Forest Biotechnology: Its Place in the World

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Abstract: Forest biotechnology is on the cusp of scientific breakthrough. Great advances have been made in two of the three components of the science: asexual propagation and association genetics. The third component, genetic engineering, is in various stages of development. Genome sequencing, a segment of association genetics, has been done for only one forest tree, *Populus tricocarpa*, but a concerted effort is being made to gain the resources for sequencing loblolly pine (*Pinus taeda*). Sequencing of that species, with a genome about seven times larger than that of the human genome, was once thought to be a prodigious task, but with advances in technology the prognosis is that the job can be completed in two to three years at a cost of $25 to $30 million. However, sequencing is only a start. The association and function of the genes, the bread and butter of the effort, can only be determined by meticulous laboratory and field testing.

Progress has been made in engineering the traits for tree growth, wood properties, cold, drought and herbicide tolerance, and insect resistance. However, the only genetically engineered tree species that has been released for commercial use is Bt-resistant European black poplar in China. There is opposition to commercial application of trees, engineered specifically for fast growth and increased yields, by those whose stance is that the value accrues only to 'big companies'. It will remain for traits that have broad societal benefits, such as conservation of threatened and endangered species and biofuels, for acceptance to be gained. Even then some countries will benefit before others, not because of the science, which is universal, but because of organized resistance. Regardless, forest biotechnology continues to progress and, combined with conventional tree breeding programs, will be hugely beneficial in housing and feeding the world's population in centuries to come.

INTRODUCTION

The objective of this paper is to give a thumbnail sketch of forest biotechnology at its present state of development and tie it to its background and to the future. As is becoming evermore obvious, forest biotechnology will become commonplace in our lifetime. The voice of present-day adversaries of the science will soothed because the results of technology will produce benefits on which society is dependent while real and perceived adverse effects to ecological systems and the environment will be neutralized. The catalyst for those accomplishments will be the cellulosic products from trees that will range from transportation fuels to foodstuff. As with all advancing technology, the developed economies will be the first to reap the benefits of the science, but only a short time will elapse before the developing economies of the world will also enjoy the benefits.

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Tree Improvement

Planting of forest trees has a historic basis in every society where native forests once covered the terrain: Middle East, Western Europe, northern Africa, Asia inclusive of Japan and India. It wasn't until the last vestiges of the 19^{th} Century that the practice became highly successful on a broad scale. Until that time, the emphasis was on planting native species, but with the source or provenance of the seeds being generally ignored. Beginning about 1900 a major effort was devoted to planting trees of exotic origin in places far removed from their indigenous populations. Examples include the movement of sitka spruce (*Picea sitkensis*) and Douglas-fir (*Pseudotsuga menziesii*) from the Pacific Northwest (U.S.) to Western Europe, radiata pine (*P. radiata)* from California to Australia, Chile and New Zealand, yellow pines from the southern U.S. and Mexico to southern Africa, and the shuffling of poplars (*Populus* spp.) to and from all parts of the world. In more recent times, consistent with attention being given to species and provenances, there has been a mass movement of the North American southern pines (*P. ellottii, P. taeda)* being established in extensive plantations in southern South America and southern China. To that list, the eucalypts (*Eucalyptus* spp.) and on a more limited basis, acacias (*Acacia* spp.) have been dispersed to nearly every country within the Tropics and Warm Temperate zones of the world (Burdon and Libby 2007).

Along with the mass movement, tree improvement programs, inclusive of selection of the species and provenances most fit for the area being planted, were initiated. Refinement of tree improvement programs with selection and interbreeding of plus-tree phenotypes had its origin in Scandinavia during the World War II years. It was patterned on the principle of agronomic-crop breeding. Beginning in the 1950s that effort was copied in nearly every region of the world where plantation forestry was a priority, and that included indigenous as well as exotic forestry. A microcosm of the indigenous-forest effort exists right here in the southern United States where successful tree improvement programs of loblolly and slash pines, and to a limited extent of other conifers, are housed at the universities of Texas A& M, Florida, and North Carolina State. In only the third generation of breeding, huge genetic gains have been achieved in adaptability, volume production, tree form, pest resistance and wood properties (McKeand *et al.* 2006).

Forest Biotechnology

A limitation of rapid genetic gain in tree improvement programs is the long time to sexual maturity and the comparable time for genetic testing of the selections and their offspring. Enter forest biotechnology, which is an extension of tree improvement with the work being done at the gene rather than the tree level. In this treatment of the subject, biotechnology has three components: asexual propagation, genomics and genetic engineering (Yanchuk 2001).

