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Biotechnology in the Southern Research Station: A Problem Analysis

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Abstract

We provide an analysis of opportunities and challenges for biotechnology in forest research in the southern United States. Four major areas of biotechnology were identified and described and then rated for priority among three groups of researchers—private sector, public sector, and the USDA Forest Service, Southern Research Station (SRS). The four areas of biotechnology were vegetative propagation, genetic transformation/modification, genomics/bioinformatics, and molecular marker applications. We concluded that these three groups have different research priorities with respect to biotechnology and that these differences complement each other. In particular, the SRS should continue its work in molecular marker technology development for applications in tree improvement, conservation genetics, forest health, and basic science. Also, the SRS should increase its efforts in genomics/bioinformatics while decreasing its research on vegetative propagation. Finally, the SRS should work on assessing the potential risks and impacts of planting and managing clones and/or genetically modified trees.

Keywords: Bioinformatics, clonal forestry, forest biotechnology research, genetic transformation/modification, genomics, marker-assisted selection (MAs), molecular markers, vegetative propagation.

Contents

Executive Summary

Four major areas of forest biotechnology research—vegetative propagation, molecular marker applications, genomics/bioinformatics, and genetic transformation/modification—have different research priorities for private- and public-sector clients. The forest industry clearly favors research that promises to increase productivity and profitability. Private forest industries that have invested heavily in vegetative propagation and gene transformation research hope to commercialize these technologies. Vegetative propagation and gene transformation techniques are also important research tools in genetics studies, and both of these technologies, especially vegetative propagation, are sufficiently well developed to be useful research tools. Molecular marker applications, genomics, and bioinformatics are important supporting research areas for commercial applications, but vegetative propagation and gene transformation technology are the critical elements for commercialization. Public- and private-sector clients are likely to support research to sustain ecosystems, restore species, and preserve threatened and endangered species. Thus, both client groups are interested in molecular marker studies of population structure and dynamics and in genomics research on genes controlling traits like disease and insect resistance. Genomic studies and bioinformatic analyses provide a foundation for understanding the genetic basis for traits of commercial interest, as well as traits that enhance forest health and sustainability.

Publicly funded research should support both public- and private-sector clients. The central question is how to prioritize publicly funded research in the Southern Research Station (SRS) among the four biotechnology research areas. Private companies have developed efficacious vegetative propagation and gene transformation technology, and both technologies may soon be available for public and private organizations that want to capitalize on genetically modified (GM) trees. However, the benefits from these technologies will accrue primarily to private forest industry. Developing these techniques in publicly funded labs would

require duplicating a substantial amount of proprietary research. Furthermore, citizens' concerns may prevent planting clones or GM trees on public lands in all but a few circumstances where genetic modification and/or vegetative propagation are the only means to achieve the common goal. For example, foresters may deploy GM trees resistant to a new disease or insect pest when traditional breeding programs would take too long to protect adequately a species at risk. Publicly funded research should develop methods to evaluate and monitor the risks of deploying clones and GM trees since policymakers will require unbiased information on which to base regulation.

Molecular marker applications address many questions important to both public- and private-sector clients. Many applications have the potential to increase forest productivity and health. Molecular markers can enhance the efficiency of breeding programs through marker-assisted selection (MAS). They can also be used to deploy trees with known resistance to diseases like fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*) in high hazard areas and to monitor genetic diversity as it changes in commercial breeding populations. Molecular markers permit us rapid inference of genetic structure and mating patterns. Such population information is important for commercial species, but is especially valuable for policymakers considering the fate of threatened and endangered species. Molecular markers may also resolve taxonomic issues for conservation prescriptions and form the basis for constructing genomic maps, which add value to many of these analyses. Because of the broad application for both public and private clients, molecular marker applications are a high research priority in the SRS. Furthermore, molecular marker application is fast and relatively inexpensive biotechnology.

Genomics studies and bioinformatic analyses explain the regulation and expression of genes. Structural genomics involves genetic and physical mapping and large-scale DNA sequencing that clarify the overall organization of the genome. Functional genomics involves large-scale gene analysis to discover key genes and

regulatory networks. Bioinformatics integrates data from structural and functional genomics research. With increasing capability in genomics and bioinformatics, the SRS can apply the *Populus* genome sequence and sequences from other plant species in its own research. Discovering genes related to forest productivity and health and understanding their regulation will be critical for addressing emerging research issues. Both public and private groups can use genomics and bioinformatics to understand the genetic basis for wood formation, disease and insect resistance, flowering, and stress response.

Recommendations

The SRS should continue to emphasize the application of molecular marker technology to a broad range of questions about forest genetics and tree improvement and should expand its capability in genomics research and bioinformatics.

The SRS should deemphasize its own research in vegetative propagation and gene transformation methods, but should seek opportunities to support the research of others in these areas.

The SRS should evaluate the risks of clonal and GM tree deployment.

Introduction

The U.S. Department of Agriculture Forest Service must align its research priorities in forest biotechnology to meet the needs of both public and private forest landowners. Their interests overlap significantly in many areas, but we perceive a dichotomy in the hierarchy of importance that both groups give to different research areas. The private sector is likely to support research to increase profits, while the public sector supports research on ecosystem sustainability or restoration. This report will identify and prioritize research that both groups need over the next 5 years. It will provide a broad overview of currently available biotechnology for forest research and serve as a planning tool for prioritizing biotechnology research within the SRS.

