plantations: a global perspective*. In S.H. Strauss & H.D. Bradshaw, eds. *Proceedings, 1st International Symposium on Ecological and Societal Aspects of Transgenic Plantations*, pp. 176–186. College of Forestry, Oregon State University, Corvallis, OR, USA.

Krutovskii, K.V. & Neale, D.B. 2001. *Forest genomics for conserving adaptive genetic diversity*. Forest Genetics Working Paper FGR/3. Forest Resources Development Service, Forest Resources Division, FAO. Rome.

Libby, W.J. 1973. Domestication strategies for forest trees. *Can. J. For. Res.*, 3: 265–276.

Owusu, R.A. 1999. *GM Technology in the forest sector: a scoping study for WWF* (available at www.panda.org/resources/publications/forest/gm/gm-risk.html)

Pew Initiative on Food and Biotechnology. 2001. *Biotech branches out: a look at the opportunities and impacts of forest biotechnology*. Proceedings of a workshop. Atlanta, GA, USA, Pew Initiative on Food and Biotechnology, Society of American Foresters, Ecological Society of America. 28 pp.

Pilate, G., Guiney, E., Holt, K., Petit-Conil, M., Lapierre, C., Leplé, J.-C., Pollet, B., Mila, I., Webster, E.A., Marstorp, H.G., Hopkins, D.W., Jouanin, L., Boerjan, W., Schuch, W., Cornu, D. & Halpin, C. 2002. Field and pulping performances of transgenic trees with altered lignification. *Nat. Biotechnol.*, 20: 607–612.

Ritland, C. & Ritland, K. 2000. DNA-fragment markers in plants. In A.J. Baker, ed. *Molecular methods in ecology*, pp. 208-234. Oxford, UK, Blackwell Scientific.

Schmidt, L. 1997. *Tree improvement glossary*. Technical Note No. 46. Humlebæk, Denmark, Danida Forest Seed Centre.

Sedjo, R.A. 1999. *Biotechnology and planted forests: assessment of potential and possibilities*. Discussion paper 00-06, Resources for the Future, Washington, DC, 28 pp.

Stone, G.D. 2002. Both sides now: fallacies in the genetic modification wars, implications for developing countries, and anthropological perspectives. *Curr. Anthropol.*, 43(4) Aug.–Oct. 2002.

United Nations Environment Programme (UNEP). 1992. *The Convention on Biological Diversity*. Article 2. New York and Geneva, United Nations, Environmental Law and Institutions Programme Activity Centre.

Yanchuk, A.D. 2001. The role and implications of biotechnological tools in forestry. *Unasyuva* 204(52): 53–61.

Table 2.4.1. Current global applications and projected future importance and trends of forest tree biotechnology

Broad technologies	Components ¹	Current applications ²		Projected trends
		A	B	
Bioinformatics	Large databases	3	1	The storage, retrieval, analysis, and interpretation of large amounts of biological data will cross boundaries of all broad biotechnologies. Capability with and application range of this tool will continue to increase dramatically. Mining the massively increasing amounts of data at all scales, and integrated analyses and syntheses of these data will greatly increase power to detect genes and understand their functions. Bioinformatics research requires resource-intensive, multidisciplinary teamwork, naturally leading towards more international cooperation over a range of study scales and systems. Opportunities are opening up for developing countries and countries in transition.
	Targeted DNA sequence	3	0	
	Proteomic	2	0	
	Gene mapping & markers	3	2	
	Microarray	2	0	
Diversity measurement	Phenotypic	3	2	The use of molecular markers for studying natural and artificial forest tree populations has undergone unprecedented expansion due to the vast array of population genetics applications they have enabled. These topics include measurement of genetic diversity within and among populations, comparisons among taxa, historical reconstruction and prediction of species' range shifts, gene flow, assessment of natural and artificial (e.g. seed orchard) population mating system parameters, introgression and hybridization. Markers have also been used to evaluate the impacts of domestication and silviculture. Genomic and QTL mapping have recently expanded owing to the development of unlimited numbers of markers. Anticipated development cost reductions for SNPs will trigger an increase in their use in all forest genetics resources applications.
	Integrated applications	1	0	
	mtDNA	2	0	
Gene discovery	cpDNA	2	0	Phenotypic and quantitative trait measurements represent the backbone of all conventional tree breeding. Their proven efficacy and ease of use has resulted in significant gains in many species worldwide.
	RAPD, AFLP, RFLP	3	2	
Gene discovery	Microsatellite (SSR, STS)	1	0	QTLs will likely increase in importance, particularly for important or hard to measure traits, especially those which require older material for assessments (e.g. all wood properties, disease and insect resistance). The focus is shifting from flanking region markers to markers within the actual gene or QTL of interest.
	SNP, ESTP	3	1	
Gene discovery	Phenotypic traits	3	3	Massive, redundant conifer genomes will restrict sequencing to a few regions of interest. EST sequencing for commercially important species will accelerate within 5 years. Whole genome sequencing has begun for <i>Populus</i> ; the data will be freely available; other economically and ecologically important species will follow. International collaboration is important for species whose ranges cross international borders.
	QTL mapping	3	1	
Gene discovery	Genome & EST sequencing	2	0	As microarrays become available for gene discovery for growth and yield, wood quality and adaptive attributes (e.g. disease and insect resistance and stress tolerance), more reliable oligo-based arrays will likely replace cheaper clone-based arrays. The potential benefits and challenges will spur international collaboration. This new field is likely to prove most cost-effective for understanding gene function, and for rapid production technology development for advanced breeding programmes and in high yield plantations.
	Microarray analysis	2	0	
Gene discovery	Proteomic analysis	2	0	Notes for microarray analysis also apply here. Proteomics aims to elucidate protein variation beyond simple transcriptional regulation, including levels of expression, interactions and post-translational modification.
	Phenotypic traits	3	3	

