

# HARVEST ON THE HORIZON: FUTURE USES OF AGRICULTURAL BIOTECHNOLOGY

PREPARED BY THE PEW INITIATIVE ON FOOD AND BIOTECHNOLOGY

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## PREFACE

Over the last decade, scientists' ability to alter the traits of plants and animals by moving genes from one organism into another has come out of the laboratory into mainstream domestic agriculture. To date, scientists have largely used this technology to create crops that benefit farmers, such as corn and cotton capable of fending off destructive pests, and soybeans resistant to chemical herbicides. Now, however, in numerous universities and company laboratories, the power of biotechnology is being used to modify agricultural plants and animals for a wider array of purposes. Among other things, scientists are exploring whether it is possible to increase cancer-fighting ingredients in food, to harvest organs from animals for transplantation to humans, to deliver vaccines in fruit and to rescue threatened species such as the American chestnut tree. While many future applications will continue to be aimed at solving age-old agricultural problems like protecting crops from pests, genetic engineering may create new opportunities—and challenges—for the future.

This report, the first published by the Pew Initiative on Food and Biotechnology, provides an illustrative overview of what could be the “next generation” of genetically engineered agricultural products. It should not be considered a comprehensive inventory of everything in the R&D pipeline, or a forecast of what is to come. Much of the research cited is at an early stage, and many of the applications face significant technical, economic, marketing and regulatory issues before they can be brought to market. Instead, this report is intended to underscore the broad scope of current agricultural biotechnology research and to illustrate some of its potential uses, many of which are dramatically different than those commercially available today.

The report is not intended to be an endorsement for these future applications. Indeed, it is likely that many of them will generate strong debate on their relative risks and benefits. *Harvest on the Horizon* has deliberately avoided a discussion of those issues to provide a clear snapshot of the breadth of today's agricultural biotechnology research efforts. Future reports and activities of the Pew Initiative on Food and Biotechnology will examine the broader public policy issues raised by agricultural biotechnology. This report is intended to help inform that debate by illustrating the range of potential uses of agricultural biotechnology being explored by scientists.

We would like to acknowledge the contribution of Joyce A. Nettleton, DSc, RD, who combined both published work and the results of interviews she conducted with researchers to create the research profiles at the heart of this report. We also thank Dr. Ralph Hardy for his thoughtful review of early drafts of this report.

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## INTRODUCTION

Anything one man can imagine, other men can make real.

~ Jules Verne

### THE “HOW?” OF BIOTECH: PUTTING BIOTECHNOLOGY IN CONTEXT



The ability of modern biotechnology to change the characteristics of a plant or animal through the direct manipulation of genetic material is a remarkable scientific achievement. While scientists may not yet be able to accomplish the vision of Jules Verne, the tools of biotechnology developed over the last thirty years have clearly opened up dramatic opportunities to create new varieties of plants and animals.

At the same time, the novelty of biotechnology has raised questions. Some view biotechnology as a logical and modest extension of conventional plant and animal breeding technologies. Others see it as a novel technology that is dramatically different from traditional breeding. How different is it from traditional plant and animal breeding? How is it being used? What kind of problems is it attempting to solve? What are some of the likely future uses of agricultural biotechnology? These are some of the questions this report attempts to answer.

#### *What does the term “biotechnology” mean?*

The term “biotechnology” was first coined in 1917 by Karl Ereky, a Hungarian engineer, to describe the large-scale production of pigs that were fed sugar beets. For much of the last century, it has been the broad term applied to the use of any living organism for a practical purpose—anything from the selective breeding of plants and animals to fermentation of beer or treatment of sewage with organic materials.

For the purposes of this report, however, the term “biotechnology” refers to the use of recombinant DNA technology to take genes from one organism and insert them into the DNA of another plant or animal. Unless otherwise stated, the report uses the terms genetic engineering, bioengineering, genetic modification, genetic engineering, and biotechnology interchangeably.

### *What is Biotechnology?*

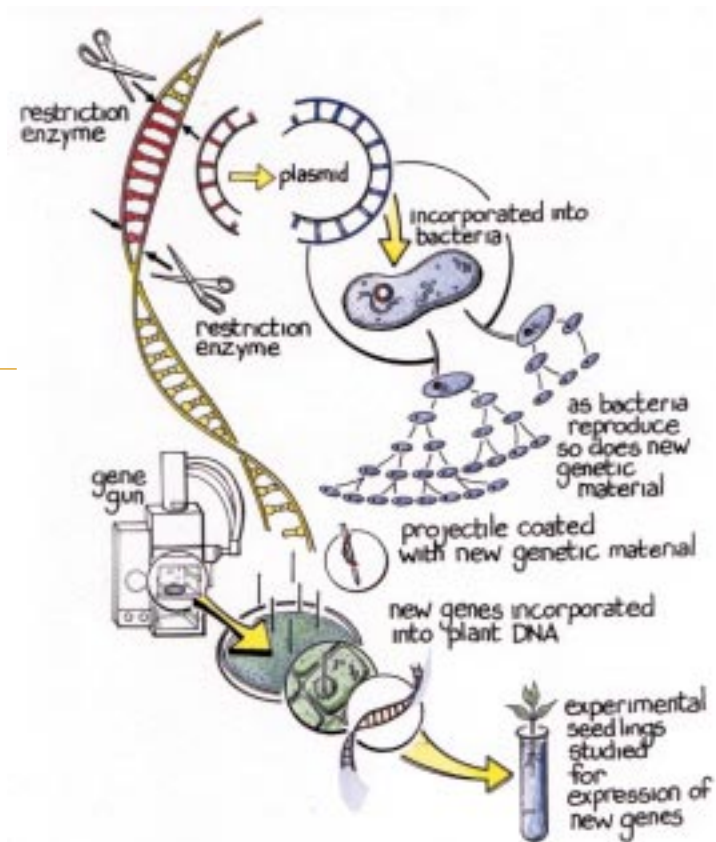
In the last fifty years, since the discovery of the structure of deoxyribonucleic acid, or DNA, by American biochemist James Watson and British biophysicist Francis Crick, scientists have made enormous strides in understanding how genes work. Genes are segments of long DNA strings wrapped into chromosomes and present in most cells, whether plant, animal or human. Through a series of molecular “messengers”, genes make—or “express”—the thousands of proteins responsible for virtually every living process. In general, each gene directs the production of a specific protein that has a specific function. For example, a single gene produces the human blood clotting protein known as Factor VIII. People with mutations in this single gene cannot make functional Factor VIII, which causes hemophilia. Gene expression is regulated by different DNA segments that cause genes to turn on or off, starting or stopping the protein production.

In the 1970s, scientists learned how to cut a specific gene out of a DNA string by using biochemical “scissors” called restriction enzymes (shown on page 6). They were able to take the isolated gene and insert it into circular pieces of DNA known as plasmids that are found in bacteria. The bacteria rapidly reproduced, making thousands of copies of the inserted gene. Scientists developed several ways to insert the copies of the isolated gene into the DNA of a different bacteria, plant or animal. When successfully inserted into the new organism, the gene began to make the same protein it did in its original donor organism.

The first successful effort of this “recombinant DNA” technology involved cutting a gene from a virus and inserting it into a bacterium, creating the first “transgenic” organism—that is, an organism that combined the DNA from two different species. Today, recombinant DNA technology is widely used to create transgenic bacteria that produce useful proteins, such as human insulin to treat diabetes, or chymosin, an enzyme widely used in making cheese.

Scientists have been able to create transgenic plants in a similar way. First, they identify a gene in an organism that is responsible for a particular trait—for example, pest resistance. After isolating and making copies of the gene, scientists insert it into the target plant's DNA, generally through one of two techniques. One involves a “gene gun”, which shoots microprojectiles coated with the isolated gene into the target plant's tissues (shown below). Another widely used method allows the isolated gene to hitchhike into a plant's chromosome on the back of a common soil bacterium that infects plants. In either case, scientists must test the plant to see whether the gene has been successfully inserted and whether it functions as expected. Once the gene has been inserted, the bioengineered plant cells are grown in a special culture that causes the cells to differentiate into the unique types of cells that make up the plant. The small plants are transferred from the laboratory culture to soil, where they are grown like conventional seedlings. The genetically engineered plants are then bred back with traditional crop varieties using conventional breeding techniques. Scientists test the resulting transgenic plant to make sure it continues to grow as well as the conventional variety and that the new trait works as expected.

#### HOW A GENE IS TRANSFERRED



For animals, scientists use a variety of different techniques to insert the isolated gene into the DNA. As with plants, they must carefully test the modified animal to be sure the trait is present and stable, and does not have an adverse effect on the animal.

### *How new is biotechnology?*

Some scientists argue that modern biotechnology is just the next step in a progression of increasingly scientific efforts by humans to selectively breed better food crops and domesticated animals. Other experts, however, take the view that recombinant DNA technology is very different from anything we have done before.

Changing the genes of plants and animals to better meet human needs is not a recent development. Farmers have known for centuries that they could gradually improve their crops by saving and replanting seeds from the best plants. Likewise, they knew, they could improve their animal stock by breeding the best pairs. Some also realized they could create new plant and animal varieties with desirable traits by carefully selecting individual plants for subtle differences. It wasn't until Mendel's 19th Century work began to unravel the mechanisms of inheritance, however, that breeding became more scientific and deliberate.

Over the centuries of crop cultivation and domestication of animals, the process of artificial (human) selection and selective breeding has created a diversity of food crops and animals with a wide variety of traits. For example, kale, cabbage, cauliflower, broccoli and Brussels sprouts are all vegetable varieties derived from a single species (Bailey and Bailey 1976). Hybridization—the process of breeding genetically different parents with contrasting characteristics to produce a hybrid offspring with the useful characteristics of both parents—has resulted in higher yields and more disease resistant crops. For example, improved varieties of rice with significantly higher yields than traditional varieties have helped meet the developing world's food needs.







A consequence of selecting for traits through conventional breeding has been the gradual change in the genes of domesticated plants and animals. The genes of an Angus cow, for example, differ from the genes of a Holstein, just as they both differ from a common undomesticated ancestor. As a consequence, most of the food we eat today comes from plants and animals that are genetically different from their early ancestors.

While modern biotechnology follows in the same tradition of improving crops and animals for human uses, its approach and techniques are quite different. In the past, breeders selected for traits without knowing which genes were responsible for the trait; the transfer of genetic material was controlled, for the most part, by the usual mechanics of sexual reproduction. The breeders had little power over, or even knowledge of, which genes were actually transferred. In contrast, biotechnology transfers only selected genetic materials, such as the gene for the specific trait, and other genetic materials to help track the gene and make it work effectively in the target plant or animal.

Some scientists argue that this precision makes the effect of creating new varieties through biotechnology more predictable than conventional breeding, where genes with unknown and possibly undesirable functions can also be transferred with the genes responsible for the desired trait (Pueppke, 2001). Others disagree. They argue that most significant traits are likely to be affected by a complex interaction among numerous genes, about which there is limited knowledge. According to these scientists, conventional breeding using sexual reproduction is more likely to pass on all of the genetic material needed for a trait to work successfully in a plant or animal than recombinant DNA techniques (Palumbi, 2001).

Further, since recombinant DNA techniques insert genetic material through direct manipulation rather than through sexual reproduction, scientists are not limited to moving genes between members of the same species. (See Box: *Is the ability to cross species lines new?*) They can take a gene from one plant or animal species, directly insert it into the genes of a different plant or animal species, and find it expressing the same protein in the second organism as it did in the first. In this way, for example, it has become possible to take the gene from a bacterium that makes a protein toxic to insect pests and insert that gene in corn, so the corn now makes the same insect-killing protein in its tissues.

While modern biotechnology falls within the long tradition of the human manipulation of the genetic materials of plants and animals, it also greatly expands the ability of scientists to move traits across species lines, and makes possible for the first time the ability to move genes across distant species, phylas or even kingdoms. It is precisely because the technology is so potentially powerful and capable of novel uses that a number of issues have been raised. These include concerns about the safety of food made from genetically modified plants and animals and concerns about the impact on the environment, as well as the ethical and moral implications of the technology.

*Is the ability to cross species lines new?*

The ability to cross the species boundary is not entirely new. In the wild, tree species such as poplar and oak have been known to naturally create hybrids. Scientists using conventional hybrid breeding techniques have also been able to cross species. For example, a German experimenter in the 19th century developed a hybrid of rye and wheat, two different species. In addition, grafting—physically fusing two plants together so they grow as one—often involves the joining of different species. However, most conventional breeding is done within a species. Modern biotechnology, through its ability to directly transfer selected genetic material, greatly increases the potential to move genes between species and creates new possibilities to move genes across very distant species, phylas, or kingdoms.



*The “What?” and “Why?” of Biotech: Its Purposes*

Today, biotechnology researchers are developing new products that they believe will offer better solutions to traditional agricultural problems: making food production easier; growing food with improved quality and nutrition; and using agriculture to meet non-food needs, such as fiber, fuel and other products.

**Making Food Production Easier.** Several different strategies to increase crop yields or make food production easier are being explored by industry and university researchers. One approach is to attack the causes of crop losses, such as pests (insects, viruses, and disease), stress (weather variability like drought and frost) and competitors for soil nutrients (weeds). Another is to find ways to improve the plant's own efficiency to create more food, or to produce the same amount of food with fewer inputs and resources. A third strategy focuses on improving a plant's ability to grow in soils that are nutrient-deficient or that have excesses of minerals or salinity. Increasing the efficiency of animal production relies on similar strategies: animals can be selected that produce more meat or milk, or are more disease-resistant.

The same strategies are also being pursued through conventional breeding and the use of agricultural chemicals. Many technology developers believe, however, that biotechnology may reduce costs for farmers and in some cases reduce the use of chemical pesticides compared to conventional farming.



Existing and emerging biotechnology applications, some of which are discussed in this report, are addressing all of these strategies to improve agricultural yields and make food production easier. Examples include:

- ◆ Modifying corn to contain the Bt pesticide, thus building in resistance to the European corn borer, a significant corn pest in the U.S.;
- ◆ building resistance in channel catfish to enteric septicemia, a serious disease in the aquaculture industry which can kill fish within five days of exposure;
- ◆ modifying crops to resist commercial herbicides, thus enabling the plants to grow while competitive weeds are killed;
- ◆ creating plants that can grow in drought or unfavorable soil conditions to generate productive agriculture on previously uncultivated land; and
- ◆ altering salmon to make them grow, on average, three to five times faster than their non-transgenic counterparts, allowing them to be brought to market more quickly and less expensively.

**Improved Quality and Nutrition.** Industry and university researchers have also been working to develop food products with improved quality and nutritional values. In recent years, for example, researchers have used conventional breeding techniques to develop cattle and pigs with lower fat, providing leaner and more healthful cuts of meat. Biotechnology research is also aimed at improving the quality, nutritional value and other product attributes through genetic modification. Examples of biotech products under research and development include:

- ◆ Vegetable staples such as cassava and plantains with improved total protein content and quality;
- ◆ canola oil with more nutrients like lutein that may help prevent eye disease;
- ◆ soybean oil with 80 percent more oleic acid, one-third less saturated fatty acid than olive oil, and no trans-fatty acids;
- ◆ cow's milk with reduced lactose content to improve the digestibility of milk for people with lactose intolerance;
- ◆ pigs carrying a gene for insulin-like growth factor, leading to a more lean body mass; and
- ◆ beans with characteristics that are more suitable for processing and canning, such as firmer texture and seed coats that do not split.





**Meeting Needs for Fiber, Fuel and Other Products.** Plants and animals have always served needs other than food. Trees provide lumber for building materials, as well as pulp for paper. Plants supply fibers useful for textiles like cotton, as well as chemicals and oils for industry, such as jojoba oil. Examples of genetic engineering of plants and animals for non-food uses include:

- ◆ increasing the efficiency of pulp production from trees;
- ◆ modifying fatty acids and oils for paints and manufacturing;
- ◆ creating plastics from corn for use in consumer packaging;
- ◆ introducing pigment-producing genes to make flowers bloom in colors not possible through other breeding methods;
- ◆ producing spider silk from the milk of goats;
- ◆ turning plants into biosensors that can detect or monitor hazardous materials in the environment; and
- ◆ modifying turf grass to increase its tolerance to drought, salt and cold.

Industry and university researchers are also modifying traditional crops and livestock through genetic engineering to make products with medical applications. Examples include:

- ◆ plant-based, edible vaccines for the prevention of human and animal diseases as varied as Hepatitis B, traveler's diarrhea and tooth decay;
- ◆ human proteins produced in plants for therapeutic use, such as hirudin, an anticoagulant used to treat blood clots; and
- ◆ tissue and organs grown in animals for use in human beings, a process known as xenotransplantation.

### *The “Who?” of Biotech: End-users*

As outlined above, agricultural biotech products are designed for specific functions that are sometimes invisible to the end-user, the consumer. In fact, few genetically engineered whole foods are sold directly to consumers but instead are further processed into ingredients used by food manufacturers. (See Box: *Flavr Savr Tomato*.) Some corn, for example, goes to animal feed, while some is used to create processed corn products and ingredients like corn syrup. As a result, the Grocery Manufacturers of America estimate that as much as 70 percent of the processed foods available in American grocery stores may contain ingredients derived from genetically modified plants.



### *The Flavr Savr Tomato*

The first genetically modified food product sold directly to consumers was brought to market in 1994. The Flavr Savr tomato was bioengineered to remain on the vine longer and ripen to full flavor before harvest. Conventional tomatoes are harvested while green and firm so they can get to market without being crushed; after shipment, they are force-ripened with ethylene gas, the natural ripening agent in tomatoes. However, the Flavr Savr tomato was not a market success; it was expensive and some consumers did not like the taste. The product is no longer sold, although other similar bioengineered tomatoes are used in processed tomato products.

### *The “When?” and “So What?” of Biotech: Today and Tomorrow*

Today, genetic engineering provides a set of new tools for agriculture. In addition to continuing research and development on basic crops, there are also hundreds of potential novel uses for biotechnology being researched across the entire agricultural spectrum—from trees to grass and flowers, mammals, fish, and even insects. Significant research on plant and animal genomics will likely lead to new applications, while marker-assisted breeding may accelerate conventional, non-transgenic animal and plant breeding. (See Box: *Agricultural Genomic Research*.)

### *Agricultural Genomic Research*

The rate of progress toward understanding the individual function of genes and the structure of plant and animal genomes—the master blueprint for the total set of genes belonging to an organism—is remarkable.

A major focus of current agricultural science is on genomics—the systematic investigation of animal and plant genomes. Scientists are hard at work mapping the entire DNA structure of various plants and animals. Mapping and determining the sequence of animal and plant genomes serves several purposes. As a complete genome of an organism becomes known, the position of genetic markers (easily identifiable segments of DNA), specific DNA sequences and specific genes can be determined. As genes become mapped, they can be studied more closely to determine their function. Once identified, they can become candidates for isolation and insertion into other plants or animals through recombinant DNA techniques, if they provide desirable traits. Alternatively, scientists are increasingly interested in the prospect of altering the traits of a plant or animal by directly changing the gene itself or modifying how it is regulated within the original plant or animal.

Gene sequences by themselves provide limited information, but they are useful for revealing where the genetic control for the expression of certain characteristics resides. For example, in virus-resistant soybeans, resistance occurs in a cluster of genes. Study of both single genes and gene clusters reveals much about what governs the development and expression of virus resistance and other traits.

Furthermore, scientists have found that knowing the location of genes associated with a particular trait in one species can aid in finding similar genes for that trait in another organism (Gura, 2000). Mapping genomes makes it easier for scientists to locate comparable genes in different species.



The expanding number of genome maps reveals striking genetic commonality among living organisms. For example, some 10 percent of human genes are clearly related to fruit fly and worm genes; about 99 percent of the overall DNA sequence in humans is similar to that of chimpanzees (Paabo, 2001). To date, scientists and researchers have sequenced forty-eight genomes. These include not only the human genome, but also the flowering mustard plant (*Arabidopsis thaliana*), a plant referred to in this report because of its extensive utilization in agricultural biotechnology research, as well as the fruit fly (*Drosophila melanogaster*), pathogenic bacteria and the nematode (GOLD 2001).