Two groups of landowners have different land management objectives. Currently, the South has about 200 million acres of forests. The Forest Service manages about 6 percent, other Government agencies 5 percent, forest industries 19 percent, and nonindustrial private forest (NIPF) landowners 70 percent (Wear and Greis 2002). The South produces about 60 percent of the Nation's timber products, and a large share (41 percent) of the net annual growth comes from the small proportion (11 percent) of growing-stock volume in non-Federal plantations (Wear and Greis 2002). Industry owns 54 percent and NIPF landowners own 40 percent of these plantations, the majority of which are of loblolly (*Pinus taeda* L.) and slash (*P. elliottii* Engelm.) pines. By 2040, the total acreage of pine plantations is forecast to rise by 67 percent (Wear and Greis 2002).

Virtually all of the 1.2 billion loblolly pine and 150 million slash pine seedlings planted each year are genetically improved (McKeand and others 2003). Private forest companies, three southern universities, and all Southern State forestry organizations conduct cooperative breeding programs for loblolly and slash pine. The Forest Service—SRS and Region 8 of the National Forest System (NFS)—also support research in tree improvement and genetics of these two important commodity crops as well as other species of pines and hardwoods.

The benefits of genetic improvement are available to both private forest industry and public growers who purchase seedlings from private and State nurseries. Biotechnology research promises to increase productivity and add value to commodity crops. The private sector (to include those NIPF landowners whose primary objective is timber production) wants research on technologies that increase the profits and long-term productivity of plantation forestry.

Population growth threatens rare forest communities, and rare plant and animal species. The Southeast has the highest number of endangered ecosystems of any region in the country, and 30 percent of these are critically endangered (Wear and Greis 2002).

Laws protect many of these communities, plants, and animals on public and private lands, and every Southern State has both threatened and endangered species (Wear and Greis 2002). Many species at risk occur on public lands, which represent a relatively small percentage of forested land in the South (Wear and Greis 2002). Government land is an important component in preserving endangered species. To preserve sensitive ecosystems and the plant and animal species dependent upon them, the forest industry has transferred ownership of some critical natural lands to public or private conservation organizations (Wear and Greis 2002). Clearly, on publicly owned lands, there must be different emphases among management objectives, including the status of both commodity and noncommodity species.

A dichotomy of land management objectives creates two different client groups for biotechnology research in the South. Although many areas of biotechnology research overlap, private landowners are more likely to favor research to increase forest productivity and/or profitability. A broader constituency representing the public sector favors research on both commodity and noncommodity species with the aim of ecosystem sustainability or restoration.

We define biotechnology as any technological application that uses biological systems, living organisms, or derivatives thereof to make or modify products or processes for specific use. Biotechnology in forestry generally falls into one of four main topics: (1) vegetative propagation, (2) molecular marker applications, (3) genomics/bioinformatics, and (4) genetic transformation/modification. Private forest owners and public forest managers have different priorities for research. Forest industry is primarily interested in increasing profitability while maintaining sustainability of productivity for commodity crops. The public sector is primarily interested in sustaining or restoring ecosystems and species at risk. The four main topics for biotechnology research are ranked for public- and private-sector users and for the SRS. Biotechnology research areas prioritized for public and private sectors and for the SRS are:

Biotechnology research area	Research priority		
	Private	Public	SRS
Vegetative propagation	1	3	4
Genetic transformation/modification	2	4	3
Genomics/bioinformatics	3	2	2
Molecular marker applications	4	1	1

The potential for application to both public- and private-sector clients dictates the general rationale for the SRS ranking. Supporting analysis for these rankings appears in the body of this analysis. Both public and private constituents benefit from research in molecular marker applications and in genomics and bioinformatics. Vegetative propagation and genetic transformation/modification technology provide useful research tools for ecosystem sustainability and forest health, such as clonal propagation of threatened or endangered species. However, the benefits of large-scale applications will accrue primarily to private-sector landowners.

Recommendations for the Southern Research Station

The SRS should continue to develop molecular genetic marker technology for a broad range of applications in tree improvement, conservation genetics, forest health, and basic forest genetics.

The SRS should significantly increase research in genomics and bioinformatics to take advantage of new data from complete genome sequences and gene discovery projects on model plants and forest trees.

The SRS should deemphasize research into vegetative propagation methods. The benefits from successfully implementing vegetative propagation for commodity species in the South will accrue primarily to the private sector, which has a significant lead in developing this technology. A significant Federal effort in this area would duplicate proprietary research already done by private industry. Vegetative propagation techniques are already sufficient for use in research.

The SRS should assess the risks and potential impacts of clonal and GM tree deployment on southern forest ecosystems. It should not study transformation methods, per se, because other groups are better positioned to conduct this research.

Although vegetative propagation and genetic transformation/ modification technology are not primary objectives, publicly available methods for these would be valuable. Thus, the SRS should seek opportunities to support such research by others.

Opportunities for the Application of Biotechnology in Forestry

Vegetative Propagation

Currently, the most significant barrier to clonal forestry and the application of GM technology is the economic production of large numbers of vegetative propagules from trees old enough to have been adequately tested. For decades, forest tree improvement programs have used vegetative propagation in its simplest forms (grafting and rooting cuttings). But large-scale reforestation by vegetative propagation is limited to species for which rooting cuttings is easy for mature trees or for which rejuvenation techniques are available.

For two primary reasons, finding an economical method to produce clones of tested genotypes is a high priority for foresters who want to increase profits through biotechnology. First, the ability to produce clones of mature genotypes of loblolly and slash pines with proven genetic value would increase economic returns from traditional tree improvement programs. Current methods of producing large numbers of rooted cuttings permit breeders to capitalize on genetic differences among families by planting rooted cuttings from immature and untested progenies of tested parents. This method is genetically equivalent to producing large numbers of control-pollinated seeds by conventional means (Frampton and others 1999) while cloning the best individual in the best family maximizes the realized gains from these traditional programs (Frampton and others 1999). Second, capitalizing on genetic

transformation/modification technology depends on transforming tested genotypes and transforming these in large numbers. Successful GM clones are proven genotypes into which genes from the same or different species have been introduced.

