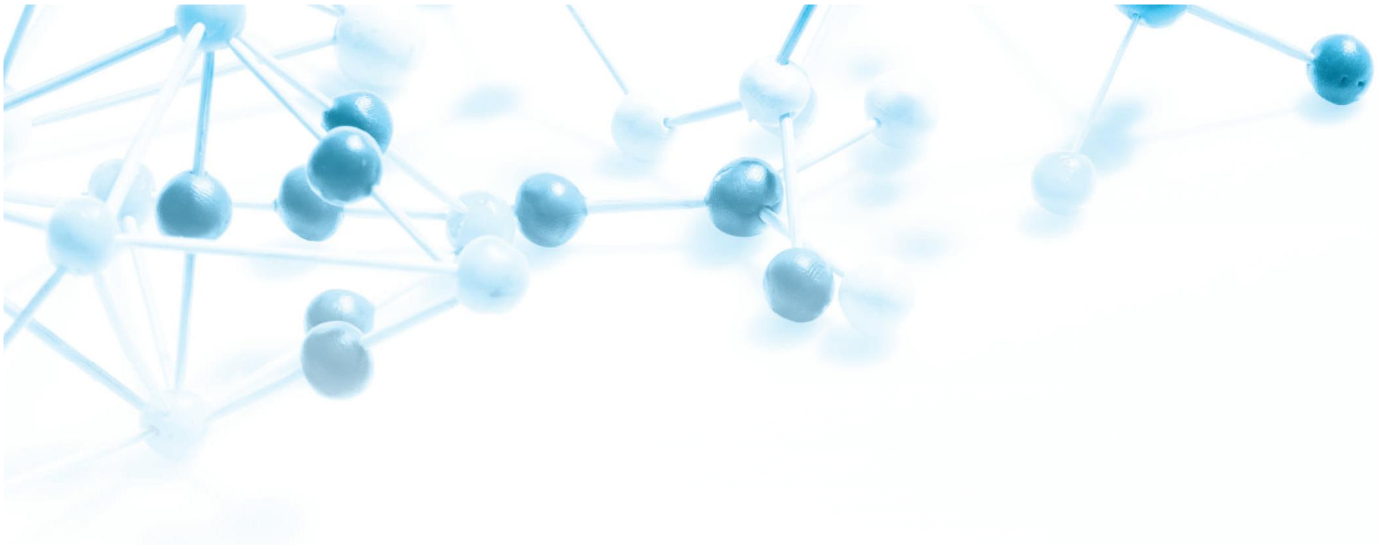
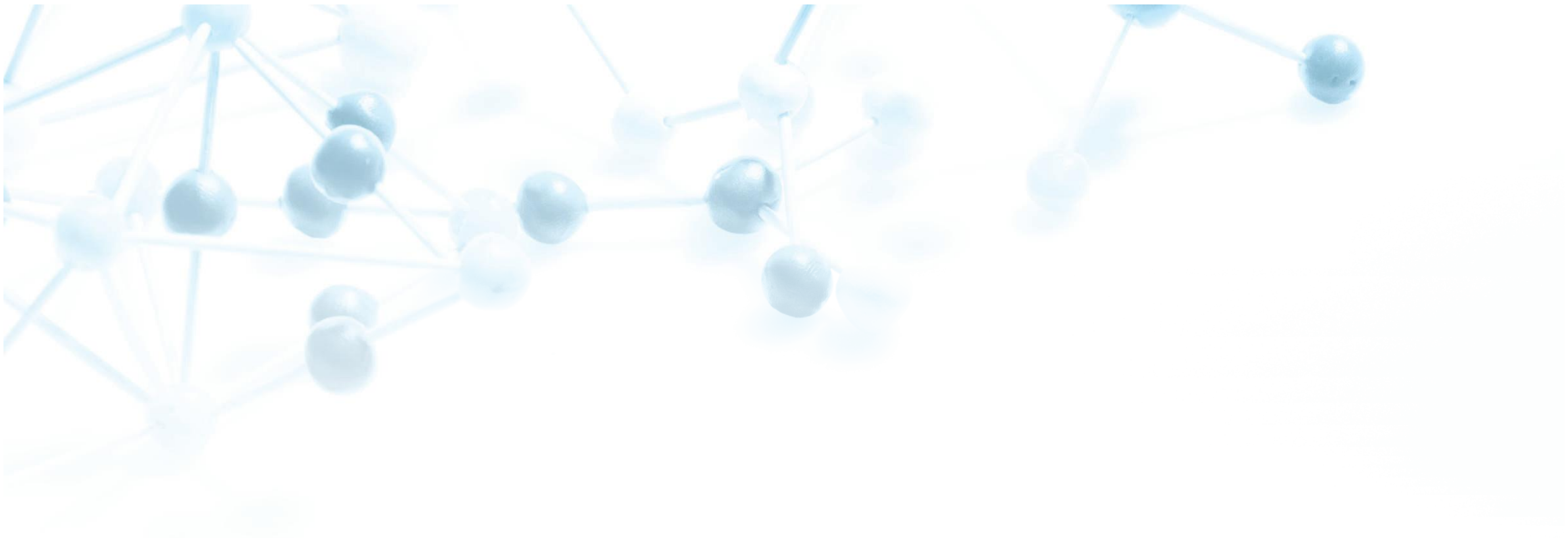


Plant Biotechnology

From Chapter 6 of *Introduction to Biotechnology*, Third Edition. William J. Thieman, Michael A. Palladino.



Plant Biotechnology

After completing this chapter, you should be able to:

- Describe the impact of biotechnology on the agricultural industry.
- Discuss the limitations of conventional crossbreeding techniques as a means of developing new plant products.
- Explain why plants are suitable for genetic engineering.
- List and describe several methods used in plant transgenesis, including protoplast fusion, the leaf fragment technique, and gene guns.
- Describe the use of *Agrobacterium* and the Ti plasmid as a gene vector.
- Define antisense technology and give an example of its use in plant biotechnology.
- List some genetically engineered crops.
- Outline the environmental impacts, both pro and con, of biotechnologically enhanced crops.
- Describe the phytochemical opportunities in plant biotechnology.
- Outline several ways in which biotechnology has the potential to reduce hunger and malnutrition around the world.

Bioengineered plants being evaluated for quality control at a plant biotechnology company.

Bill Thieman.

The red juicy tomatoes on sale at the grocery store are a true feat of engineering. Countless generations of selective breeding have transformed a puny acidic berry into the delicious fruit we know today. In the last few decades, conventional hybridization (by cross-pollination) has produced tomatoes that are easy to grow, quick to ripen, and resistant to disease. Pioneering efforts in biotechnological research have created tomatoes that can stay on store shelves longer without losing flavor. The future holds the possibility of an even more amazing transformation for the tomato: it, and other foods, could someday supplement or possibly replace inoculation as a means of vaccination against human disease. For example, researchers have successfully vaccinated volunteer patients against Norwalk virus in clinical trials by having them eat transgenic potatoes that express the vaccine.

In this chapter, we will consider the role of biotechnology in agriculture. First, we'll survey the industry to clarify the motives driving biotech research and development. We will then look more closely at the specific methods used to exchange genes in plants, including how bioengineering can protect crops from disease, reduce the need for pesticides, and improve the nutritional value of foods. Next, we'll examine the future of plant-based biotechnology products, from pharmaceuticals to petroleum alternatives. Finally, we'll consider the environmental and health concerns surrounding plant biotechnology.

FORECASTING THE FUTURE

The transfer of genes to plants has been firmly established as a reliable method to meet the need of future generations for food and energy. Fourteen million farmers in 25 countries currently benefit from genetically engineered (GE) crops, according to the International Service for the Acquisition of Agri-biotech Applications (ISAAA). There are limitations, however, to the entry of new plants to the market, and only a small number of crops have done so. These limitations are due in part to the fact that most of the thousands of existing patents for transgenic plants are held by only three companies: Syngenta, Monsanto, and DuPont. The high royalty fees and restrictions these companies have in place make the costs of developing crops based on these patents prohibitive for most farmers and researchers. This has led several biotech companies to develop their own novel gene transfer methods, and now nonprofit research groups such as the Public Sector Intellectual Property Research for Agriculture (PIPRA) are involved. The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) is also assisting. They have launched a pilot program to improve compliance with APHIS field trials and restricted the movement of regulated organisms by certifying companies through the new Biotechnology Quality Management Service (BQMS). A company that qualifies for BQMS certification can overcome many of the expensive barriers to marketing GE crops by testing the effectiveness of transgenic plants in the field and focusing on products that are safe, affordable, and driven by public need. The future of plant biotechnology is brighter with changes that make producing genetically engineered crops less expensive and improve consumer perceptions of new crops.

1 The Future of Agriculture: Plant Transgenics

Over the past 40 years, the world population has nearly doubled while the amount of land available for agriculture has increased by a scant 10%. Yet we still live in a world of comparative abundance. In fact, world food production per person has increased 25% over the past 40 years. How has it been possible to feed so many people with only a marginal increase in available land? Most of that improved productivity has depended on crossbreeding methods developed hundreds of years ago to provide animals and plants with specific traits. Recently, however, the development of new, more productive crops has been accelerated by the direct transfer of genes, as shown in **Figure 1**.