In addition to identifying genes for possible use in recombinant DNA technology, mapping animal and plant genomes also helps conventional breeding by identifying genetic markers. A genetic marker is an easily identifiable fragment of DNA associated with a particular trait or characteristic. Scientists can use such markers to help them track whether conventional breeding has successfully transferred the desired genes all the way through the various steps of breeding and cross-breeding. Genetic markers can also help scientists determine that plants of poor crop value may contain valuable genes that would not be expected based on the characteristics displayed in the plant, which can then be successfully transferred to breeding lines. Using marker-assisted breeding, new plant varieties can be developed within 3-5 years, as opposed to 10-15 years without marker-assisted breeding. However, marker-assisted breeding is still an emerging technology.





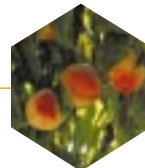


While some applications such as insect resistance and herbicide tolerance are already on the market and widely used, others may be a decade or more away. Certain uses of biotechnology, particularly those that improve agricultural production, have been on a “fast track” for commercialization. Other applications, such as enhancing the nutritional value of food, are the subject of active research and development. But many years of testing and government approval await other possible applications, such as controlling diseases in aquatic organisms, reducing the ability of insects to spread diseases to humans, and creating drug therapies harvested from transgenic animals and plants.

In addition, some biotechnology products might be technically feasible in the lab, such as plants that can grow plastic, but may not be economically feasible to bring to market. It is not just science but also the marketplace that will ultimately determine which biotechnological applications are successfully commercialized.

Biotechnology is a tool. It is not the only tool for addressing a particular set of problems, and it is not necessarily a better tool than conventional, or other, approaches or applications. It is beyond the scope of this report to weigh the costs and benefits of any particular agricultural technology or to compare the relative merits of potential alternatives.

Whether today's research projects become tomorrow's products depends on many factors not considered here. Social, political, regulatory, legal, environmental and economic questions continue to be debated. Before we make these kinds of decisions as a society—in our respective roles as consumers, regulators, producers, commentators and shareholders—we should understand where the technology is pointed.



PART I | GENETICALLY ENGINEERED PLANTS

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## AGRONOMIC IMPROVEMENTS

Agronomy is the application of the various soil and plant sciences to soil management and crop production. Agronomic improvements make crops more productive and easier to grow and harvest, while minimizing costs and negative effects on the land and environment.

One example of an agronomic improvement is selective breeding to promote desired traits in plants. Many botanists believe selective breeding over thousands of years by native people transformed the small teosinte plant, with tiny “ears” consisting of a single row of six or more kernels, into the productive multi-kernel modern corn plant.

Other examples include methods of crop rotation, such as planting legumes to replace nutrients like nitrogen that are depleted with the growing of some grain crops. Still other agronomic improvements include the development of hybrids, such as a hybrid tomato that can tolerate mechanical harvesting, and is therefore cheaper and easier to pick.

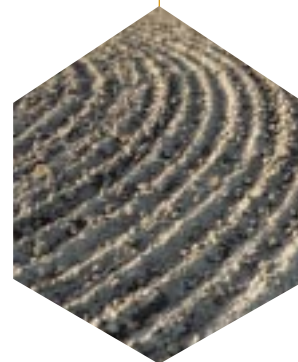
### PEST AND DISEASE RESISTANCE

Nationally, pests destroy about one-third of our crops and are an increasingly serious constraint to production, in spite of the advances in pest control technology over the last half century (Rosenzweig et al. 2000). A wide range of diseases and pests, including viruses, fungi, bacteria, insects, mites and plant nematodes are involved in significant crop losses each year. Some types of worms cause an estimated \$7 billion in annual crop losses in the U.S.; the damage from insects is even more severe (National Research Council, 2000).

Farmers have been trying to minimize losses from crop pests for hundreds of years. In the past, they have used conventional breeding practices, such as hybridization, to develop crops with better pest resistance, or chemical insecticides or biological control systems, such as predator insects that attack the targeted crop pests. Scientists can now make plants more pest resistant by inserting specific resistance genes from other plants or organisms. In some cases, recombinant DNA techniques were the first methods used to do this. This first generation of genetic engineering techniques for disease resistance relied mainly on affecting single gene traits. However, many resistance traits, such as those for fungal resistance, involve the interaction of several genes. Thus, future genetic engineering strategies are concentrating on means to control multiple gene transfers. Efforts to develop resistance to several pests or pathogens will require the use of many gene transformations.

The development of pest resistance in plants remains an ongoing effort, however, as pests themselves acquire new invasive strategies and become resistant to control measures. While development of pest-resistant plants is underway in public and private laboratories, the time required to create resistant strains, breed them into stable varieties, perform field-testing and obtain regulatory approval has so far limited the number of genetically engineered varieties commercialized.

Efforts to build crops resistant to diseases are occurring on multiple fronts. One approach is to find varieties of plants that demonstrate resistance to a specific infection-causing organism, and then determine the genetic components responsible for this natural resistance. The responsible genes can then be transferred to plants that don't have them. Other strategies rely on identifying the genes within a plant responsible for generating substances that fight pathogens, and then learning how to enhance the plant's ability to make them. Still other transgenic manipulations may aim to destroy insects that damage crops and transmit pathogenic viruses and fungi.

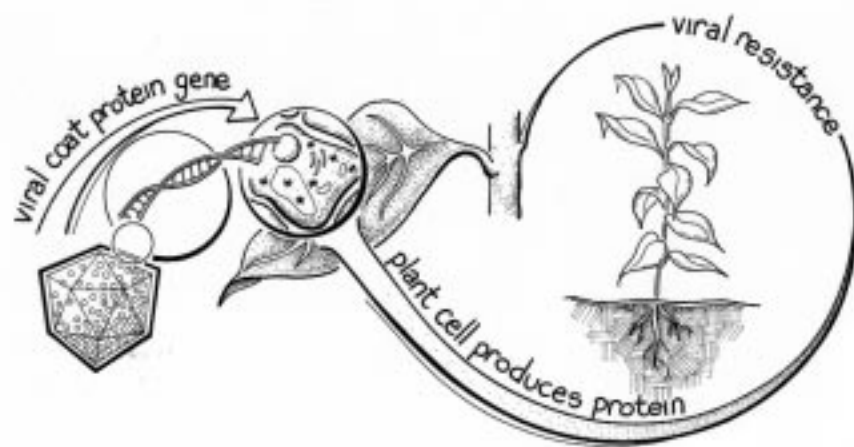


### Viruses

Viruses are among the most ubiquitous pests in agriculture. Scientists are working to develop viral resistance in a variety of crops including squash, potato, sweet potato, wheat, papaya and raspberries. Viruses are studied widely because they not only cause disease in humans, plants, animals and insects, but also are used as tools in the study of molecular biology and, in some cases, in the development of vaccines to fight the diseases they can cause.

Several techniques for virus resistance have been developed. These include viral coat protein technology and multiple gene transfers. A viral coat protein acts like a vaccine, causing the plant to develop resistance to the particular virus (illustration shown below). Transferring the gene for a viral coat protein, a part of the outer shell of a virus that does not cause disease, into a plant acts like a vaccine for the plant. The plant is then able to resist the virus, analogous to the way vaccines keep us from getting certain diseases like measles. The advantage of introducing only the coat protein is that it induces resistance without the introduction of the actual virus (Powell-Abel et al. 1986, Beachy et al. 1990). The technique has been used successfully in many plants against several different viruses.

#### VIRAL COAT PROTEIN TECHNOLOGY



The first genetically engineered virus-resistant food crop in the marketplace was yellow crookneck squash. Using the viral coat protein approach, this squash was engineered to resist the watermelon mosaic virus and the zucchini yellow mosaic virus (Animal Plant Health Inspection Service, 2000).

Potatoes are highly susceptible to many viruses, including the potato mosaic virus and the potato leaf roll virus (shown right). A leaf roll virus epidemic in 1996 was responsible for heavy potato crop losses in Idaho. The virus, spread by aphids, damaged the potatoes to the point that they were unmarketable. Scientists in Mexico, in collaboration with researchers at Monsanto, have developed potatoes resistant to several forms of this virus. Research on disease-resistant potatoes is continuing at other laboratories.

The feathery mottle virus has a damaging effect on sweet potatoes. In 1991, researchers began genetically engineering varieties of sweet potato grown in Africa, where it is an important subsistence crop. The sweet potato was engineered with coat protein from this virus and replicase genes. Replicase is an enzyme involved in the duplication of certain viral RNA molecules. Current field-testing has demonstrated successful gene transformations and the desired development of resistance to sweet potato feathery mottle virus.

Although wheat is an important food source, development of genetically engineered varieties has been slower than in corn, soy and cotton. A major pest in wheat is barley yellow dwarf virus, which can cause damage in major wheat-growing regions such as North Dakota, because no resistant strains are known. Work is in progress to engineer resistance to this disease using the viral coat protein technique. The wheat genome is highly complex—ten to twenty times larger than that of cotton or rice—and carries an exceptionally large amount of repetitive DNA sequences. Thus, targeting particular genes is challenging, and transgenic wheat biotechnology has advanced more slowly than that of other crops.

The papaya crop in Hawaii was nearly wiped out in the 1950s by the papaya ringspot virus (PRSV). Transmitted by aphids, this virus causes one of the most serious diseases of papaya worldwide (Gonsalves et al. 1998). Work to develop a transgenic virus-resistant variety began in the late 1980s. By 1992, resistant lines were field-tested; approvals for commercialization were granted in 1997. The transgenic-resistant papaya is now in wide use in Hawaii, and similar work is in progress in the Philippines, Malaysia, Thailand, Vietnam and Indonesia to enhance resistance in local papaya varieties where ringspot virus is a major pest.

Researchers are also modifying other fruits for virus resistance. Field tests of transgenic raspberries engineered for resistance to the raspberry bushy dwarf virus began in Spring 2000 (Stelljes, 2000).



potato leaf roll virus

## Fungi

In fruit and vegetable crops, fungal diseases cause significant damage to plants and are characterized by wilting, moldy coatings, rusts, blotches, scabs and rotted tissue. The search for genetic engineering tactics to combat fungi has intensified with the need to find adequate substitutes for fungicides such as methyl bromide, widely used on fruit and vegetables but being phased out due to its links to ozone depletion.

One emerging area is directed at a plant's production of defensins, a family of naturally occurring antimicrobial proteins which enhance the plant's tolerance to pathogens, especially bacteria (Garcia-Ollmedo et al. 1998). Certain defensins also demonstrate an ability to fight fungal infections.

Defensins are found throughout nature in insects, mammals (including humans), crustaceans, fish and plants. Defensins from moths and butterflies, the fruit fly, pea seeds and alfalfa seeds all show potent antifungal activity (Landon et al. 1997, Lamberty et al. 1999, Almeida et al. 2000 and Gao et al. 2000). The first transgenic application of defensins was the incorporation into potatoes of the antifungal defensin from alfalfa (Gao et al. 2000). Laboratory and field trials showed that the transgenic potatoes were as resistant to the fungal pathogen *Verticillium dahliae* as non-transgenic potatoes treated with fungicide. Although studies are continuing, the chance that fungi will build resistance to defensins is thought unlikely. No known resistant strains of bacteria or fungi have yet evolved that can overcome these highly protective, pesticidal proteins.

Ongoing research involving banana and cassava is directed to cloning resistance genes for major tropical diseases such as black sigatoka, a leaf fungus that widely infects bananas (shown left), cassava mosaic disease and cassava bacterial blight. In bananas, transgenic lines combining several antifungal genes have been generated. Selected lines are currently being tested for resistance to black sigatoka and Panama disease under greenhouse and field conditions.



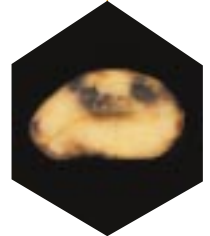
black sigatoka

Scientists are devising protection against the plant fungus *Botrytis cinerea*, a serious pathogen in wheat and barley. The strategy uses the gene for a natural plant defense compound named resveratrol (Lemaux and Qualset, 2000). Scientists have also introduced a gene from a wine grape into barley to confer resistance to *Botrytis cinerea*. Field trials are underway.

Resistance to potato late blight (shown right), a disease caused by *Phytophthora infestans*, receives high priority in potato research. Plant disease from this fungus can be destructive to crop production, as was dramatically illustrated in the Irish potato famine. In 1995, a U.S. late blight epidemic (caused by new aggressive strains of *Phytophthora infestans*) affected nearly 160,000 acres of potatoes, or about 20 percent of domestic production. Research is underway to genetically engineer potatoes that express the enzyme glucose oxidase and develop resistance to *Phytophthora* blights (Douches undated). At present, however, no products are close to commercialization.

Potatoes are also being transformed using a soybean gene for a protein (beta-1, 3-endoglucanase) that confers resistance to infection by *Phytophthora* (Borkowska et al. 1998). Other studies report that transgenic potatoes expressing a protein called osmotin showed reduced damage from lesion growth in leaves inoculated with the *Phytophthora infestans* pathogen (Li et al. 1999). Still other research is attempting to boost fungal resistance in potatoes by transferring resistance genes from peas. Infection of these transgenic potatoes with the fungus triggers hormone-like signals in the potatoes that turn on the pea resistance genes. One substance that is produced, chitosan, stops fungal growth and activates the potato's own natural defense systems.

In rice, blast (shown right) and sheath blight are major fungal diseases. Scientists created transgenic strains resistant to sheath blight that are currently being field-tested. Other researchers are working on engineering rice strains for multiple resistances to both the fungus sheath blight and the stem borer, an insect pest.



potato late blight



rice with blast



### *Bacteria*

Most bacteria living in or on plants are not harmful to their hosts, and may, in fact, be beneficial. However, some bacteria will invade their hosts and cause disease. Most food crops are susceptible to bacterial diseases, but bacteria rarely attack certain plants, such as mosses, ferns and conifers. Bacterial infections in plants may cause leaf and fruit spots (lesions), soft rots, yellowing, wilting, stunting, tumors, scabs or blossom blights. When tissue damage occurs on the blossoms, fruit or roots of food crops, yields may be reduced.

Potatoes are susceptible to blackleg and soft rot diseases caused by the bacterial pathogen *Erwinia carotovora*. To combat these bacteria, scientists have exploited the family of enzymes known as lysozymes that catalyze the breakdown of bacterial cell walls. Using cloned lysozyme genes and a promoter, transgenic potatoes were created that produced lysozyme. In laboratory tests, the transformed potatoes exhibited substantially enhanced resistance to *Erwinia carotovora*. Field tests and further development of resistant lines are in progress.

A different transgenic strategy to combat *Erwinia carotovora* was demonstrated in tobacco engineered to overexpress a peptide that kills bacteria (Ohshima et al. 1999). The genetically engineered tobacco plants were resistant to both *Erwinia carotovora* and *Pseudomonas syringae pv tabaci*, the pathogen responsible for wild fire disease in rice. Scientists have also successfully transferred a bacterial resistance gene from wild rice to cultivated rice.

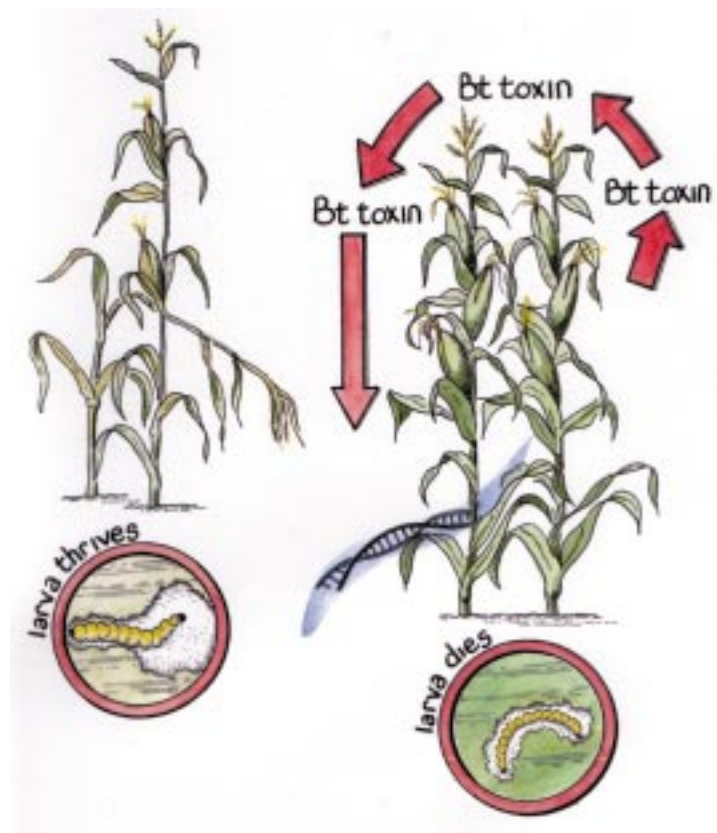
### *Insects and Mites*

Control of insect pests such as flies, aphids, borers and insect larvae is the aim of considerable research. There are several different combat tactics, including engineering for the expression of toxins in plants that kill insects when they consume the plant material, but are non-toxic to other species that eat the plant. Other alterations focus on inducing sterility in the pest organism or affecting the digestion or metabolism of the pests. In addition, attempts to enhance a plant's natural ability to produce leaf wax could make the plant more difficult for insects to consume.



tobacco plant

The best known and most widely used transgenic pest-protected crops are those that express insecticidal proteins derived from genes cloned from the soil bacterium *Bacillus thuringiensis*, more commonly known as Bt. Crystal (Cry) proteins or delta-endotoxins formed by this bacterium are toxic to many insect species. Delta-endotoxins bind specifically in the insect gut to receptor proteins, destroying cells and killing the insect in several days (shown below). There are several different Bt strains containing many different toxins. Scientists have identified and isolated the genes for several toxin proteins from different Bt strains. In recent years, these genes have been introduced into several crop plants in an effort to protect them from insect attack and eliminate the need for spraying synthetic chemical pesticides. There are more than 100 patents for Bt Cry genes. Bt field corn, sweet corn, soy, potato and cotton are commercialized in the U.S., and one or more of these are commercialized in at least 11 other countries.



HOW BT CORN WORKS

Bt controls the larvae of butterflies and moths (*Lepidopteran* insects) that eat the plants. It is especially effective against the larvae of the European corn borer (shown left), a significant corn pest in the U.S., as well as the Southwestern corn borer and the lesser cornstalk borer. In sweet corn, Bt toxins effectively deter corn earworm and fall armyworm (Bhatia et al. 2000). Recently, a different strain of Bt, *Bacillus thuringiensis tenebrionis*, was used as a gene source to confer resistance to corn rootworm, another major pest in cornfields. The resistant corn is currently in field trials (Ferber, 2000). Bt hybrid rice is also undergoing field-testing and is showing considerable effectiveness in resisting major pests in Asia such as the leaf folder, yellow stem borer and striped stem borer. Bt canola is also under development (Tu et al. 2000).



european corn borer

Borers also create a good environment for fungi to grow. Where *fusarium* fungi grow, they reduce plant quality and generate fumonisins—toxins that can be fatal to farm animals and have been linked to liver and esophageal cancer in African farmers (Marasas et al. 1988, Betz et al. 2000). Thus, one way to reduce fungal contamination is to control pests. Scientists have measured reductions in fumonisin levels in Bt corn of 90 percent or greater (Munkvold et al. 1997, Masoero et al. 1999). Bt works against insects that eat plant tissue. However, those pests that do not eat the leaves, but rather pierce and suck nutrients from the plant, require different defense strategies. These insects include aphids, white flies and stink bugs. White flies are a major pest in poinsettias, sweet potatoes and cotton. Because these insects do not consume large amounts of plant material, a leading way to combat them is the genetic expression of toxic proteins that are strong enough to kill the pest, yet safe for the plant and non-target organisms.

Avidin in transgenic corn demonstrates a different approach. Avidin is a glycoprotein, an organic compound composed of both a protein and a carbohydrate, and is usually found in egg whites. Avidin is known for chemically tying up the vitamin biotin, making it unavailable as a nutrient. Insects eating transgenic corn modified to produce avidin die from biotin deficiency. Although this corn was not toxic to mice (Kramer et al. 2000), further evaluation of its potential for insect toxicity and safety for human consumption is awaited. Transgenic corn engineered to produce avidin for commercial uses is described in the Industrial Products section of this report.

