APPLICATIONS OF BIOTECHNOLOGY IN FOREST TREE IMPROVEMENT

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Abstract.--An assessment of the role of the "new biotechnology" in aiding tree breeders achieve their goal is presented. The array of tools now available include gene and organelle transfer systems, doubled haploids, gene sequencing, restriction fragment length polymorphism, somaclonal variation, in vitro screening and others. A model single gene system for 3-carene synthesis is used to illustrate some procedures and problems to be encountered in creating transgenic trees. Finally, a case history examination of a biotechnology firm is presented to show the problems involved in commercializing new technologies.

Additional keywords: Tissue culture, molecular markers, organelle transfer, in vitro screening, 3-carene.

INTRODUCTION

Much is being said and written these days about the applications and potentials of the "new" biotechnology in improving agricultural crops and forest trees. Viewpoints among foresters range from skepticism (Libby 1983) through unguarded optimism (Krugman 1985) to apparent fantasy (Durzan 1980). From my perspective, all three viewpoints are appropriate depending upon the time-frame chosen. However, my purpose here is to examine the role of biotechnology in tree improvement within a "practical" time frame that is realistic for tree breeders and future tree improvement practices, over perhaps the next thirty years.

I will focus my discussion on (1) the concept of biotechnology as it relates to forest tree improvement, (2) the array of techniques available to tree breeders now and into the future, (3) a model single gene system for transferring genes in trees to show both problems and opportunities, and (4) a case history examination of a biotechnology firm to illustrate problems in commercializing new technologies.

THE "NEW" BIOTECHNOLOGY

What are we talking about when we use the term "biotechnology?" Imprecision in definitions is one reason why there is considerable confusion and disagreement about the potential for biotechnology in tree improvement.

If we accept the basic definition of biotechnology as the use of living organisms or their components in industrial processes, then all tree breeders

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are biotechnologists! So today we refer to our conventional tree improvement practices as the "old" biotechnology. The "new" biotechnology then refers to the novel techniques developed in recent years for altering the genetic composition of plants. These include recombinant DNA techniques, gene and genome transfer methods, and the many tools or techniques associated with genetic manipulation such as cell and tissue culture, molecular markers, in vitro screening and metabolite production, etc. So although all tree breeders may take some comfort in the fact that we are biotechnologists, my discussion and in fact the rest of the world considers only the "new" biotechnology as the biotechnology.

BIOTECHNOLOGY TIMEFRAME

It may be useful at the outset to provide an assessment of what, how and when I believe the various biotechnologies may impact on tree breeding and forest genetics well into the future. This will serve to focus on at least some of the major tools, methods, or goals of biotechnology and how they may benefit tree improvement from my perspective.

Although the tree breeding efforts of the last 40-50 years are now rapidly coming to fruition in terms of commercial use of improved seed, we are still at what must be considered to be a "low" overall level, especially compared with the status of agricultural crop breeding today. With the new biotechnology incentives (funds, tools, and knowledge), tree improvement could rise above its present levels much more quickly than would have been possible without biotechnology. The real danger, however, lies in overemphasizing the new at the expense of the more conventional approaches to tree breeding and genetic analysis. It is absolutely imperative that sound, traditional tree breeding efforts also be accelerated or at lease maintained in order to capture the potential benefits of tree improvement in the foreseeable future. Moreover, the new biotechnologies are heavily dependent on ongoing breeding programs for their success. Unfortunately, there are clear signs that breeding programs are being sacrificed in favor of biotech programs both in forestry and in horticulture (Acuff 1987).

Thus, Figure 1 depicts an increasing level of activity in both tree breeding and forest biotechnology as necessary to raise tree improvement practices from low to high over the next 50 years. At the present time we see a significant stimulation in basic research in forest biology as a result of new commitments to biotechnology. Next on my hierarchy of implementation would be the commercial tissue culture production of select genotypes of important tree species. The role of tissue culture in forest improvement has been clearly outlined by Haissig, et al. (1987). Development of culture systems is important not only for tree production but also as a vehicle for doing the other techniques of genetic manipulation later on. It is interesting to note that it has been more than 50 years since the pioneering tissue culture work of Gautheret (1934, 1940) and Jacquiot (1947, 1949) in France on tree species. In spite of a large research effort on a variety of tree species over these 50 years, we still do not have a commercial system on a forest tree species in the United States. But we may be getting close for such species as loblolly pine, Douglas-fir, Western white pine, and black locust. Nevertheless, the experience with tissue culture serves to illustrate the difference between "potential" and actual "application" for an

important tool of biotechnology.