Broad technologies	Components ¹	Current applications ²		Projected trends
		A	B	
Molecular genetic modification	Metabolomics	1	0	Still in the initial stages, metabolomics assesses the presence/absence of non-protein structural precursors of essential components in biochemical pathways.
	Gene insertion/ Sequence modification	2	1	Inserting foreign targeted genes into tree genomes has profound potential. This method will enable cross-species gene transfer in cases where it is not possible via conventional breeding. Transformation has engendered major public contention and strict biosecurity protocols for testing and deployment. Some countries and organizations have restricted testing and planting GM trees. Potential for gene escape into wild populations needs further study for risk assessment. Reproduction must be eliminated or postponed past rotation age. The technique could potentially improve fibre yield and quality as well as other important qualitative, quantitative and adaptive attributes.
	Gene targeting/ Knockout	1	0	Silencing gene function is the complement of gene insertion. This technique will expand as the results of microarray and proteomic analyses accrue. Similarly, there is a broad spectrum of potential functional genomics applications and associated ethical issues.
Product verification	Pedigree verification	3	1	Development of larger-scale, lower-cost application platforms will increase accessibility by genetic improvement programmes to this technology, expanding the prevalent uses: retroactive pedigree verification and verifying clonal identities in seed orchards. Other potential applications include determining the efficacy of seed orchard management techniques on a large scale, and consequent growth and yield determination of resulting crops.
	Quality control/ Quality assurance	1	0	Rapid developments in product description systems, commonly used in food manufacturing facilities, will result in increasing support for research and application of automated product verification systems. The main technology is PCR-based, e.g. DNA markers to detect mislabelled products (e.g. clones). Functional genomics is expected to yield suites of gene markers that can be used to check process efficiencies during mass production of clonal seedlings.
Cloning	Organogenesis	1	1	Micropropagation (organogenesis) and gametic or somatic embryogenesis require treating tissue explants with growth regulators to induce bud or shoot formation. Most of the shortcomings of organogenesis can be overcome using somatic embryogenesis, but methodology and success are species dependent. These methods are currently in production and cloning will be a significant element in high yield plantation forestry.
	Somatic embryogenesis	1	0	
Biosensing	Simple biosensors	1	0	Measuring and monitoring components of clonal production will increasingly employ sensors comprising physical, chemical and molecular markers to detect specific biological processes, e.g. expression-tagged genetic markers to quantify or detect presence/absence of metabolic pathway components of interest.

¹ AFLP: Amplified fragment length polymorphism; cpDNA: chloroplast DNA; mtDNA: mitochondrial DNA; EST: Expressed sequence tag; ESTP: Expressed sequence tag polymorphism; PCR: polymerase chain reaction; QTL: Quantitative trait locus; RAPD: Random amplified polymorphic DNA; RFLP: Restriction fragment length polymorphism; SNP: Single nucleotide polymorphism; SSR: Single sequence repeat; STS: Sequence-tagged site.

² A = developed countries; B = developing countries and countries in transition; on a scale of 0-3: 0 = nil, 3 = common.