Plants produce wax as a natural protective coating. Genetic modification can increase the expression of this inherent trait. Experiments to increase leaf wax are in the early stages, but scientists have already raised wax content by as much as 15-fold. This strategy is aimed at increasing the plant's resistance to both pests and fungal pathogens.

### Plant Nematodes

There are more than 15,000 named species of nematodes, microscopic worms about a half-millimeter long that feed on plant roots (shown right). The most common of these plant parasites found worldwide is the root-knot nematode. Probably every form of plant life, including field crops, ornamentals and trees, is attacked by at least one species of nematode. They are responsible for 10 percent of global crop losses worth an estimated \$80 billion a year (Ayivor, 2001). Transgenic strategies to combat nematodes are emerging. Nematodes are particularly destructive in bananas, soybeans, rice and potatoes. Scientists are fighting these parasitic worms in potato and banana crops using the genes for cystatins, defense proteins that occur naturally in rice and sunflowers. Incorporation of the genes in potatoes produced as much as 70 percent nematode resistance in field trials.

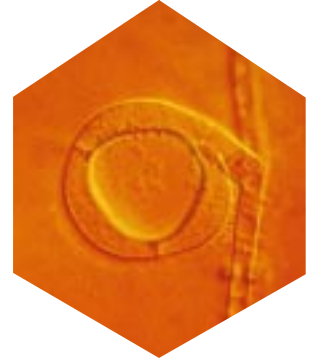
Nematodes are particularly fond of soybeans. In the U.S., the soybean cyst nematode is considered the most devastating pest. Standard plant breeding led to a highly resistant variety of soybeans from a wild strain, but it did not cross well with modern soybean lines. Using genetic markers (See Box: *Agricultural Genomic Research* in Introduction), a means of identifying cells with particular traits, scientists bred plants containing the resistance gene with domesticated varieties, circumventing the poor performance characteristics of the wild variety. While the new varieties are not transgenic, they resulted from combining the use of modern genetic markers with conventional breeding techniques.

### IMPROVEMENTS TO CROPS

Improving field-crop production and soil management is another central aim of genetic engineering technology in commodity crops. Applications include crop resistance to herbicides; improved nitrogen utilization, reducing need for fertilizer; increased tolerance to stresses such as drought and frost; regulation of plant hormones, which are key to plant growth and development; attempts to increase yield, and a multitude of other, less widespread applications.

### Herbicide tolerance and resistance

There are many negative effects when weeds grow with crop plants, the most common being competition for sunlight, water, space and soil nutrients. If weeds grow with crops, they too use these growth factors, and may cause losses great enough to justify control measures.



parasitic nematode



In addition to economic yield loss, other concerns may determine when weed control is justified. For example, eastern black nightshade in soybeans or late-emerging grasses in corn may not reduce yield, but these weeds can clog equipment, causing harvest delays. The most common method currently employed to manage weeds is the use of herbicides.

The use of genetic modification techniques has created crops that are both tolerant and resistant to herbicides, or weed killers. This technology allows herbicides to be sprayed over resistant crops from emergence through flowering, thus making the applications more effective. To date, six categories of these crops have been engineered (Hager and McGlamery, 1997) to be resistant to the herbicides glyphosate, glufosinate ammonium, imidazolinone, sulfonylurea, sethoxydim and bromoxynil.

Probably the best-known herbicide for which tolerance has been genetically engineered into crops is glyphosate, known commercially by brand names such as Roundup®, Rodeo® and Accord®. Resistance to glyphosate is the transgenic trait most common in agriculture worldwide. To date soy, corn, cotton, canola, sugar beets and, most recently, wheat, have been genetically transformed for glyphosate tolerance. Although glyphosate has been used as an herbicide for 26 years, transgenic glyphosate-resistant crops are a more recent development and are widely deployed on acres devoted to soy and cotton (Felstot 2000a,b, James 2000). Research is underway to create other glyphosate tolerant crops. To date, two weed species, annual rigid ryegrass and goosegrass, have built resistance to glyphosate (Hartzler 1998, 1999, Felsot 2000c).

Corn, soy, rice, sugar beet, sweet corn and canola have also been genetically modified to tolerate the herbicide glufosinate ammonium. The seeds for these crops are sold commercially under brand names such as Liberty Link®. Transgenic soybeans, cotton and flax with a tolerance to the herbicide sulfonylurea are also on the market. Other strains of engineered soybeans and corn are resistant to sethoxydim, the active ingredient in the commercial herbicides Poast®, Poast Plus®, and Headline®, used to control undesirable grass species.

The herbicide bromoxynil, sold under the commercial name Buctril®, is normally toxic to cotton, a broadleaf crop, and is primarily used on grass-like crops, such as corn, sorghum and small grains, to kill invading broadleaf weeds. Scientists have genetically modified cotton plants for resistance to this herbicide, allowing its use to control broadleaf weeds in cotton fields.



### *Improved nitrogen utilization*

There appear to be relatively few biotechnology applications specifically designed to enhance the characteristics of farm crops, such as size, yield, branching, seed size and number. Scientists have, however, created some enhancements. A recent example is the discovery of a gene in the alga *Chlorella sorokiniana* that has a unique enzyme not found in conventional crop plants. The enzyme, ammonium-inducible glutamate dehydrogenase, increases the efficiency of ammonium incorporation into proteins. In some plants, it increases the efficiency of nitrogen use. The practical implication is that less fertilizer would be necessary to grow these plants. When the gene was incorporated into wheat, biomass production, growth rate and kernel weight all increased, as did the number of spikes in the plant (Woods, 1999).

### *Stress tolerance*

The term “stress” applied to plants usually refers to adverse non-biological, external environmental conditions such as drought, flooding, temperature changes (hot or cold), salinity, pH (acidity or alkalinity) and heavy metals. Of these, drought and salinity are the most widespread, the latter exacerbated by irrigation practices (Cheikh et al. 2000).



drought-stricken land



rice paddies

It appears likely that stress tolerance involves a family of genes, rather than a single one. They are rapidly activated in response to cold, inducing the expression of “cold-regulated” genes, and resulting in enhanced freezing tolerance. Over-expression of these genes in *Arabidopsis*—small plants of the mustard family that are commonly used to study plant genetics—increases freezing tolerance and leads to elevated levels of proline and total soluble sugars, substances that protect against cold (Gilmour et al. 2000).

Common stress responses in plants involve water retention at the cellular level. As a result, researchers have given special attention to osmoprotectant molecules, or molecules that hold water, such as sugars, sugar alcohols, certain amino acids (proline) and quaternary amines like glycinebetaine (Cheikh et al. 2000). Various plants genetically engineered for increased levels of protectant sugar have shown increased drought tolerance. For instance, *Arabidopsis* and tobacco plants engineered to produce mannitol, a sugar alcohol, withstood high saline conditions and had enhanced germination rates and increased biomass.

Other strategies have addressed different stress factors. Improved cold tolerance and normal germination under high salt was reported in *Arabidopsis* engineered to express the enzyme choline oxidase. Transgenic rice, engineered to express the late embryogenesis abundant protein gene transferred from barley, was significantly more tolerant to drought and salinity than conventional varieties of rice (Xu et al. 1996). Another transgenic rice engineered in the laboratory for enhanced expression of the enzyme glutamine synthetase had increased photorespiration capacity (a part of the photosynthesis process) and increased tolerance to salt. Preliminary results suggested enhanced tolerance to chilling as well (Hoshida et al. 2000).

### *Plant hormone regulation*

There are five major classes of plant hormones: Auxin, cytokinins, gibberellins, abscisic acid and ethylene. Plant hormones have been targeted for genetic modification to influence plant growth and development; fruit development and ripening; stem elongation and leaf development; germination, dormancy and tolerance of adverse conditions. These hormone classes are highly interactive; the concentration of one affects the activity of another. For example, the ratio of the hormone abscisic acid to gibberellin in a plant determines whether a seed will remain dormant or germinate.

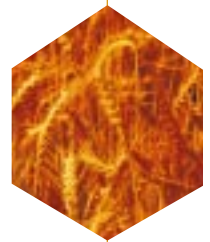


The recent discovery of an enzyme involved in the production of the hormone auxin enabled researchers to investigate the effects of moderating auxin production in determining plant characteristics. When auxin is overproduced, branching is inhibited and leaves curl down as the plant elongates, a reaction typically related to reduced light exposure. The same gene that produces this enzyme is apparently related to a gene in mammals that governs enzymes that detoxify certain chemicals.

In wheat, the hormone abscisic acid slows seed germination and improves the tolerance to cold and drought. Extending or enhancing the production of abscisic acid may also delay germination, a useful characteristic in climates where spring rain is sparse or falls late in the season. Production of abscisic acid is increased in response to environmental stress, and a family of enzymes called protein kinases stimulates its production. Selecting plant varieties high in abscisic acid, or engineering plants to produce more of the hormone, may confer greater drought and cold tolerance (Stelljes, 2001).

Introduction of dwarfed, high-yielding wheat contributed to the 'Green Revolution' of the 1960s and 1970s, during which world wheat yields almost doubled. Shorter varieties of wheat grains, with a greater resistance to damage by wind, resulted from a reduced response to the hormone gibberellin. Scientists have since shown that the gene called *Rht* can cause "dwarfing" in a range of plants, opening up the possibility of quickly developing higher-yielding varieties in several crops. Researchers believe that this strategy could be applied to a still wider range of crops through genetic engineering (Peng et al. 1999).

The plant hormone ethylene regulates ripening in fruits and vegetables. Controlling the amount and timing of ethylene production can initiate or delay ripening, which might reduce spoilage that can occur between the time produce is picked and brought to market. Transgenic techniques aim to regulate the enzyme that breaks down a precursor of ethylene production. By regulating the timing and rate of this degradation, ripening can be controlled. This technology has been applied and field-tested in tomatoes, raspberries, melons, strawberries, cauliflower and broccoli (Agritope, 2001), but has not yet been commercialized.





### *Increased Yield*

Often, increased yield—greater plant biomass, more numerous tubers, larger seeds and other characteristics—is an unexpected result of unrelated genetic modifications. For instance, transgenic potatoes with increased protein also produced more tubers and showed a 3 to 3.5 percent increase in yield (Chakraborty et al. 2000). Direct strategies to raise yields have focused on metabolic pathways such as photosynthesis that increase the activity of the plant. Other examples include transgenic rice with an antisense gene, which inhibits the formation of certain proteins and thus prolongs the grain-filling period of the plant. This rice, in its first field test, increased productivity by 40 percent (Finkel, 1999).

## APPLICATIONS FOR IMPROVED PRODUCT CHARACTERISTICS

Genetic applications to alter product quality characteristics—or “output” traits—are aimed at increasing nutrition, modifying allergens and improving various functional attributes for consumers.

For example, rice, that has been genetically engineered to have increased iron and beta-carotene (the precursor of vitamin A), has received considerable publicity for its potential benefit to developing nations, where nutrient deficiencies are responsible for widespread health problems. While promising, research on these varieties remains at a relatively early stage.

### *Nutrients*

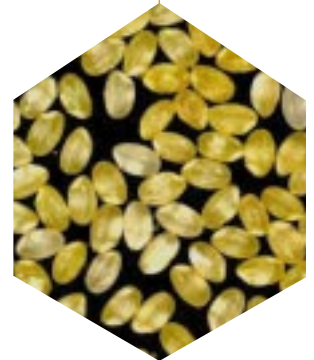
Using bioengineering, scientists have added or modified nutrients in various crops, and created several nutritionally enhanced products. Although few have reached commercialization, examples include adding iron to rice, or increasing beta-carotene and vitamin E in vegetable oils to boost the nutritional value. Other genetic modifications have altered the fatty acid composition in oils from soy and canola to create healthier fats. Plants have also been engineered to increase phytonutrients—substances exclusive of nutrients that have benefits for improving health or preventing disease. These include isoflavones in soy and lycopene in tomatoes.



Two genetic modification strategies have also been devised to increase the iron levels in cereal crops. One is the introduction of the gene that encodes for ferritin, an iron-storage protein (Deak et al. 1999). Overexpression of this gene improves the storage capacity of plants by as much as three-fold (Goto et al. 1999). Using this and other genetic technologies, rice was engineered to contain beta-carotene, which it normally lacks, and enhanced iron content. This transgenic “golden rice” (shown right) has yet to be bred into hybrid and native strains, so field testing of modified local varieties, commercial production and acceptance are still years away.

Another method for enhancing iron is reducing phytic acid content, which improves the degree and rate at which iron and other minerals are absorbed. In one experiment, corn genetically modified to be low in phytic acid was processed into tortillas. The iron absorption from these tortillas was 49 percent greater than from tortillas made with conventional corn (Mendoza et al. 1998). To further explore the effectiveness of iron absorption by reducing phytic acid, additional iron was added in the form of iron salt supplements and consumed with either strain of corn fed as porridge instead of tortillas. In this case, no absorption effect was observed. Although it is not clear why the phytic acid level had no effect, it is well known that when dietary iron levels increase, absorption decreases. Other substances in the diet may also have contributed to the reduced absorption.

While plants are the primary dietary source of vitamin E, they contain relatively low concentrations of the vitamin. Recent genetic engineering technology has been able to increase the vitamin E content of oils (Shintani and DellaPenna, 1998). As it happens, many seeds have abundant levels—up to 20-fold more of gamma-tocopherol, the immediate precursor of alpha-tocopherol, the active form of the vitamin. However, little of the gamma form is converted to the active vitamin. Researchers identified, isolated and cloned the gene responsible for expressing the enzyme that converts gamma-tocopherol to alpha-tocopherol. The gene was transferred to *Arabidopsis*, which subsequently exhibited a nine-fold increase in vitamin E. Incorporation of this gene to stimulate similar gamma-tocopherol to alpha-tocopherol conversion into soy, canola and corn is probably not far in the future.



golden rice



Seed oils—particularly mustard and canola—have also been developed to contain carotenoids, especially beta-carotene, a nutrient widely studied for its role in cancer prevention. But this project is still in the testing stage.

Protein (or rather specific amounts of essential amino acids, the building blocks of protein) is needed to fulfill human nutritional requirements for growth, health maintenance and muscle development. In regions of the world where cereal grains cannot be grown, people often rely upon starchy vegetables (roots, tubers or rhizomes) to supply most of their calories. While such crops often have high yields, the primary disadvantage is their very low protein content, less than one percent.

Researchers are seeking to improve protein content and quality in vegetable staples such as cassava and plantain through changes in amino acid profiles. For example, a non-allergenic seed albumin gene was introduced into the potato to increase its protein content. Transgenic tubers had 35 to 45 percent more protein and enhanced levels of essential amino acids (Chakraborty et al. 2000). Moreover, transgenic plants produced more tubers and a yield increase of 3 to 3.5 percent. Scientists have also altered soybeans for higher protein in tofu (Protein Technologies, 2001).

In an attempt to create healthier fats, researchers have modified the fatty acid composition of soy and canola in several ways. They have produced oils from soy and canola with reduced or zero levels of saturates; canola with medium chain fatty acids; high stearate canola oil free of trans-fatty acids; high oleic acid soybean oil, and canola with the long chain fatty acids gamma linolenic and stearidonic acid (Ursin, 2000). The latter is of interest as an indirect source of docosahexaenoic acid (DHA), one of two long chain Omega-3 fatty acids shown to be beneficial in protecting against heart attack. DHA is available almost exclusively from seafood, primarily fatty fish. The plant precursor of DHA, linolenic acid, is poorly converted to DHA.

Transgenic high oleic acid soybean oil has 80 percent more oleic acid, one-third less saturated fatty acid than olive oil, and no trans-fatty acids. Researchers have also modified sunflower oil for high oleic acid content. Another type of modified soybean oil is low in saturated fatty acids (7 percent compared with 14 percent in commodity soybean oil) and richer in linoleic acid than commodity soybean oil (64 percent compared with 51 percent). Still another has reduced linolenic acid and no trans-fatty acids, increasing its stability for use as an ingredient in processed foods (Protein Technologies, 2001).



canola plant

Another seed unique for its high level of a single fatty acid is mangosteen (*Garcinia mangostana L.*). This tropical tree, grown in India, the East Indies and Southeast Asia produces seeds (shown right) with as much as 56 percent by weight of stearic acid, a saturated fatty acid widespread in foods. Stearic acid is noteworthy from a nutritional perspective for its stability and textural properties and because it is one of the few saturated fatty acids that does not appear to raise blood cholesterol levels. Thus, it is useful in fats for manufactured and processed foods. Enzymes cloned from mangosteen have also been expressed in canola with resulting increased levels of stearic acid. This research demonstrates the potential of the technology and the unusual sources of enzymes to alter fatty acid profiles in popular food oils such as canola (Facciotti et al. 1999).



mangosteen

Biotechnology has also aimed at increasing phytonutrients—substances in plants—exclusive of nutrients, that have benefits for improving health or preventing disease. For example, new research in nutrition suggests lutein may support multiple lines of defense against eye disease, and that lycopene serves as a powerful antioxidant in cancer prevention. Also called “accessory health factors” phytonutrients include isoflavones in soy, lycopene in tomatoes and polyphenols in green tea. In the laboratory, scientists have engineered tomatoes with 2.5 times as much lycopene as traditional tomatoes (shown right) (Weaver-Missick, 2000). At least one company is developing soy with more isoflavones (Protein Technologies 2001), and canola with increased antioxidants and beta-carotenes, lutein and lycopene (Agri-Food Trade Service, 2001).



lycopene enhanced tomatoes

There are major constraints on this research, in part because there is still much about phytonutrients that is unknown. For example, some members of a class of phytonutrients may have deleterious effects while others are beneficial, as is the case with various flavonoids, water-soluble plant pigments that, while not considered essential, help maintain overall health as anti-inflammatory, antihistaminic and antiviral agents. In addition, scientists do not fully understand the biosynthetic pathways, or the succession of enzyme activities, for many phytonutrients. Another constraint is the limited scientific information about the safety and efficacy of potentially beneficial phytonutrients. However, there is considerable research activity on phytonutrients and further development and applications are anticipated.

*Anti-nutritional factors*

Some plants, especially cereals and legumes, are nutritious foods and feeds but also contain varying amounts of substances that interfere with digestibility and nutrient absorption. In excess, these materials may even be toxic. Genetic modifications are being explored to reduce these anti-nutritional substances, including phytate in cereals and legumes; glycoalkaloids such as solanine and chaconine in potatoes; tomatine, solanine, lectins and oxalate in tomatoes and eggplant; gossypol in cottonseed; trypsin and other protease inhibitors in soy, and tannins and raffinose in legumes.

Phytate is widely distributed in cereals and legumes and reduces the absorption of iron, zinc, phosphorus and other minerals in humans and other animals. Phytate is indigestible for swine and poultry because their digestive tracts lack the enzyme phytase, which releases phosphorus from phytate. Studies have shown that including phytase in the food ration improves phosphorus absorption and reduces phosphorus excretion. In the food animal industry, particularly for swine and poultry, high phytate feeds are associated with high levels of phosphorus excretion. Excess phosphorus in animal manures can be washed into streams or leach into ground water and become a serious source of water pollution. Research has indicated that poultry have substantially reduced phosphorus excretion when fed phytase as a supplement alongside ordinary soybeans or alternatively, genetically transformed soybeans expressing the phytase enzyme (Denbrow et al. 1998). Similarly, swine fed low-phytate corn showed increased phosphorus retention and reduced excretion (Spencer et al. 2000a). Genetically modified low-phytate corn contains at least five times as much available phosphorus as unmodified corn. Low-phytate corn feed was also associated with improved growth and finishing characteristics (Spencer et al. 2000b). In wheat engineered to express the enzyme phytase, seeds exhibited a two to four-fold increase in phytase activity (Brinch-Pedersen, 1999). This opens the possibility of improving the digestibility of wheat, especially among non-ruminant animals.