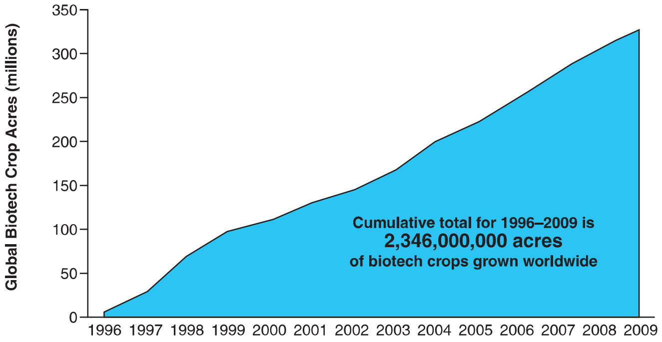


FIGURE 1 Total Acres of Biotech Crops Grown in 2009 Between 1996 and 2009, more than 2.3 billion acres of biotech crops were successfully grown as a result of approximately 70 million repeat decisions by farmers to grow these crops. Almost 93% (13 million) of the 14 million farmers growing biotech crops in 2009 were small and resource-poor, living and working in developing countries such as China, India, the Philippines, and South Africa. In terms of total acreage of biotech crops planted, the developed world (with its larger farms) planted 40% of the acreage, while developing countries accounted for 60%. 2009 is the latest year for which figures are available.

Courtesy of International Service for the Acquisition of Agri-biotech Applications.

Plant transgenesis (the direct transfer of genes to plants) permits innovations that are impossible to achieve with conventional hybridization methods. A few developments that have significant commercial potential are plants that produce their own pesticides, plants that are resistant to herbicides, and even bioproducts like plant vaccines and biofuels. Because producing transgenic plant proteins is relatively easy and the quality of the proteins is reasonably good, the prospects for future research and development in these areas look especially bright. For example, through classic breeding, the average strength of cotton fibers has been steadily increasing by about 1.5% per year. Biotechnology has dramatically accelerated this pace. By inserting a single gene, the strength of one major upland cotton variety was increased by 60%.

The prevalence of transgenic crops in world agriculture is on the rise. In 2005, the billionth acre was planted, and only 2 years later, in 2007, the second billionth was planted. Although the transgenic revolution began in the United States and other developed nations and the United States still dominates the market, other countries have continued to add transgenic crops in recent years. In 2008, some 13.3 million farmers in 25 countries planted transgenic crops—90% of them in developed countries—but just a year later, in 2009, world farmers planted 173 million acres of soybeans (70% of the total planted), 40 million acres of corn (40% of the total planted), 25 million acres of cotton (10% of the total planted), and 15 million acres of canola (5% of the total planted). To these staple crops, herbicide-resistant sugar beets were added in the United States and Canada in 2009 (59% of the beet crop was transgenic in its first year). New biotech crops such as alfalfa, wheat, and potatoes will come to the market within 10 years, as well as high-oleic-acid soybeans and vitamin A–enriched rice. Beyond foods and feed crops, plant-produced vaccines and bioplastics as well as enhanced phytoremediation plants will reach the market in the next 10 years.

Although food crops are only one aspect of biotechnology's impact, they have been the focus of considerable controversy worldwide. On the one hand, hunger continues to plague much of the world. This reality is a compelling argument for the rapid development of more productive and nutritious crops. On the other, some sectors are concerned that experimentation could be harmful to the environment and human health.

The debate is far from over. To develop an informed opinion, decision makers must understand the science behind these new products, analyze the products themselves, and be knowledgeable about the regulations that exist to monitor biotechnology research. In any case, it is unlikely that the revolution in agricultural biotechnology will stop. Protests or not, biotechnology plant products will play a key role in our society. The next section describes the methods used to create new agricultural products.

2 Methods Used in Plant Transgenesis

Genetic manipulation of plants is not new. Ever since the birth of agriculture, farmers have selected for plants with desired traits. Since then, technologies have been ever evolving to meet the world's increasing world for agricultural products.

Conventional Selective Breeding and Hybridization

Even though careful crossbreeding has continued to improve plants through the millennia—giving us larger corn cobs, juicier apples, and a host of other modernized crops—the methods of classic plant breeding are slow and uncertain. Creating a plant with desired characteristics requires facilitating a sexual cross between two different plant lines and repeated backcrossing between the hybrid plant's offspring and one of the original parent plants. Isolating a desired trait in this fashion can take years. For instance, Luther Burbank's development of the white blackberry involved 65,000 unsuccessful crosses. In fact, plants from different species generally do not hybridize, so a genetic trait cannot be isolated and refined unless it already exists in a plant strain.

Transferring genes by crossing plants is not the only traditional way to create plants with desirable features. **Polyploids** (multiple chromosome sets greater than normal, usually more than 2N or one set of chromosomes from each parent) plants have been used for many years as a means to increase desirable traits (especially the size) of many crops including watermelons, sweet potatoes, bananas, strawberries, and wheat. The utilization of the drug colchicine (which keeps a cell from dividing after it has double chromosomes) followed by hybridization is a means of introducing commercially important features of related species into potentially new cultivated crops (refer to **Figure 2**). This process results in hybrid plants where whole chromosome sets, rather than single genes, from related plants can be transferred. The additional chromosomes often produce plants that produce fruits and vegetable much larger than native wild varieties.

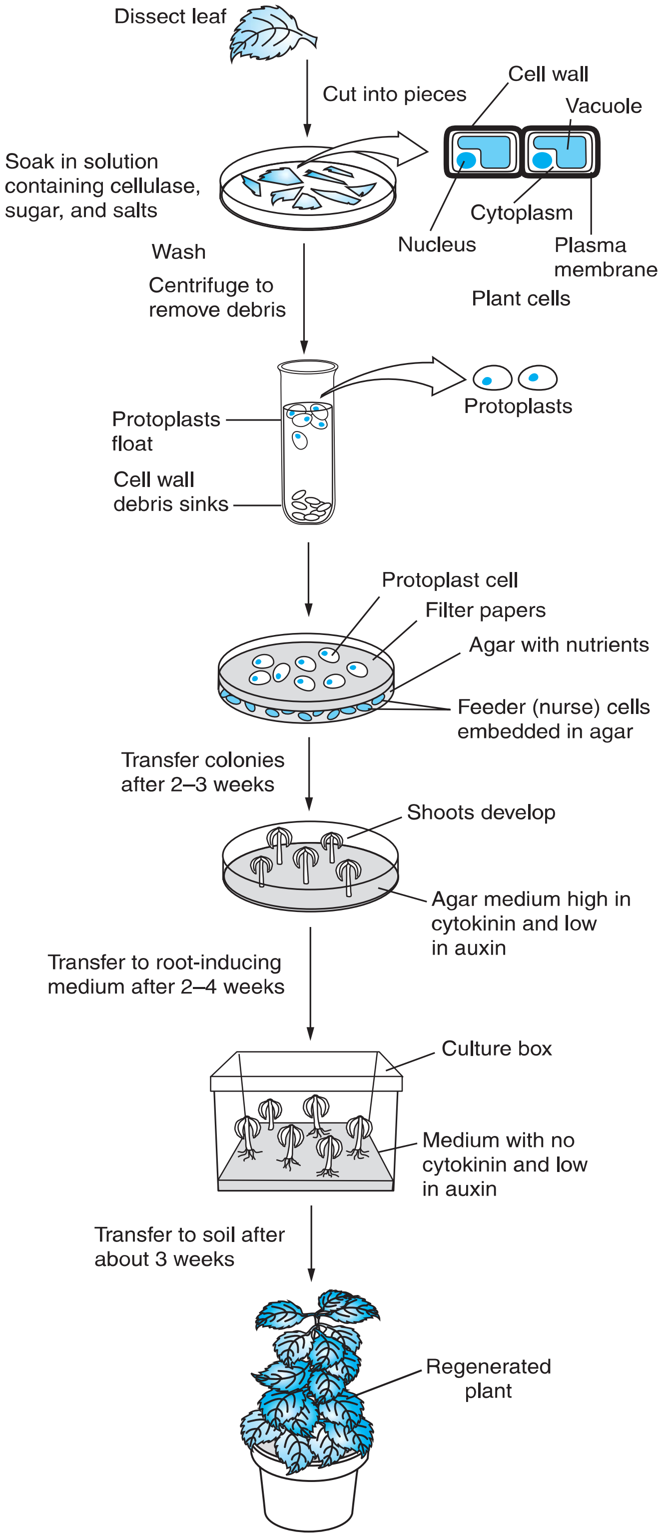


FIGURE 2 **Protoplast Fusion and Regeneration of a Hybrid Plant** After dissecting a plant leaf, it is possible to create protoplasts by digesting the cell wall with the enzyme cellulase. To create a hybrid plant, protoplasts from different plants are fused by culturing them in a sterile medium that stimulates shoots (cytokinin) and roots (auxin).

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Cloning: Growing Plants from Single Cells

Plant cells are different from animal cells in many ways, but one characteristic of plant cells is especially important to biotechnology: many types of plants can regenerate from a single cell. The resulting plant is a genetic replica—or clone—of the parent cell. Animals can be cloned too, of course, but the process is more complicated. This natural ability of plant cells has made them ideal for genetic research. After new genetic material is introduced into a plant cell, the cell rapidly produces a mature plant, and the researcher can see the results of the genetic modification in a relatively short time. Next we consider some of the methods used to insert genetic information into plant cells.

Protoplast fusion

When a plant is injured, a mass of cells called a **callus** may grow over the site of the wound. Callus cells have the capability to redifferentiate into shoots and roots, and a whole flowering plant can be produced at the site of the injury. You may have taken advantage of this capability if you have ever "cloned" a favorite house plant by rooting a cutting.

