Biotechnology

Science, Education & Commercialization

Biotechnology: Science, Education and Commercialization

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Preface

Modern biotechnology, based on a combination of cell culture and recombinant DNA technology, began in the early 1970s. In less than two decades it has become not only a fascinating and promising frontier of the biological sciences, but also one that has important practical applications, and societal and environmental implications on a global scale. Naturally therefore, biotechnology has attracted the attention of the scientific community, the public and industry. The fascination and interest in biotechnology is truly international.

Scores of national, regional and international meetings are held each year on various facets of biotechnology including science and technology, commercialization, and legal and regulatory issues. However, few, if any of the meetings, provide an opportunity to review and discuss all the aspects of biotechnology and their interrelationships on a global scale. This volume includes papers presented by a distinguished group of individuals at the Second International Symposium on Biotechnology. The symposium, Biotechnology: Science, Education and Commercialization was held at the University of Florida, December 3-6, 1989.

Thus far the most impressive advances and applications have been achieved in biomedical, animal and microbial biotechnology, supported by technical breakthroughs in bioengineering. The development of plant and aquatic biotechnologies is slower, although eventually these may have the most significant, long-lasting and global impact because of their potential for improving the quality and the quantity of food, and indirectly the environment. Sections I and II provide overviews of these advances, and several aspects of their practical application and commercialization.

It is a matter of serious international concern that the bulk of the scientific, technical and commercial development of biotechnology has and is taking place in the United States, Japan, and a few countries in Western Europe. This is particularly worrisome because there is very little effort in biotechnology in the developing countries of Asia, Africa and Latin America which could probably benefit the most, and immediately, from the applications of biotechnology because of their large populations and the attendant problems.

Glasnost and the lifting of the Iron Curtain have most recently revealed the apparently long-festering food, health and environmental problems in Eastern Europe. They too could benefit greatly from the fruits of biotechnology. There is thus an urgent and immediate need to build an infrastructure in these regions, not only to transfer the proven and working technologies developed elsewhere, but also to educate and train young scientists in order to make these countries self-sufficient in the long term, so that they can attend to their own special needs and interests. Section III describes many of the international and regional efforts directed toward overcoming these problems, particularly through the training and education of young scientists from the developing countries, and the collaborative research programs which have been setup.

One of the most serious problems that will limit the future development of biotechnology as a science as well as a commercial enterprise, is the increasing shortage of trained personnel. Section IV highlights these difficulties, and describes and discusses the initiatives being taken in the United States. These initiatives may well serve as a model for other countries.

The volume concludes with descriptions of the enormity of the Human Genome Project and its impact not only on human health, but on all aspects of biology.

The success of biotechnology in meeting human needs, in protecting and improving the environment, in economic development, etc., will also depend on the public's perception and acceptance of this new technology and its products. It is, therefore, critically important that the public and the political leadership in the developing, as well as the developed, countries be fully informed and well educated about all the ramifications of biotechnology. The responsibility for this education rests with the scientific community and the biotechnology industry.

It is my hope that the accounts of frank and open discussions of the varied facets of biotechnology included in this volume will be of special interest and use to scientists, educators, policy makers, corporations and all others who are interested in, are fascinated by, or have concerns about, biotechnology.

My task in the editing of this volume was made easy and enjoyable by the kind cooperation of each of the authors. I am especially grateful to Ms. Casey Smith for her prompt and expert assistance in the preparation of the manuscript using Desk Top Publishing. I also wish to thank Mr. Chris Leopold in preparing the art work for the book, and Ms. Lenie Breeze of the Biotechnology Institute for Technology Transfer for her support and coordination of the entire project.

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INTRODUCTION

Farmers worldwide are searching for resource efficient, cost effective and more profitable production systems. The world is looking for environmentally friendly and sustainable agriculture. It is my belief that the research accomplishments of the last ten years have shown that biotechnology can provide many of the technological innovations needed for sustainable agriculture.

The special power of biotechnology comes from genetic engineering, that is, the ability to transfer genes by non-sexual procedures. With this technique of transferring genes, scientists can take just one or several genes from one organism, a corn plant or a cow, and insert these genes into a completely different organism, such as bacteria, to produce a bacterium with those new genes.