Scientists are also seeking ways to reduce toxic substances such as glycoalkaloids. Researchers inserted antisense genes into potatoes to block the activity of the enzyme UDP-glucose glucosyltransferase, key to the production of the glycoalkaloid alpha-chaconine. This toxic substance can, at high enough levels, cause irritation of the gastrointestinal tract or impairment of the nervous system. Preliminary findings indicated that the transgenic potatoes produced fewer glycoalkaloids (Wood, 1997).



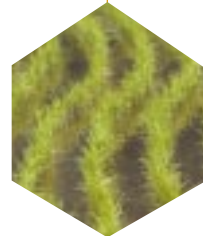
eggplant

## Allergens

Some people have an abnormally high sensitivity to certain substances, such as pollens, foods or microorganisms. These substances, known as allergens, exist in both food and nonfood plants. One out of every five Americans suffers from allergies, asthma or both, according to the National Institute of Allergy and Infectious Diseases. Common indications of allergy may include sneezing, itching and skin rashes.

Food allergies and sensitivities cause a wide variety of conditions, symptoms and diseases, a few of which can be life threatening. A food allergy or hypersensitivity is one that provokes an immune response, while a food intolerance incites an abnormal physiological reaction (Sampson, 1997). Experts estimate that 2 percent of adults, and from 2 to 8 percent of children, are truly allergic to certain foods. Food intolerance is a much more common problem than allergy. Unlike allergies, intolerances generally intensify with age (U.S. Food and Drug Administration 1994). The eight most commonly allergenic foods are milk, eggs, peanuts, soybeans, fish, crustaceans, tree nuts and wheat. There are also significant allergies to non-food plants, such as ryegrass and other plants with airborne pollens that may cause hay fever or other seasonal allergic symptoms.

Most known allergens in food are proteins, suggesting the possibility of modifying the structure, or possibly eliminating the allergenic protein from the food. In some cases, traditional plant breeding has identified hypoallergenic strains that are targets for further genetic modification to reduce allergenicity. Neutralizing the allergens in major food grains would have an enormous impact on millions of families, where one or more members cannot eat these foods that are household staples. Researchers have used this approach in rice, the first food crop with reduced allergenicity to be created through genetic engineering (Matsuda et al. 1993, Nakamura and Matsuda 1996). Further testing and development work continues to assure that people with known allergies to rice products can consume this genetically engineered food without developing their typical allergic reaction. In foods such as peanuts, however, which are highly allergenic to some sensitive individuals, the allergenic proteins constitute the majority of the plant's protein, so that elimination may not be possible (Wilkinson, 1998).



Another example where genetic modification may be used to reduce allergenicity is in wheat, one of the “big eight” allergenic foods. Although not yet commercially available, scientists have genetically engineered wheat to overexpress the gene responsible for the synthesis of thioredoxin, an enzyme that catalyzes the reduction of disulfide bonds within protein molecules, thus reducing the protein’s allergenic properties. When expressed in wheat, the enzyme reduced the bonds in the major allergenic proteins—the gliadens and glutenins—and to a lesser extent the minor ones, too, making them markedly less allergenic (Buchanan et al. 1997). At the same time, the functional characteristics of the wheat were not impaired.

Scientists are also exploring the potential of recombinant DNA technology to reduce the allergenicity of non-food allergens. For example, ryegrass is a dominant source of airborne pollen in temperate climates, and using antisense technology, scientists engineered ryegrass with reduced Lol p 5 protein levels. As this is the major allergen in ryegrass, the modification reduced the plant’s allergenicity (Bhalla et al. 1999).

Although genetic engineering has the potential to reduce allergenicity of foods, it also has the potential for unintentionally introducing new allergens. Scientists are working to establish methods to detect and assess allergenicity (Taylor and Nordlee 1996, Fuchs and Astwood 1996), and this assessment is part of the review process for new transgenic foods.

#### *Functional attributes*

Researchers are also genetically modifying crops in search of enhanced functional properties for specific purposes, such as firmer tomatoes for canning, or beans with less breakage.

One of the first applications to reach the market was the highly publicized Flavr Savr tomato discussed above, which was genetically engineered for delayed ripening. While the transformation process did delay ripening and extend shelf life, the product was expensive to produce and purchase and some consumers did not like the taste. This led to its withdrawal from the market. Other work aims to create a tomato that ripens on the vine but remains firm during harvest, handling and shipping. Firm tomatoes are preferable for canning, which consumes the largest share of tomato production. Using antisense technology, researchers have created tomatoes that are 40 percent firmer than their conventional counterparts and stay firm for at least two weeks (McBride, 2000). Scientists have also engineered beans for desirable canning characteristics such as firm texture and seed coats that do not split (Comis, 2000).





Several experiments are being conducted with soybeans. One would diminish that undesirable byproduct of bean consumption, flatulence, by creating high sucrose soybeans through reduction of the carbohydrate raffinose (Dupont 2001, Protein Technologies Inc., 2001). Another seeks to modify soybean oil to reduce the linoleic acid content so that it is more stable for industrial applications (Protein Technologies Inc., 2001).

Presently, barley is an unsuitable feed for poultry because poultry lack the enzyme to break down  $\beta$ -glucan, the predominant polysaccharide (a type of carbohydrate) in endosperm cell walls. Scientists have created transgenic malt that can depolymerize  $\beta$ -glucan. Adding transgenic malt to barley-based poultry feed enabled poultry to metabolize barley, grow as well as poultry fed a corn-soybean diet, and produce more hygienic droppings (von Wettstein et al. 2000). The digestibility of feeds can also be improved with modification of starch levels in different crops. For example, cattle can more readily digest amylose-free wheat in feed (Nakamura et al. 1995).

Starch is also widely used as a thickener and sweetener in foods, and for multiple manufacturing uses (see section on Industrial Products). Extensive research has been directed toward altering the properties, quantity and distribution of starch in many plants, for a variety of purposes. The principal forms of starch are either linear (amylose) or branched polymers amylopectin. Using genetic engineering technology to influence the amount and length of chain branching and polymerization increases the availability of starches with different properties. It also enables the development of novel starches. However, plants differ widely in where they store different types of starch; thus modification of starch production must be tailored to the particular plant. What works in the potato, for example, may not work in wheat or rice. Moreover, results in one variety of a crop may not be obtained in another.





A well-known example of the modulation of starch synthesis has been the development of transgenic potatoes engineered to contain a gene for an enzyme affecting starch synthesis. The transgenic potatoes had up to 60 percent more starch than non-engineered strains. The increased starch content made the potatoes take up less fat during frying, resulting in a lower-fat product.



About 40 percent of tapioca starch is used for the production of modified starch, sweeteners and the flavor enhancer monosodium glutamate. In processing tapioca, a significant amount of starch remains in the waste material and wastewater. It is estimated that even after extraction, the waste still contains 50 percent starch. Some starch can be used in animal feeds, but the low level of protein in waste tapioca makes it unsuitable for feeds requiring higher protein. Bioengineered improvements in tapioca, such as reduction in water content and higher starch concentration, may increase the ease of processing the plant material into a finished starch product. Further, raising the efficiency of starch utilization in the processing of sweeteners reduces the amount of starch reaching the waste stream. The possibility of converting the starch content of wastewater to energy, using high rate anaerobic digestion, is promising. However, a number of factors remain to be overcome, including the effect of environmental sulfates in the waste stream and the efficiency of energy production. The use of transgenic organisms offers potential solutions.

When the enzyme thioredoxin is overexpressed in barley endosperm, the activity of the enzyme pullulanase, a rate-limiting enzyme in breaking down starch, increases four-fold. Breaking down starch is a key part of the barley malting process, and tests with this engineered variety showed that the time required could be reduced by up to a day. Overexpression of thioredoxin also hastened barley germination, of special interest to growers of this normally slow-germinating grain (Cho, 1999).



SUMMARY CHART OF FOOD CROP PRODUCTS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
Arabidopsis	30	Use this flowering mustard plant as a model organism to research how plants can withstand adverse environmental conditions such as flooding, drought and salinity.	Arabidopsis is currently being engineered to produce compounds that help plants survive in soils with high salt levels.	Being tested in research greenhouses.
Banana	22	Find a way to make banana plants resistant to black sigatoka, a leaf fungus that widely infects the fruit and can destroy the entire plant.	Clone several anti-fungal genes and incorporate them into the DNA of trial banana plants.	Being tested in research fields and greenhouses.
Cereal crops	33	Enhance the iron content in cereal crops such as rice.	Ferritin, a protein that causes plants to store iron, has been introduced to the rice genome; preliminary research shows a three-fold improvement in the iron storage capacity of the rice plants.	Being tested in research fields.
Field and sweet corn, soybeans, potatoes and cotton	25	Engineer crop plants so they are resistant to worms (including the European corn borer, the Southwestern corn borer, the cornstalk borer, corn earworm and fall armyworm), which eat through the stalks and devastate entire acreages.	Several delta-endotoxin genes have been cloned from <i>Bacillus thuringiensis</i> (Bt) and incorporated in the DNA of these crops. The crops release a toxin that kills worm larvae when they try to eat the plant stalks.	Available commercially
Papaya	21	Develop resistance to papaya ringspot virus, which devastated the Hawaiian crop in the 1950s.	Use viral coat protein technology to create resistance in transgenic papaya by using a gene from the virus itself to disarm the pathogen.	Available commercially; in use in Hawaii since 1997.
Potato	22	Stop wilting and death of the plant, caused by infection with a fungal pathogen.	Incorporate anti-fungal defensins from alfalfa.	Being tested in research greenhouses.
Potato	23	Eliminate or curb late potato blight, a destructive plant fungus associated with the Irish potato famine that causes severe plant and leaf damage.	Use a gene from soybeans to create a protein that confers resistance to blight.	Undergoing laboratory investigation.
Potato	24	Stop blackleg and soft rot diseases caused by a bacterial pathogen.	Develop transgenic potatoes that produce a substance that breaks down the cell wall of bacteria.	Undergoing laboratory investigation.

SUMMARY CHART OF FOOD CROP PRODUCTS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
Potato	40	Reduce fat absorption during frying, to create a lower-fat fried potato.	Engineer transgenic potatoes to contain a gene for an enzyme affecting starch synthesis. The resulting potatoes had up to 60 percent more starch than non-engineered strains, causing the potatoes to take up less fat during frying.	Being tested in research greenhouses
Potato and banana	27	Eliminate or reduce plant damage from nematodes, microscopic worms that feed on roots and are among the most abundant parasites in the world.	Incorporate genes for defense proteins that occur naturally in rice and sunflowers.	Being tested in research fields.  (So far tests indicate a 70 percent nematode resistance).
Potato	21	Eliminate or curb potato leaf roll virus, which damages potatoes.	Use viral coat protein resistance strategy and anti-sense technology to develop resistance to the virus.	Available commercially.
Potato	32	Maximize yield.	Produce transgenic potatoes with more protein, to increase both tubers and yield.	Being tested in research laboratories and greenhouses.  (So far, an increase of 3 to 3.5 percent has been achieved).
Rice	23	Reduce major fungal diseases such as blast and sheath blight, which cause from 11 to 30 percent of crop losses annually.	Develop transgenic strains with multiple resistances to both sheath blight and stem boring insects.	Being tested in research fields.
Rice	32	Maximize yield.	Modify transgenic rice with a gene that inhibits formation of certain proteins and, thus, prolongs the grain-filling period of the plant.	Being tested in research fields.  (In the first trial, this rice demonstrated a 40 percent increase in productivity).
Rice (aka "Golden Rice")	33	Overcome lack of beta-carotene, a nutrient widely studied for its role in cancer prevention, as well as iron deficiency.	Genetically engineer the rice to contain beta-carotene, as well as enhance its iron content.	Being tested in greenhouses and research fields.
Soy, corn, cotton, canola, sugar beet and wheat	28	Eliminate weeds that compete with crops for soil nutrients, water and sunlight.	Genetically engineer the crops so they can tolerate the herbicides used to kill weeds, and survive herbicide applications.	Available commercially.
Soybean and canola oils	34	Improve fatty acid profiles in these oils so they are more nutritious.	One example has been the creation of transgenic soybean oil that has 80 percent more oleic acid and no transfatty acids.	Available commercially.
Soybeans	27	Eliminate or reduce damage from nematodes, microscopic parasitic worms.	Use genetic markers, a means of identifying genes with particular traits, to create soybean plants resistant to the nematode.	Being tested in research greenhouses.
Sweet potato	21	Fight sweet potato feathery mottle virus, which can cause heavy crop losses.	Use viral coat protein resistance strategy to develop virus-resistant plants.	Being tested in research fields.

## SUMMARY CHART OF FOOD CROP PRODUCTS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
Tomatoes	32	Increase the anti-oxidant properties of tomatoes.	Engineer tomatoes with more lycopene, a powerful anti-oxidant, and increased levels of lutein, known to help fight eye disease.	Undergoing laboratory investigation.  (Scientists have already produced tomatoes with 2.5 times as much lycopene, as well as higher levels of lutein).
Tomatoes	38	Control the ripening process to reduce spoilage caused by over-ripening that may occur during transport of produce to market.	Use transgenic techniques to regulate production of the plant hormone ethylene, which confers the ability to initiate or delay the ripening process.	Being tested in research fields.
Vegetable staples such as potato, cassava and plantain	34	Increase total protein content.	Introduce a non-allergenic seed albumin gene to increase protein content.	Being tested in research greenhouses.  (Transgenic tubers produced so far had 35 to 45 percent more protein and enhanced levels of essential amino acids, as well as a yield increase).
Wheat	21	Combat barley yellow dwarf virus, which can destroy the plants and prevent seed formation.	Use the viral coat protein approach to develop resistance to the virus.	Undergoing laboratory investigation.
Wheat	38	Reduce allergenicity in one of the most commonly allergenic foods.	Genetically engineer wheat to overexpress the gene that controls the enzyme that modifies the protein in wheat that causes allergic reactions.	Being tested in research greenhouses.
Wheat and barley	23	Eliminate a serious plant fungus, <i>Botrytis cinerea</i> , which causes severe damage to the plant and grain kernels.	Insert the gene for a natural plant defense compound that is found in certain wine grapes.	Being tested in research fields.
Yellow crookneck squash	21	Curb or kill mosaic virus that can cause blemishes and rot.	Viral coat protein approach was used to develop resistance to the virus.	Approved for commercial use.

## SECTION 2: GENETICALLY MODIFYING TREES

In addition to their recreational and ecological value in forests, trees provide useful products such as paper, fuel, lumber, fruits and nuts. Growers face many of the same challenges as crop producers: pests and diseases, shortfalls in quality and efficiency and environmental stresses. In the same way scientists seek to modify crop characteristics, so, too, do they seek to alter trees. However, the bioengineering of trees is less developed. The biology of trees is different from most crop plants. For example, the gymnosperms, a class of trees that includes conifers, are distinctly different from flowering plants. The long lifecycle of trees also makes genetic engineering more challenging. Conifers, for example, have a complex reproductive cycle (up to two years) and it can take years to obtain enough seed for large-scale propagation (Charest, 1995). Generation times in the populus species (poplar, aspen, cottonwood) range from four to eight years (James et al. 1999). Another difference that sets trees apart from crops and flowers is their ability to cross-hybridize within the same genus, which complicates patterns of gene flow. For instance, different species of maple trees, such as sugar maple or red maple (shown right), can be bred together to create hybrid maples, and a gene alteration to one could more easily spread to others. Thus, interaction of trees with their environment is more pronounced and their ecosystems more complex (Charest, 1995).

Further advances in shaping transgenic trees will require additional knowledge about tree development to identify useful genes. Other considerations are the need for management strategies to minimize or avoid unwanted outcropping, and to preserve existing gene diversity (Strauss et al. 1999). However, research is underway, and while the specific tactics differ somewhat from those of the more familiar food and feed crops, the applications are similar and include pest, disease and herbicide resistance; modifications in product quality, and stress tolerance.



red maple

### *Pest, Disease and Herbicide Resistance*

Transgenic cottonwood trees expressing the Bt gene have been created and are being tested for resistance to the cottonwood leaf beetle, a major pest of the populus species throughout North America (James et al. 1999). Trees modified with several Bt genes, showed resistance to the cottonwood leaf beetle, but the level of resistance varied depending on the stage of the beetle's development. Adults, for example, did not appear susceptible to the Bt proteins expressed by the trees. Beetles from different geographic regions also varied significantly in sensitivity.

Another example of genetic modification to resist pests is the transgenic hybrid poplar, altered to increase resistance to the gypsy moth. Researchers used an insect-specific scorpion neurotoxin gene and observed higher mortality among gypsy moth larvae (Wu et al. 2000). Scientists are also working with poplar trees to introduce resistance to herbicides, including glyphosate and chlorsulfuron (Schulz et al. 1990).

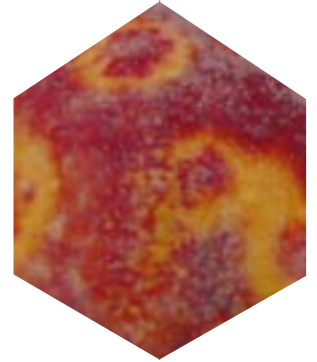
The codling moth, also known as the apple cutworm, is a major pest infesting fruit trees such as apples and pears. The larvae burrow in fruit (shown left) and can destroy over 80 percent of an apple crop and up to 60 percent of pears. Recently, scientists used a Bt gene to engineer pest resistance in Gala apples.

Resistance to a variety of viruses, bacteria and fungi in trees has also been engineered using various methods successfully applied in crops (Charest, 1995). Much of the work has been done on fruit trees, where these pests can be a major cause of crop loss. It is estimated, however, that transgenic fruit trees remain many years away from commercialization, in part because of the difficulty in achieving successful transformations and also because of costs (Warner, 1995). Resistance to fire blight, a bacterial disease in apple trees, was created using transgenes for the expression of cecropins, a small protein native to the giant silk moth. Using transgenic technology to express the enzyme chitinase from insects, scientists also developed resistance to apple scab fungus in McIntosh apple trees (Aldwinckle and Norelli, 2000).



apple cutworm larvae

Plum pox virus (PPV), the cause of sharka disease, is a major pathogen in stone-fruit (plum, peach, nectarine, apricot and *Prunus* ornamentals). It is spread rapidly by aphids and can cause heavy orchard losses. The disease (shown right) can severely disfigure fruit, reduce fruit quality and compromise the health of trees. Among fruit trees, plum pox virus is a serious scourge, and cultivated trees are highly susceptible. The virus can affect some leaves and fruit. To date, no highly resistant varieties have been identified or developed with traditional breeding. Scientists have used viral coat protein technology described earlier in an attempt to produce resistance to the plum pox virus (Tree Genetic Engineering Research Cooperative, Oregon State University, 2001). Resistance to the virus was achieved in transgenic plums that were transformed with plum pox virus coat protein. The altered plants were susceptible to another aphid-transmitted virus, however, but the susceptibility was overcome by further engineering the virus coat protein (Jacquet et al. 1998). Other scientists recently reported success in developing a transgenic plum using the coat protein from plum pox virus (Ravelonandro et al. 2000). Field tests of the transgenic lines were resistant to plum pox virus for three years, whereas control trees became infected in the first year. Hybrids developed from the transgenic trees were also virus-resistant.



plum pox virus (ppv)

Transgenic lines of hybrid aspen have been developed to be resistant to crown gall disease, the disease caused, ironically, by one of the most widely used agents for genetic transformations, *Agrobacterium tumefaciens*. Researchers are using different molecular strategies to build disease resistance, and field trials will permit evaluation. Because the trees used are imported from Japan, they are grown for two years under quarantine before being released for field trials (Iowa State University, 1995).