Other biotechnologies shown in Figure 1 are likewise in the "potential" category and some must in all probability await many years before they are feasible commercially. My estimation of a time frame for each could be substantially out of order, but that is not too important. What is important is that research is being done to varying degrees on each item and basic knowledge is accumulating. This is the key to application no matter how far in the future each is likely to have an impact on our breeding results.

In my view the development of molecular markers such as terpenes, flavonoids, isoenzymes, and DNA restriction fragment length polymorphisms could influence our tree breeding progress before too many years have elapsed. Linkage of these molecular markers with important traits could greatly facilitate selection for both single gene and polygenic traits. They may also be used for strain identification and plant variety protection, as probes for genetic diversity and for constructing genetic maps (Beckmann and Soller 1986).

Whole tree regeneration from single cells will be a key procedure in cell culture allowing the proliferation of genotypes that have been transformed using recombinant DNA, microinjection, or protoplast fusion techniques.

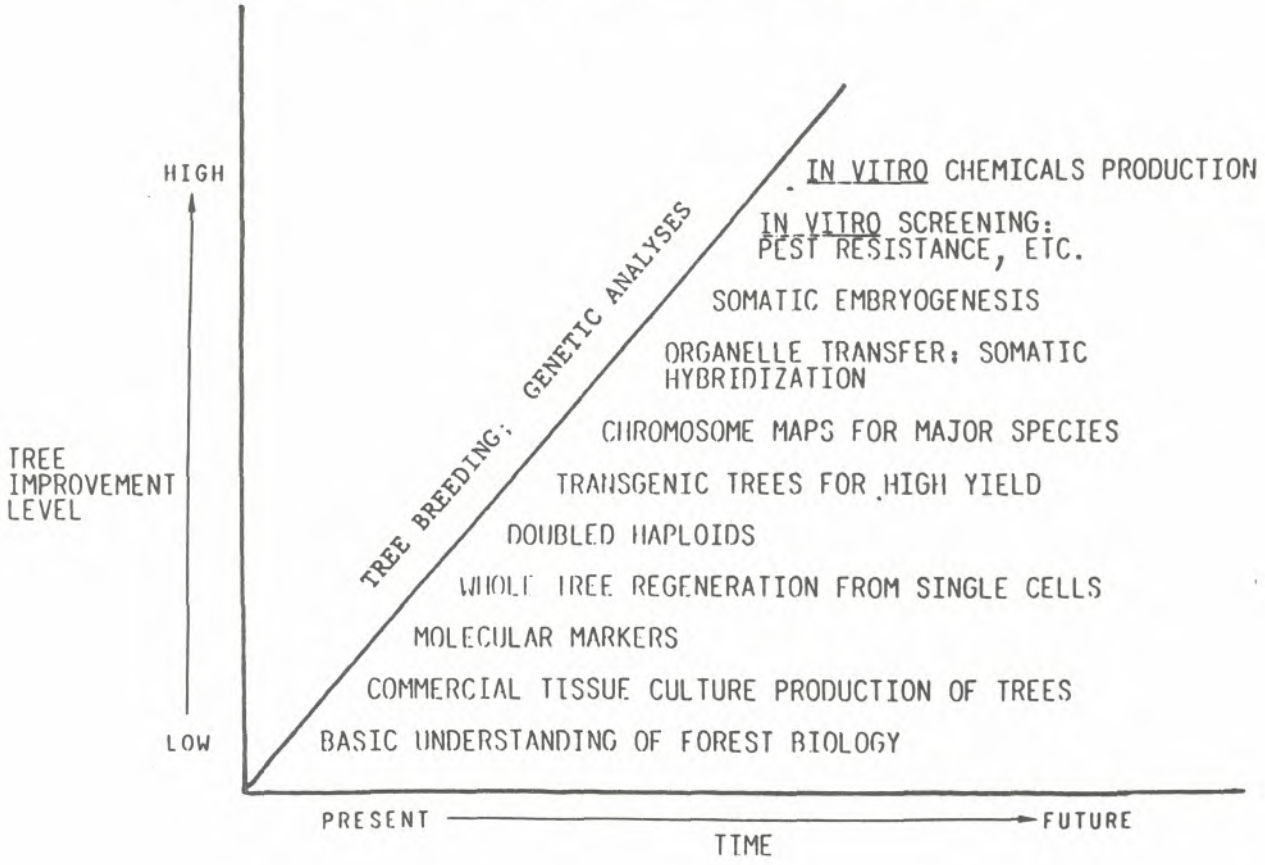
The development of doubled haploids of tree species could be a useful tool in studying gene effects in a homozygous background and perhaps for increasing the efficiency of selection for quantitative traits (Choo 1981). However, many tree species, especially the conifers, may not survive the homozygous state because of an excess genetic load or other genic imbalances, so this procedure may have limited application.