Vegegative propagation. Vegetative propagation, a form of asexual propagation, has been with us for thousands of years, as evidenced by the inhabitants of Mesopotamia ripraping the banks of the Tigris and Euphrates Rivers and their tributaries with clonal poplars and willows to help control flooding and soil salinization. Those genera are relatively easy to vegetatively propagate, but not so with many others species of both angiosperms and gymnosperms. Some of the species of commercial importance, such as loblolly pine, can be vegetatively propagated in their juvenile state, but once they progress toward the equivalence of puberty in humans they become progressively harder to clone, and at some point most genotypes become recalcitrant to vegetative propagation.

In case of uncertainty about the value of vegetative propagation, clonal forestry offers significant genetic gain in uniformity that translates into added volume gains, ease in plantation management and manufacturing efficiency. In addition, the technology is absolutely essential if genetic engineering is to be accomplished in forest trees. As opposed to agronomic crops, which can be inbred and then outcrossed to distribute a single genetic modification into its progeny, forest trees are generally resistant to inbreeding and, as a result, are powerless to set seeds with the intact genetic modification. The only procedure for genetic engineering to be successful in forest trees is for each genotype to be modified at the embryonic stage.

To overcome the recalcitrance of selected genotypes, somatic embryogenesis offers a viable alternative and, in the process, it adds the benefit of 'having your cake and eating it too'. That benefit is the ability to store a portion of the manufactured embryos in cryopreservation while another portion is used to test the genetic worth of the clone of which the plantets are a part. Somatic embryogenesis is accomplished by selecting plant material in the blastocyst stage of the embryo, *i.e.,* undifferentiated tissue. By coddling the tissue through various laboratory procedures, mature embryos can be produced *en masse,* all of the same genotype. The naked embryos, *i.e.,* without a seed coat can be coaxed to germinate and develop into plantlets suited for plantation establishment (Pait 2004).

Genomics. Genomics is the study of the arrangement of genes on chromosomes. The exercise in genomics that most people can associate with is the huge effort expended on sequencing the human genome. Despite the millions of dollars and multitude of years spent on the project the job is only partially complete. Knowing the location of a gene on a specific chromosome has little value until the function of that gene is known. The correlation between the genes and their functions and interactions are being slowly developed, but it will take years before the task is complete.

Other organisms with smaller and less complicated genomes have been or are being sequenced, most notably *Arabidopsis thaliana*, a species of the mustard family. Good correlations exist between the genotypes of the least and the most advanced plants, and also between the least and most advanced animals. To that end, *A. thaliana* has served as a good model for rice (*Oryza sativa*), wheat (*Triticum* spp.), corn (*Zea mays*), soybean (*Glycine max*) and other food, forage, and fiber crops, inclusive of trees. To date the only forest tree species to have its genome sequenced is black cottonwood (*Populus tricocarpa*). Efforts are now in progress to have the genome of a conifer sequenced. The species selected for that endeavor is loblolly pine because it has the most advanced quantitative breeding base of any conifer in the world. The advancement results from the ongoing tree improvement programs of the three major cooperative programs in the South, each of which has been in progress in excess of 50 years. The genotyping of a tree of this species will serve as the template for all other pines and, in fact, all other conifers because of genome similarity of the gymnosperms.

The number of genes in loblolly pine is about seven times larger than that of the human genome. With the time and money expended for the much smaller human genome, why take on the colossal task for a conifer tree? The answer, in addition to the available genetic base from tree breeding and the evolutionary aspects of a long-lived organism, is that the technology for gene sequencing has developed so rapidly within recent years that the job will require only a fraction of the time and resources that it would have consumed several years ago. The initial estimate was that the five-year effort would cost $130 million for partial sequencing and related research. Now the estimation is that complete sequencing can be done for $25 million in half the projected time. One day we will know the gene or genes responsible for fusiform rust (*Cronartium fusiforme*), for example, and will be able to silence those genes by over expression or under expression or by inserting a gene from an unrelated plant, such as *A. thaliana,* into loblolly pine to give resistance to the disease.