Vegetative Propagation Is an Important Tool in Experimentation

Cloning individuals to control the inherent variation among genotypes is a powerful experimental tool (Loegering 1984). For example, we need to understand the underlying genetic interactions between host and pathogen in fusiform rust disease in loblolly and slash pines (Nance and others 1992). Although controlling fusiform rust infection has significant economic benefits, cloning can increase the efficiency of experiments (Shaw and Hood 1985). Cloning is more feasible for experimentation than for producing commodity crops since success rates and economic returns are less important. In fact, experiments currently use clones of several species, including loblolly and slash pines, that are difficult to propagate vegetatively in large numbers (Anon. 2000). Vegetative propagation already occurs at a practical scale and cost for experimentation. Also, because the ability to clone is not required to address many important questions about forest ecosystems, it is of secondary importance for public-sector research.

The Future of Clonal Forestry Is Near

The successful development of micropropagation is the future of clonal forestry, especially for species that root poorly as older trees. The most promising technique, somatic embryogenesis (SE), produces clonal lines from immature seeds. SE is the process of culturing explants from immature zygotic embryos to produce embryogenic masses from which somatic embryos are produced and germinated. Emblings thus generated, i.e., somatic seedlings, are genetically identical members of a clone of the original embryo. Successful SE for several species, including the southern pines, is currently under commercial development. Coupled with cryopreservation while genotypes are being tested, SE is the most promising method for clonal forestry in many species (Vendrame and others 2001).

However, substantial barriers remain to successful clonal forestry in the southern pines (Pullman and others 2003). In the South at present, vegetative propagules of selected genotypes are being planted only in experimental trials. Not every tested genotype can be successfully vegetatively propagated with current SE technology, and the success of maintaining or restoring juvenility in stock plants for rooted cutting production of clones is still under study. Currently, the cost of producing vegetative propagules remains high enough to deter both rooted cuttings and SE. Furthermore, researchers have not adequately assessed the risks of clonal deployment.

Molecular Marker Applications

Molecular genetic markers are genes or other identifiable portions of DNA with traceable inheritance. Markers have many applications in forest genetics and tree improvement. A researcher can select among several kinds of DNA markers with different attributes that best suit an experiment (reviewed in Cervera and others 1997; Nelson, in press; Wang and Szmidt 2001). As gene-sequence information becomes more readily available for forest trees, sophisticated marker techniques developed for other organisms will aid forest genetics (Harry and others 1998). These new markers, called expressed sequence tag polymorphisms (ESTP or EST), derive from complementary DNA (cDNA) libraries that represent expressed genes. EST markers provide most of the attributes desired in molecular markers. More recently, single nucleotide polymorphisms (SNP) have been developed in forest trees (summarized in Plomion and others 2003). Abundant SNPs may have application in genetic mapping studies that do not rely on pedigree structure such as mapping by association or linkage disequilibrium mapping (Kwok 2001, Roses 2000).

Molecular genetic markers have applications in “fingerprinting” genotypes; DNA sequence variations detected by examination of multiple markers produce a unique identifier for a genotype. Fingerprinting unambiguously identifies specific genotypes and can also provide evidence for the origin of a genotype with respect to

provenance and species as well as species hybrids. A common use in tree improvement is to certify the parentage of controlled crosses (Lin and others 1997). All of these uses have practical applications in forest management.

Molecular genetic markers provide information about populations. Markers are useful for estimating population genetic structure, level of inbreeding, gene flow, and type of mating system (Adams and others 1990). They also provide phylogenetic (Tsumura and others 1996) and paleobotanical (Marshall and others 2002) insights. Genetic markers can produce population genetics information more quickly than common garden studies. The combination of markers and common garden studies such as seed source or provenance tests provides the most powerful information for understanding population genetic structure, mating system, and gene flow. However, in situations requiring rapid decisions about the conservation or management of species at risk, genetic markers alone provide timely and useful information for policymakers and forest managers. Genetic markers can also resolve taxonomic questions to determine appropriate conservation prescriptions (Gordon and Kubisiak 1998).

Molecular genetic markers may directly enhance traditional breeding. Statistical associations between markers and genes can be made for traits of interest. Subsequently, the presence or absence of markers can indicate the presence or absence of the desired gene. Using markers to guide selection and breeding in traditional tree-breeding programs is termed MAS (Williams and Neale 1992). Although the concept is straightforward, the application of MAS is problematical and is not routinely applied in forestry because the conditions are restrictive for economic viability and economic success is not assured (Byram 2000). However, genetic markers can direct planting decisions for loblolly pine families that carry major genes for resistance to fusiform rust (Wilcox and others 1996). Markers are also an effective way to monitor genetic diversity in tree-improvement programs as they move through successive cycles of selection and breeding (Williams and Hamrick 1996).

Molecular genetic markers are valuable in maintaining forest health. Used to determine the origin of introduced pathogens or insects, markers can reduce the possibility of future introductions. As diagnostic tools, molecular markers can be used to detect trace amounts of pathogens in samples (Blomquist and Kubisiak 2003) and measure gene flow among pathogen populations providing a means to help manage risk to forest ecosystems (Kinloch and others 1998, Klopfenstein and others 2001). As we learn more about the genetic basis for disease development in host:pathogen systems (Kinloch and others 1998), it will become possible to detect and monitor resistance and virulence genes in the host and pathogen populations, thus providing a valuable tool for controlling forest diseases.