The natural potential of these cells to be reprogrammed makes them ideal candidates for genetic manipulation. Like any plant cells, however, callus cells are surrounded by a thick wall of cellulose, a barrier that hampers any uptake of new DNA. Fortunately the cell wall can be dissolved with the enzyme cellulase, leaving a denuded cell called a **protoplast.** The protoplast can be fused with another protoplast from a different species, creating a cell that can grow into a hybrid plant. This method, called **protoplast fusion,** as shown in Figure 2, has been used to create broccoflower, a fusion of broccoli and cauliflower, as well as other novel plants.

Leaf fragment technique

Genetic transfer occurs naturally in plants in response to some pathogenic organisms. For instance, a wound can be infected by a soil bacterium called *Agrobacterium tumefaciens* (recently reclassified as *Rhizobium radiobacter* by genome analysis, but the name *Agrobactor* is still in common usage; we use it throughout our discussion). This bacterium contains a large circular double-stranded DNA molecule called a *plasmid,* which triggers an uncontrolled growth of cells (tumor) in the plant. For this reason, it is known as a **tumor-inducing (TI) plasmid.** The resulting tumor is known as **crown gall.** If you have

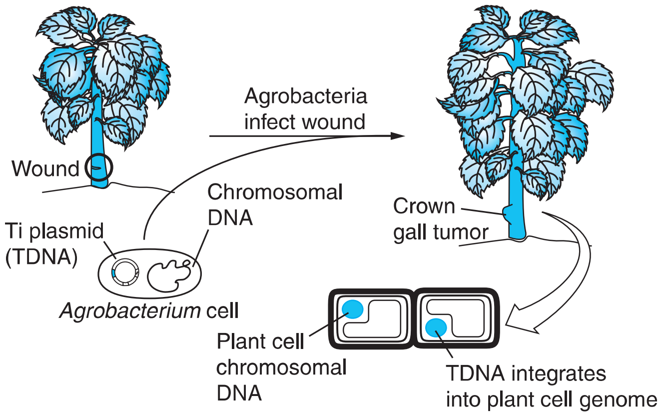

FIGURE 3 **Process of Crown Gall Formation** The Ti plasmid of *Agrobacter* causes "crown galls" in susceptible plants. Through genetic engineering, it is possible to silence the tumor-inducing genes and insert desirable genes into the plasmid, making it a vector for gene transfer.

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ever seen a swelling on a tree or rose bush, you may have seen *Agrobacter*'s effects (see **Figure 3**).

The bacterial plasmid gives biotechnologists an ideal vehicle for transferring DNA. To put that vehicle to use, researchers often employ the **leaf fragment technique.** In this method, small discs are cut from a leaf. When the fragments begin to regenerate, they are cultured briefly in a medium containing genetically modified *Agrobacter,* as shown in **Figure 4**. During this exposure, the DNA from the TI plasmid integrates with the DNA of the host cell, and the genetic payload is delivered. The leaf discs are then treated with plant hormones to stimulate shoot and root development before the new plants are planted in soil.

The major limitation to this process is that *Agrobacter* cannot infect **monocotyledonous** plants (plants that grow from a single seed embryo) such as corn and wheat. **Dicotyledonous** plants (plants that grow from two seed halves)—such as tomatoes, potatoes, apples, and soybeans—are all good candidates for the process, however.

Gene guns

Instead of relying on a microbial vehicle, researchers can use a **gene gun** to literally blast tiny metal beads coated with DNA into an embryonic plant cell, as shown in **Figure 5**. The process is rather hit or miss—and more than a little messy—but some of the plant cells will adopt the new DNA.

Gene guns are typically used to shoot DNA into the nucleus of a plant cell, but they can also be aimed at the **chloroplast,** the part of the cell that contains chlorophyll. Plants have between 10 and 100 chloroplasts per cell, and each chloroplast contains its own bundle of DNA. Whether they target the nucleus or the chloroplast, researchers must be able to identify the cells that have incorporated the new DNA. In one common approach, they combine the gene of interest with a gene that makes the cell resistant to certain antibiotics. This gene is called a **marker gene** or reporter gene. After firing the gene gun, the researchers collect the cells and try to grow them in a medium that contains a specific antibiotic. Only the genetically transformed cells will survive. The antibiotic-resistant gene can then be removed before the cells grow into mature plants, if the researcher so desires.

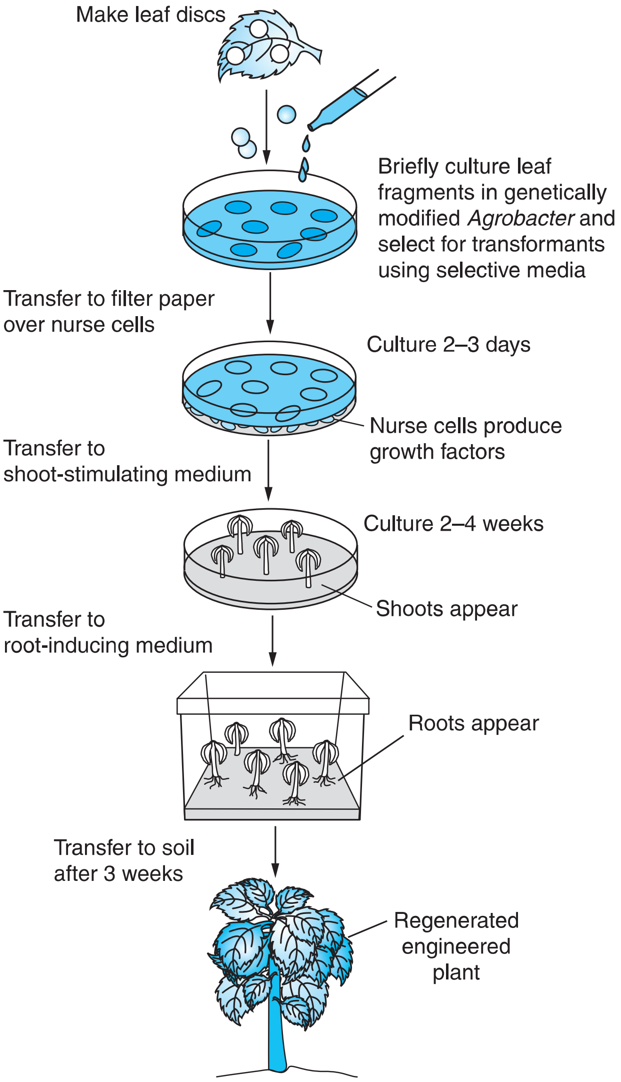

FIGURE 4 **Transfer of Genetically Modified Ti Plasmid to Susceptible Plants through Tissue Culture**

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Chloroplast engineering

The chloroplast can be an inviting target for bioengineers. Unlike the DNA in a cell's nucleus, the DNA in a