Even today genomics is having a positive effect on plant breeding through at least two technologies: marker aided selection (MAS) and quantitative trait loci (QTL). Through quantitative genetics, MAS has application for a number of traits, including vegetative propagation. Even though the gene or genes for rooting, for example, are unidentified the association can be made by the presence of a marker gene in a genotype that roots well. In like vein, QTLs operate on the principle that the location of a gene or genes on various chromosomes might account for a percentage of the gain to be achieved by their presence. The scenario might be that the identified genes account for only 46% (or some other number) of the variation of the trait, but that assurance is money in the bank when dealing with recombinant genetics.

Genetic engineering. Genetic engineering is the only component of forest biotechnology that has generated opposition to the science. A major reason for the concern is the visualized grotesqueness of the offspring that might be generated from the insertion of a gene from a mouse into a tree, for example. In reality, it will be a gene from a soil bacterium such as *Bacillus thuringiensis* that connotes insect resistance or *Agrobacterium tumefaciens* that gives resistance to glyphosate, the active ingredient in Roundup Ready® that will be the transferred gene. Both of the bacterial genes for insect resistance and glyphosate tolerance have been successfully inserted in trees for experimental purposes. Gene insertions or modifications have also been made for lignin modification (Chiang 2003), tree growth, and cold and drought.

The only genetically engineered tree species that has been released for commercial use is *Populus nigra* in China, with the inserted gene being *B. thuringiensis.* Initial reports from 2003 revealed that about 400 acres of such trees had been planted, but the information since then of additional area established or success of the original plantations has been muted (Wang 2004)

Of The Future

I predict that, in the U. S., genetically engineered trees will not find a use for increased growth in the foreseeable future. Increased tree growth and yield will primarily come from tree improvement programs, inclusive of asexual propagation. To that end, tree improvement and forest biotechnology fit together like hand and glove. Genetic engineering is nothing without asexual reproduction and asexual reproduction is nothing without breeding programs to produce ever-improved genetic recombinants.

I further predict that the first benefits from genetic engineering to commercial forestry in our society will be for insect resistance and tolerance to glyphosate. The research has been largely completed for those traits with the use of *B. thuringiensis* and *A. tumefaciens.* Beyond that, value-added products such as pharmaceuticals, carbon sequestration, bioremediation, pulp and paper manufacture, and most importantly conservation of threatened and endangered tree species and bioenergy will gain priority over traits specific to growth and yield. The concentration on species conservation and bioenergy will conclude this paper.

Tree conservation. A number of tree species in the U.S. are threatened by extinction from attacks by foreign pests, either insects or diseases. The tree species that has attracted most attention as threatened or endangered is American chestnut (*Castanea dentata).* Occupying a range throughout the Appalachian Mountains, the eastern portions of the Central and Lake States and into southern Ontario with a tree count in the overstory crown class of approximately three billion, the species was killed to ground level during a 40-year period beginning about 1900. The pest was a fungus (*Cryphonectria parasitica*) of Oriental origin that was accidentally introduced to New York in the late 1800s on ornamental stock of Chinese chestnut (*C. mollisima*) (Sisco 2004). Attempts to find trees resistant to the fungus and to introduce hypovirulent strains of the fungus have largely met with failure even though some research on those subjects is still in progress.

The program that is showing progress for restoration of the species was initiated in 1989 by The American Chestnut Foundation (TACF) with the goal of producing a tree with 15/16 American chestnut and 1/16 Chinese chestnut through a backcross breeding program (Sisco 2004). The program is nearing its completion through the fourth backcross.

To supplement the backcross breeding program, research has been initiated to identify the two or three genes in Chinese chestnut that connote resistance to the disease. Funded by the National Science Foundation, the anticipated identification of the causal genes will allow the results to be used in a conventional tree improvement program or it will allow insertion of the genes for resistance from Chinese chestnut into American chestnut. Utilizing forest biotechnology to restore the icon of forest tree species to its natural range will be of significant positive social and environmental value.

Bioenergy. Unsettled conditions in the Middle East, Nigeria and Venezuela, from whence the U. S. obtains most of its imported crude oil, have driven the price upwards to $60/barrel. At that price, alternative fuels such as ethanol begin to look very promising for transportation needs.

Corn has been the sole feedstock for commercial ethanol manufacture in the U. S. largely because of favorable legislation that imposes $0.51 per gallon tariff on imported ethanol and a federal subsidy of $0.54 per gallon. The federal subsidy is complemented by an added subsidy in a number of states in the Midwest where corn is the major agricultural crop. The economics has driven the price of corn from $2.00/bushel in March 2006 to futures of $4.38/bushel in March 2007. With the incentives and crude oil at $55 to $60 per barrel ethanol producers could pay from $3.65 to $4.54 per bushel of corn and still realize a 12% return on investment. (Runge and Senauer 2007).