This is the basis of one of the first applications of biotechnology to agriculture, the use of somatotropin to enhance the efficiency of protein production in livestock. Somatotropins are proteins normally produced by the pituitary gland of a domestic animal, which are now being produced by the ton in fermentation tanks by genetically transformed *E. coli* bacteria. Somatotropin can increase the productivity of milk-producing and meat-producing animals.

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In other experiments, scientists have inserted various foreign genes into plants and endowed these plants, called genetically modified or transgenic plants, with desirable new traits. The potential to modify crops in this fashion will likely impact crop agriculture as significantly as other past innovations such as farm mechanization, hybrid crops, and crop chemicals.

Each of these past technical innovations has produced major industries and we expect the same from biotechnology. One way to measure the tremendous progress made in applying genetic engineering methods to crop plants is to examine the number of crops that are now amenable to genetic modification. Over 30 different crops can now be genetically modified.

Certainly within the next two years, every major crop will be routinely improved using genetic engineering techniques. This opens the door for improving the productivity, the safety and nutritional quality and literally, the value of crops. Another measure of the rate of advancement is the startling fact that a little more than 10 years ago the first plant gene was isolated. Within the next 10 years, scientists will have characterized every gene in a plant! Finally, perhaps a sign of progress which hits home to everyone is that today sophomores in high schools are doing experiments on gene cloning that scientists were receiving Nobel prizes for only a few years ago.

BACKGROUND

The rapid progress that has been made in the development of gene transfer systems for higher plants has surprised even the most optimistic researchers in the field. Today, nearly three dozen species of crop plants can be routinely manipulated using available *Agrobacterium tumefaciens* transformation systems (Gasser and Fraley 1989). Almost all major dicotyledonous crop species are accessible to improvement using transformation technology, including soybean and cotton. Recently, free DNA delivery methods based on the use of electroporation (Fromm et al. 1986), particle gun (Klein et al. 1988) and calcium phosphate precipitation methods have been successfully used to produce transgenic cells and plants. This has facilitated the development of transformation systems for rice, corn and other monocotyledonous crops.

Genetically Modified Plant Species

tomato	alfalfa	petunia
potato	soybean	arabidopsis
lettuce	cotton	
peas	sunflower	pear
carrot	oil seed rape	poplar
cauliflower	flax	walnut
cabbage	lotus	apple
celery	sugarbeet	cucumber
tobacco		
horseradish		corn
		rye
		asparagus
		rice

There is no intrinsic difference between DNA introduced by breeding and that introduced through genetic engineering. In terms of the genetic stability of genes introduced by these two means there again does not seem to be any difference. Once a gene is inserted via transformation or by a genetic cross, it is stably maintained and expressed in the plant. On the basis of this similarity, a recent National Academy of Sciences report concluded, "Crops modified by molecular and cellular methods should pose risks no different from those modified by classical genetic methods for similar traits."

CROP IMPROVEMENT

The individual merits of genetic engineering and plant breeding are complementary. The primary virtue of genetic engineering is the ability to move traits from any organism into plants, and the ability to directly introduce traits into elite plant varieties that have been optimized by plant breeding. Genetic engineers are limited by the need for identification and isolation of the specific genes responsible for the traits that they wish to introduce. In contrast, plant breeders are able to transfer traits, like nematode resistance, by functionally assaying for the trait during each step of a breeding program without any information on the mechanism responsible for the trait. However, such traits may only be moved between species which can be made to interbreed. Engineering and breeding are currently being used in concert to produce genetically modified seeds with all of the most desirable traits in combination. While efforts to identify and transfer genes of agronomic importance into plants are just beginning, some degree of success has already been achieved in engineering selective tolerance to herbicides, viral diseases and insect pests. I would like to briefly update you on the progress of three Monsanto lead commercial candidates: Glyphosate tolerance, insect control and virus resistance.

Glyphosate Tolerance

Engineering herbicide tolerance into crops represents a new alternative for conferring selectivity and enhancing crop safety of herbicides. Research in this area has largely concentrated on those herbicides with minimal environmental impact due to properties such as high unit activity, low toxicity, low soil mobility and rapid biodegradation and with broad spectrum activity against a variety of weeds. The development of crop plants which are tolerant to such herbicides would provide for more effective, less costly and more environmentally attractive weed control.