Genomics and Bioinformatics

Genomics permits geneticists to make the connection between genes and phenotypes. Genomics is the study of genomes, the total DNA and RNA in cells. Genetics is the study of single genes inferring their presence and effect from phenotypes. Genomics considers genes as a complete, dynamic system as they interact with one another in different tissues and over time, linking gene expression to phenotype and allowing phenotype to be predicted from genotype. Structural genomics studies genome organization. Functional genomics studies gene regulation and expression, and their role in cell function and phenotype determination. Bioinformatics is necessary to manage and analyze the large, complex datasets that result from structural and functional genomics research.

Genetic maps provide a framework for integrating diverse types of genetic information. Forest geneticists have used molecular markers for decades (Neale and Harry 1994). Several new types of markers have attributes that suit different applications, but all markers are cumulatively informative. Positioning all available markers on a single reference map integrates past and future genetic maps (Sewell and others 1999). Such a reference map

shows how genomes are organized and provides insight into how they function. These maps are also critical components of MAS, map-based cloning, and gene discovery projects.

Genetic linkage maps rely on estimating crossover, i.e., recombination, frequencies during meiosis. Genes located near one another are linked since they tend to be inherited together. Because crossing over occurs, the crossover frequencies can serve as surrogates to order markers into linkage groups and construct maps of their relative positions within these groups (Hartl and Jones 2001, chapter 5). Physical mapping measures the number of base pairs of DNA between markers rather relying on recombination frequency. Physical maps are based on sequences of overlapping segments of DNA (contigs) that provide measures of distances (base pairs) between probes or markers within the contigs. Physical mapping is done by hybridizing probes against a library of DNA clones that are subintervals of a larger piece of DNA. If a clone contains a sequence complementary to the probe, the clone and probe will hybridize and produce a signal. By comparing and ordering the probe sequences, it is possible to determine the order of probes (and their DNA complements) in the larger piece of DNA. This information can identify genes and their relative positions along chromosomes. Physical maps can also be visualized when specific chromosome segments are labeled with compounds that can be seen with a light microscope (Hartl and Jones 2001, chapter 13). There is a current effort to map *Populus* using bacterial artificial chromosome (BAC) libraries (U.S. Department of the Interior, Oak Ridge National Laboratory 2003).

Genetic linkage mapping shows the relative positions of chromosomal segments and genes, but does not identify the exact physical location of the segments on chromosomes. Other techniques do permit the visualization of the physical location of segments on chromosomes such as fluorescent *in situ* hybridization (FISH). FISH techniques use genomic probes, which are single-stranded DNA or RNA of a specific base sequence labeled in some way so they can be visualized; FISH probes are labeled with

fluorescing dyes, hence the name. Probes hybridize when they pair with their complementary strands of DNA to form a double-stranded molecule. Once the probes have hybridized, the physical location of the sequences that complement the sequences of the probes can be visualized, providing a karyotype that depicts an individual's chromosomes arranged in a standard format showing the number, size, and shape of each chromosome (Doudrick and others 1995). Physical mapping has several potential advantages over linkage mapping. First, there are genomic regions in which little crossing over occurs. Since linkage maps rely on crossing over, physical maps are required to study these chromosomal regions. Second, as genetic distance gets smaller, crossover rates decline. Thus, very large populations of individuals must be mapped to locate genes separated by small genetic distances. Physical mapping does not require recombination, so it can provide useful information from a single genotype.

Map-based cloning can be used to identify and clone a gene of interest. Map-based cloning requires a sufficiently dense genetic map to locate markers flanking a genomic region that codes for a phenotype of interest. The region flanked by the markers is then isolated, sequenced, and examined for differences in individual genes expressing different phenotypes. In addition to a dense genetic map, a good physical map is invaluable to guide the "walk" to the gene, especially in large, complex genomes such as *Pinus* and *Populus.* Map-based cloning is relatively simple in plants like *Arabidopsis* where there is a wealth of genetic and physical map information available (Jander and others 2000). Map-based cloning may soon be feasible for *Populus,* as it is the first tree genome to be sequenced (DOE Joint Genome Institute 2002) and it has a good physical map based on BAC contigs. Again, map-based cloning is problematical for conifers such as *Pinus* due to their large, complex genomes (Murray 1998) and because a complete sequence awaits improvements in technology (Lev-Yadun and Sederoff 2000). However, new technology may significantly ease the task (Peterson and others 2002).

Sequence tagged sites (STS) offer a way to physically map genes. STSs are DNA sequences that occur once in a haploid genome. Once they have been sequenced, appropriate primers can be designed that permit their detection by polymerase chain reaction amplification (Hartl and Jones 2001, chapter 13), which amplifies a DNA sequence inclusive of the primers. Given adequate numbers and distribution in the genome, STSs can be used to assemble contigs of BAC clones to provide a physical map of the markers. If STSs are derived from DNA of expressed genes, they are even more valuable as they tag a specific gene. An EST can be used to assemble contigs of expressed regions if they are unique and do not belong to a gene family with more than one gene of the same, or similar, sequence. ESTs may also be used to construct microarray experiments to couple gene expression profiles with phenotypes (Sterky and others 1998).