Genetic transformation of trees offers enormous potentials for impacting future tree improvement progress. Methods of genetic manipulation which bypass the sexual process would enable the transfer of specific genes from species to species or tree to tree without the long generation intervals now required. A precedent has already been set for such a feat: the insertion of a gene for tolerance to the herbicide glyphosate into a hybrid poplar has been accomplished (Fillatti et al. 1986). The testing and commercialization of this technique may require many more years of research, but it should serve as a model for other similar approaches where the bacterium Agrobacterium tumefaciens is a suitable vector or where other techniques for incorporating foreign DNA into a recipient cell are available.

I will return to the subject of gene transfer in more detail to illustrate the techniques and problems that are associated with this exciting development which certainly epitomizes the new biotechnology in the minds of most scientists and lay people.

Moving further up the hierarchial scale of Figure 1, I have noted that cytological research and particularly chromosome maps for tree species are needed. At this time I know of no known gene that has been located on the chromosome of a tree. At a time when we are witnessing a gigantic effort to map the entire human genome, it seems appropriate to redirect some effort towards this goal in trees.

FIGURE 1. ASSESSMENT OF THE IMPACTS OF SELECTED BIOTECHNOLOGIES ON FOREST TREE IMPROVEMENT



Cell fusion, in which the DNA complement of organelles such as chloroplasts, mitochondria, or entire protoplasts of contrasting genotypes are enticed to fuse, is another technique for bypassing sex to accomplish somatic hybridization. These methods are fraught with practical difficulties but may have some impact later in the 21st century (Hansen et al. 1986).

Somatic embryogenesis, or the derivation of embryos and thence new trees form somatic tissues, is a tool which could greatly benefit tree improvement in the distant future (El-Nil 1980; Gupta and Durzan 1986, 1987). Presently, occasional embryos have been induced, usually from tissues predisposed to form embryos. Repeatability, consistency, and high frequency regeneration from normal somatic tissue is not possible. If it could be achieved, another method for mass propagation of superior genotypes developed by the tree breeder would be available. Regeneration of trees from single cells or protoplasts is probably even further in the future.

The final two technologies I wish to mention as having potential for benefiting tree improvement depend upon highly sophisticated procedures of tissue culture. If we can develop in vitro screening techniques for creating new (somaclonal) variation, or for early evaluation of resistance to pests, such as insects and diseases, or to environmental stresses, such as temperature and drought, the efficiency of selection would increase by orders of magnitude. Such screening techniques would likely depend heavily on detection of chemical genotypes in individual cells, perhaps using diagnostic probes such as poly- or monoclonal antibodies (Haugen and Tainter 1987). This is a difficult task and perhaps far in the future, barring some spectacular breakthrough in the underlying research. Probably equally as difficult as the cell screening technology would be the last technique I will mention which is the actual production of tree products, e.g., high value chemicals, cellulose, perhaps even fiber, in vitro. Trees are known for the vast array of diverse chemicals they produce in vivo. Yet chemists have barely scratched the surface in describing and developing new substances or processing methods that may have commercial use. Once discovered, high value chemicals could conceivably be produced in large, controlled culture systems with far greater efficiency than in the living tree itself. The cellulose fiber may indeed be the ultimate product of an in vitro system. Clearly, a development such as this would drastically alter the tree breeder's protocol!