The commercial strategy in engineering herbicide tolerance is to gain market share through a shift in herbicide use, but not to increase the overall use of herbicides as is popularly held. In fact, herbicide resistant plants will have the positive impact of reducing overall herbicide usage through substitution of more effective and environmentally acceptable products.

Advances have been particularly dramatic in the engineering of selective herbicide tolerance because existing knowledge of herbicide mode-of-action and metabolism has permitted rapid identification of key target genes. It seems clear now that, in the next 5 to 10 years, commercial level, selective tolerance will be available for many major existing herbicides, including Roundup^R, sulfonylureas and imidazolinones as well as several newly introduced products. Glyphosate, the active ingredient in Roundup[R] herbicide, is a broad spectrum non-selective herbicide produced by Monsanto which is used to control annual and biennial sedges, grasses and broad-leafed weed species. It is rapidly absorbed by foliar tissue and is quickly translocated to various plant organs. Glyphosate is rapidly metabolized in the soil and has no residual activity. The shikimate pathway enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), involved in aromatic amino acid biosynthesis has been identified to be the specific target of glyphosate in bacteria and in higher plants.

Tolerance to glyphosate has been engineered into a variety of crops by introducing genetic constructions for the production of EPSPS enzymes (Shah et al. 1986). Experiments have been carried out with a variant EPSPS gene which encodes an enzyme that is 6,000 fold less sensitive to glyphosate inhibition than the wild-type enzyme. Transformed tobacco, canola, cotton, soybean and sugarbeet plants expressing the glyphosate-resistant EPSPS gene were found to be significantly more tolerant to glyphosate than control plants. By making these crops resistant to glyphosate, a new market will be created for glyphosate. At the same time farmers are provided with improved and environmentally safe weed control. Glyphosate, quite simply is the best chemical in the world for controlling weeds and biotechnology will make it even better!

Insect Tolerant Plants

Another application of genetic engineering with important implications for crop improvement has been the production of insect resistant plants. Progress in engineering insect resistance in transgenic plants has been achieved through the expression in plants of the insect toxin gene of *Bacillus thuringiensis (B.t.)*.

B.t. is an entomocidal bacterium which produces a parasporal protein crystal. Most strains of *B.t.* are toxic to lepidopteran larvae (Dulmage 1981) although some strains with toxicity to dipteran or coleopteran larvae have also been described. The insect toxicity of *B.t.* resides in the parasporal protein crystal which, in the case of lepidopteran-active strains, is composed of toxin protein subunits of approximately 130,000 kDa. The *B.t.* proteins have absolutely no effect on humans, animals or beneficial insects. Genes encoding the lepidopteran-specific toxins from several strains of *B.t.* have been cloned, sequenced and introduced into plants. The first attempts to express the full length gene in plants were unsuccessful, but genes containing only the amino terminus of the protein were able to demonstrate weak insecticidal activity.

Recently, using knowledge of the rules which govern plant gene expression, Monsanto scientists have literally redesigned the bacterial gene to work in plants. In doing so, its expression has been improved one thousand fold. The excellent insect control observed under field conditions indicates this technology may have commercial application in the near future (Delannay et al. 1989). Early market opportunities for caterpillar resistance are leafy vegetable crops, cotton and corn.

Insect resistant crops represent a significant opportunity because of the environmental and regulatory pressures on certain insecticides and because of the resistance that some insect populations have developed toward certain classes of chemicals. There are many benefits including: reduced pesticide residues, reduced groundwater concerns, less worker exposure, and even fewer insect parts in food!

Virus Tolerant Plants

Significant resistance to tobacco mosaic virus (TMV) infection, termed "coat protein-mediated protection," has been achieved by expressing only the coat protein gene of TMV in transgenic plants (Powell-Abel et al. 1986). Using this approach, similar results were obtained for transgenic tomato, tobacco and potato plants against a broad spectrum of plant viruses, including alfalfa mosaic virus, cucumber mosaic virus, potato virus X and potato virus Y. The mechanism for coat protein-mediated cross protection is likely to involve interference with the uncoating of virus particles in cells prior to translation and replication. Transgenic tomatoes carrying the TMV coat protein gene have been evaluated in greenhouse and field tests and shown to be highly resistant to viral infections (Nelson et al. 1988). The transgenic plants showed no yield reduction after virus inoculation whereas the control plants suffered 23 percent to 69 percent yield losses. This summer, the first test of potato plants engineered to resist two key viruses, PVX and PVY, was carried out with excellent results (Lawson et al. 1990).