Table 2.4.2. Anticipated contribution to and scale of impact of each broad area of biotechnology on elements of natural and artificial forest populations

Applicable forestry component	Spatial scale	Development elements relevant to biotechnology	Broad technologies						
			Molecular applications						Cloning/ Regeneration
			Bioinformatics	Diversity measurement	Gene discovery	Genetic modification	Simple 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	progeny testing	X	X	X			X	X
	tree	attribute assessments	X	X	X		X		
	population	diversity management		X	X				
Production populations	population	mating system	X	X					X
	population	gene flow/contamination	X	X				X	X
	population	seed orchard management	X	X				X	
	population	seed orchard design	X	X				X	X
	population–landscape	silvicultural impact assessment		X			X		X
Regeneration	stand	natural		X	X			X	X
	stand	artificial		X	X	X		X	X
Domestication	population	native species diversity	X	X	X		X	X	
	population–landscape	native species growth/yield			X		X	X	
	population	exotic species risk assessment	X	X	X		X	X	
	population–landscape	exotic species growth/yield			X		X	X	
Gene conservation	population	diversity assessment	X	X					
	population	gene flow/contamination		X			X		X
	population	effective population size		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
	stand	IPM options					X	X	
	tree–landscape	other pest control		X	X	X	X		X
Processing/ Value added	stand	pulp processing	X		X	X	X	X	
	stand	wood treatment	X		X	X	X	X	
Marketing	tree	chain of custody	X		X			X	
	stand–landscape	certification		X		X	X	X	X
	stand	product description	X	X	X			X	

2.5 Acronyms

AAB	African Agency of Biotechnology (Agence Africaine de Biotechnologie)
AB	Antibiotic
AFLP	Amplified fragment length polymorphism
AFOCEL	Association Forêt-Cellulose, France
AGRICOLA	US National Agricultural Library – Article Citation Database
AGRIS	International Information System for the Agricultural Sciences and Technology (FAO)
APHIS	US Animal and Plant Health Inspection Service
ASFA	Aquatic Fisheries and Science Abstracts (FAO)
BioDeC	Biotechnology in Developing Countries (FAO)
<i>Bt</i>	<i>Bacillus thuringiensis</i>
C4H	Cinnamate 4-hydroxylase
CAF	Chinese Academy of Forestry
CAMCORE	International Cooperative for Tree Conservation and Domestication
CAS	Chinese Academy of Sciences
cDNA	Complementary DNA
CHH	Carter Holt Harvey, New Zealand
CIRAD	Centre de Coopération Internationale de Recherche Agronomique pour le Développement (Agricultural Research Centre for International Development), France
CONABIA	Comisión Nacional Asesora de Biotecnología Agropecuaria (The National Advisory Committee on Agricultural Biosafety), Argentina
CONAFOR	Comision Nacional Forestal (National Forestry Commission), Mexico
cpDNA	Chloroplast DNA
CSIRO	Commonwealth Scientific and Industrial Research Organisation, Australia
ELISA	Enzyme-linked immunosorbent assay
ERMA	Environmental Risk Management Authority, New Zealand
EST	Expressed sequence tag
ESTP	Expressed sequence tag polymorphism
ETFRN	European Tropical Forest Research Network
EUFORGEN	European Forest Genetic Resources Programme
FSC	Forest Stewardship Council
FSTA	Food Science and Technology Abstracts
GM	Genetically modified
GMO	Genetically modified organism
GUS	β -glucuronidase

INRA	Institut National de la Recherche Agronomique (National Institute for Agricultural Research), France
IP	Intellectual property
IPM	Integrated pest management
ISAAA	International Service for the Acquisition of Agri-biotech Applications
IUFRO	International Union of Forest Research Organizations
MAS	Marker-assisted selection
METLA	Finnish Forest Research Institute
MMG	Mapping, marker-assisted selection and genomics
mtDNA	Mitochondrial DNA
NGO	Non-governmental organization
NZFRI	New Zealand Forest Research Institute Ltd
PCR	Polymerase chain reaction
PMGC	Poplar Molecular Genetics Cooperative
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RURAL	Database of social and economic development in rural areas of developing countries, Royal Tropical Institute, Netherlands
SE	Somatic embryogenesis
SEMARNAT	Secretaria de Medio Ambiente y Recursos Naturales (Ministry of the Environment), Mexico
SNP	Single nucleotide polymorphism
SOFA	<i>The State of Food and Agriculture 2003–2004</i> (an FAO publication)
SSH	Suppressive subtractive hybridization
SSR	Single sequence repeat
STS	Sequence-tagged site
TGERC	Tree Genetic Engineering Research Cooperative, Oregon State University, USA
TROPAG	Database of agriculture and environment for developing regions, Royal Tropical Institute, Netherlands
UNDP	United Nations Development Programme
USDA	United States Department of Agriculture
VIB	Vlaams Instituut voor Biotechnologie, Zwijnaarde, Belgium