### *Product characteristics*

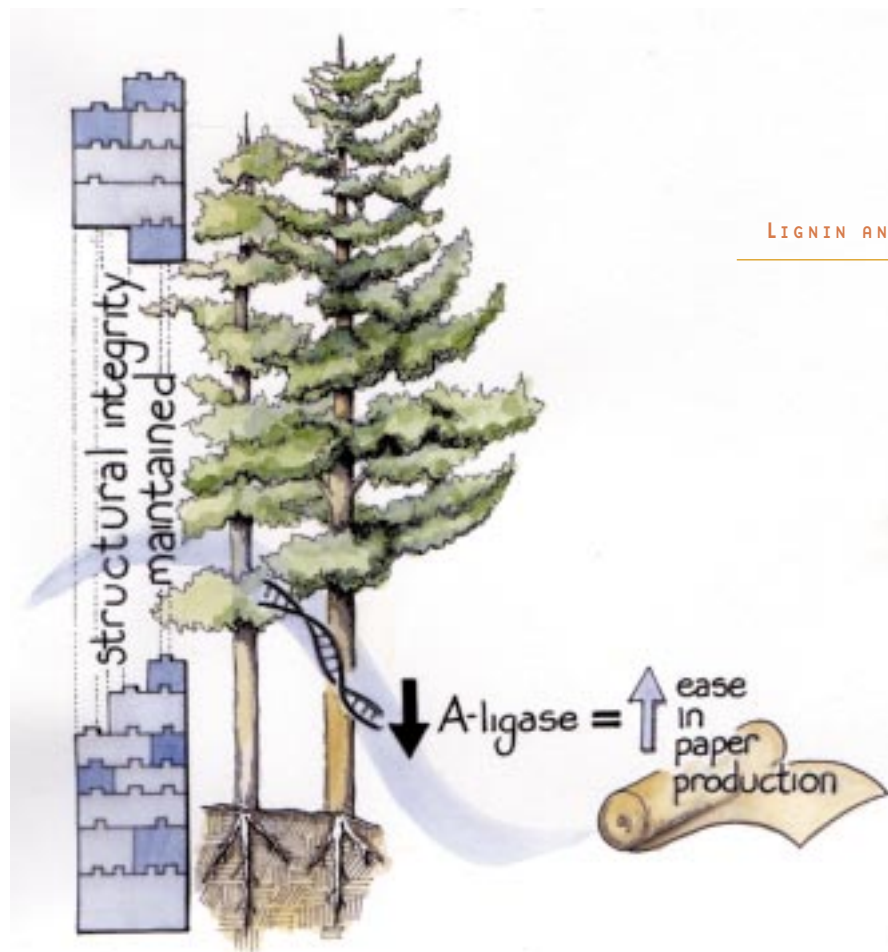
How wood characteristics are modified is determined by the ultimate use of the tree—for paper pulp, plywood, fuel, etc. Because hardwoods are easier to genetically manipulate than conifers, more work has been done in this class. Fast-growing trees with reduced lignin, a polymer related to cellulose that provides rigidity, have potential benefits for improving the ease and efficiency of processing and producing paper. For lumber purposes, however, increased lignin may be desirable; likewise for firewood, where increasing lignin content would likely increase energy production. Thus, advances in understanding the biology of lignin production underlie some of the strategies for genetic manipulation in forestry.

In the paper industry, finding ways to increase the efficiency of pulp production by making it easier to break down lignin has stimulated considerable transgenic research. Recently, the application of antisense genetic engineering technology in aspens to reduce the expression of A-ligase, a key enzyme in lignin biosynthesis, resulted in transgenic trees with up to 45 percent less lignin (Hu et al. 1999). Leaf, root and stem growth were enhanced and the structural integrity of the tree was maintained. Another possible modification of lignin production for the paper industry deals with a different enzyme, cinnamyl alcohol dehydrogenase (CAD). The reduced expression of this enzyme facilitates solubilization and fragmentation of lignin during pulping (Lapierre et al. 1999).

Modifying the characteristics of edible tree fruits is also an aim of biotechnology research. For example, in citrus crops, limonoids are responsible for bitterness and their production is associated with quality and value in citrus juices. Limonoids have also been associated with both anti-carcinogenic properties in laboratory animals and with discouraging insect pests in the field. Research is in progress using transgenic techniques to control limonoid bitterness in citrus (Hasegawa and Miyake, 1996).







## SUMMARY CHART OF TREE PRODUCTS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
<b>Apple trees</b>	46	Stop or reduce fire blight, a destructive bacterial disease that kills blossoms, shoots, limbs and, sometimes, the entire tree. Fire blight is common throughout the Mid-Atlantic region.	Insert a gene from the giant silk moth into the DNA of apple trees to make them resistant to fire blight.	Being tested in research fields.
<b>Populus Species (includes: Poplar, Aspen and Cottonwood trees)</b>	48	Reduce the lignin content, a structural component of trees that provides rigidity and is difficult to break down during the process of producing paper pulp.	Apply anti-sense genetic engineering to reduce the lignin biosynthesis.	Being tested in research fields.
<b>Cottonwood trees</b>	46	Reduce damage from the cottonwood leaf beetle, a major pest of the Populus species throughout North America.	Modify trees with several Bt genes.	Being tested in research fields.  (Early results show resistance to the pest but the level varied depending on the state of the beetle's development).
<b>Fruit trees</b>	46	Reduce or eliminate damage from the codling moth (also known as the apple cutworm). The larvae can destroy over 80 percent of an apple crop and up to 60 percent of pears.	Use a Bt gene to engineer resistance to the pest.	Being tested in greenhouses and limited research fields.  (Scientists recently accomplished this objective in Gala apples).
<b>Stonefruit (plum, peach, nectarine, and apricot)</b>	47	Combat plum pox virus, a major disease carried by aphids and responsible for heavy orchard losses. The disease can severely disfigure fruit, reduce its quality and compromise the health of trees.	Use viral coat protein technology to create transgenic fruit by using a plum pox virus coat protein.	Being tested in research fields.  (Transgenic lines were resistant to plum pox virus for three years. Hybrids developed from the transgenic trees were also virus-resistant).

## SECTION 3: GENETICALLY MODIFYING GRASS AND FLOWERS

Turf in golf courses and commercial landscaping requires enormous quantities of water, pesticides and herbicides to preserve its appearance and functionality. Thus, most modifications of turf grass and other ground cover plants are aimed at developing pest, disease and herbicide resistance, and increasing tolerance to stresses such as drought. Scientists are also looking to engineer flowers and potted plants for such characteristics as different shades of color and color intensity; size and shape; flower size and number; color pattern, and extended shelf life of cut flowers.

### *Herbicides, pests and disease*

A major challenge, especially in golf courses, is invasion by annual bluegrass. Control of this weed is accomplished with herbicides, but bluegrass is particularly vigorous and persistent. Scientists are working to engineer bentgrass to resist herbicides such as glyphosate, that may allow for better bluegrass and weed control. Researchers are also forming disease resistant plants with an emphasis on protection against the fungal genus *Fusarium*. Transgenic lines of bentgrass expressing five potential disease resistance genes have been field tested for enhanced resistance to what is known as dollar spot disease (Belanger et al. 2000).

### *Stress tolerance*

Modification of turf grass using the gene encoding for the enzyme betaine aldehyde dehydrogenase (BADH) is being investigated because of the potential for conferring enhanced tolerance to drought, salt and cold. BADH catalyzes the last step in the production of the osmoprotectant, glycine betaine. Scientists have found that tobacco and plants expressing the gene for BADH exhibit increased tolerance to salt, high light and cold (Holmstrom et al. 2000).

### *Product Characteristics*

The first application of transgene technology in flowers was the creation of an orange petunia developed by introducing a pigment-producing gene from corn (Meyer et al. 1987). Since then, intense research efforts have been applied to developing new colors, particularly blue. None of the five leading cut flower species—rose, gerbera, lily, chrysanthemum and carnation—can be bred for blue color using traditional techniques, because none contains the enzyme pathways to produce those pigments (Mol et al. 1995). Transgenic flowers, however, can have colors in the blue to mauve range. The first mauve carnation, “Moondust” was introduced in 1996 and is now commercially available in Australia. Scientists have since applied this technology, which required several gene transformations, to other species, and are attempting to extend the range of this palette.



Beautiful, durable and long-lasting flowers are in great demand by the floral industry. Several approaches to increasing flower life have been pursued, including modification to control ethylene synthesis, control of cytokinin levels and modulation of the expression of senescence genes. A patent exists for the “long vase length” gene, which has been applied to several varieties of carnation and other species. A long stem strong enough to support the flower bloom is desirable for use in vase presentation and floral arrangements.

## SUMMARY CHART OF GRASS AND FLOWER PRODUCTS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
Turf grass (often used for golf courses)	51	Control invasion by annual bluegrass	Engineer bentgrass to resist herbicides, such as glyphosate, which may allow for better bluegrass and weed control.	Being tested in research fields.
Turf grass	51	Increase tolerance to drought, salt and cold.	Modify turf grass using the gene encoding for the enzyme betaine aldehyde dehydrogenase (BADH), which has the potential to confer enhanced tolerance to these conditions.	Being tested in research fields and laboratories.
Petunia	51	Create a non-traditional color of petunia.	An orange petunia was developed in 1987 by introducing a pigment-producing gene from corn.	Available commercially.
Flowers	51	Create transgenic flowers in non-traditional colors, particularly blue. Parallel objectives: Increase flower life and create longer flower stems strong enough to support the bloom and long enough for vase presentations.	The first mauve carnation, “Moondust,” was introduced in 1996 and scientists are now attempting to extend the range of this palette. Several transgenic approaches to increasing flower life are being pursued, including modification to control ethylene synthesis. “Long vase length genes” have been applied to several varieties of carnation and other species.	Greenhouse testing continues for a wider range of cut flower colors. The mauve carnation, “Moondust,” is now available commercially. A patent also now exists for the “long vase length” gene.

Biotechnology is also being applied to plants to create industrial products and pharmaceuticals. Industrial products under development include proteins and enzymes, modified starches, fats, oils, waxes, plastics and specialty substances. Pharmaceutical products include enzymes, antibodies, vaccines and specialty proteins for use in therapy and diagnostic techniques.

Using plants to make non-food chemicals requires additional precautions to ensure that the chemicals do not get into the food supply. In addition to more conventional containment techniques, two other technologies demonstrate alternative approaches. One involves a proprietary technology developed by CropTech Corporation (Blacksburg, VA), whereby the transgenic plant does not express the protein until it is injured when it is shredded for processing. Thus, plant production and protein expression are separated. Another method of containment is to grow plants hydroponically in a closed system, where product is obtained from plant root exudates (Borisjuk et al. 1999). Such a system also ensures continuous production of the substance of interest in a form that is easily purified.

### INDUSTRIAL PRODUCTS

Petroleum-based products have replaced many industrial uses for plants, but desirable materials are still derived from plants, including cotton, rubber, wool, silk, linseed oil, cork, wood and paper (Somerville and Bonetta, 2001). Attempts to modify certain properties of plant materials using traditional methods have achieved limited success; thus, biotechnology offers a new method to expand modifications for industrial use. Commercial applications of transgenic plants under development include proteins for diagnostic, therapeutic and manufacturing purposes; modified fatty acids and oils for paints and manufacturing; biopolymers as substitutes for plastics, and specialty substances, such as pigments and fragrances.

#### *Proteins*

The potential market for plant-produced industrial proteins is huge. A key target category is enzymes, which are widely used in detergents, pulp and paper production, and the manufacture of food ingredients. In the laboratory, the industrial enzyme bacterial cellulase has been produced in *Arabidopsis* (Ziegler et al. 1999). The enzyme, used in the production of alcohol, degrades plant cell walls. Because it requires high temperatures for activity, it remains stationary in the live transgenic plant and is not activated until the harvested plant is processed. A similar enzyme, fungal xylanase, was produced in the oil bodies of transgenic canola, but expression levels were too low for commercial production (Hood and Jilka, 1999).



The first plant-produced commercial products were recombinant avidin and beta-glucuronidase, produced in transgenic corn. As described earlier, avidin is a glycoprotein, an organic compound composed of both a protein and a carbohydrate, usually found in egg whites. Transgenic corn can produce avidin, which is used in medical and biochemical diagnostic kits, at levels many times greater than those found in egg whites. Beta-glucuronidase is also used as a biochemical diagnostic protein (Kusnadi et al. 1998, ProdiGene 1999).

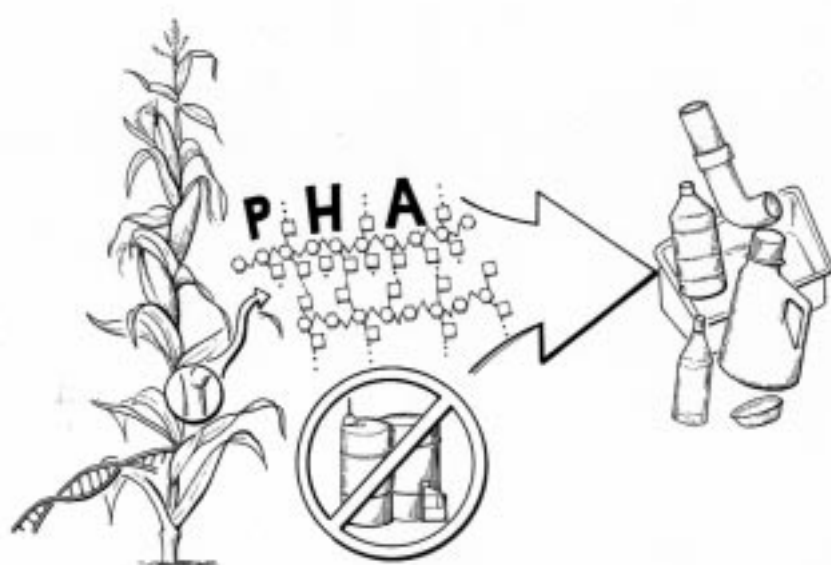
### *Biopolymers and Plastic*

Petroleum-based chemicals are currently used in the manufacture of today's polymers and plastics. Scientists and researchers have been working to develop more renewable sources for these compounds that have less impact on the environment. Corn offers one solution.

Until recently, the goal of synthesizing a plant-derived plastic, polyhydroxyalkanoate (PHA), directly in the plant remained elusive. In 1992, scientists isolated the genes necessary for plastic production, transferred them into *Arabidopsis*, and produced the first plant-synthesized PHA (Poirier et al. 1992). The technology was further adapted in corn so that the product would be expressed only in the leaves and stalk, leaving the ears free for food or feed. Scientists have had trouble refining the technology to make production feasible, in part because product synthesis occurs best in the chloroplast, the site of photosynthesis (Gerngross and Slater, 2000). Inhibiting photosynthesis, the engine of plant metabolism, hinders plant growth and reduces yield. Subsequent purification and processing also require costly inputs of both chemicals and energy. To date, the energy cost of producing plastic in corn exceeds that of fossil fuel-based production by 300-fold. As a result, Monsanto, the primary commercial company producing PHA, abandoned the development of plant-based plastic.



Other potential plant-based polymers, particularly for fiber applications, are being explored. Bacteria have been engineered to synthesize polymers that closely resemble the natural fibers silk, elastin, collagen and keratin (Somerville and Bonetta, 2001). Extending the technology, it may be possible to engineer plants to produce totally unique polymers similar to those of natural fibers. Production remains at low levels, however.



PLASTIC FROM CORN

### *Fatty acids, oils and waxes*

As mentioned in the section on improved nutrition, the ability to modify the fatty acid composition and oil content of plants presents several opportunities to produce novel fatty acids of commercial value in crops. Such fatty acids may be useful in foods and feeds, or purified as precursors for the synthesis of more complex materials such as nylon (Somerville and Bonetta, 2001). Through the use of biotechnology, researchers have successfully modified the fatty acid content of edible vegetable oils (high oleic acid soybean oil) and these adaptations were directed toward changing the pattern, rather than the quantity, of fatty acids.

Some fatty acids such as linoleic acid are essential for health while others like erucic acid can be toxic. Thus, manipulation of fatty acid content must be carefully evaluated for changes in non-target fatty acids. Concentration of single fatty acids in plant oils is complicated by the fact that, with few exceptions, oils contain mixtures of fatty acids and other lipids and are usually stored in seeds with considerable amounts of protein. Thus, efficiency of production has inherent limitations. Oil production in roots or tubers has already been demonstrated in *Arabidopsis*, and future modifications may allow oil production in plants to be independent of seed quality and protein (Ogas et al. 1997).

Industrial applications focus primarily on boosting the total content of oil that is highly enriched in a particular fatty acid. Although scientists have reported the fatty acid composition of some 8,000 seed oils, less is known about individual biosynthetic pathways for the approximately 500 fatty acids. Progress was made in the 1990s, when genes for certain key enzymes were identified and cloned. Many possibilities exist because the structure of fatty acids ranges from simple eight carbon chains to complex 24 carbon chains with double bonds, hydroxyls and other constituent groups. One product that has reached the market is high lauric acid canola oil (Murphy, 1999), used in the confectionery industry, simulated dairy products, icings and frostings. As described earlier in another processed food application, researchers have also created soybean oil with reduced linolenic acid to improve its stability and avoid the need for partial hydrogenation, which creates undesirable trans-fatty acids.

The castor plant is unique in producing approximately 90 percent of its oil as ricinoleic acid. Castor oil is used mostly in lubricants, paints, plastics and cosmetics. But castor beans (shown left) are lethal, and consuming even small amounts can be fatal. The danger lies not with the oil, but with the protein ricin contained in the seed. Moreover, castor beans are highly allergenic, especially to those who must handle them. Thus, commercial cultivation of castor plants in the U.S. is limited. Biotechnology might revive U.S. castor bean production, as scientists are using antisense technology to disarm the ricin protein and thus remove the allergen. Another potential application of genetic engineering technology in castor plants is for epoxy oil production (Wood, 2001). Epoxy oil is similar in structure to ricinoleic acid and only a relatively minor modification may be needed to stimulate its production. The market for epoxy oil is estimated at about \$300 million, with the potential for manufacturing premium oil-based paints that would not require organic solvents. A patent for transgenic castor has been filed.



castor beans



Another plant of specialized interest is jojoba, or goat nut. Jojoba (shown right) is a desert plant grown primarily in the Southwestern U.S., Argentina and Israel, unique for its high content of wax esters. Jojoba oil is used in cosmetics, lubricants and surfactants, and its use has grown rapidly with the decline in sperm whale oil. Jojoba wax is highly stable and does not become rancid or degrade in high temperatures. Jojoba seeds contain 44 to 56 percent wax. Most jojoba oil comes from wild plants, but cultivation is expanding. Scientists have cloned enzymes from jojoba, and when expressed in *Arabidopsis*, these transgenic plants produced seeds having from 49 to 70 percent of the oil present as waxes (Lardizabal et al. 2000).

Jojoba has also contributed to the fundamental understanding of fatty acid chain elongation. Because of its ability to produce long chain fatty acid derivatives, jojoba was examined for enzymes involved in the production of very long chain fatty acids. A gene encoding for beta-Ketoacyl-CoA synthase (KCS) was cloned and engineered into canola with low erucic acid (Lassner et al. 1996). Transgenic plants subsequently produced very long chain fatty acids, revealing the importance of KCS in the production of these fatty acids.

### Specialty products

Astaxanthin is a naturally occurring red carotenoid pigment found in the shells of shrimp, crawfish, crabs and lobster. It is often used in aquaculture salmonid feeds to make the flesh more pink and appealing to consumers (Mann et al. 2000). While still in the testing phase, bioengineering of tobacco has produced astaxanthin by altering the carotenoid pathway using a gene from algae. The application also has relevance for horticulture and floriculture.

Specialty applications in the research stage include genetic strategies to enhance flavor and fragrance. Increased fragrance has particular value in the floriculture and cosmetics industries. Enhancing flavor is useful not only in edible plants or fruits, but also in the production of essential oils used in fragrances and processed foods. Creating flavors and fragrances entails highly complex mixtures of substances. One example is the identification of a novel strawberry alcohol gene acyltransferase (SAAT) that plays a crucial role in the development of flavor in ripening fruit (Aharoni et al. 2000), as evidenced by the production of the transgenic bacteria *Escherichia coli* bearing the SAAT gene.