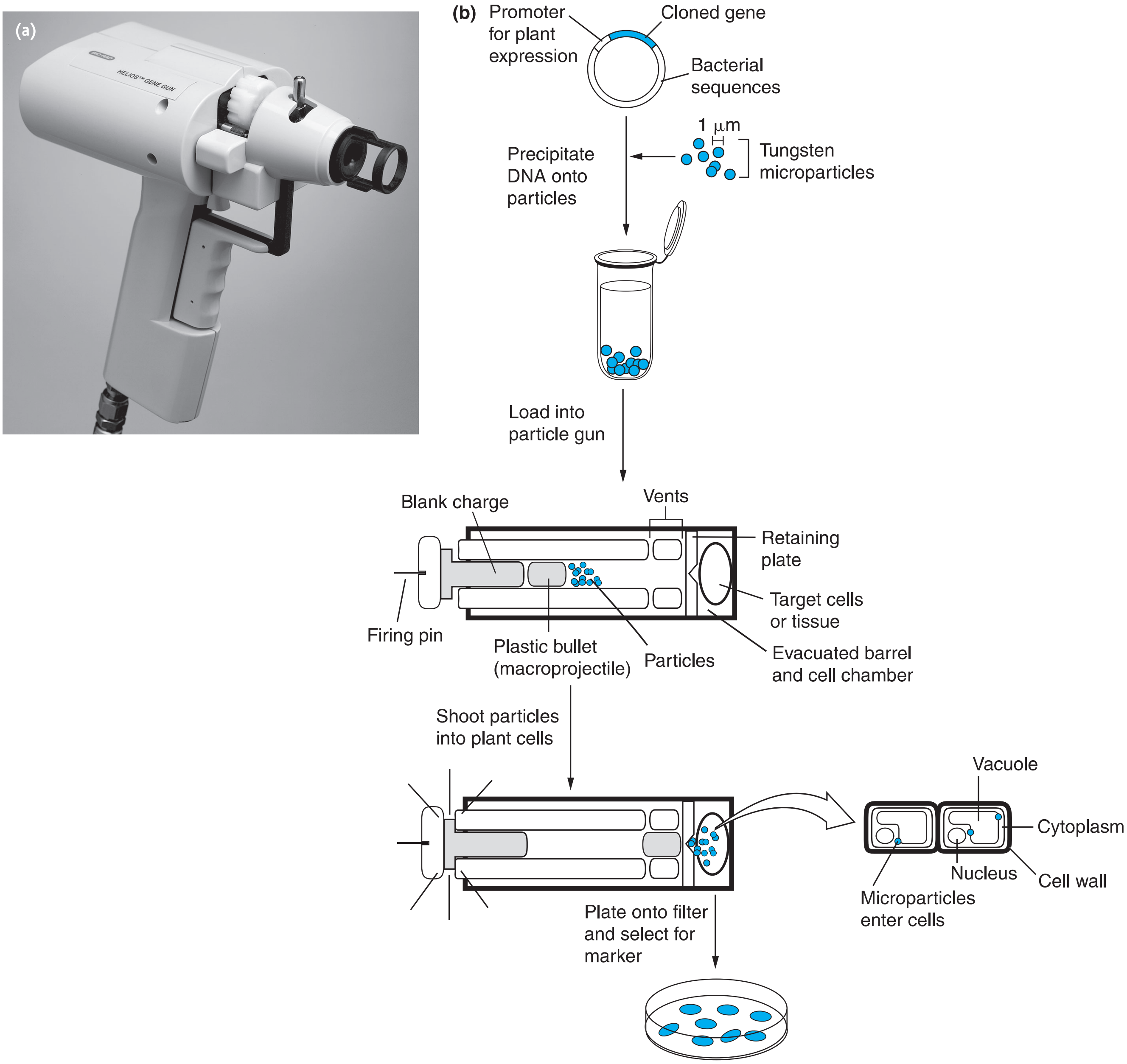

FIGURE 5 **Gene Guns** The "bullets" of the gene gun (a) are tungsten beads 1 micrometer in diameter. (b) DNA is coated on the surfaces of the beads and fired from guns with velocities of about 430 meters per second. The targets can include suspensions of embryonic cells, intact leaves, and soft seed kernels.

(a): Bio-Rad Laboratories Diagnostics Group.

chloroplast can accept several new genes at once. Also, a high percentage of genes inserted into the chloroplast will remain active when the plant matures. Another advantage is that the DNA in the chloroplast is completely separate from the DNA released in a plant's pollen. When chloroplasts are genetically modified, there is no chance that transformed genes will be carried on the wind to distant crops. This process is shown in **Figure 6**.

Antisense Technology

Recall the tomato. It is red, juicy, and tasty—and extremely perishable. When picked ripe, most tomatoes will turn to mush within days. But the Flavr Savr tomato, introduced in 1994 after years of experimentation, stayed ripe and fresh for weeks. The Flavr Savr tomato was the first genetically modified food approved by the U.S. Food and Drug Administration

(FDA) and, although it was not an economic success and is no longer available, it is common to find other varieties of genetically modified food, including tomatoes, on the market today. These foods were developed using antisense technology, in which a gene that encodes for a specific trait is removed from plant cells, used to produce a complementary copy of itself, and transferred back to the original cells using *Agrobacter* as a vector organism.

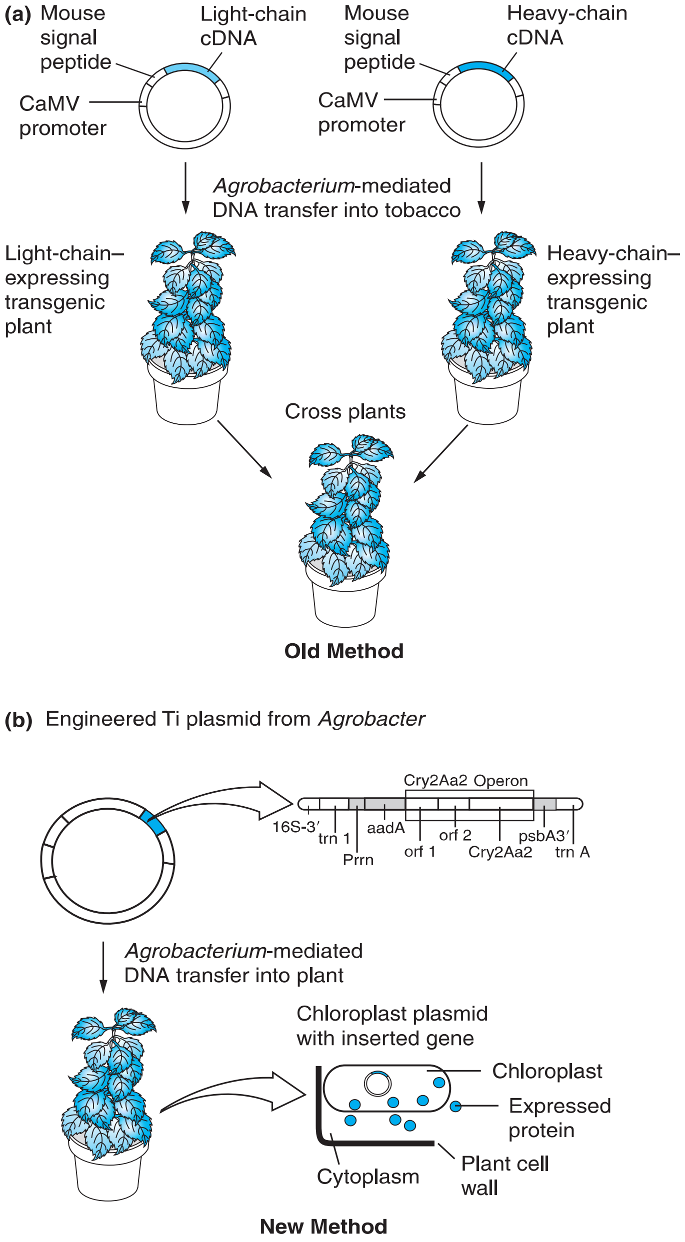

FIGURE 6 Old versus New Method for Cloning Multiple Genes into Plants In the past, when more than one gene was to be expressed, two plants—each with its own inserted gene—had to be produced. (a) Standard crossing by pollen transfer was required to produce the hybrid plant. (b) Now it is possible to insert more than one gene by stacking them in the chloroplast DNA.

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Ripe tomatoes normally produce the enzyme **polygalacturonase** (or PG), a chemical substance that digests pectin in the wall of the plant. This digestion induces the normal decay that is part of the natural plant cycle. The gene that encodes PG was identified, removed from plant cells, and used to produce a complementary copy of itself. Using *Agrobacter* as a vector, the gene was then transferred into tomato cells. Once in the cell, the gene encoded an mRNA molecule (**antisense molecule)** that united antisense RNA with and inactivated (complementary sequence) the normal mRNA molecule (the sense molecule) for PG production. With the normal mRNA inactivated, no PG is produced, no pectin is digested, and natural "rotting" is slowed. This process is shown in **Figure 7**.

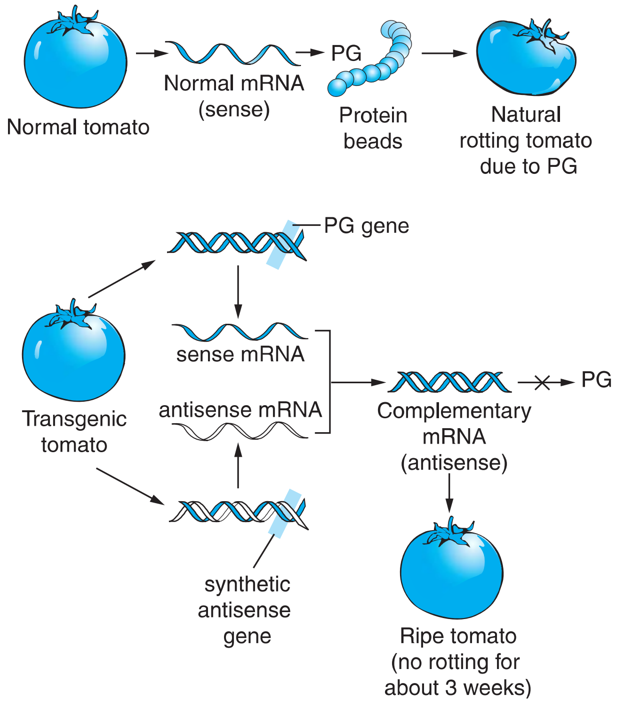

FIGURE 7 One of the First Commercial Transgenic Plant Products Tomatoes that rot only slowly are produced by first isolating the gene that encodes polygalacturonase. This gene encodes normal (sense) mRNA, which is translated into PG. After inducing it to produce a cDNA (complementary DNA) counterpart, it is possible to insert the PG into a vector for transfer to tomatoes. Transgenic plants with the new insert will produce PG mRNA and antisense mRNA, canceling the production of PG. This slows the rotting process and produces a tomato that will last for about 3 weeks after ripening.

Gene Silencing: An Alternative to Antisense Technology

We can expect to see more gene silencing advancements in the future. Researchers are working on a potato that resists bruising. They have removed a gene responsible for producing an enzyme that promotes color changes in peeled potatoes. It is a subtle improvement, but market analyses have shown that consumers prefer potatoes that are not bruised by handling. Researchers are also working on ways to increase the protein content of plants like potatoes by using genes from chickens. This improvement in the nutritional value of a common food could help many people worldwide to get the protein they need in their diets.

A Purdue University researcher has discovered a fountain of youth for tomatoes that extends shelf life by about a week. He found that by adding a yeast gene, he could increase production of a compound that slows aging and delays microbial decay in tomatoes. The organic compound spermidine is a polyamine found in all living cells. Polyamines such as spermidine enhance the nutritional and processing quality of tomato fruits. Fully ripe tomatoes from those plants, compared with nontransgenic plants, last about 8 days longer before shriveling. Symptoms of decay associated with fungi were delayed by about 3 days. Shelf life is a major problem for any type of produce, especially in countries such as those in Southeast Asia and Africa, which cannot afford controlled-environment storage.