The level of capsid protein in the engineered plants (typically 0.01 percent to 0.5 percent of the total protein) is well below the levels found in plants infected with this endemic virus. This fact should facilitate registration and commercialization of virus resistant plants. Since there is currently no method of controlling virus diseases in crops, it meets a true unmet need. Again, there are numerous benefits including: reduced insecticide application and improved food quality. Virus resistance could provide significant yield protection in important crops such as vegetables, corn, wheat, rice, and soybean.

Future Research

The results described above indicate the potential for using gene-transfer technology for crop improvement. However, with all the attention focused on agricultural applications, it is important to emphasize the impact this powerful tool will have on advancing basic research in all aspects of the plant sciences. Precise localization of the regulatory sequences for tissue-specific and environmentally modulated expression of genes has just begun for a small number of plant genes. The availability of specific molecular probes is already extending research in plant physiology, biochemistry and cell biology. All the traits that I have covered so far are agronomic traits. They create value on the input side of crop production; they are valuable to the farmer. These genes represent the first wave of new products. They will be closely followed by crops engineered to improve nutrition, taste, storage and consumer appeal. In a little longer term, we will see crops modified to serve as sources of specialty chemicals and pharmaceutical products.

Perhaps new value-added crops of the 21st Century will include crops grown for pharmaceutical purpose. (Perhaps we will harvest human insulin from the "North Forty" instead of from tanks of genetically transformed *E. coli.*). There are obviously many other applications of gene transfer to agriculture that have not yet been mentioned. For example, gene transfer can help halt the decline in the genetic diversity of crops which makes most modern agriculture vulnerable to attack by rapidly evolving plant disease and pest organisms. In the next two decades the plant breeder will have the capacity to quickly introduce important new diversity into key crops and, ultimately, to introduce new crops. In certain crops which have a narrow germplasm base such as soybean, gene transfer would hasten the use of new varieties in a manner unobtainable by traditional breeding methods or tissue culture-induced variation.

Plant Biotechnology - Long-Term Goals

Agronomic Traits	Quality Traits	Specialty Chemicals
Herbicide tolerance	Oil composition	Plastics
Insect control	Solids content	Detergents
Virus resistance	Nutritional value	Pharmaceuticals
Fungal resistance	Consumer appeal	Food additives

KEY ISSUES

The impact of this new gene transfer technology in agriculture promises to be stunning. By the mid-1990s several useful transgenic seeds will be available to farmers and by the year 2000, dozens should be available. While only a few important traits have been transferred to important grain and vegetable crops, only time and not great technical obstacles, stands in the way of future improvements. Perhaps the only key obstacles are gaining necessary regulatory and public approval. At Monsanto we are working closely with the food industry and the federal government to register engineered crops. We are also putting considerable effort into increasing the understanding and public support for agricultural biotechnology products.

Public Perception

The field testing of genetically engineered plants containing herbicide, virus and insect tolerance genes represents an important step in the commercialization of plant biotechnology research. In view of recent research breakthroughs, increased public awareness and the existence of new regulatory policies, such tests have undoubtedly taken on greater significance than deserved. It is important to remember that many of the first tests that have been conducted are not with commercial cultivars and that several years of plant breeding efforts, field evaluations and scale-up lie ahead before improved crops will be marketed. At the same time though, the technology is developing faster than most realize and already issues such as regulatory costs and registration time-lines are becoming key concerns to companies attempting to develop improved, genetically engineered crops. While the commercial significance of current field tests will only be determined after much additional evaluation, several important observations are noted below:

- Field experiments were carried out with local community support.
- No adverse impacts of engineered plants on test site environment.
- Introduced traits did not affect plant growth or reduce yield.
- Field performance was as good or better than greenhouse tests.

It will be important that the process for evaluating field testing of genetically engineered plants recognizes and responds quickly to the need for testing of additional plants at multiple locations. It will also be important in subsequent evaluations that normal agronomic practices be employed in field tests, including completion of crop reproduction cycles and testing in normal production areas. Finally, it is critical that regulatory requirements dealing with the commercialization of genetically engineered plants be formulated in such a fashion that recognizes the inherent low risk of the technology and which does not draw undue attention to the particular biotechnology process used to improve plants.