Jojoba tree



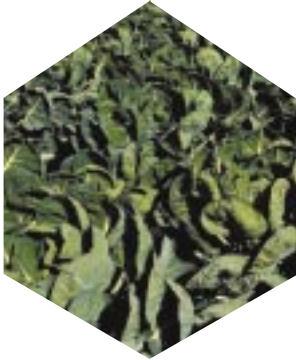
## PHARMACEUTICALS

Modern medicine is continually trying to produce more effective and affordable pharmaceuticals to prevent or treat disease. Examples of genetically engineered plant pharmaceuticals in various stages of research and production include edible vaccines, plant-produced antibodies and human proteins. To date, there are no commercial plant-produced pharmaceuticals, but three vaccines and two therapeutic proteins are in clinical trials, and many others are in various stages of research. With years of testing for safety in animals and then humans still ahead, along with the regulatory approval process, these products will not reach the marketplace for some time.

### *Plant-produced and edible vaccines*

Vaccines constitute one of the greatest achievements of modern medicine. Traditional vaccine preparations consist primarily of a killed or weakened version of a pathogen (the causative agent) or of some part of that agent. By tricking the immune system into behaving as if the pathogen had already infected the body, the immune system homes in on foreign antigens, usually proteins or protein fragments that are produced uniquely by the causative agent and not by a host. Beyond eliminating invaders, activation of the immune system against specific antigens can lead to the creation of memory cells that will later repel pathogens.

Another potential application of biotechnology is the production of vaccines and drugs in transgenic plants. Technology developers believe that edible vaccines could offer advantages over conventional immunization programs by eliminating both the need for purification and the hazards associated with injection. Scientists are developing vaccines grown or harvested from plants that can prevent human and animal diseases. Research has demonstrated that bioengineered plants can express antigens capable of stimulating an immune response. In addition, plant-expressed antigens elicit an appropriate antibody response in the host and these plant-produced proteins can confer immunity. Some of the plants used to develop the vaccines include corn, spinach, tobacco, lettuce, tomato, soybean, cowpea and potato (Langridge, 2000).



spinach field



The first demonstration that plants could express biologically active antigens was shown in a 1990 patent application (Curtiss and Cardineau, 1990). In this work, tobacco was genetically engineered to express an antigen for the surface protein of the dental bacterium, *Streptococcus mutans*. When fed to mice, biologically active antibodies were induced to fight off these bacteria. Since then, several other vaccines, or edible vaccines, have been expressed in various plants (Walmsley and Arntzen 2000). Examples of human or animal diseases for which target vaccine proteins have been expressed in transgenic plants are rabies, mink enteric virus, piglet diarrhea, colon cancer, pulmonary infections, cholera, traveler's diarrhea, tooth decay, hepatitis B, *Helicobacter pylori* (a bacterial cause of ulcers), human cytomegalovirus, Norwalk virus, foot-and-mouth disease and other opportunistic infections (Hood and Jilka 1999, Walmsley and Arntzen 2000).

The first demonstration of effective edible vaccines was reported in 1995 (Arntzen, 1995) and the first account of a human clinical trial of a plant-derived edible vaccine appeared in 1998. This trial demonstrated that following the consumption of transgenic potatoes containing a vaccino-gen, there was subsequent induction of an immune response (Tacket et al. 1998). Ten of the eleven test subjects produced specific antibodies to the toxin used, while none was produced in control subjects. These results are comparable to those measured in volunteers exposed to live organisms. The study was crucial for demonstrating that edible vaccines could survive digestion and effectively stimulate an immune response.

Development of a variety of acceptable foods for vaccine delivery is underway. Emphasis is given to foods that are well liked, consumed raw and have a long shelf life so that acceptability and effectiveness are enhanced. Scientists have synthesized edible vaccines in several foods including the banana, potato, sweet potato and tomato (Kapusta 1999, Tacket et al. 2000).



Also under development is an edible vaccine in transgenic bananas that fights hepatitis B, a potentially fatal disease often transmitted through blood (Richter et al. 2000). A recent report showed the effectiveness of an edible vaccine for the virus in a study using mice. The mice responded to a diet of transgenic potatoes containing the hepatitis B surface antigen with increased hepatitis B antibodies. Research is also underway to create other edible vaccines in transgenic corn for AIDS prevention and treatment, using the simian immunodeficiency virus.

In May 2001, scientists at Thomas Jefferson University announced they are working on genetically engineering spinach to express HIV-suppressing proteins, believing this may be a viable way to deliver a safe and inexpensive AIDS vaccine. The researchers introduced a gene expressing the protein into a common plant virus, which allowed for easy insertion of the genetic material into the spinach plant. The plant then began to produce the desired protein. Vaccines grown in plants could be cheaper to harvest than those produced in the lab. In addition, using plants to generate vaccines could increase manufacturing safety. Traditional production of vaccine components in animal cultures, or sometimes even in human tissue cultures, carries the remote possibility of contamination, a risk greatly reduced in making plant-based vaccines.

Researchers are also working on edible vaccines for animal health. In April 2000, it was announced that a patented edible vaccine was created in transgenic corn that confers protection in pigs against a common gastrointestinal virus (Anon. 2000). This transgenic corn is not expected to be commercially available for several years, pending further trials and regulatory approval.

In a variation on the concept of using edible vaccines to develop immunity, researchers are exploring ways to use transgenic foods to suppress autoimmunity—the body’s development of immunity against its own cells. Autoimmune responses underlie several diseases, including insulin-dependent diabetes, psoriasis, systemic lupus erythematosus, Graves’ disease and rheumatoid arthritis. In test animals, “autoantigens” (proteins derived from normal tissue in a treated individual) given orally can sometimes suppress immune activity, although no one knows why. Researchers are working to develop plant-based diabetes vaccines, such as potatoes containing GAD (glutamic acid decarboxylase, an enzyme involved in autoimmunity) linked to the innocuous B subunit of the *Vibrio cholerae* toxin (to enhance uptake of the antigens). Mice susceptible to diabetes that were fed the transgenic potatoes were able to suppress an immune attack and had a delay in the onset of high blood sugar, an indication of diabetes (Arakawa et al. 1998).



## Antibodies

In addition to inducing the production of antibodies in people and animals consuming edible vaccines, researchers are also working to create plant-produced antibodies. To date, all applications remain in laboratory and demonstration phases. Plant-produced antibodies will most likely be applied in diagnostic testing prior to animal and human therapeutic uses.

Actual success in preventing disease through the use of plant-produced antibodies was first reported in 1998. A published study described the first human clinical trial in which a monoclonal antibody was produced in a transgenic plant and then topically applied to the teeth, instead of being consumed as an edible vaccine. This treatment prevented colonization by *Streptococcus mutans*, demonstrating the potential for delaying or preventing dental disease and cavities.

Antibodies to fight cancer are also being developed through agricultural biotechnology. Stoger et al. (2000) announced the expression in wheat and rice of a single-chain Fv antibody against carcinoembryonic antigen, a marker antigen for tumors. Effectiveness of the antibody was retained in the grain for at least five months in storage without appreciable loss, an important consideration in handling and distribution.

Using transgenic plants to produce antibodies for diagnostic testing has been reported in Japan (Tsuda et al. 1998). To reduce the costs of testing donor blood for certain antibodies, researchers produced transgenic tobacco plants engineered to express the hepatitis B core antigen, which is used to screen blood to determine if donors have been infected with the virus. The antigen produced in tobacco performed as well as the bacterial antigen. Estimates are that one fully expanded transgenic tobacco leaf could be used to test anywhere from 64,000 to 102,000 people, depending on the concentration of antigen in the leaf.

## Therapeutic proteins

There is an expanding demand for complex human proteins for therapeutics and diagnostics. Transgenic animals, and more recently plants, are the production methods of choice for many complex human proteins. Biopharmaceuticals, such as insulin identical to that produced by humans, were first produced using genetically engineered microorganisms in huge fermentation tanks. This led to a search for other production methods, once it was learned that some human proteins are not biologically active when produced in microbial systems. Mammalian cell culture soon followed, but this approach was limited by long growing times, potential contamination by pathogenic viruses and relatively small-scale production.

tobacco plant





The list of human proteins that can be developed in transgenic plants grows daily. These proteins are important because they can be used in diagnostic testing and in quantity for therapeutic purposes. Examples include enkephalins,  $\delta$ -interferon, human serum albumin, human hemoglobin, erythropoietin and angiotensin-1-converting enzyme (Giddings et al. 2000). Applied Phytologics (Sacramento, CA) has modified rice to produce human  $\delta$ -1-antitrypsin, a protein of therapeutic potential in cystic fibrosis, liver disease and hemorrhages. A pharmaceutical product made from this protein is undergoing clinical trials.

In tobacco, researchers have produced two human lysosomal proteins, beta-glucocerebrosidase and iduronidase, and several other proteins (CropTech 2001, Naj 1995). The enzyme beta-glucocerebrosidase is missing or insufficient in people with Gaucher's disease, a genetic disorder that can result in pain, fatigue, jaundice, bone damage, nerve damage or even death. It may be present in 10,000 to 20,000 Americans. The disease manifests as a lipid storage disorder in which lipids accumulate in bone, causing mineral loss. The enzyme is very expensive because it is available in natural form only from a human placenta and it takes 10 to 12 tons of placentas to make enough protein for a single patient. Scientists have created the enzyme in mammalian cell culture, but the product is slightly different from the native enzyme. Production of this rare enzyme in native form in transgenic plants could reduce the cost of this drug and make treatment of Gaucher's disease accessible to more patients.

The second enzyme, alpha-L-iduronidase, is a lysosomal enzyme that is deficient or absent in people with a mutation in gene coding. The result is a disorder named mucopolysaccharidosis (MPS-I) that severely affects normal growth and development and is eventually fatal. Treatment relies solely on providing the enzyme.

Another protein of interest, currently being produced in transgenic canola grown commercially in Canada, is hirudin, an anticoagulant used to treat blood clots. Production is novel in that hirudin is fused to oleosin and accumulates in the oil bodies of the plant. Separation from oil bodies is more easily accomplished than from seeds (Giddings et al. 2000). SemBioSys Genetics Inc., of Calgary, Canada, developed the transgenic plants. Other research at Integrated Protein Technologies, a division of Monsanto, is using transgenic corn to manufacture a variety of therapeutic agents such as cytokines, structural proteins, blood products, hormones, monoclonal antibodies and antibody fragments.

## ENVIRONMENTAL REMEDIATION AND CONSERVATION

Scientists are working on ways to use biotechnology for environmental preservation and remediation. Certain plant species have the ability to absorb or detoxify metals and hazardous substances, and thus may be useful for remediation efforts. Transgenic plants are also being used as biosensors, detecting or monitoring for the presence of certain hazardous materials. Efforts are also underway to restore endangered trees and plants, such as the American chestnut, which has been virtually wiped out by blight.

### *Metal Contaminants*

Metals such as copper, cadmium, cobalt, aluminum, manganese, nickel, selenium and zinc are essential in small amounts in the human diet and to maintain health in plants. In high concentrations, however, these substances are toxic. At least 45 plant families are known to accumulate metals in large amounts relative to other plants (Reeves and Baker, 2000). The ability to genetically engineer plants to accumulate heavy metals offers the possibility for cleaning up soils with high levels of contaminants (Guerinot and Salt, 2001).

Plants have various strategies for increasing their uptake of minerals from deficient soils. One involves the ability to secrete organic acids, such as citrate, to increase the solubility of the metal. Another is the release of amino acids that bind to the soluble iron from deficient soils. Overproduction of these compounds may increase mineral uptake. The gene encoding a transporter that can absorb the bound iron has recently been identified in corn (Guerinot and Salt, 2001). Plant transporter proteins are also involved in moving minerals from the soil to the root, and several genes have been identified that encode for these substances. It is likely that the metal-accumulating function of plants involves a suite of genes.



american chestnut tree





jellyfish

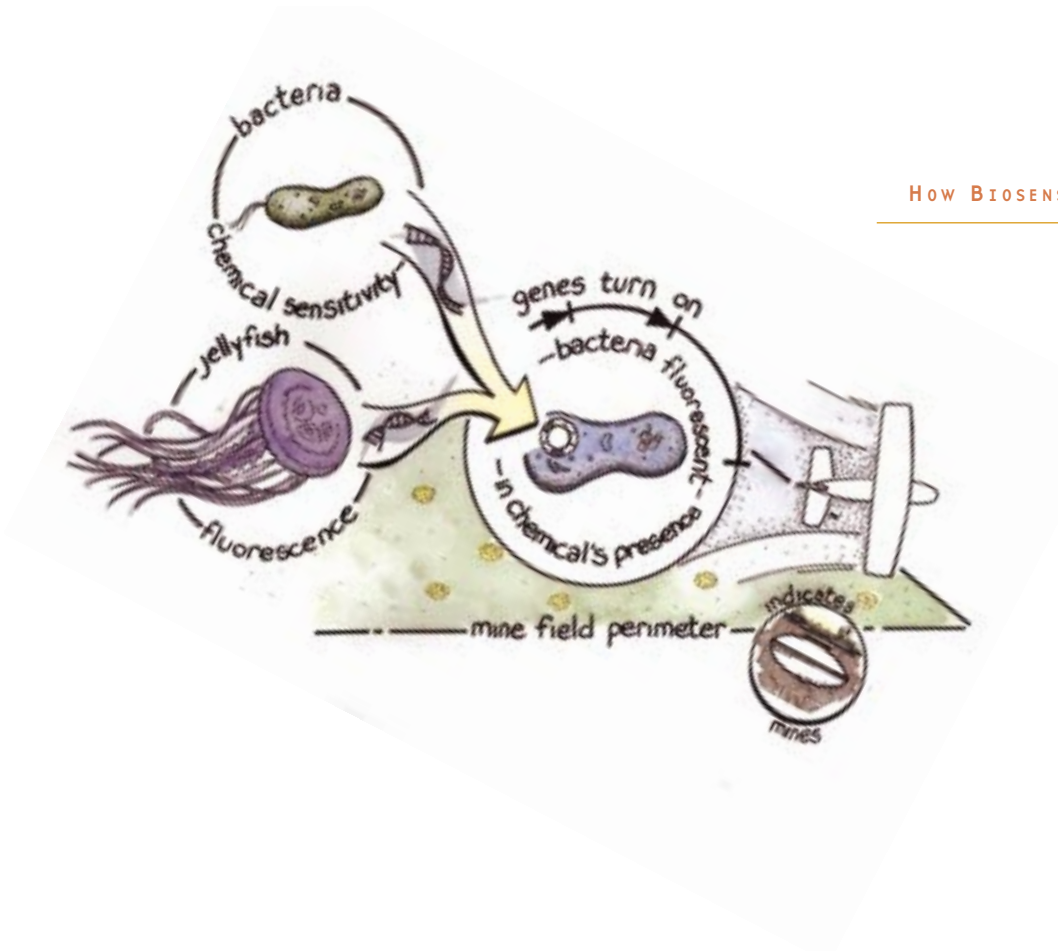
Subsequent steps related to metal accumulation in plants involve storage mechanisms, and it appears that different types of metals are stored and handled differently. For example, plant materials will bind the metals cadmium and zinc, which are stored in the vacuoles, small cavities or spaces in the tissues of the plant. Iron, on the other hand, is stored as the protein ferritin in other parts of plant cells. In still other circumstances, metals may be released as gases. Through genetic modifications, researchers are attempting to optimize plants' abilities to sequester contaminants. Transgenic trees have been developed that can be successfully grown in regions of heavy metal contamination. The roots sequester and incorporate heavy metals, yet the trees remain healthy. Although much has been learned about metal binding, uptake, transport and storage in the plant, much also remains to be accomplished before metal accumulation can be enhanced in target plants.

### *Biosensors*

Scientists are working to genetically modify plants to act as warning systems or biosensors that can detect or monitor hazardous materials. For instance, they engineered the bacteria, *Pseudomonas putida*, for sensitivity to trinitrotoluene (TNT), the material commonly used in explosives. The sensitive bacteria were then further modified to contain the protein from jellyfish that creates a green fluorescent color so that it would alter the bacterial color when triggered. When the bacteria were field tested, they detected all landmines without fail (Burlage, 1999). Although the use of bacteria as detective agents has many drawbacks, particularly with regard to handling and storage, the principle applied to plants would offer advantages. Scientists also showed that incorporating an enzyme derived from an explosive-degrading bacterium into tobacco plants enabled the plants to grow in the presence of TNT (French et al. 1999). In this alternate use for tobacco plants, the addition of the fluorescent protein would enable plants to be readily monitored. Cultivating such transgenic plants in fields with land mines presents many logistical challenges, but it is possible that it could be accomplished with aerial seeding and care. A remote detection system based on laser-induced fluorescence spectroscopy could then be applied in the aerial detection of activated TNT-rich plants (Di Benedetto, 1999). Using similar technology, it is possible that transgenic plants altered for sensitivity to radioisotopes or other organic toxins could be used to monitor radioactivity around nuclear plants or waste sites, or sites contaminated with organic toxicants.



HOW BIOSENSORS WORK



### *Species Recovery*

Biotechnology may play a role in the recovery of certain endangered plant species. For instance, genetic engineering technology is being used in the recovery of the American chestnut (*Castanea dentata*). Once the dominant hardwood of eastern forests and Appalachia, the American chestnut was virtually eliminated from native forests by chestnut blight, a disease caused by the fungus *Cryphonectria parasitica*. A few surviving specimens, along with trees found in isolated stands in Europe and other naturally resistant strains from China, provided stock for building resistant varieties. The discovery of hypovirulent fungi (*Endothia parasitica*) in isolated stands in Italy made it possible to engineer genetic resistance in some trees that, in field trials, have shown resistance to the pathogenic fungus. It was later discovered that some stands in Michigan had also survived the blight because of hypoviruses (Bunk, 1999). The example of the American chestnut demonstrates the possibility of using transgenic trees to help resurrect wild forest species that have been devastated by exotic pests (Strauss et al. 1999).



SUMMARY CHART OF INDUSTRIAL, PHARMACEUTICAL AND REMEDIATION PRODUCTS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
American chestnut tree	66	Extend the recovery process for the American chestnut that was virtually eliminated by chestnut blight, a fungal disease.	A hypo-virulent fungus, discovered in chestnut trees in Italy made it possible to engineer resistance in some transgenic trees.	Ongoing testing in research fields; improved hypovirulent viruses and transgenic fungi are being developed.
Anti-bodies	61	Develop anti-bodies that can be used in diagnostic testing.	Researchers produced transgenic tobacco plants engineered to express the Hepatitis B core antigen. This antigen is used to screen blood for Hepatitis B.	Undergoing investigation in laboratories.
Avidin	54	Achieve efficient production of avidin, which is usually found in egg whites. Avidin is used in medical and biochemical diagnostics.	Transgenic corn was developed that can produce avidin at a level many times greater than that found in egg whites.	Available commercially.
Biosensors	64	Develop biosensors capable of detecting landmines.	Scientists engineered bacteria for sensitivity to TNT, the material commonly used in explosives. The bacteria were then further modified to contain a jellyfish protein that creates a green fluorescent color when TNT is detected.	Being tested in research fields.
Castor oil	56	Reduce toxicity and allergenicity of castor seeds.	Anti-sense technology is being used to disarm the ricin protein and thus remove the protein that causes the allergen.	Undergoing investigation in laboratories; a patent for transgenic castor oil has been filed.
Edible vaccines	60	Improve the safety and availability of vaccines, particularly in developing countries.	Develop transgenic plants that produce the vaccine, enabling it to be consumed directly by humans.  (Researchers recently introduced a gene expressing an HIV-suppressing protein into a spinach plant. The plant then began to produce the desired protein.)	Undergoing investigation in laboratories.
Enzymes for industrial purposes	53	Enhance the production of the industrial enzyme cellulase, which is used to make alcohol.	Cellulase has been produced in transgenic Arabidopsis. Because it requires high temperatures for activity, it remains inactive in the live transgenic plant and is not activated until the harvested plants are processed.	Undergoing investigation in laboratories.
Epoxy oil	56	Increase production for use in premium oil-based paints that require no organic solvents.	Stimulate epoxy oil production in castor beans; excess oil is then available for incorporation in specialty paints	Undergoing investigation in laboratories.