TRANSGENIC TREES

As stated earlier, the development of methods by which tree breeders could transfer desirable genes directly from one tree to another, thus bypassing the breeding process completely, is a most intriguing concept. In order to show how this might be done and what the major barriers are, I will use a model system, the gene determining 3-carene concentrations in trees. This gene has been well studied (Hanover 1966; Hiltunen 1975; Baradat, et al. 1972) and is recognized as a single gene trait of which there are very few described for tree species. It has proven to be useful in population studies and fingerprinting species and hybrids (Schaefer and Hanover 1986; Bongarten and Hanover 1982). Moreover, 3-carene is a very interesting and potentially useful chemical for other reasons, as shown in Figure 2. For example, there is evidence (Reed, et al. 1986) that 3-carene may be involved in tree resistance to bark beetle attack. If this proves to be the case or if another analogous single gene chemical affecting tree quality is discovered,

FIGURE 2. CHARACTERISTICS OF 3-CARENE

- A UNIQUE MONOTERPENE (ONLY COMMONLY OCCURRING MONOTERPENE WITH CARANE SKELETON)

- CONCENTRATION CONTROLLED BY SINGLE GENE (Cc) IN ALL SPECIES STUDIED. INCOMPLETE DOMINANCE

- GENE EXPRESSED IN SOME TISSUES (BARK, FOLIAGE) AND NOT OTHERS (CONES. XYLEM) DEPENDING UPON SPECIES

- ENVIRONMENTALLY STABLE

- ANTIBIOTIC PROPERTIES

- ALLERGEN PROPERTIES

- IMPLICATED AS INSECT REPELLANT

- ALLELE FREQUENCIES VARY GREATLY BETWEEN SPECIES. POPULATIONS. RACES AND WIND-POLLINATED FAMILIES

then we may wish to transfer such a gene into trees lacking the ability to synthesize the compound of interest. If we choose to transfer the 3-carene gene today, would it be possible and how might it be done? The answer to the first question is no. Therefore, the answer to the second question is based on a number of assumptions that existing barriers to gene transfer can be overcome by future research.

In judging the practicality of transferring a single portion of DNA (gene) from one tree to another, we should be aware of the complexity of gene regulatory controls which must be in place and synchronized with other cell functions in order for new DNA to be expressed when and where we want it to be. Figure 3 provides a concise summary of gene regulation in eukaryotic organisms which is, of course, much more complex than that of the more intensively studies prokaryotes.

On the basis of both facts and theories, I have summarized several procedures that may be employed for transferring the 3-carene gene or another discrete DNA entity from one tree to another (Figure 4). Many of these procedures have been developed on lower organisms or with species that are more easily manipulated than are most woody plants. Nevertheless, progress is being made with trees so that some of the procedures are now feasible even though the whole process is not.

Let me point out some of the major obstacles which prevent us from transferring genes of importance in trees at this time. To begin with, isolation of the enzymes which catalyze synthesis of 3-carene in cells and which are membrane bound will be a formidable task. It is also possible that high 3-carene synthesis is the result of regulatory gene action which would make the isolation of the gene even more difficult. In addition, although the synthesis of oligonucleotides to construct DNA sequences (genes) deduced from protein sequencing is now routine for reasonable small portions of the DNA, creating very large segments may be impossible. Therefore, I opted to go for direct isolation of the mRNA for 3-carene cyclase.