International Competitiveness

A problem that faces U.S. farmers, in particular, is ensuring that their farm products can compete in world market places, including the United States. Unless we plan on a future in which we protect, to a much higher degree, the price of U.S. grown commodities, American farmers must use some of the new technologies to increase their productive efficiencies. Failure to adopt new technologies, such as biotechnology, environmentally safe pesticides, environmentally safe synthetic fertilizer formulations and other new technologies that can significantly increase the efficiency of production and ensure high product quality, could cause a permanent crippling of U.S. agriculture.

It is also important to recognize that these new technologies will hasten the restructuring of U.S. agriculture whether or not they are adopted by U.S. farmers. For the technologies will certainly be adopted in other countries and this will increase their productive efficiency, reduce their costs of goods, and make offshore commodity products more attractive to users in the United States than high-priced, local products. American farmers need the same tools as their competitors to produce food at a competitive price, and on a sustainable basis. A country or an industry can survive for only a short period of time by erecting barriers to competition and by not investing in innovation. Eventually that industry must adopt new technology to survive; examples are the steel and automobile industries in the United States, which may have waited too long to adopt new technology.

Third World Application

The majority of the five billion additional people that will live on our planet in 2030 will be in the less developed countries of the humid tropics. Because of the problems of food distribution, agriculture in the humid tropics must be indigenous and very productive. Improved agricultural practices and biotechnology can help address some of the more urgent agricultural problems of the humid tropics. In fact "plant biotechnology" has been identified by Unesco and several other international scientific groups as a number one priority to meet the future agricultural production needs of many less developed countries.

For example, genes for increased protein could be transferred into important root crops, like cassava and taro. Genes for virus-resistance, insect-resistance, and fungal-resistance have also to be inserted into important tropical crops (cassava, sorghum, millet, cotton) to enhance yield and reduce input costs. Moreover, the technology of plant gene transfer must be developed *in situ* in some tropical countries, to ensure that it responds to local conditions and thereby to ensure that the technology is more readily accepted by local populations and governments, so that ultimately the plant breeder and the farmer in the tropics use plant gene transfer as part of their agricultural practice.

From the forgoing, I do not wish to imply that biotechnology alone can provide a quick technology fix to the problems of germplasm preservation and world hunger. Economic and political reform, education, solutions to rural landlessness, relief from overburdening international debt, and agricultural infrastructure for research, teaching and extension, new ways to limit deforestation, realistic levels of government controlled food prices, population control through family planning, and many other things will be needed. But the new technologies can be powerful and we believe necessary tools, to ensure a sustainable supply of adequate food.

Sustainable Agriculture

Sustainable agriculture means different things to different people. In fact, arguments over the diversity of definitions have created confusion over the concept. In part this confusion stems from misunderstanding. In other cases it reflects the different motives of particular advocates. The definition adopted by the American Society of Agronomists in 1988 has many elements that seem appropriate: "A sustainable agriculture is one that over the long term, a) enhances environmental quality and the resource base on which agriculture depends, b) provides for the basic human food and fiber needs, c) is economically viable, and d) enhances the quality of life for the farmer and for society as a whole."

The time dimension is a key characteristic of sustainable agriculture. It implies the ability to meet our need and to endure indefinitely albeit with appropriate evolution, for agricultural practices change with time. Sustainable agriculture must evolve over the next forty years as the population of the world doubles, and as the demand for meat, milk, eggs and fish increases. Production of animal protein for food requires the use of more agricultural resources than the production of plants for food. For example, today it takes five or six times as many agricultural resources to produce a calorie of pork as to produce a calorie of grain.

In 2030, because of the doubling of world population and the upgrading of the diet of many people, the world must be able to produce significantly more than twice as much food as it now does. A troubling question emerges: how will agricultural systems evolve by 2030 to meet the needs of five-billion additional human beings and the additional demands of dietary improvements? The agricultural systems of today and certainly those of the past are unlikely to be adequate. The farming practices of 1930 when the world's population was only two-billion, will not be able to provide the more than five-fold amount for food needed in 2030. We cannot rely on past or even current technologies alone to provide food for a rapidly-increasing population and for dietary upgrading. Investment in new agricultural technologies, particularly biotechnology, is one of the keys to sustainable agriculture.