SUMMARY CHART OF INDUSTRIAL, PHARMACEUTICAL AND REMEDIATION PRODUCTS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
Human proteins	62	Meet the expanding demand for complex human proteins used in therapeutics and diagnostics.	Corn, rice canola and tobacco have been modified to produce specific human proteins.  (Researchers have modified rice to produce a protein of therapeutic potential in cystic fibrosis, liver disease and hemorrhages.)	Undergoing investigation in laboratories.  (A pharmaceutical product made from the rice protein is undergoing clinical trials.)
Human proteins	62	Make anti-coagulants that can be used to treat blood clots.	Hirudin, an anti-coagulant, has been produced in transgenic canola.	Available commercially in Canada.
Plants that can accumulate metals	63	Clean up soil contaminated by high concentrations of metals such as copper, cadmium, cobalt, aluminum, manganese, nickel, selenium and zinc.	Transgenic plants and trees capable of absorbing many hazardous metals have been developed.	Being tested in research fields.
Plastics	54	Develop renewable resources as alternatives for petroleum-based products.	Scientists isolated the genes necessary for plastic polymer production, transferred them into corn and Arabidopsis, and produced the first plant-synthesized plastic. This source of plastic, however, is not yet economically feasible.	Undergoing investigation in laboratories.

PART II

GENETICALLY ENGINEERED ANIMALS

## SECTION 1: GENETICALLY ENGINEERED MAMMALS

### BASIC GENETIC RESEARCH

The first transgenic mouse was produced in 1981 (Gordon and Ruddle, 1981), and was genetically engineered to include the gene for human growth hormone. Compared with its normal litter mate, the transgenic mouse was much larger and had levels of growth hormone in its blood serum that were several hundred times higher than in control mice. Transgenic mice with “knock out” genes—specific genes that have been deleted—were developed in 1987 and have become valuable in research for understanding gene function and expression. Several strains of transgenic mice have been developed for the production of human proteins used to study infections, inflammatory conditions and cancer (Yang X-D et al. 1999a and 1999b, Russel et al. 2000). Transgenic mice are also used to produce monoclonal antibodies and anti-inflammatory agents (Green, 1999), products with potential use in treating infection and disease.

### PRODUCTION OF HUMAN PROTEINS

Work on the modification of animals to produce human proteins is aimed at expanding the range of proteins suitable for human medical therapy. To date, scientists have produced human proteins usually in sheep, pigs or goats, generally targeted to genetic disorders for which there are few, if any, alternative therapies. Several of these proteins are in various stages of clinical trials prior to application for FDA approval (Teutonico and McKown, 1999).

Alpha-1-antitrypsin (AAT), also known as alpha-1-protease inhibitor, is a human blood protein whose prime physiological target is the enzyme neutrophil elastase. Many respiratory diseases, including cystic fibrosis and chronic obstructive pulmonary disease, are characterized by an imbalance of AAT and elastase in the lungs. Severe AAT deficiency (hereditary emphysema) is thought to affect around 150,000 to 200,000 people in the U. S. and Europe. Approximately one in 2,000 children in the Western hemisphere is born with the cystic fibrosis genetic defect. Administration of supplemental AAT is therefore expected to alleviate the deleterious effects of elastase in the lungs of those afflicted with these diseases. PPL Therapeutics, PLC, of Edinburgh, Scotland, has used transgenic animals to produce the human protein ATT. The ATT product is in Phase II clinical trials for cystic fibrosis and early clinical study for those with congenital AAT deficiency.



Genetic modification of animals is also being used to produce a human pancreatic enzyme that will aid in lipid digestion. Difficulty with lipid digestion interferes with early growth and development. Bile salt stimulated lipase, also known as BSSL, is an enzyme produced in the pancreas and in human milk that breaks down lipids during digestion. Several conditions are characterized by deficiency of this enzyme, including pancreatic insufficiency, chronic pancreatitis and cystic fibrosis. Pre-term infants also have difficulty digesting lipids and unless they are breast fed, have no access to this enzyme. PPL Therapeutics has reported that a proof of concept trial to create BSSL in animals is now underway in Belgium. PPL also notes that the creation of fibrinogen, a major component in blood clotting, is in the development stage, using transgenic sheep. This protein would be used in tissue sealants to treat wounds.

Researchers are also bioengineering animals to produce milk with human proteins that would be useful for medical therapy. Genzyme Transgenics, in Framingham, MA., has successfully produced in transgenic goat milk more than seven human immunoglobulins, proteins that improve the immune response. By the end of 2000, the company had demonstrated successful expression of 65 proteins in transgenic animals, many of them monoclonal antibodies that may be used in cancer treatment. None are commercially available.

Bioengineered animals may also be useful in creating new vaccines for diseases such as malaria. In 1998, researchers at Genzyme Transgenics in the U.K., in conjunction with scientists at the American National Institute of Allergy and Infectious Diseases, reported successful production of the MSP-1 antigen in the milk of transgenic mice. MSP-1 elicits a human antibody response to the malarial parasite *Plasmodium falciparum*. The production of this protein would be the basis for the development of a malaria vaccine. The MSP-1 antigen is currently undergoing purification and if it can be demonstrated to be effective in laboratory monkeys, it may lead to further development of the protein in goats.

Cow's milk is another area of study, since it is such a large component of human diets, especially in children. Lysozyme is an antimicrobial protein found in human milk. The application of transgenics has the potential to increase bovine production of lysozyme in cow's milk to nearly 3,000 times the level found in human milk. This could enhance health protection in human infants fed cow's milk, as well as increasing product shelf life.



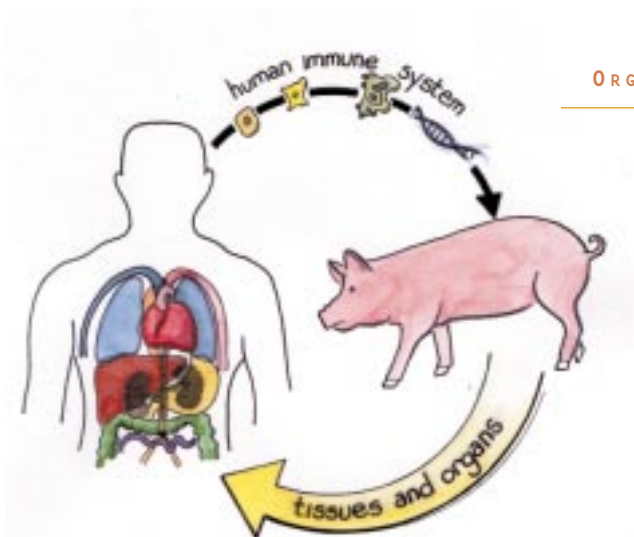
To date, several human proteins have been produced in sheep, pigs or goats but no commercial development of these proteins has been reported. Some of them and their intended applications include:

- ◆ TPA, or tissue plasminogen activator, produced in goats for treatment of blood clots
- ◆ Factor VIII and Factor IX, produced in sheep for the treatment of blood clotting disorders such as hemophilia
- ◆ Antithrombin III, produced in goats, also for the prevention of blood clotting
- ◆ CFTR, or cystic fibrosis transmembrane conductance regulator, for treatment of cystic fibrosis
- ◆ Human protein C produced in pigs for use as an anticoagulant



## XENOTRANSPLANTATION

Another area of transgenic animal research is xenotransplantation the production of tissue and organs in animals for use in humans. Research centers on the pig because of its many biological similarities to human beings. The ability to clone pigs, first announced by PPL Therapeutics in March 2000, is the first step. A major challenge is to form pig tissues without 1-galactosyl transferase, a protein in swine linked to human tissue rejection. Being able to “knock out” production of this protein in pigs would enhance the compatibility of pig tissue for human transplantation by reducing the rejection response. Research efforts with this focus are proceeding.



## ORGAN TRANSPLANTS FROM ANIMALS



## FARM ANIMAL PRODUCTION

Applications of genetic modification in animals include inducing more rapid growth or weight gain, altering milk to reduce lactose or improve shelf life, or increasing disease resistance. The first transgenic farm animals were reported in 1985 (Hammer et al. 1985). In 1987, scientists produced the first transgenic poultry and, in 1991, the first transgenic goats.

An early approach to improve growth efficiency in animals entailed administering growth hormone (GH), either through injection, ingestion or through transgenes. GH transgenic pigs matured sooner and had less fat; however, they subsequently developed severe health problems. More recently, scientists in search of reduced-fat meats for human diets produced pigs carrying a transgene for insulin-like growth factor (IGF-1) (Weaver, 1998). These pigs had, on average, 18 to 23 percent less carcass fat, greater lean body mass, unchanged levels of GH and none of the health problems associated with the GH transgene. One constraint that may hinder further development is the need to demonstrate that the higher levels of IGF-1 in muscles have no adverse effects when eaten. Another application of transgenic technology in animals is altered functionality and composition of milk. For example, a reduction in the lactose content of cow's milk would diminish the likelihood of sensitivity responses in people with lactose intolerance.

Transgenic technology may also be used to produce milk with reduced water content. This change has economic implications for milk production and transportation, and any processing that involves removing water, such as cheese and milk powder production. Using transgenic techniques to reduce the beta-lactoglobulin content of cow's milk would also make cow's milk less allergenic. Increased antimicrobial proteins might also prolong the shelf life of milk and reduce the potential for milk-associated gastrointestinal disturbances.

Another goal is to reduce the risk of disease in dairy cows. For example, the ability to produce the bacteriocidal enzyme lysostaphin has potential to reduce the incidence of mastitis, a disease of the udder (Oldam and Daley 1991, Agricultural Research Service 2001). Currently, antibiotics are used to treat mastitis, and the milk cannot be used while the cows are on the drugs. It has recently been shown that mice containing the transgene for the enzyme lysostaphin were resistant to infection from *Staphylococcus aureus*, a cause of mastitis, with no effect on their milk profiles (Kerr et al. 2001). And scientists have just successfully cloned a heifer with a transgene to produce lysostaphin (shown left) (Suszkiw, 2001).

cloned heifer



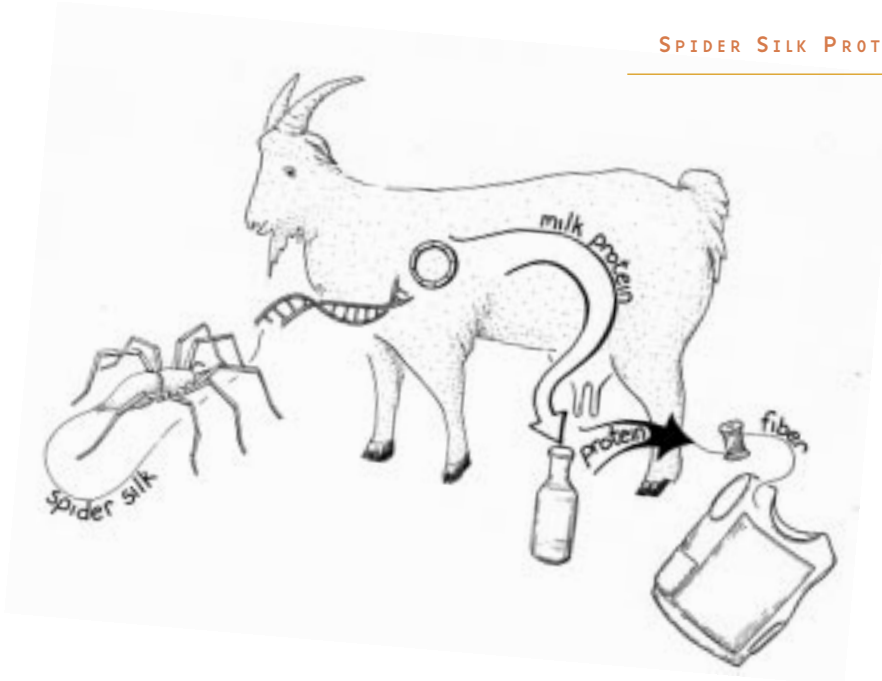
## INDUSTRIAL PRODUCTS

A novel application of transgenic technology is the production of spider silk proteins in specially bred transgenic goats. The frame silk of spiders is an ultra-strong material that can hold 400,000 pounds per square inch without breaking and is acknowledged to be the world's strongest material. Because this silk is highly prized, yet cannot be cultivated commercially, scientists have sought other ways to replicate the material. Although genes for spider silk have been identified, their application in microbial or cellular production systems has been unsuccessful, mainly because the genes are large and contain many repetitive units. The ability of the mammary gland to produce these proteins was the key to silk production in the milk of transgenic goats (Huynh et al. 1991). Transgenic goats are now being used to produce spider silk proteins in their milk. Using traditional dairying techniques, milk is collected and the proteins are extracted from the milk then spun into fibers. These fibers will be sold under the commercial name BioSteel®, which will be used in a variety of medical device products, such as superfine ophthalmic applications and prostheses. BioSteel® will also be used in industrial applications, including light weight, flexible body armor for military and law enforcement and high performance technical sporting equipment, for example, bio-degradable fishing line.



spider silk

### SPIDER SILK PROTEIN FROM GOAT MILK



SUMMARY CHART OF MAMMALS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
Goats	75	Produce an ultra-strong material similar to the frame silk of spiders, which is highly prized, yet cannot be cultivated commercially in spiders.	Breed transgenic goats whose mammary glands produce milk with proteins derived from spider genes that can be used to create spider silk.	Soon to be commercially available.
Pigs	73	Produce tissue and organs for use in human beings.  (Transplanting animal organs to humans is known as xenotransplantation.)	Scientists have demonstrated the ability to clone pigs and are now working to better understand the factors that contribute to tissue rejection and viral transmission, factors critical to successful transplantation.	Undergoing investigation in laboratories.
Sheep, pigs or goats	73	Modify animals to create human proteins that might be used in expanding the range of proteins available for medical therapy.	Produce proteins generally targeted for genetic disorders that have few, if any, alternative therapies.	Undergoing investigation in laboratories.  (Several of these proteins are in clinical trials prior to application for FDA approval).

## SECTION 2: GENETICALLY ENGINEERED AQUATIC ORGANISMS

According to the Food and Agriculture Organization of the United Nations, world fishery production is over 122 million tons per year, with over 93 million tons destined for human consumption. Nearly one-third of all fish produced for food (including shellfish) comes from aquaculture. It is estimated that in 1997, the value of aquaculture production was about \$46 billion, compared to about \$83 billion for commercial fisheries. The growth of aquaculture production is heavily concentrated in Asia, with most of the increase in China, whose share of the world production has increased to nearly 70 percent (FAO, 2000).


It is not surprising then, that the first transgenic fish were produced in China in 1986. Since that time, scientists have engineered at least ten species (Hew and Fletcher, 1997). An advantage of working with transgenic fish is that large numbers of homogeneous fish can be obtained from each experiment. Biotechnology applications are being created for use in cultivated shrimp, but in general, developments in shellfish have lagged behind those in farm animals. Part of the reason for this relates to the lack of basic knowledge about shrimp. As the genetics of shrimp become better understood and gene mapping advances, the application of biotechnology to shrimp could expand.

Most genetic engineering of fish and other aquatic species has focused on enhanced growth, stress resistance, disease resistance and sterility (the latter being an important technique to control the unintentional release of genetically modified organisms into the environment). A few research efforts have concentrated on other applications and uses. For instance, scientists have sought to genetically modify fish such as tilapia for the production of pharmaceuticals. A patent has been granted for the technology, developed by AquaGene Inc., Alachua, FL., but so far no product has been created. Scientists have also applied transgenic techniques to create fish that act as biosensors. By incorporating an easily detectable “reporter” gene such as luciferase, a light-emitting enzyme that can be activated at low concentrations, such fish could be used as early detection environmental monitors. Researchers have also engineered zebrafish for enhanced sensitivity to different categories of environmental pollutants, including aromatic and halogenated hydrocarbons (dioxin, PCBs), electrophile response elements (quinones) and metal response elements (mercury, copper, cadmium and zinc) (Carvan et al. 2000). Fish with sensitivity to estrogens and retinoic acid are also being developed.



sockeye salmon

## ENHANCED GROWTH



One motivation for enhancing the growth rate of farmed fish is the economic benefit of improved production. In salmonids (salmon, trout, char), faster growth means that smoltification, or maturation, is reached more quickly. The first transgenic salmon contained a salmon growth hormone gene and resulted in salmon that grew, on average, three to five times faster than their non-transgenic counterparts (Hew and Fletcher, 1997). The effectiveness of the transgenic growth hormone gene derives from its ability to generate growth hormone in tissues other than the pituitary (Hew and Fletcher, 1997). Other investigators using the salmon growth hormone gene have obtained similar enhanced growth in tilapia (Martinez et al. 1996). To date, at least ten species of fish have been genetically modified for enhanced growth: the common carp, Crucian carp, channel catfish, loach, tilapia, pike, rainbow trout, Atlantic salmon, Pacific salmon and Sockeye salmon (Hew and Fletcher, 1997). None of these transgenic fish has been approved for commercialization.

Basic studies on shrimp at the University of California seek to understand the hormones that regulate the molting process, or shedding of their shells. Stimulation of molting increases growth rate. One approach would use genetic engineering to inhibit hormone production so growth continues and molting is accelerated, creating larger shrimp in a shorter period of time. Practical applications of this research may take as long as a decade.

### *Stress Tolerance*

Transgenic fish containing an “anti-freeze” gene from other fish able to survive freezing temperatures have shown enhanced ability to withstand cold, but have not become freeze-tolerant. The research continues, and is of particular importance in aquaculture. Modifying salmon for tolerance to sub-freezing temperatures, for instance, would extend the geographic range where caged salmon could be farmed. Likewise, modest increases in cold tolerance of farmed fish, such as catfish, offer potential advantages where occasional cold weather can cause massive fish kills. Other factors, primarily containment and risk to wild stocks of unintentional escape, are being studied before cold-adapted transgenic fish can be introduced to the environment.

### *Disease resistance*

From the aquaculture perspective, probably the most urgent concern is the control of disease and parasites, which tend to be species specific. Fish reared in the high-density conditions of aquaculture are generally more susceptible to infection than those found in the wild. Because a disease outbreak in aquaculture has potentially devastating economic consequences, improving control and disease resistance are high priorities for the industry.

In channel catfish, enteric septicemia of catfish (ESC) caused by the bacterium *Edwardsiella ictaluri* is the most serious disease in the industry and can be fatal to fish as quickly as five days after exposure (Thune et al. 1997). Researchers have sought resistant strains of catfish with little success and have found no effective treatment or preventive strategy (Camp et al. 2000). Intense research efforts to produce ESC-resistant catfish using genetic engineering strategies are underway, but further details are proprietary. Another major pathogen in channel catfish is the protozoan parasite *Ichthyophthirius multifiliis* that causes white spot disease. Fish can acquire immunity against the parasite, but genetic engineering technologies have the potential to enhance resistance (Lin et al. 1996).

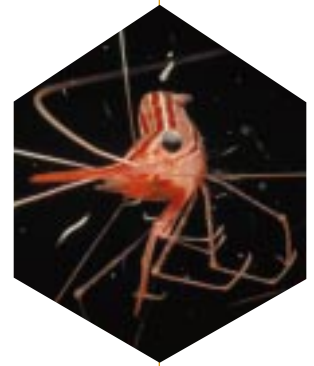
Salmonids host several different viruses, two of which, salmon anemia virus and infectious haematopoietic necrosis virus, cause extensive mortality. Several laboratories are seeking ways to create virus resistance in salmon using such different approaches as antisense and ribozyme technology, expression of viral coat proteins and expression of bactericidal or antimicrobial proteins such as lysozyme (Hew and Fletcher, 1997).

Looking again at shrimp, research mainly addresses two issues: Improved genetic stock and disease resistance. The primary pathogens afflicting shrimp are the viruses that cause yellow-head disease and white-spot syndrome. Disease problems are aggravated by the concentration of shrimp in aquaculture. Current work is aimed at the genetic improvement of tiger prawns, *Panaeus monodon*, and the development of virus resistance in the improved shrimp.

Researchers at the University of California, San Diego, have recently filed for a patent on creating transgenic shrimp with resistance to viruses. This group has pioneered the use of retroviruses for the delivery of genes across kingdoms and for simplifying gene cloning. They have worked with cultured shrimp, *Panaeus stylirostris*, using primarily retroviruses for genetic modifications (Shike et al. 2000). Another application of genetic technology is reflected in a patent filed in 1998 for the use of genetic markers to select shrimp with superior growth characteristics (Bagshaw et al. 1998).