The escalation in corn prices has adversely affects the price of ethanol, but it also causes a ripple effect in the economy. Specifically impacted are the cattle, hog and poultry producers, and the effect is beginning to play out in food prices. In addition to the economy being adversely affected, the realists are questioning the environmental impacts of a corn crop that is estimated to cover 90.5 million acres in 2007, the highest acreage since World War II. Lands with low soil fertility, poor internal drainage, high erosion potential, and forest conversion are being brought into the program. The majority of those lands will require added nutrients, a product of fossil fuels, to make them economically productive. In addition, monocultural farming with corn planted year-on-year requires additional nutrients and greater application of pesticides to control competition from weeds, insects and diseases than occurs with rotational cropping.

The limitations of corn for ethanol have resulted in the search for alternative crops. Switchgrass (*Panicum vergatum)* has received considerable attention as a cellulosic resource that could help solve the energy dilemma, as have trees and other plants. The limitation to those crops is the time and cost of extracting the sugars from the cellulose of plant fibers and then the fermentation of the sugars to ethanol. To date, a half dozen or so enzymes were needed for the sugar extraction even before the fermentation process. As plant biotechnology intensifies, however, more efficient enzymes are being discovered or designed. Organizations such as Novozymes and Genencor are devoting their whole energy to the identification and manufacture of such enzymes. It will be only a matter of time until the extraction and fermentation process is greatly expedited.

Switchgrass and plant residues such as corn stover and wheat straw are championed by some enthusiasts as the cellulosic materials for ethanol production. Acknowledging the benefits from those materials, they have the serious limitations of collection and storage on an annual basis. The calculation of one ethanol plant manager was for one tractor-trailer load of switchgrass every six minutes to equate to the capacity of the plant for corn ethanol production (Runge and Senauer 2007). With such limitations, the subject turns to trees. A major advantage of trees is that they store on the stump. No need for huge storage facilities of the material that is essential for annual crops.

Research is in progress to increase the cellulose content of trees with a commensurate reduction of lignin. Positive results have been achieved with aspen (*Populus tremuloides)* (Chiang 2003) and research is in progress to do the same with a conifer, such as loblolly pine. The latter species with its wide adaptability to diverse sites and a wide geographic base, which ranges from Maryland and Delaware south to Florida and west to Texas makes it a suitable candidate for bioenergy plantations. The vision is that those plantations will surround a decommissioned pulp mill, many of which dot the southern landscape, that will be retrofitted for ethanol production. Estimates are that the retrofitting of such a pulp mill could be done at about 20% of the cost of a new ethanol plant (Kelley 2006). After all, the facilities are in place for everything but the cellulosic conversion: wood supply, chipping operation, power plant, digesters, refiners, transportation, etc.

Even with the advances being made in converting cellulose to fuel the benefits need not stop there. The host of products that can be made from fossil fuels can also be made from plant material. And, on top of that, the sugars from plants, especially from genetically engineered trees with their elevated cellulosic content, are candidates for the manufacture of foodstuff. One day we will be competing with termites for the carbohydrates sequestered in trees.

Summary

Biotechnology is the coming science of the 21st century. The involved scientists have only nipped the tip of the iceberg in ferreting out the cause and effect of genes that inhabit every living thing on earth. Forest biotechnology will parallel the advances made in the farming of flora and fauna on which civilization depends, but it will do so only in conjunction with related sciences such as conventional tree breeding.

The emphasis in the short run will be concentrated on disciplines that benefit society as a whole. In this treatise, I've addressed conservation of threatened and endangered species and bioenergy as the two disciplines that will most rapidly get public support. Engineered trees for faster growth and greater yields per unit area of time will, in the short run, continue to get negative publicity because of the perception that the benefits will accrue to 'big companies'. Following acceptance of specialty crops for the good of the whole will set the stage for acceptance of value-added products such as trees engineered for fast growth, tolerance to adverse sites, and exotic plantations. The application of forest technology will first accrue to the owners of large industrial tracts of land, then to the REITs and TIMOs, and lastly to the non-industrial private landowners.

Because of the internationalization of biotechnology one country will not benefit to the exclusion of another one so far as the science is concerned. The difference in application will come in acceptance. The adversaries of the science will delay acceptance in some countries while it is readily implemented elsewhere. Worldwide acceptance will come only when there is worldwide need.

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