The isolation of messenger RNA is like searching for a needle in a haystack, but theoretically could be done if the 3-carene cyclase can be distinguished from other enzymes catalyzing monoterpene conversions. If sufficient quantities of 3-carene cyclase can be isolated, antibodies to the protein could be produced which would allow synthesis of cDNA which would then be amplified in plasmids and transferred into recipient tissue via the Ti plasmid of Agrobacterium tumefaciens. Alternatively, the plasmids could be inserted by microinjection. Insertion directly by microinjection has not been demonstrated yet. Insertion using Agrobacterium as the vector has been demonstrated only for Populus (Fillatti et al. 1986) as mentioned before but is by no means routine or even feasible for any species at this time. Regardless of which method of insertion is eventually employed, there still remains the task of regenerating the single transformed cell into a new tree. This too is a formidable task and not likely to be possible for many years.

As indicated before, there are many difficulties associated with almost every step outlined here even though most of the basic procedures have been demonstrated for lower organisms. Finally, once the gene is in the recipient tree there is no assurance that: its enzyme product will have the proper substrate, the cell will be able to tolerate the new chemicals, or that it

FIGURE 3. SUMMARY OF GENE REGULATION

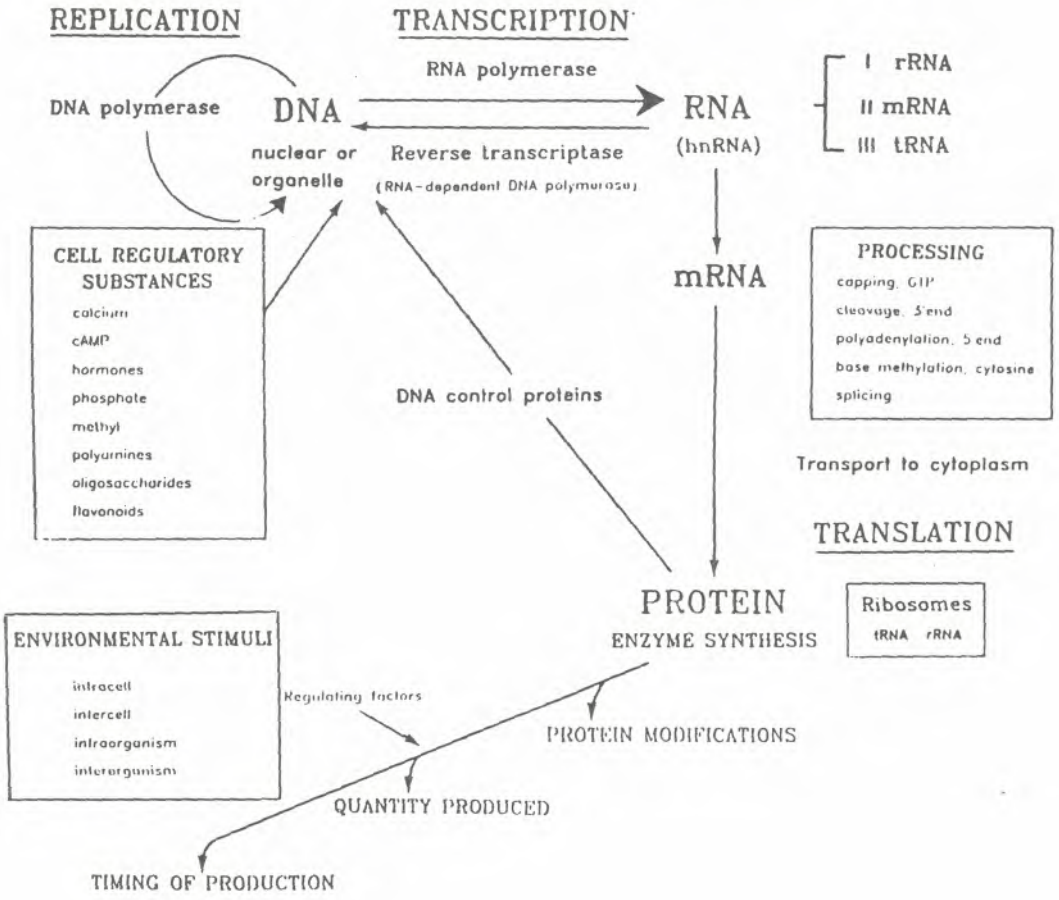


Figure 4. PROCEDURES FOR TRANSFERRING THE 3-CARENE GENE FROM ONE TREE TO ANOTHER

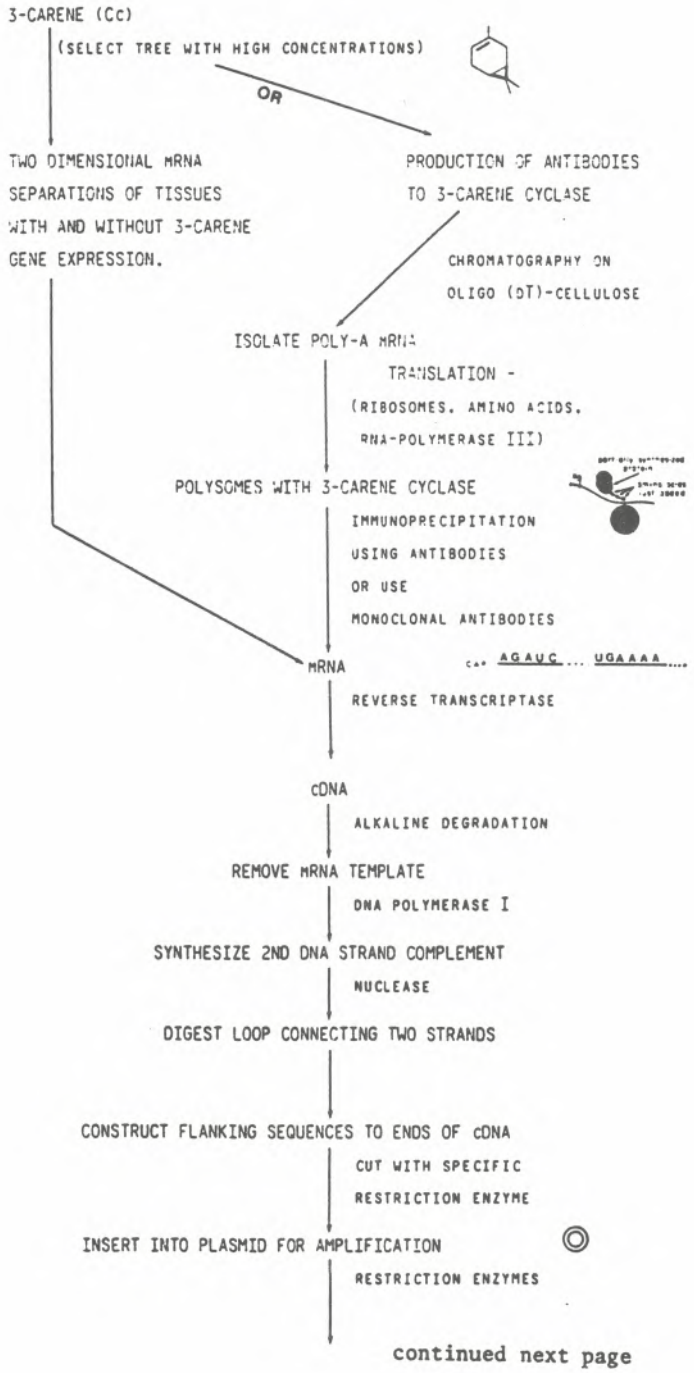


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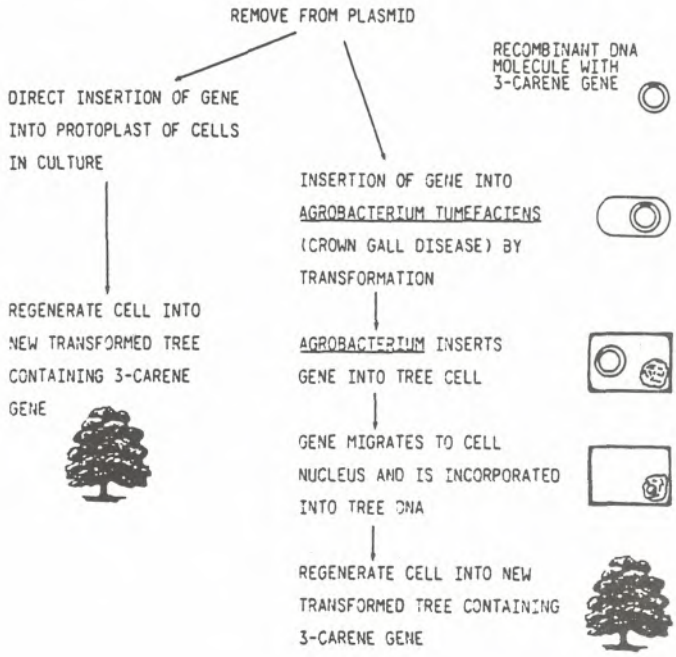
Figure 4 continued



will be properly regulated.

The potentials for gene transfer are great and the possibilities are exciting. Realistically, it may be 50 years before there is a significant impact on practical tree breeding through recombinant DNA technology.