A candidate gene approach offers another method for gene discovery and studying their function in large genomes. This approach compares the sequence homology of genes that are expressed in tissues of interest with proteins of known or predicted function. Gene expression describes the transcription of DNA into messenger RNA (mRNA) that is subsequently translated into proteins that perform cell functions. Thus, the kinds and amounts of mRNA produced by a cell reflect which genes are expressed and permit the study of how the cell responds to different situations. The process begins with isolating mRNA from the appropriate tissue, such as developing xylem to study wood formation, and reverse transcribing them into cDNA. After random selection, cDNA clones are partially sequenced to produce ESTs whose sequences are compared to sequences of proteins of known or predicted function in public databases. As the sequences available in public databases increase, the candidate gene approach will assume greater importance in genomics studies of forest trees. To enable the genetic transformation/modification of existing commercial lines of forest trees, gene discovery projects in progress try to identify and map the genes active during different stages of xylem formation (Chen and others 2001, Lev-Yadun and Sederoff 2000)

and flowering (Rottmann and others 2000), among other traits. Hundreds of thousands of ESTs for many species (for a list, see The Center for the Advancement of Genomics 2000) are stored in databases accessible through the Internet (National Center for Biotechnology 2003, Pittsburgh Supercomputing Center 1999). These sequences represent an unprecedented opportunity for forest geneticists, and our challenge is to use this information effectively.

Microarray and sequencing technology enable the simultaneous study of the expression of many genes. With this technology, we can determine not only whether genes are switched “on” or “off,” but also the degree to which they are expressed. Microarrays are solid supports made of various materials upon which an organized array of spots of genomic DNA, cDNA, or oligonucleotides (DNA usually < 40 base pairs) is immobilized. The microarray is coincubated with cDNA probes derived from tissues of interest and labeled with a fluorescent tag. The fluorescent tag enables the construction of a digital image of the array that varies depending on the amount of cDNA hybridized with the target DNAs on the microarray. Thus, the greater the fluorescence, the greater the gene expression for a given sample. By using different colors of fluorescent tags, a single experiment can differentiate more than one sample (Hartl and Jones 2001, chapter 13). For example, microarray expression analysis with different colored tags can label diseased and healthy tissue, and the expression of each sequence can be quantified on each immobilized DNA spot (Morse and others 2004). Researchers can determine which sequences are expressed more or less under different conditions, e.g., stressed and nonstressed, and infer which sequences are involved in defending against stresses (Wu and others 1999). Conceptually, this same technology identifies which genes are involved in any other processes. Gene expression is dynamic and varies among different tissues and over time, so the experimental design of microarray experiments must adapt accordingly. Microarray mutation analysis can detect small changes in DNA sequence such as SNPs. The SNP expression patterns generated are powerful molecular markers (Kwok 2001).

The very large databases generated from genomics studies require a capability in bioinformatics. Bioinformatics employs computational science to manipulate the huge databases containing nucleotide and amino acid sequences and associated information. Using bioinformatics, researchers can efficiently assess relationships among members of very large datasets. With these computational tools, they can locate genes within sequences, generate computerized genomic maps, and compare candidate genes with similar sequences from organisms that have been better characterized. A viable bioinformatics capability is a critical component of genomics research.

Genetic Transformation/Modification

Genetic transformation/modification has the potential to confer significant genetic changes in plants more rapidly than conventional breeding. Genetic transformation/modification is the introduction of a heterologous (foreign) or homologous (conspecific) gene or genes into a plant's genome by artificial means. Traditional breeding methods take many generations of selective mating to produce changes in trees that genetic engineering may accomplish in a few years. Introducing new genes into an existing genetic background would clearly benefit growers interested in improving profits from forest trees.

Transgenic trees have important commercial applications. *Populus* have been transformed to incorporate genes from a natural insect pathogen, *Bacillus thuringiensis*, to confer resistance to insects (McCown and others 1991) and to modify wood composition (Hu and others 1999). Extending these results to more commercially important species, e.g. loblolly pine, may provide a large economic impact. Biolistics (Charest and others 1997) can produce GM trees, and other organisms such as *Agrobacterium* (Kim and others 1997) can serve as vectors for introducing new genes. Efficient transformation systems that include vertically integrated gene function testing can test novel gene constructs in forest trees (Hinchee and others 2003). Large numbers of promising gene constructs are first screened in an easily transformed model

species, such as *Populus*. Promising constructs are then used to transform more intractable, but economically important, such as *Pinus* or *Eucalyptus* spp. In the near future, the technology to produce transgenic southern pines will become more efficient (Connett and others 2002).

There is significant public debate about planting GM trees. In British Columbia and Alberta, citizens' objections to planting GM trees are so great that moratoria have been adopted or are pending (British Columbia Ministry of Forests 2000). In Europe, a moratorium on planting GM crops is effectively in place (McCord and Gartland 2002). Such moratoria may end if public perception changes. At present, the United States may not permit the planting of GM trees, particularly on public lands. In May 2003, a "Stakeholder Forum on Biotechnology," convened by the Pew Initiative on Food and Biotechnology, "generally agreed to outcomes, principles, and components for a regulatory system for agricultural biotechnology that protects public health and the environment" (Pew Initiative on Food and Biotechnology 2003). However, the forum was unable to reach consensus on the "full range of issues in sufficient detail" on a package of regulatory reforms (Pew Initiative on Food and Biotechnology 2003). The U.S. Government retains an interest in developing consensus on policies regarding the use of biotechnology in agriculture. Thus, an advisory committee to examine the long-term impacts of biotechnology on the U.S. food and agriculture system and provide guidance to the U.S. Department of Agriculture on pressing individual issues was established by the Secretary of Agriculture (National Archives and Records Administration 2003).