3 Practical Applications

Protecting plants from viruses, insects, and weeds while improving their nutrition and properties is the goal of commercial growers, and biotechnology has produced some interesting examples.

Vaccines for Plants

Crops are vulnerable to a wide range of plant viruses. Infections can lead to reduced growth rates, poor crop yields, and low crop quality. Fortunately farmers can protect their crops by stimulating a plant's natural defenses against disease with vaccines. Just like a human vaccine for polio, plant vaccines contain dead or weakened strains of the plant virus that turn on the plant's version of an immune system, making it resistant to the real virus.

Vaccinating an entire field is not easy, but it is no longer necessary. Instead of injecting the vaccine, the vaccine can be encoded in a plant's DNA. For example, researchers have recently inserted a gene from the tobacco mosaic virus (TMV) into tobacco plants. The gene produces a protein found on the surface of the virus and, like a vaccine, turns on the plant's immune system. Tobacco plants with this gene are immune to TMV. The tomato mosaic virus is similar enough to be stopped by this technique. **Figure 8** shows this process.

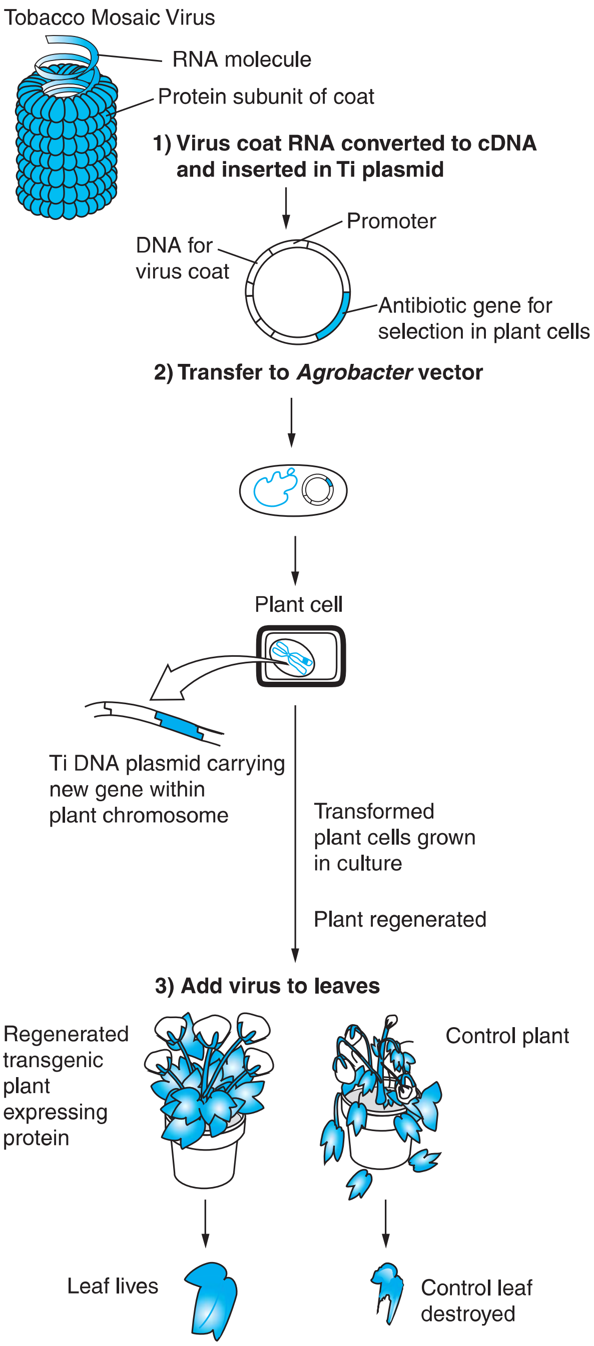

FIGURE 8 **Plant Vaccines** Transgenic plants expressing the TMV coat protein (CP) were produced by *Agrobacterium*-mediated gene transfer. When the generated plants expressing the viral CP were infected with TMV, they exhibited increased resistance to infection. Whereas control plants developed symptoms in 3 to 4 days, transgenic CP-expressing plants resisted infection for 30 days.

Genetic vaccines have already proven themselves in a wide variety of crops. The development of disease-resistant plants has revitalized the once-ravaged papaya industry in Hawaii. In addition, disease and pest-resistant strains of potatoes offer many advantages to both growers and consumers.

Genetic Pesticides

For the last 50 years, many farmers have relied on a natural bacterial pesticide to prevent insect damage to crops. *Bacillus thuringiensis* **(Bt)** produces a crystallized protein that kills harmful insects and their larvae. The crystalline protein (from the *Cry* gene) breaks down the cementing substance that fuses the lining cells of the digestive tract in certain insects. Insects subjected to this protein die in a short time from "autodigestion." The *Cry* gene causing this event is the subject of an expanding market of "insect-resistant" genetically engineered plants. By spreading spores of the bacterium across their fields, farmers can protect their crops without using harmful chemicals.

Now, instead of spreading the bacterium directly across their fields, farmers can grow plants containing Bt genes. Plants that contain the gene for the Bt toxin have a built-in defense. This biotechnologically enhanced pesticide has been successfully introduced into a wide range of plants, including tobacco, tomatoes, corn, and cotton. In fact, most soybean seeds planted today contain the gene for Bt toxin, which effectively kills cotton-infesting insects. Bt with its insecticidal protein is shown in **Figure 9a**.

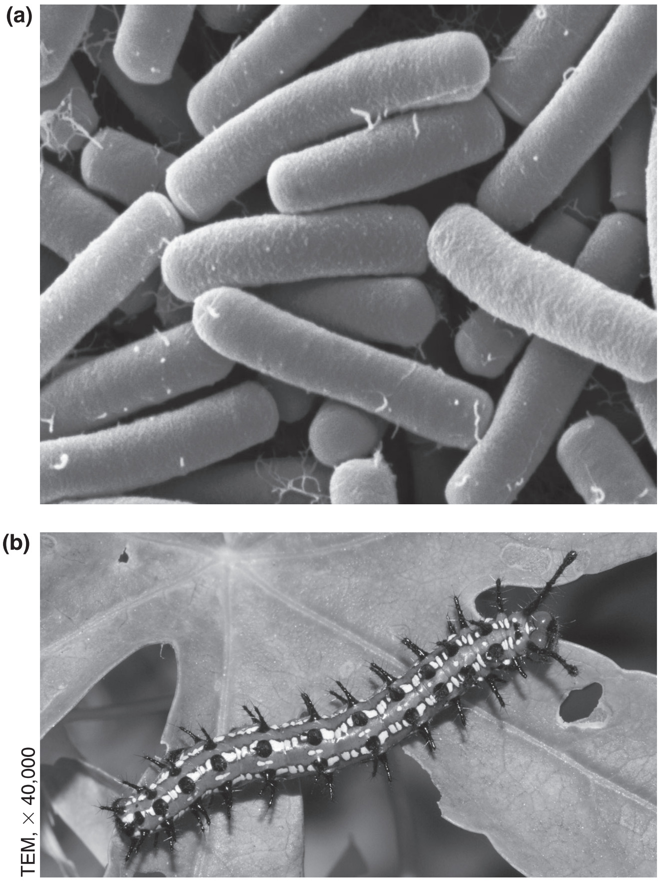

FIGURE 9 Bt with Crystal of Insecticidal Protein Figure (a) shows the crystalline protein as a crystal within the *Bacillus thuringiensis* bacterium. Genetically engineered plants expressing the *Cry* gene are "insect resistant" owing to the production of a small amount of this bacterial protein. Insect larvae (b) that would normally consume plant tissue will die if they consume the crystalline protein (*Cry* gene product).

(a): Mediscan/Corbis., **(b):** Nature's Images, Inc./Photo Researchers, Inc.

The widespread use of the Bt gene is one of the most remarkable success stories in biotechnology. It is also one of the biggest sources of controversy. Cornell researchers conducted a laboratory experiment in 1999 suggesting that the pollen produced by bioengineered corn could be deadly to monarch butterflies. The results were expected. Researchers had known for years that, in large doses, the toxin naturally produced by *B. thuringiensis* could be harmful to butterflies. Still, the report set off a firestorm of controversy. It was the first tangible evidence that genetically altered food could harm the environment, and the monarch butterfly quickly became the unofficial mascot of opponents of genetic engineering.

When researchers took their experiments out of the laboratory and into the field, many of their concerns quickly faded. Several studies found that few butterflies in the real world would be exposed to enough pollen to cause any harm. In fact, butterflies are unlikely to ingest toxic amounts of pollen even if they feed on milkweed plants less than 1 meter from the typical field of genetically modified corn. Still, scientists speculate that a small percentage of butterflies will inevitably get dusted with a lethal amount of pollen. Some of the monarchs that survive the exposure may then be unfit for their long migrations. On the whole, however, concerns that genetically altered corn could devastate monarchs seem to have been disproven: after 2 years of study, the Agricultural Research Service (a division of the USDA) announced in 2002 that Bt toxin posed little risk to monarch butterflies in real-world situations.