### **Sterility**

There is interest in creating sterility in transgenic species as a way to control the environmental impact of engineered fish. A more “traditional” method already available for producing sterile fish is through polyploidy, or giving the fish more than the usual number of sets of chromosomes. This approach, however, does not guarantee complete sterility, particularly because not all the treated eggs produce sterile fish. Also, the technique cannot be applied to all species of fish and in some cases the fish do not perform as well as conventional fish. Thus, polyploidy is not always commercially feasible. As a result, scientists have also considered different genetic engineering approaches that regulate hormones to confer sterility in transgenic fish (Hew and Fletcher, 1997).



SUMMARY CHART OF AQUATIC ORGANISMS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
Farmed catfish	79	Eliminate enteric septicemia, a serious disease in the industry, which can be fatal to fish as soon as five days after exposure.	Use genetic engineering technologies to enhance resistance to the disease.	Undergoing investigation in laboratories
Farmed fish	78	Enhance growth to reduce production time and increase food availability.	Development of transgenic salmon, which contained a growth hormone gene that caused the fish to grow 3 to 5 times faster than their non-transgenic counterparts.	Undergoing investigation in laboratories  (To date, at least nine species of fish have been genetically modified for enhanced growth).
Farmed fish	78	Increase tolerance to cold to extend geographic range for fish farming and reduce losses in aquaculture from sudden climate change.	Improved cold tolerance has been accomplished by transferring the gene which controls freeze tolerance from the genome of another fish.	Undergoing investigation in laboratories
Farmed fish	79	Produce transgenic species that are sterile, thus avoiding the environmental risk of releasing the modified fish into the general aquatic population.	Scientists are working on genetic engineering approaches that regulate hormones to confer sterility in transgenic fish.	Undergoing investigation in laboratories  (A method called polyploidy is already available for producing sterile fish, but it does not guarantee 100 percent of the fish will be sterile).
Farmed shrimp	79	Curb pathogenic viruses that cause yellow-head disease and white-spot syndrome.	Current work uses retroviruses to genetically modify tiger prawns and for virus resistance.	Undergoing investigation in laboratories

## SECTION 3: GENETICALLY ENGINEERED INSECTS

Insect activity in agriculture can be both beneficial and adverse. Insects are essential for pollination, control of predators and food for other creatures. On the other hand, they transmit pathogens to plants and animals, consume plants and facilitate infestation by other organisms. Researchers are looking for alternatives to synthetic chemical pesticides, because of concern about the potential impact of pesticides on the environment and human health. More benign strategies to limit insect damage have evolved that use biological mechanisms already found in nature. Researchers are also genetically engineering insects in an effort to make them more effective predators, less virulent pests or ineffective carriers of disease. Efforts have centered on limiting insects' interaction with other pests, controlling their ability to survive in the wild and ensuring a high degree of target specificity, so that unwanted environmental effects can be minimized. This strategy has advanced slowly, in part because of environmental concerns and the importance of determining whether or not transgenic insects introduce unintended damage.

Genetic engineering techniques are also being applied to insects for other purposes, such as developing insect viruses for use as biopesticides and controlling the transmission of diseases like malaria.

### *Control of Insect Populations*

In 1995, researchers at the University of Florida created a transgenic mite, the Western orchard predatory mite (*Metaseiulus occidentalis Nesbitt*), by adding a marker gene to it (Hoy, 2000). The mites were not viable in Florida, although they were effective predators of the spotted spider mites that infest strawberries and ornamentals. Mites genetically improved through selective breeding techniques had previously been shown to be more effective in controlling pests in California almonds, where their use resulted in fewer pesticide applications. Thus, transgenic pesticide-resistant mites could become more highly effective predators.

The pink bollworm *Pectinophora gossypiella* is a destructive cotton pest that currently infests much of the Southwestern U.S. Pink bollworm larvae feed inside the growing cotton boll, destroying the cotton and costing farmers millions of dollars in crop losses and control costs. One existing pest management program involves releasing sterile bollworm moths to prevent the pest from reproducing successfully. In June 2000, the Animal and Plant Health Inspection Service (APHIS), a part of the U.S. Department of Agriculture, received an application for the field release of transgenic pink bollworm engineered to contain the green fluorescent protein from jellyfish. The purpose of this marker gene is to aid in monitoring and assessing the distribution of sterile pink bollworms used in insect control. This transformation is not intended to affect the behavior of the insect.





A 1996 application to the APHIS sought permission to test a transgenic nematode that occurs widely in nature, but is not a plant pest. The genetic transformations include insertion of a gene from jellyfish for the green fluorescent protein to make successful transformations easily detectable, and another gene to modestly increase nematode tolerance to heat. The latter was designed to ensure better survival against unexpected temperature changes during transportation and storage, not to confer additional survival value. While designed mainly for research purposes, such transformations could lead to other strategies for effective pest control.

### *Baculoviruses for biopesticides*

A novel approach to disarming insect pests employs naturally occurring insect viruses called baculoviruses. Baculoviruses infect only insects and have potential as biopesticides because they act only on invertebrates (arthropods), are highly specific and lethal to the target insect species and have no known resistance (Carstens, 1996). Baculoviruses act slowly, requiring 5 to 14 days to kill their host insect, and they are not highly virulent. The long period of activation means that substantial damage may accrue to crops before the pest is eliminated. In addition, baculoviruses are relatively expensive to produce compared with chemical pesticides. However, as pressures increase to abandon synthetic chemical pesticides and as pesticide resistance builds, such highly specific environmentally benign agents may become more economically attractive. Brazil has successfully produced baculoviruses from infected insects and used them for 10 years on as much as 40 percent of its soybean crop.



The U.S. Environmental Protection Agency (EPA) has approved wild baculoviruses for use as biopesticides for more than 25 years (EPA, 2000). Today, scientists have demonstrated ways to engineer baculoviruses to accelerate their activity. By incorporating hormones that affect insect behavior, such as reducing the insect's appetite or accelerating its metamorphosis, the insect may die sooner and inflict less damage. One example is a baculovirus engineered to contain the hormone Hez-HK-II, cloned from the corn earworm (Suszkiw, 1998). When insects ingest the virus, the virus replicates and produces large amounts of the hormone, which disrupts the larva's metabolism. The insect stops eating in about two days and dies. The baculovirus is quickly degraded by sunlight.

Another approach is to use the baculovirus to deliver a poison such as scorpion or mite toxin (Treacy et al. 2000). Extensive research and field testing using scorpion toxin engineered into a baculovirus showed the engineered virus to be as effective as Bt, or more so (Henahan undated). In field tests, the transgenic baculovirus did not affect spiders, honeybees or other beneficial insects and acted more quickly than native baculovirus in killing the target cotton bollworms and tobacco bud-worm pests.

### *Control transmission of pathogens*

One of the most compelling applications of genetic engineering in insects is the potential to reduce the ability of mosquitoes to spread disease. *Aedes aegypti* is the primary insect transmitting dengue and yellow fever; malaria is delivered with the bite of *Anopheles gambiae* mosquito. The ability to infect mosquitoes with transgenic viruses or to make them resistant to the malaria parasites they host could reduce mosquitoes' ability to transmit the diseases. Scientists aim to interfere with development or infectivity of the avian malaria parasite *Plasmodium gallinaceum* and hence make the mosquito, the parasite's host, less infectious (James et al. 1999, James 2000). Researchers have also done extensive work identifying and cloning genes specific to female mosquito salivary glands that interfere with blood coagulation and render delivery of the parasite ineffective. They have engineered a mosquito with immunity to the pathogen conferred by expression of defensin A, an insect immune factor that is produced when the insect feeds on blood (Kokoza et al. 2000). This kills the pathogen within the mosquito and interrupts its transmission to humans. In other studies, antibodies were used to block sporozoite invasion of the mosquito salivary glands, as well as block the infectivity of the sporozoites once the insect has contacted its human host. Researchers continue to involve new genes and strategies, compare effects in other mosquito species and develop new technologies for testing antiparasite genes.



SUMMARY CHART OF INSECTS

PRODUCT	PAGE	THE OBJECTIVE	THE SOLUTION	STATUS OF RESEARCH
Mites	81	Control insect pests.	Researchers have created a transgenic mite shown to be effective in controlling pests in California almonds.	Undergoing investigation in laboratories and research fields.
Mosquitoes	83	Control malaria and other mosquito-borne diseases by reducing the insect's ability to spread disease.  (Mosquitoes are the primary insects responsible for transmitting dengue and yellow fever, as well as malaria).	Infect mosquitoes with transgenic viruses that might make them resistant to the malaria parasites they host, kill the parasite, or otherwise interfere with its ability to infect humans.	Undergoing investigation in laboratories  (Scientists have engineered a mosquito that kills the parasite within its own body, thus preventing its transmission to humans).
Pink bollworm	81	Curb destruction of cotton crops.  (Bollworm larvae feed inside the growing cotton boll, destroying the cotton and costing farmers millions of dollars for control techniques and crop losses).	One existing pest management program involves releasing sterile bollworm moths to prevent the pest from reproducing successfully.	Being tested in research fields.

## CONCLUSION

Industry and university scientists are applying the new tools of biotechnology across a broad range of plants and animals for a wide variety of possible future uses. Much of this research remains at early stages. The broad scope of current research suggests challenges ahead. As new products emerge, state and federal regulators tasked with the responsibility to protect the environment and ensure the safety of food are likely to face novel questions. Public attitudes about biotechnology will be affected both by the adequacy of the regulatory response and the perceived benefits and risks of the particular products brought to market. In that context, understanding the potential uses of this technology can help us anticipate and prepare for these coming questions. Whether today's research projects become tomorrow's products will depend not only on continued scientific progress, but also on addressing the public's concerns about the technology and on the realities of the marketplace.

## GLOSSARY

**abiotic** arising from non-living organisms.

**adjuvant** a pharmacological agent added to a drug to enhance its effect.

*Agrobacterium tumefaciens* a bacterium used in the process of creating genetically modified plants.

**allele** one of two or more copies of a gene in plants or animals.

**allergen** something that causes an allergy.

**allergy** an excessively sensitive state involving the immune system as a result of exposure to certain substances, usually proteins.

**anadromous** fish that return from oceans to fresh water to spawn (e.g. salmon).

**anaphylaxis** an acute, severe, sometimes fatal allergic reaction affecting two or more body systems.

**antibiotic resistance markers** *see selectable marker gene*.

**antibody** neutralizing proteins generated in reaction to foreign proteins in the blood and producing immunity against certain microorganisms or their toxins.

**antigen** a substance which, when introduced into the body, is capable of inducing specific immune responses, including antibody formation or immunity against disease.

**antinutrient** an undesirable substance in food that can inhibit nutrient metabolism or absorption.

**antisense** in general, the complementary strand of a coding sequence of DNA. They interact with their complement and affect its function, usually preventing it from being translated.

*Arabidopsis* small plant of the mustard family commonly used to study plant genetics and plant genomics.

**base pair** two bases that form a rung of the "DNA ladder." A DNA strand consists of a chain of nucleotides, each of which is made of a molecule of sugar, a molecule of phosphoric acid and a molecule called a base. The four bases used in DNA (A, T, G and C) are the "letters" that spell out the genetic code (*see DNA*) that determine individual hereditary characteristics.

**biodiversity** the number and types of organisms in a region or environment.

**biological invasion** the introduction of an organism into a new environment or geographical region, followed by rapid multiplication and spread.

**biotechnology** a set of biological techniques developed through basic research and now applied to research and product development. In particular, the use of recombinant DNA techniques.

**biotic** relating to life or specific life conditions.

**broodstock** the group of males and females from which fish are bred for aquaculture.

*Bt*, *Bacillus thuringiensis* a soil bacterium that produces toxins that are deadly to some insects.

**carrying capacity** the maximum number of organisms of a given species that can be supported in a given area or habitat.

**cellularity** characterizes the physical and chemical properties of cells found within a specific tissue.

**cellulolytic** the capacity to digest the components of plant tissues and fibers used in making paper and textiles.

**chimera** an organism containing two or more genetically distinct cell or tissue types.

**chromatography** a technique for separating complex mixtures of chemicals or proteins into their various constituents.

**chromosome** a threadlike strand of DNA and associated proteins that is in the nucleus of a cell.

**clone** an exact replica of another; organisms asexually derived by division from a single cell.

**confined field trial** field trial carried out with specific restrictions on location, plot size, etc.

**congeneric** belonging to the same generation.

**conspecific** belonging to the same species.

**cross-compatible** the ability of two related organisms to exchange genes through sexual reproduction.

**Cry** designation of a gene encoding insecticidal crystal proteins in the soil bacterium *Bacillus thuringiensis*.

**delta-endotoxins** *Bt* insecticidal proteins.

**developmental asynchrony** a pattern of development that allows different sub-populations to reach sexual maturity at different times.

**DNA (deoxyribonucleic acid)** the molecule that encodes genetic information.

**DNA sequence** the specific order of bases in a DNA molecule, whether in a fragment of DNA, a gene, a chromosome or an entire genome.

**dormancy** a delay in the growth of viable seeds because of unfavorable environmental conditions.

**eclosion** the emergence of an insect larva from the egg.

**ecological amplitude** the range of environmental conditions in which an organism can survive and reproduce.

**epiphytic** one organism living within or upon another without causing harm.

**epistatic** a dependent relationship between genes.

**epitopes** separate antigenic areas within a given protein.

**erucic acid** a fatty acid having 22 carbons and one double bond that is common to traditional rapeseed oil. Canola oil contains less than 2 percent erucic acid.

***Escherichia coli* (*E. coli*)** a bacterium found in the intestine of animals and humans used extensively in genetic engineering. *E. coli* in undercooked meat can be fatal to humans if digested.

**Exotic non-native;** refers to an organism that has been introduced into an area.

**expression (as in gene expression)** generation of a messenger RNA (mRNA) copy of a gene encoded in an organism's DNA.

**fibroblasts** irregularly shaped, branching cells distributed throughout vertebrate connective tissue.

**field trial** tests of the ability of a new crop variety to perform under normal cultivation conditions.

**fitness** the genetic contribution of an individual to the next generation.

**flow cytometry** a technique for rapid automatic separation of suspensions of living cells into defined sub-populations.

**gamete** the products of cells divided in sexually reproducing organisms.

**gene** the fundamental physical and functional unit of heredity.

**gene construct** a sequence made by splicing several genes together.

**gene flow** the movement of genes from one population to another.

**gene gun** a device for propelling DNA molecules into living cells.

**gene knockout strategy** used to determine the function of a specific gene by inactivating (knocking out) that gene in the intact organism and then studying the consequences.

**gene product** proteins resulting from the transcription of a gene.

**gene stacking** the process of inserting two or more different genes into an organism.

**genetic drift** the random change in gene frequencies in populations.

**genome** the master blueprint for the total set of an organism's genes.

**genomics** the study of genomes.

**genotype** the hereditary constitution of an organism.

**germplasm** hereditary material.

**gill irrigation** oxygen transfer from water to the blood in fish.

**glycoalkaloids** toxic secondary organic compounds found in the potato family.

**glycolysis** the process by which sugars are converted to acids.

**heat-labile** easily destroyed by heat.

**heterozygous** having two different genes at a given location on the chromosome map.

**homology** structural similarity due to descent from a common ancestor or form.

**hybrid** the offspring produced by breeding plants or animals of different varieties, species or races.

**immunoglobulin (Ig)** *see antibody.*

**immunoglobulin E (Ig E)** an antibody produced by an allergen which has specific structural and biological properties.

*in utero* within the uterus.

*in vitro* outside the living body; in a laboratory or test tube.

*in vivo* within the living body.

**intellectual property (IP)** the legal rights associated with inventions, artistic expressions and other products of the imagination (patent, copyright and trade-mark law).

**introgression** movement of a new gene into a population.

**irradiation** a process involving the use of low levels of radiation to reduce the presence of disease-causing agents, for example during the processing of food products.

**leptokurtic** a normal statistical curve that is quite steep or sharp.

**lipogenesis** the conversion of carbohydrates and organic acids to fat.

**luciferase** an enzyme from firefly tails that catalyses the production of light.

**lysozyme** a relatively small enzyme that catalyzes the breakdown of cell walls of certain bacteria.

**mass spectrometry** a technique for determining the composition of a molecule and its fragments.

**mating system** the mode of transmission of genes from one generation to the next through sexual reproduction.

**meiosis** cell division by which eggs and sperm are produced.

**methanogenesis** the process of creating methane gas during metabolism.

**mitosis** the process by which the equal partitioning of replicated chromosomes into two identical groups takes place.

**mutagenesis** the process of changing the DNA base sequence at a specific site.

**mycorrhizae** a group of fungi that grow in close association with plant roots.

**ontogenetic delay** a delay in the course of growth and development to maturity.



**ontogeny** the course of growth and development of an individual to maturity.

**operons** gene clusters under common control in bacteria.

**organoleptic** the taste and aroma properties of a food or chemical.

**outcrossing** mating between different individuals/species.

**phage** a virus specifically attacking bacteria.

**phenotype** the genetically and environmentally determined appearance of an organism.

**plasmids** non-chromosomal pieces of DNA that code for a subset of cellular functions.

**pleiotropic response** multiple changes to an organism's appearance associated with a single change at the genetic level.

**pollination** the transfer of pollen between the male germ cell of a plant (anther), and the female reproductive system (stigma) in seed plants.

**polyphagous** herbivores that feed on a wide variety of host plants from many different families.

**precautionary principle** an approach to the management of risk when scientific knowledge is incomplete.

**prion** normal cell protein present on nerve cell membranes. It is found in most mammals, but its normal function is unclear. Abnormal prions are thought to cause certain diseases, including mad cow disease.

**proteinase inhibitors** a class of proteins capable of inhibiting insect feeding.

**proteome** the complete complement of proteins made by a given species in all its tissues and stages.

**proximate analysis** chemical analysis of the main constituents of food.

**rate-limiting enzyme** an enzyme that controls the overall flow of activity through a sequence of reactions.

**recombinant DNA (rDNA)** DNA molecules created by splicing together two or more different pieces of DNA.

**reporter gene** a gene whose gene product is easily detected.

**restriction enzymes** DNA-cutting enzymes that recognize and bind to specific short sections of DNA sequence.

**rhizobacteria** bacteria closely associated with plant roots.

**rhizosphere** the soil zone immediately surrounding a plant root system.

**salmonids** members of the fish family "Salmonidae," including salmon, trout and char.

**secondary metabolite** a chemical produced by a plant that does not appear to have a direct role in its growth.

**secondary pests** those species within an ecosystem that are normally kept in check by natural enemies.

**seed shattering** the spontaneous dispersal of mature seed from a plant following ripening.

**selectable marker gene** a gene whose product protects the cell containing it from a toxic chemical (e.g. antibiotic) used to identify the modified cells.

**selfing** mating by a single individual containing both female and male reproductive systems.

**single nucleotide polymorphism (SNP)** single-base variations in the genetic code between different individuals of the same species.

**smoltification** the combination of physiological, behavioral and morphological changes that members of the fish family "salmonid" experience when they migrate from fresh water rivers into the ocean.

**somaclonal variation** altered characteristics in plant tissues by extended growth in a laboratory test tube (possibly a form of mutation).

**totipotency** the ability to regenerate a fully differentiated organism from a single cell.

**transcription** the synthesis of RNA (ribonucleic acid) molecules concerned in translating the sequence of DNA into the structure of protein molecules.

**transgene** a gene from one organism inserted into the genome of another.

**transgenic** an organism that has had genes from another organism put into its genome through recombinant DNA techniques.

**transposons** short stretches of DNA with the capacity to move between different points within a genome.

**triploidy** three copies of genetic information in each cell rather than the normal two copies found in most plants and animals.

**vaccinogen** antigens capable of invoking a protective immune response.

**vector** any organism or DNA construct that enables movement or transmission of another organism or gene.

**volunteer plant** crop plants that persist for a few seasons without deliberate cultivation.

**wide cross** a sexual cross between distantly related species that normally would not breed.

**xenotransplantation** the surgical removal of an organ or tissue from one species and transplantation into a member of a different species.

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