Policymakers need sound science upon which to base their decisions. The primary risk of planting GM trees is the unknown consequence of GM trees mating with undomesticated trees. Novel genes, such as those carrying insect resistance, will likely escape into wild tree populations with unknown impacts on both target and nontarget species. Most domesticated crops will hybridize with their cross-compatible wild relatives (Ellstrand and others 1999). Since

genetically improved forest trees are only one to a few generations of breeding and selection removed from undomesticated relatives, they will certainly hybridize. If efforts to genetically modify trees reduce or prevent flowering, this concern may be ameliorated. However, some scientists fear that adverse effects may surface in 50 to 100 years (Kaiser 2001). Since many GM organisms are not regulated after they are commercialized, private industry, which will most benefit from their use, may not take adequate steps to contain and monitor GM trees for their impacts on ecosystems. Furthermore, Mexico currently has no policy against planting GM trees.[1] If transgenic trees are planted in Mexico, wind can easily transport pollen into the United States. Public agencies should, therefore, assess risks and impacts for GM trees to provide credible information to direct policy decisions.

Genetically modified organisms may more readily gain public approval in certain situations. Many exotic pests have been introduced into the United States, and the rate of introductions is increasing (National Biological Information Infrastructure 2003). Genetic transformation/modification of forest trees to rapidly introduce sources of resistance to exotic pests may be the only efficient way to respond to new introductions quickly enough to prevent ecological disasters. The long generation time of most forest trees complicates response to newly introduced exotic pests with traditional breeding methods. For example, it may be possible to introduce genes for resistance to the balsam wooly adelgid (BWA) [*Adelges piceae* (Ratzeburg)] into Fraser fir [*Abies fraseri* (Pursh) Poir.] from other true firs (*Abies* spp.). BWA has been decimating adult Fraser fir populations in the Southern Appalachian Mountains since the early 1950s, and there is no known resistance to BWA in Fraser fir populations. However, some related species of *Abies* are apparently more resistant than Fraser fir (Mitchell and others 1970). Genetic transformation/modification of American chestnut [*Castanea dentata* (Marsh.)] with heterologous genes from

[1] Personal Communication. 1993. Dr. Francisco Garcia Garcia, Director of Research and Development, National Forest Agency, Mexico.

other *Castanea* spp. may also offer a faster means than traditional backcross breeding of deploying trees resistant to the chestnut blight (*Cryphonectria parasitica*) (Kubisiak and others 1997).

Genetic transformation/modification is the standard for demonstrating gene function. The clearest way to demonstrate gene function is to remove it from its background, intentionally modify its expression, and create transgenic lines in which to study the altered expression. The altered expressions can inactivate a gene ("loss of function" or "knockout" mutations), activate a gene when or where it is not expected to be expressed ("gain-of-function" mutations), or alter its expression so it is overexpressed or underexpressed. Thus, the ability to transform plants for genomics research is critical.

Research Status and Opportunities for the Southern Research Station

Each area of biotechnology research has potential value to our public- and private-sector clients, and we recommend the future involvement of the SRS in each area. This discussion assumes that the SRS through the Southern Institute of Forest Genetics (SIFG) wants to continue or assume a significant leadership role in a particular research area or to occupy a particular niche such as conducting research in a unique area or working on species not under significant study by others. However, collaborations that bring unique talents or resources to the same researchable question will greatly facilitate the proposed research.

The SIFG has a strong capability in marker applications. In the early 1990s, the SIFG developed the capability to employ molecular markers in research. It used markers effectively to study the host: pathogen system of the southern pines and fusiform rust (Nance and others 1992; Nelson and others 1993; Stelzer and others 1997, 1999). The SIFG leads the study of the genetics of the fusiform rust fungus (Doudrick and others 1993a, 1993b; Hamelin and others 1994; Kubisiak and others 2004). Molecular markers can diagnose dangerous exotic diseases (Blomquist and Kubisiak 2003) and may be used to study diseases of less economic importance (Nelson

and others 2003b). In the South, research on fusiform rust disease is the forest industry's primary interest. Molecular markers have supported programs on loblolly and slash pines (Kubisiak and others 2000, Nelson and Echt 2003, Nelson and others 2003a), as well as a variety of less important forest species (Nelson, in press; Troggio and others 2001; Weng and others 2002) and other crops (Hawkins and others 2001; Huang and others 2000, 2002, 2003).

The SIFG will continue to study the genetics of the southern pine:fusiform rust pathosystem (Nance and others 1992; Nelson and others 1993; Stelzer and others 1997, 1999). Molecular markers will be an important tool in that effort. Future plans include using markers to (1) facilitate studies on the stability and predictability of fusiform rust resistance when deployed operationally, (2) compare differential responses of infected and noninfected seedlings using microarray technology, and (3) map avirulence genes in the fusiform rust pathogen.

SIFG scientists also use molecular markers to study the populations of threatened and endangered species (Auckland and others 2001, Gordon and Kubisiak 1998, Weekley and others 2002); they are currently studying the endangered pondberry [*Lindera melissifolia* (Walt.) Blume]. Also, in collaboration with The American Chestnut Foundation, the SIFG uses molecular genetic markers to aid in the restoration of American chestnut (Huang and others 1998; Kubisiak 1996, 1999; Kubisiak and Roberds 2003). Molecular markers continue to provide data on threatened and endangered species so policymakers and managers can decide how best to conserve populations at risk. The number of species at risk is expected to increase, and the NFS will continue to harbor a significant number. Increasingly, forest managers will need timely information to make decisions based on sound science.