Herbicide Resistance

Traditional weed killers have a fundamental flaw: they often kill desirable plants along with weeds. Today, biotechnology allows farmers to use herbicides without threatening their livelihood. Crops can be

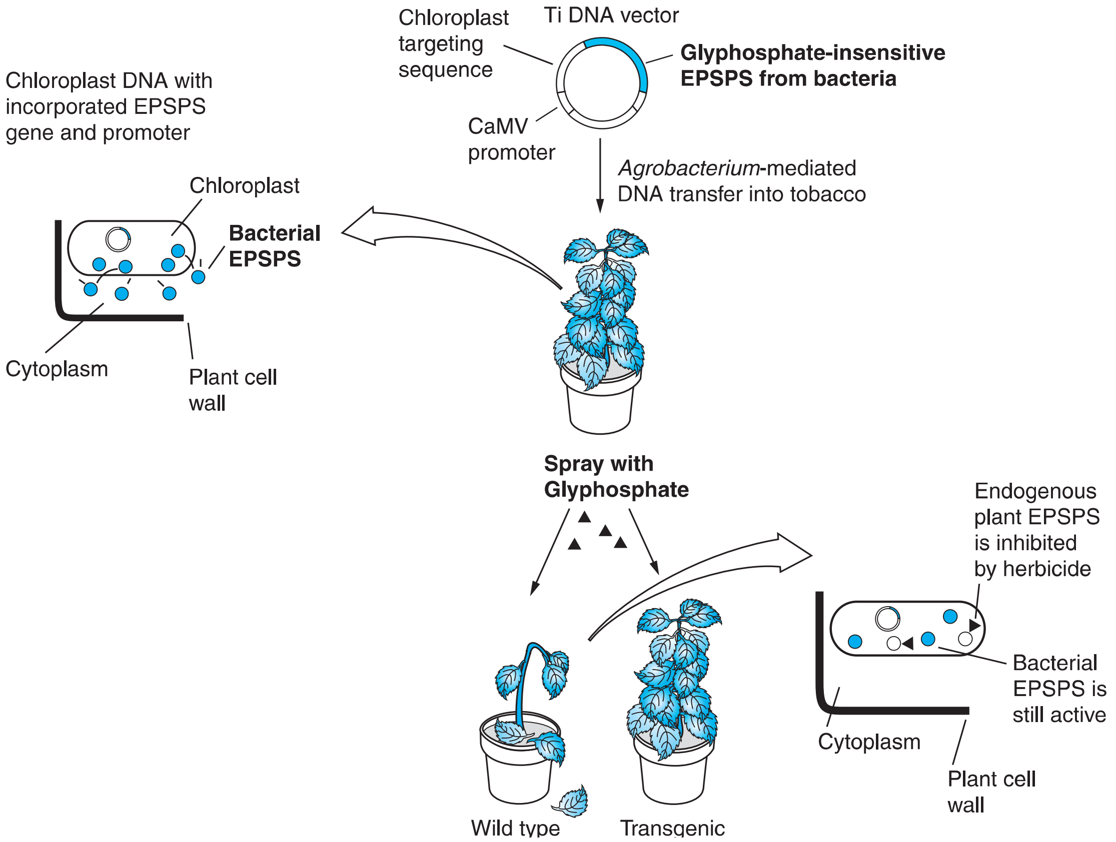

FIGURE 10 **Engineering Herbicide-Resistant Plants** Glyphosphate is a compound that blocks a key enzyme in plant protein synthesis. Plants sprayed with this compound (e.g., Roundup) will die because a key enzyme is blocked. Genetically engineered plants that express an alternative gene (*EPSPS*) can bypass this blockage with an enzyme that uses an alternative chemical pathway. Transferring the TDNA vector (from Agrobacter) with the *EPSPS* gene (from bacteria) to plants and activating the promoter (with the help of a chloroplast-targeting sequence) can lead to incorporation of the EPSPS gene in the plants, making them resistant to the glyphosphate. This process has been used in many commercial plant varieties, making them herbicide-resistant and allowing the grower to spray susceptible weeds and resistant plants (and substituting glyphosphate for more harmful herbicides).

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genetically engineered to be resistant to common herbicides such as **glyphosphate.** This herbicide works by blocking the enzyme EPSPS, which functions in a biochemical pathway responsible for the synthesis of aromatic amino acids and other compounds vital to plant growth and survival. Through bioengineering, scientists have created transgenic crops that produce an alternative enzyme that is not affected by glyphosphate, meaning that weeds are susceptible while desirable plants are not. This approach has been especially successful in soybeans. Most soybeans grown today contain herbicide-resistant genes. The process is shown in **Figure 10**. Glyphosphate is currently the world's most widely used herbicide, effectively controlling of a wide group of unwanted plant species. Since 1996, the high usage of transgenic glyphosphate-resistant crops in the Americas has led to exclusive use of glyphosphate for weed control over very large areas.

Unfortunately, in regions where transgenic glyphosphate-resistant crops dominate, glyphosphate-resistant populations of damaging weed species have now evolved. A single-site mutation of a proline amino acid at position 101 in *EPSPS* has been implicated in these glyphosate-resistant weeds, which have been found in the United States, Argentina, and Brazil. As more transgenic glyphosphate-resistant crops are planted over the next few years, it is anticipated that other glyphosphate-resistant weed species will evolve. In response to this development, Monsanto has added a broad-spectrum weed killer to reduce the development of resistant weeds. Future use of glyphosphate-resistant plants will require engineering to combat this event. Other companies are at work on similar products to combat glyphosphate-resistant weeds. Glyphosphate is considered essential for present and future world food production, and any action to secure its sustainability has become a global imperative.

Farmers who plant herbicide-resistant crops are generally able to control weeds with chemicals that are milder and more environmentally friendly than typical herbicides. This development is significant because, before the advent of resistant crops, U.S. cotton farmers spent $300 million per year on harsh chemicals to spray on their fields, exclusive of the large cost in human manual labor necessary to keep cotton plants weed-free, which is no longer necessary.

Enhanced Nutrition

Of all the potential benefits of biotechnology, nothing is more important than the opportunity to save millions of people from the crippling effects of malnutrition. One potential weapon against malnutrition is **Golden Rice**—rice that has been genetically modified to produce large amounts of beta carotene, a provitamin that the body converts to vitamin A. According to recent estimates, 500,000 children in many parts of the world will eventually become blind because of vitamin A deficiency. Currently health workers carry doses of vitamin A from village to village in an effort to prevent blindness. Simply adding this nutrient to the food supply would be much more efficient and, in theory, much more effective.

Biotechnology may not, however, be the magic bullet that ends malnutrition. Although promising, genetically modified foods have their limitations. For instance, the provitamin in Golden Rice must dissolve in fat before it can be used by the body. Children who do not get enough fat in their diets may not be able to reap the full benefits of the enriched rice. Some groups would like to see more conventional breeding techniques used to combat world hunger. For example, although Golden Rice was ready to appear within 2 years of its development, no farmers have, as of 2011, yet planted the rice, largely because of concerns voiced by environmental organizations. These groups endorse programs such as Harvest Plus in place of the introduction of transgenic crops in developing countries. Harvest Plus is a collection of 12 crops that aim to boost levels of vitamin A, iron, and zinc, and it relies on conventional breeding. However, other groups support transgenic crops in these same locations: the Bill and Melinda Gates Foundation, for example, is spending $36 million to support Golden Rice, GM cassava, sorghum, and bananas.

The Future of Plant Biotechnology in Pharmacology

Recall that plants can be ideal protein factories. A single field of a transgenic crop can produce a large amount of commercially valuable protein. At this time, transgenic corn has the highest protein yield per invested dollar of any bioreactor organism. The possibilities are practically endless.

In the not-so-distant future, farmers will grow human medicine along with their crops. It is already possible to harvest human growth hormone from transgenic tobacco plants. Plants can also manufacture vaccines for humans, as we've seen. Edible vaccines can be produced by introducing a gene for a subunit of the virus or bacterium. The plant expresses this protein subunit, and it is eaten with the plant. When the subunit antigen enters the bloodstream, the immune system produces antibodies against it, providing immunity. The need for inexpensive vaccines that do not require refrigeration was first voiced by the World Health Organization in the early 1990s and has resulted in studies of vaccines in bananas, potatoes, tomatoes, lettuce, rice, wheat, soybeans, and corn. Researchers at Cornell University have recently created tomatoes and bananas that produce a human vaccine against the viral infection hepatitis B. Researchers are actively studying the tomato as another source of pharmaceuticals. Through engineering of the chloroplast (abundant in green tomatoes), scientists hope to create an edible source of vaccines and antibodies, as shown in Table 1.

Plants express a wide range of chemical compounds called phytochemicals, and biotechnologists are converting plants into small-scale factories to produce chemicals useful to human health. Biotechnology can alter the production of complex technical therapeutic proteins via plant pathways, with examples including antibodies, blood products, cytokines, growth factors, hormones, and recombinant enzymes. This "molecular farming" will likely bring several products to market in the near future with applications to the treatment of diseases and conditions such as cystic fibrosis, non-Hodgkin's lymphoma, hepatitis, Norwalk virus, rabies, and a range of gastrointestinal illnesses.