COMMERCIALIZING BIOTECHNOLOGY

The model presented above showed how even the biological feasibility of recombinant DNA technology is probably far from our grasp at this time. Granted, there are some encouraging developments coming from research on several tree species and we are gaining a wealth of basic knowledge about tree biology from the surge of interest in biotechnology. But what are the problems with actual commercialization of the new biotechniques?

To answer this question, I thought it might be informative to take a brief look at an actual situation in commerce today--a case study, if you will. The case is one in which a pioneer biotechnology scientist left the comforts of his University position in 1981 to attempt to commercialize some of the new developments in the fields of tissue culture and genetic engineering. The goal of his company, Crop Genetics International, Inc., was to develop, manufacture and market products that improve crop productivity. Specifically, the company uses cell culture technology to produce "disease free" (not resistant) sugarcane seed products. Also, it is attempting to develop a microbial delivery system for insecticides and fungicides to protect cotton, corn, soybeans, wheat, etc. Crop Genetics International has recently issued a prospectus (February 18, 1987) through Drexel, Burnham, Lambert for the first public sale of common stock to further finance its operations. The principals of the company are competent, dedicated people who are striving hard to bring plant biotechnology into commerce. But the road is not an easy one, judging from the following facts about CGI:

1. Since its formation in 1981 CGI has accumulated a deficit of $7,065,255.
2. CGI has paid no dividends and does not foresee paying any.
3. CGI had sales of seedcane of $964,449 in 1985-86; the costs of production exceeded revenues.