Recognizing the importance of molecular genetic markers in forest research, the SIFG has recently invested in new high-throughput marker technology to enable the rapid production of data from very large studies. The Loblolly Pine Founder Project

aims to provide a baseline for genetic variation in the population of loblolly pine that will enable monitoring changes in that population through successive cycles of genetic improvement (Williams and others, in press). When available, these data will be a valuable public resource for a variety of studies, including population genetics, phylogeny, and paleobotany. They will also provide a database that has the potential for identifying and mapping markers associated with quantitative trait loci that code for traits of commercial interest. Molecular markers offer an unambiguous way to diagnose certain diseases such as sudden oak death, caused by *Phytophthora ramorum* (Blomquist and Kubisiak 2003), and to track the spread of introduced pests. Applications for molecular markers in protecting forest health are likely to increase as the numbers of exotic pests increase (National Biological Information Infrastructure 2003).

The sequence data from the International Populus Genome Consortium will soon be available to the scientific community (Plomion and others 2003). This effort has spawned several functional genomics projects in Canada, France, Sweden, and the United States. These large research projects are searching for candidate genes that code for growth and development, wood properties, and defense responses. The SIFG should position itself to take advantage of the wealth of information forthcoming from these model species programs.

In summary, uses for molecular marker applications in a wide variety of research projects will continue and will likely increase in the future. The high-throughput capability of the SIFG marker lab provides the means to provide data quickly for both public- and private-sector clients. The SIFG should continue to maintain and enhance a strong capability in molecular markers by frequent upgrades of essential equipment and employing and training skilled technical staff.

The SIFG is revitalizing research in structural genomics. In the early 1990s, the SIFG initiated a significant effort in genomics

research and produced the first molecular map (Nelson and others 1993) and karyotype (Doudrick and others 1995) for slash pine. This research entered a hiatus that ended in 2001. The renewed effort has produced the first FISH-based karyotype of loblolly pine and introduced improvements in the existing slash pine karyotype (Islam-Faridi and others 2003). These karyotypes are fundamental to genomics studies that will explain genome organization, permit molecular mapping of regions that have little recombination, and encourage comparative genomics studies among species. Future work will generate the first FISH-based karyotypes for longleaf (*P. palustris* Mill.) and shortleaf (*P. echinata* Mill.) and enable comparative genomics studies among the southern pines. Exploratory work will study the feasibility of employing FISH with BAC clones (BAC-FISH) for physical mapping and genome analysis. BACs are DNA fragments 100 to 300 kb in size that can increase the accuracy and efficiency of physical mapping. Combining BAC and FISH provides a technique for rapidly determining the chromosome location for a group of genomic clones and greatly simplifies map-based cloning (Islam-Faridi and others 2002). These techniques may prove to be invaluable in studying the very large genomes of conifers.

The use of FISH technology holds great promise for advancing our knowledge of genome organization in the southern pines and other forest trees. The SIFG will fill this unique niche in the forest genetics community.

The SIFG must develop greater capability in bioinformatics to use the sophisticated new computational biology tools effectively and to access genomic and sequence data from other better characterized organisms. These goals will require additional funding for new equipment and technical staff.

The SIFG should collaborate to assess risks from deploying clones and/or GM trees. This effort will require coordinating the resources of several research work units to examine the genecological risks, the sociological implications, and a cost-benefit analysis.

Summary of Recommendations

The SRS should continue to emphasize the application of molecular marker technology to a broad range of biological and ecological questions.

The SRS should expand genomics research to include microarray analyses of gene expression, particularly for traits that affect forest health, to explore the application of FISH technologies to physical mapping, and to add bioinformatics capabilities.

The SRS should deemphasize research in vegetative propagation and gene transformation methods, but should seek opportunities to support the research of others.

The SRS should evaluate the risks of deploying clones and/or GM trees.

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Glossary[2]

allele: alternative form of a genetic locus; a single allele for each locus is inherited from each parent.

amplification: an increase in the number of copies of a specific DNA fragment.

bacterial artificial chromosome (BAC): a vector used to clone DNA fragments (100- to 300-kb insert size; average, 150 kb) in *Escherichia coli* cell.

bioinformatics: the science of managing and analyzing biological data using computing techniques (especially important in analyzing genomic research data).

candidate gene: a gene located in a chromosome region suspected of coding for a trait of interest.

centimorgan (cM): a unit of measure of recombination frequency. One centimorgan is equal to a 1-percent chance that a crossover will occur between two genetic loci in a single generation.

cDNA library: a collection of DNA sequences that code for genes that generated from mRNA sequences.

clone: an exact copy made of biological material such as a DNA segment, a whole cell, or a complete organism.

comparative genomics: the study of genetics by comparisons between organisms.

complementary DNA (cDNA): DNA that is synthesized from a messenger RNA template.

contig: group of cloned (copied) pieces of DNA representing overlapping regions of a particular chromosome.

crossing over: the exchange during meiosis of corresponding sections of DNA, resulting in an exchange of alleles between chromosomes.

DNA (deoxyribonucleic acid): the molecule that encodes genetic information. DNA is a double-stranded molecule held together by weak bonds between base pairs of nucleotides. The four nucleotides in DNA contain the bases adenine (A), guanine (G), cytosine (C), and thymine (T). In nature, base pairs

[2] Many definitions were taken verbatim from the following Web site and altered for context where necessary (http://www.ornl.gov/TechResources/Human_Genome/glossary/glossary.html#genemapping), the Human Genome Project [Date accessed: September 16, 2003].

form only between A and T and between G and C; thus the base sequence of each single strand can be deduced from that of its partner.

expressed sequence tag (EST): a short strand of DNA that is a part of a cDNA molecule and can act as identifier of a gene.

fingerprinting: in genetics, the identification of multiple specific alleles in a genotype's DNA to produce a unique identifier for that genotype.