Rather than growing human or animal cells on expensive nutrient-rich media, biopharmers insert genes into the cell of plants and the plants do the work of transcribing and folding the protein. Since plants can be grown in larger quantities than cell cultures, they can offer a much greater volume of prod-

TABLE 1

Beneficial Crop Product Traits	Crops Available Now	Crops That May Be Available in the Future
Bt crops are protected against insect damage and reduce pesticide use. The plants produce a protein—toxic only to certain insects—found in *Bacillus thuringiensis*.	Corn, cotton, potatoes	Sunflower, soybeans, canola, wheat, tomatoes
Herbicide-tolerant crops allow farmers to apply a specific herbicide to control weeds without harm to the crop. They give farmers greater flexibility in pest management and promote conservation tillage.	Soybeans, cotton, corn, canola, rice	Wheat, sugar beets
Disease-resistant crops are armed against destructive viral plant disease with the plant equivalent of a vaccine.	Sweet potatoes, cassava, rice, corn, squash, papaya	Tomatoes, bananas
High-performance cooking oils maintain texture at high temperatures, reduce the need for processing, and create healthier food products. The oils are either high-oleic or low-linoenic types. In the future, there will also be high-stearate oils.	Sunflower, peanuts, soybeans	
Healthier cooking oils have reduced saturated fat.	Soybeans	
Delayed-ripening fruits and vegetables have superior flavor, color, and texture; are firmer for shipping; and stay fresh longer.	Tomatoes	Raspberries, strawberries, cherries, tomatoes, bananas, pineapples
Increased-solids tomatoes have superior taste and texture for processed tomato pastes and sauces.	Tomatoes	
Nutritionally enhanced foods will offer increased levels of nutrients, vitamins, and other healthful phytochemicals. Benefits range from helping developing nations meet basic dietary requirements and boosting disease-fighting and health-promoting foods.		Protein-enhanced sweet potatoes and rice; high-vitamin-A canola oil; increased antioxidant fruits and vegetables

Source: BIO member survey.

uct than a manufacturing plant would. However, since the first human-like enzyme was first produced in transgenic tobacco plants in 1992 at Virginia Polytechnic Institute, the biopharma industry has had a wave of trials with no approvals. Nevertheless, the first plant-based pharmaceutical products may be on the market before long.

Protalix, a biotech company in Israel, has FDA approval for a drug to treat Gaucher's disease. The disease has no cure, but the product in development breaks down the accumulating fatty substances, and patients would have to take this drug throughout their lives. Since there is no other ready supply, the drug received fast-track approval. The drug is manufactured by producing proteins from carrot cell cultures in disposable plastic bioreactors. Another company, Medicago, a U.S. company, is developing flu vaccines in tobacco plants (see **Figure 11**) grown in greenhouses. After the plants are transformed by *Agrobacter*, they produce the necessary protein shell, which is then harvested and made into a vaccine.

The Future of Plant Biotechnology: Fuels

Biofuels (fuels produced from biological products, such as plants) can be produced almost anywhere in the world from homegrown raw materials and may be an important use of plant biotechnology in the future. As the need for alternatives to fossil fuels increases, the U.S. government is looking toward biotechnology

FIGURE 11 **Botanical Biopharming** Genetically transformed water plants being transformed to produce pharmaceutical proteins.

Fancy/Alamy.

to offer solutions. The 2007 Biofuels Initiative increased federal funding 60% over the 2006 budget with the stated intention of replacing 30% of U.S. current fuel with bioethanol by 2030 (see **Figure 12**).

Bioethanol refineries have sprung up all over the Midwest (resulting largely from subsidies and incentives) and can be used to convert sugars from any cellulosic source. However it takes 7 gallons of gasoline to produce 10 gallons of kernel corn ethanol, which represents a relatively modest net gain. For this reason biotechnology is needed to convert the readily available cellulosic sources into biofuels—perhaps by developing biofuel-producing organisms—and thus making this procedure more economically viable.

Biofuels from Plant Waste

In the future, there may be other opportunities for plants to provide fuels. Specifically, scientists are developing methods to capture energy trapped in plant wastes. The solar energy captured through photosynthesis enables the storage of energy in plant cell wall polymers (cellulose, lignin, and hemicelluloses in straw, husks, hulls, and trees). This energy remains trapped unless plants are burned. Despite the increasing use of starch-based ethanol and biodiesel, fuel produced from plant by-products has been unavailable up to now. If that energy could be released, grasses, wood, and crop residues would offer the possibility of a renewable, geographically distributed source of sugars for conversion to fuel. This process would include collection, destruction of the cell walls (pretreatment), and conversion of the sugars to biofuels.

One such sugar is hemicellulose—a family of polysaccharides composed of five- and six-carbon sugars fibrils. Lignin is the glue that crosslinks these fibrils to provide strength. One challenge in the development of plant wastes as fuel has been finding enzymes that can function in the high acid conditions needed for the pretreatment of hemicellulose in order to break down lignin. Research continues; the refineries developed

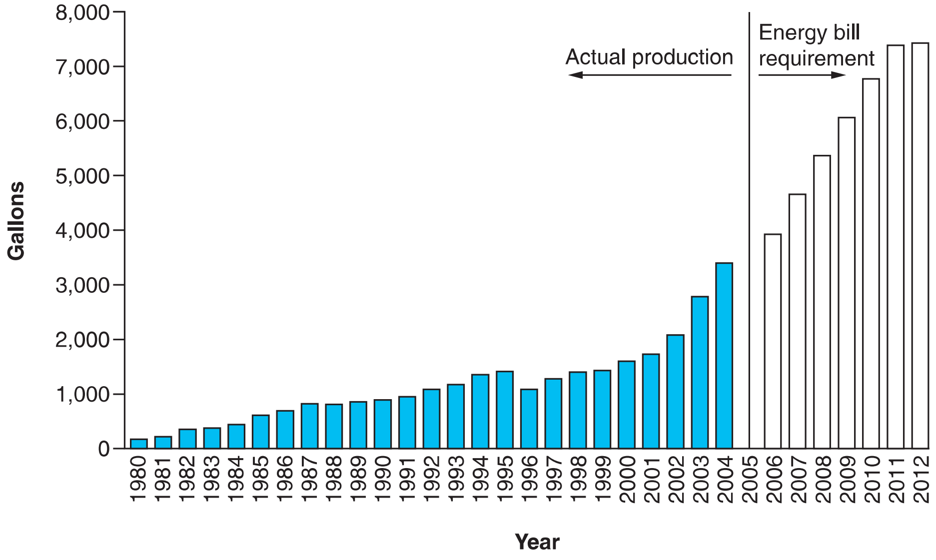

FIGURE 12 **U.S. Energy Policy Act of 2005** Calls for 7.5 billion gallons of ethanol and biodiesel to be added to America's fuel supply by 2012.

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for bioethanol production from sugars can potentially be converted to convert these plant wastes to fuel.

Biofuels from algae

The most recent group of biofuel producers are looking beyond corn and French-fry grease to microscopic algae for the next alternative to petroleum. Microalgae naturally produce and store lipids (vegetable-like oils). If they can be biochemically altered to become more efficient, biofuels may result. The U.S. Department of Energy (DOE) explored the potential of algae prior to 1996, but when crude oil prices dropped from $50 to $20 per barrel that year, the DOE lost interest. With the passage of the Biofuels Initiative in 2007 and the rising cost of crude oil, interest in algae has returned. The challenge is to produce at least a million gallons of fuel per day. Solazyme, in San Francisco, has developed a closed bioreactor system of proprietary algae that eat anything from waste glycerol to sugar pulp and produce triacylglycerides and methanol. After chemical modification and concentration, the alkanes produced resemble biodiesel. Yields of 50% to 60% of oil per gram of algal cells are considered excellent, and Solazyme's algae are producing 75% oil per gram of dry weight. This company is the first to produce a barrel of microbial fuel oil and is confident that the goal of a million gallons of fuel per day can eventually be achieved.

4 Health and Environmental Concerns

Ever since the inception of transgenic plants, people have worried about potentially harmful effects to humans and the environment. In an age when "natural" is often equated with "safe," these decidedly unnatural plants carry an air of danger. Activists have staged protests against companies producing genetically modified plants (GMOs, or genetically modified organisms) (see **Figure 13**).

Such fears have the power to shake up an industry. In 2000, potato-processing plants in the Northwest stopped buying genetically modified potatoes. There was never any sign that these potatoes—engineered to be pest-resistant—were inferior or dangerous. They looked and tasted just like non–genetically modified potatoes, and farmers did not need to use gallons of chemicals to get them to grow. They were able to survive aphids and potato bugs but not the tide of public opinion (see **Figure 14**).

What are some opponents of plant biotechnology saying, and what are some of the other points of view? What are the pros and cons of GMO crop production?

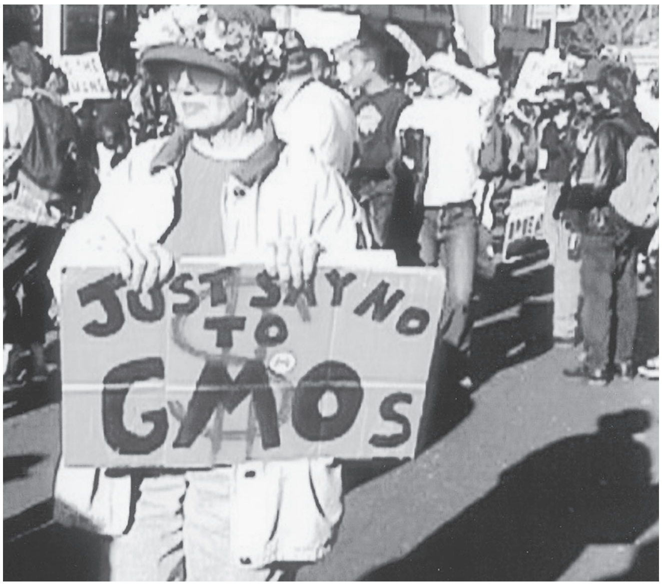


FIGURE 13 A Parade in Boston Protesting Genetically Modified Food at the 2000 BIO Conference

Bill Thieman.