Any new biotechnology company faces a number of risks. These risks for CGI are listed in the prospectus and are undoubtedly typical of those faced by entrepreneurs attempting to capitalize on the new plant biotechnologies. They include:

1. Losses during early development; no assurance of profitability.
2. First sales of seedcane made in 1985-86; cost of production exceed revenues.
3. Company's products are still to be developed, field tested, approved by government agencies, manufactured in production quantities, and marketed successfully.
4. No assurance of product performance.
5. Government regulation; slow and costly process.
6. Potential for product liability; high insurance costs.
7. No assurance of patent protection essential for profitability of products and processes.

8. Highly competitive: other companies may "scoop".
9. High dependence on key personnel who may be mobile.
10. High dependence on universities and research laboratories for appropriate microorganisms.
11. Volatility of sugar prices.
12. Dilution factor: buy shares at $16.00 each; worth $5.53 after offering.

As I stated initially, my purpose here in providing both a model gene transfer scenario and a company case history is not to discourage the pursuit of potentially useful tools of biotechnology but to put these pursuits in their proper perspective in relation to other more conventional tools available to tree breeders, especially natural variation, selection, hybridization, seed production, and cloning. In fact, these tools are even more critical to successful tree improvement today. I am confident that we are indeed in the midst of a revolution in forest genetics and forest biology as well. However, this revolution is not going to be a rapid one in terms of practical applications that tree breeders, seed companies and nurseries can incorporate into their operations in the near future.

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