fluorescence *in situ* hybridization (FISH): a physical mapping approach that uses fluorescent tags to detect hybridization of probes with metaphase chromosomes and with the less-condensed somatic interphase chromatin.

functional genomics: the study of genes, their resulting proteins, and the role played by the proteins in a biochemical process.

gene: the fundamental physical and functional unit of heredity. A gene is an ordered sequence of nucleotides on a chromosome that encodes a specific functional product such as a protein or RNA molecule.

gene expression: the process by which a gene's coded information is converted into the structures present and operating in the cell.

gene mapping: determination of the relative positions of genes on a DNA molecule and of the distance, in linkage units or physical units, between them.

gene regulation: the process by which gene expression is controlled, i.e., turned on and off by the cell.

genetic marker: a gene or other identifiable portion of DNA whose inheritance can be followed.

genome: all the genetic material in the chromosomes of a particular organism.

genomic library: a collection of clones made from a set of randomly generated overlapping DNA fragments that represent the entire genome of an organism.

genomics: the study of genes and their function.

homologous chromosome: chromosome containing the same linear gene sequences as another.

hybridization: the process of joining two complementary strands of DNA or one each of DNA and RNA to form a double-stranded molecule.

karyotype: a representation of an individual's chromosomes arranged in a standard format showing the number, size, and shape of each chromosome type.

linkage: the proximity of two or more alleles on a chromosome.

linkage map: a map of the relative positions of genetic loci on a chromosome, determined on the basis of how often the loci are inherited together. Distance is measured in centimorgans (cM).

meiosis: the process of two consecutive cell divisions in the diploid progenitors of sex cells resulting in four daughter cells, each with a haploid set of chromosomes.

messenger RNA (mRNA): RNA that serves as a template for protein synthesis.

microarray: sets of miniaturized chemical reaction areas that may also be used to test DNA fragments, antibodies, or proteins.

mitosis: the process of nuclear division in cells that produces daughter cells genetically identical to each other and to the parent cell.

molecular genetics: the study of macromolecules important in biological inheritance.

nucleotide: a subunit of DNA or RNA consisting of a nitrogenous base (adenine, guanine, thymine, or cytosine in DNA; adenine, guanine, uracil, or cytosine in RNA), a phosphate molecule, and a sugar molecule (deoxyribose in DNA and ribose in RNA). Thousands of nucleotides are linked to form a DNA or RNA molecule.

oligonucleotide: a molecule usually composed of 40 or fewer nucleotides; used as a DNA synthesis primer.

physical map: a map of the locations of identifiable landmarks on DNA such as restriction enzyme cutting sites, or genes. Distance is measured in base pairs.

polymerase chain reaction (PCR): a method for amplifying a DNA base sequence using a heat-stable polymerase and two 20-base primers, one complementary to the (+) strand at one end of the sequence to be amplified and one complementary to the (-) strand at the other end. Because the newly synthesized DNA strands can subsequently serve as additional templates for the same primer sequences, successive rounds of primer annealing, strand elongation, and dissociation produce rapid and highly specific amplification of the desired sequence. PCR also can be used to detect the existence of the defined sequence in a DNA sample.

polymorphism: difference in DNA sequence among individuals that may underlie differences among phenotypes.

population genetics: the study of variation in genes and their frequencies among a group of individuals.

primer: short preexisting polynucleotide chain to which new deoxyribonucleotides can be added by DNA polymerase.

probe: single-stranded DNA or RNA molecules of specific base sequence, labeled either radioactively or immunologically, that are used to detect the complementary base sequence by hybridization.

repetitive DNA: sequences of varying lengths that occur in multiple copies in the genome.

RNA (ribonucleic acid): a chemical found in the nucleus and cytoplasm of cells that plays an important role in protein synthesis and other chemical activities of the cell.

segregation: the normal biological process whereby the two pieces of a chromosome pair are separated during meiosis and randomly distributed to the germ cells.

sequencing: determination of the order of nucleotides (base sequences) in a DNA or RNA molecule or the order of amino acids in a protein.

transcription: the synthesis of an RNA copy from a sequence of DNA (a gene); the first step in gene expression.

transformation: a process by which the genetic material carried by an individual cell is altered by incorporation of exogenous DNA into its genome.

transgenic: an experimentally produced organism in which DNA has been artificially introduced and incorporated into the organism's germ line.

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We provide an analysis of opportunities and challenges for biotechnology in forest research in the southern United States. Four major areas of biotechnology were identified and described and then rated for priority among three groups of researchers—private sector, public sector, and the USDA Forest Service, Southern Research Station (SRS). The four areas of biotechnology were vegetative propagation, genetic transformation/modification, genomics/bioinformatics, and molecular marker applications. We concluded that these three groups have different research priorities with respect to biotechnology and that these differences complement each other. In particular, the SRS should continue its work in molecular marker technology development for applications in tree improvement, conservation genetics, forest health, and basic science. Also, the SRS should increase its efforts in genomics/bioinformatics while decreasing its research on vegetative propagation. Finally, the SRS should work on assessing the potential risks and impacts of planting and managing clones and/or genetically modified trees.

Keywords: Bioinformatics, clonal forestry, forest biotechnology research, genetic transformation/modification, genomics, marker-assisted selection (MAs), molecular markers, vegetative propagation.

The Forest Service, United States Department of Agriculture (USDA), is dedicated to the principle of multiple use management of the Nation's forest resources for sustained yields of wood, water, forage, wildlife, and recreation. Through forestry research, cooperation with the States and private forest owners, and management of the National Forests and National Grasslands, it strives—as directed by Congress—to provide increasingly greater service to a growing Nation.