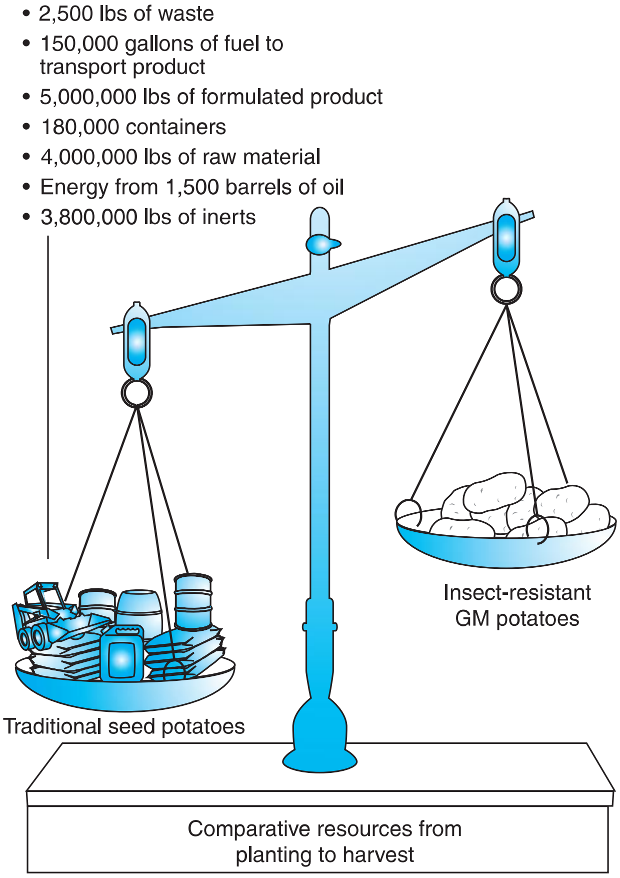
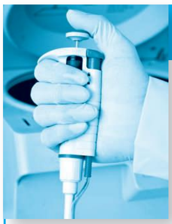


FIGURE 14 Comparison of Resources for Production of GMO and Traditionally Grown Potatoes Considerably more raw materials and energy are expended in the production and application of this insecticide compared with having insecticidal genes in the plant itself.



TOOLS OF THE TRADE

Excision of Reporter Genes

Researchers know that a gene has been transferred to a plant cell because antibiotic-resistant genes (for example, kanamycin antibiotic resistance) are usually used as "reporters" for commercial plant engineering. They allow only those transformed plant cells that can live on the antibiotic medium to be selected. The presence of antibiotic genes (and small amounts of antibiotics) in plants has caused some public concern. However, we know that it is possible to remove specific genes after transformation and selection because four scientists at Rockefeller University have developed the process. It involves the use of a promoter that can be activated to stimulate excision through naturally existing mechanisms in the plant embryo or plant organ tissue *after* the antibiotic selection for the transformed cells has occurred.

Concerns about Human Health

Every plant contains DNA. Whenever you munch a carrot or a bite into a slice of bread, you're eating more than a few genes. Opponents of genetic engineering have nothing against genes per se. Instead, they fear the effects of *foreign* genes, bits of DNA that would not naturally be found in the plant. A 1996 report in the *New England Journal of Medicine* seemed to confirm at least some of those fears. The study found that soybeans containing a gene from the Brazil nut could trigger an allergic reaction in people who were sensitive to Brazil nuts. Because of this discovery, this type of transgenic soybean never made it to market.

We can look at this incident in two different ways. Opponents say that this case of the soybean clearly demonstrates the pitfalls of biotechnology. They envision many scenarios where novel proteins trigger dangerous reactions in unsuspecting customers. Supporters see it as a success story: the system detected the unusual threat before it ever reached the public.

At this time, most experts agree that genetically modified foods are unlikely to cause widespread allergic reactions. According to a recent report from the American Medical Association, very few proteins have the potential to trigger allergic reactions, and most of them are already well known to scientists. The odds of an unknown allergen "sneaking" into a genetically modified food on the grocery shelf are very small. In fact, biotechnology may someday help prevent allergy-related deaths. Researchers are now working to produce peanuts that lack the proteins that can trigger violent allergic reactions.

Allergies are not the only concern. Some scientists have speculated that the antibiotic-resistant genes used as markers in some transgenic plants could spread to disease-causing bacteria in humans. In theory, these bacteria would then become harder to treat. Fortunately bacteria do not regularly scavenge genes from our food. According to a recent report in the journal *Science,* there is only a "minuscule" chance that an antibiotic-resistant gene could ever pass from a plant to bacterium. Furthermore, many bacteria have already evolved antibiotic-resistant genes.

If you scan the antibiotechnology literature, you'll see many more accusations. Headlines such as "Frankenfoods may cause cancer" are common. To this date, however, science has not supported any of these concerns. The National Academy of Sciences recently reported that the transgenic food crops on the market today are perfectly safe for human consumption.

Concerns about the Environment

Recall from the section on genetic pesticides that recent studies have put to rest fears that bioengineered corn could kill large numbers of monarch butterflies. However, worries about the environment have not disappeared. For one thing, genetic enhancement of crops could lead to new breeds of so-called superweeds. Just as genes for antibiotic resistance could theoretically spread from plants to bacteria, genes for pest or herbicide resistance could potentially spread to weeds. Because many crops—including squash, canola, and sunflowers—are close relatives to weeds, crossbreeding occasionally occurs, allowing the genes from one plant to mix with the genes of another. At this time, however, few experts predict any sort of explosion of genetically enhanced weeds. Further studies are needed to gauge the full extent of this threat and develop ways to minimize the risk.

The potential ecological hazards of biotechnologically enhanced crops must be weighed against the clearly established benefits. First and foremost, biotechnology can dramatically reduce the use of chemical pesticides, as seen in Figure 14. One of the key environmental benefits of biotech crops is the reduction in insecticide and herbicide applications to crops. In countries where biotech crops have been planted, pesticide use on four biotech crops—soybeans, corn, cotton, and canola—has fallen by 791 million pounds per year. (8.8%). This has resulted in a 17.2% reduction in the associated environmental impact.

YOU DECIDE

The StarLink Episode

In 2000, traces of genetically modified StarLink corn turned up in taco shells sold in grocery stores. Intended for animal consumption, the corn contained the gene for herbicide resistance, which breaks down in the soil or the stomach of cattle. On the surface, this news was not shocking. Many processed foods on market shelves contain genetically altered corn or soybean products. This particular type of corn, however, had never been approved for human consumption because of lingering concerns over potential allergic reactions. The Environmental Protection Agency (EPA)—the agency that regulates the use of all pesticides—had approved StarLink only for animal feed and industrial use. The discovery of StarLink corn in the food supply triggered a massive recall of potentially "tainted" products. Soon after the recall, Aventis, the company that produced StarLink, struck a deal with the EPA and agreed to stop planting the corn.

Had this "unapproved pesticide" really done any harm? The Centers for Disease Control and Prevention (CDC) took immediate steps to find out. A handful of people had complained of allergic-type reactions after eating the genetically altered corn, and the CDC investigated each case closely. A total of 28 people were found to have had symptoms consistent with an allergic reaction. However, blood tests showed that none of these people were sensitive to the Bt protein.

How should this episode have been handled? Was the public adequately protected from harm? Did the government overreact? What is the best balance between regulations and commercial interests? You decide.

Forests of genetically altered trees could pull billions of tons of carbon from the atmosphere each year and reduce global warming, according to researchers at Lawrence Berkeley National Laboratory and Oak Ridge National Laboratory. They claim that it might be possible to alter trees genetically so that they would send more carbon into their roots, keeping it out of circulation for centuries. These innovations could substantially boost the amount of carbon that vegetation naturally extracts from air. This change would require a modification of the current regulatory climate for producing genetically engineered trees in the United States and require a change in societal perceptions of the issues surrounding the use of genetically altered organisms. The potential exists, but the implementation depends on greater acceptance of genetically modified organisms in our environment.

On the whole, biotechnology does not seem to be taking us to the brink of ecological disaster and may actually offer some solutions to environmental problems. Indeed, the National Academy of Sciences recently reported that biotechnologically enhanced crops pose no greater environmental threat than traditional crops.

Regulations

Biotechnology is not a lawless frontier. As we have already seen, several different agencies regulate the production and marketing of genetically modified foods. The FDA regulates foods on the market, the USDA oversees growing practices, and the EPA controls the use of Bt proteins and other so-called pesticides. The approach of these agencies has changed over the years, especially in the case of the FDA, but they are actively involved in approving plant crops.

As early as 1992, at the beginning of the biotechnology revolution—the FDA announced that genetically altered food products would be regulated by the same tough standards applied to regular foods—nothing more, nothing less. Even though they were not bound by law, food companies voluntarily consulted with the FDA before marketing any product. In 2001, the agency adopted a stricter, more formal approach. Under these rules, companies must notify the FDA at least 120 days before a genetically altered food reaches the market. The manufacturer must also provide evidence that the new product is no more dangerous than the food it replaces. The determining factor for plant-based foods or products will be the attitude of consumers. The Center for Food Safety and the Grocery Manufacturers Association and Food Products Association have informed the USDA of their "strong opposition to the use of food crops to produce plant manufactured pharmaceuticals in the absence of controls and procedures that assure essentially 100% of the food supply." There are many nonfood plants and contained-growth plant systems that will satisfy this concern, and no manufacturer has violated these rules to date.