Throughout the ages, humans have used selective breeding techniques to create plants and animals with desirable genetic traits. By selecting organisms with naturally occurring or mutagen-induced variations and breeding them to establish the phenotype, we have evolved varieties that now feed our growing populations and support our complex civilizations.

Although we have had tremendous success shuffling genes through selective breeding, the process is a slow one. When recombinant DNA technologies emerged in the 1970s and 1980s, scientists realized that they could modify agriculturally significant organisms in a more precise and rapid way—by identifying and cloning genes that confer desirable traits, then introducing these genes into organisms. Genetic engineering of animals and plants promised an exciting new phase in scientific agriculture, with increased productivity, reduced pesticide use, and enhanced flavor and nutrition.

Beginning in the 1990s, scientists created a large number of genetically modified (GM) food varieties. The first one, approved for sale in 1994, was the Flavr Savr tomato—a tomato that stayed firm and ripe longer than non-GM tomatoes. Soon afterward, other GM foods were developed: papaya and zucchini with resistance to virus infection, canola containing the tropical oil laurate, corn and cotton plants with resistance to insects, and soybeans and sugar beets with tolerance to agricultural herbicides. By 2012, more than 200 different GM crop varieties had been created. Worldwide, GM crops are planted on 170 million hectares of arable land, with a global value of $15 billion for GM seeds.

Although many people see great potential for GM foods—to help address malnutrition in a world with a growing human population and climate change—others question the technology, oppose GM food development, and sometimes resort to violence to stop the introduction of GM varieties (ST Figure 5.1). Even Golden Rice—a variety of rice that contains the vitamin A precursor and was developed on a humanitarian nonprofit basis to help alleviate vitamin A deficiencies in the developing world—has been the target of opposition and violence. On August 8, 2013, 400 protesters broke through security fences surrounding a field trial of Golden Rice in the Bicol region of the Philippines. Within 15 minutes, they had uprooted and trampled most of the GM rice plants. The attackers argued that Golden Rice was a threat to human health and biodiversity and would lead to Western corporate control of local food crops.

Opposition to GM foods is not unique to Golden Rice. In 2013, approximately two million people marched against GM foods in rallies held in 52 countries. Some countries have outright bans on all GM foods, whereas others embrace the technologies. Opponents cite safety and environmental concerns, while some scientists and commercial interests extol the almost limitless virtues of GM foods. The topic of GM food attracts hyperbole and exaggerated rhetoric, information, and misinformation—on both sides of the debate.

So, what are the truths about GM foods? In this Special Topic chapter, we will introduce the science behind GM foods and examine the promises and problems...
of the new technologies. We will look at some of the controversies and present information to help us evaluate the complex questions that surround this topic.

What Are GM Foods?

GM foods are derived from genetically modified organisms (GMOs), specifically plants and animals of agricultural importance. GMOs are defined as organisms whose genomes have been altered in ways that do not occur naturally. Although the definition of GMOs includes organisms that have been genetically modified by selective breeding, the most commonly used definition refers to organisms modified through genetic engineering or recombinant DNA technologies. Genetic engineering allows one or more genes to be cloned and transferred from one organism to another—either between individuals of the same species or between those of unrelated species. It also allows an organism’s endogenous genes to be altered in ways that lead to enhanced or reduced expression levels. When genes are transferred between unrelated species, the resulting organism is called transgenic. The term cisgenic is sometimes used to describe gene transfers within a species. In contrast, the term biotechnology is a more general one, encompassing a wide range of methods that manipulate organisms or their components—such as isolating enzymes or producing wine, cheese, or yogurt. Genetic modification of plants or animals is one aspect of biotechnology.

In 2012, it was estimated that GM crops were grown in approximately 30 countries on 11 percent of the arable land on Earth. The majority of these GM crops (almost 90 percent) are grown in five countries—the United States, Brazil, Argentina, Canada, and India. Of these five, the United States accounts for approximately half of the acreage devoted to GM crops. According to the U.S. Department of Agriculture, 93 percent of soybeans and 88 percent of maize grown in the United States are from GM crops. In the United States, more than 70 percent of processed foods contain ingredients derived from GM crops.

Soon after the release of the Flavr Savr tomato in the 1990s, agribusinesses devoted less energy to designing GM foods to appeal directly to consumers. Instead, the market shifted toward farmers, to provide crops that increased productivity. By 2012, approximately 200 different GM crop varieties were approved for use as food or livestock feed in the United States. However, only about two dozen are widely planted. These include varieties of soybeans, corn, sugar beets, cotton, canola, papaya, and squash. ST Table 5.1 lists some of the common GM food crops available for planting in the United States. Of these GM crops, by far the most widely planted are varieties that are herbicide tolerant or insect resistant. At the time of writing this chapter, no GM food animal was approved for consumption, although a GM salmon variety was nearing market approval in the United States (Box 1). A number of agriculturally important animals such as goats and sheep have been genetically modified to produce pharmaceutical products in their milk. The use of transgenic animals as bioreactors is discussed earlier in the text (see Chapter 22).

Herbicide-Resistant GM Crops

Weed infestations destroy about 10 percent of crops worldwide. To combat weeds, farmers often apply herbicides before seeding a crop and between rows after the crops are growing. As the most efficient broad-spectrum herbicides also kill crop plants, herbicide use may be difficult and limited. Farmers also use tillage to control weeds; however, tillage damages soil structure and increases erosion.

Herbicide-tolerant varieties are the most widely planted of GM crops, making up approximately 70 percent of all GM crops. The majority of these varieties contain a bacterial gene that confers tolerance to the broad-spectrum herbicide glyphosate—the active ingredient in commercial herbicides such as Roundup®. Studies have shown that
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Glyphosate is effective at low concentrations, is degraded rapidly in soil and water, and is not toxic to humans. Farmers who plant glyphosate-tolerant crops can treat their fields with glyphosate, even while the GM crop is growing. This approach is more efficient and economical than mechanical weeding and reduces soil damage caused by repeated tillage. It is suggested that there is less environmental impact when using glyphosate, compared with having to apply higher levels of other, more toxic, herbicides.

Recently, evidence suggests that some weeds may be developing resistance to glyphosate, thereby reducing the effectiveness of glyphosate-tolerant crops. (This and other concerns about herbicide-tolerant GM plants are described later in this chapter.) One method used to engineer a glyphosate-tolerant plant is described in the next section.

Insect-Resistant GM Crops

The second most prevalent GM modifications are those that make plants resistant to agricultural pests. Insect damage is one of the most serious threats to worldwide food production. Farmers combat insect pests using crop rotation and predatory organisms, as well as applying insecticides.

Despite these assurances, environmental groups are planning to fight the sale of GM salmon. Some grocery chains in the United States have banned GM fish, and legislators in several western U.S. states are trying to block the approval of the AquAdvantage salmon based on fears that the accidental release of these fish could contaminate wild salmon populations with transgenes and disturb normal ecosystems.

Supporters of GM fish point out that the GM salmon are very unlikely to escape their facilities, and if any did escape, they would be poorly adapted to wild conditions. Critics of the new GM salmon point out that the technique used to create sterile triploids (pressure-shocking the fertilized eggs) still allows a small percentage of fertile diploids to remain in the stock. They state that even a few fertile fish, if they escaped into the wild, could have long-term effects on wild populations. A study published in 2013 shows that it is possible for the AquAdvantage salmon to breed successfully with a close relative, the brown trout. In laboratory conditions, the hybrids grew more quickly than either the GM or non-GM varieties, and in closed stream-like systems, the hybrids outcompeted both parental fish varieties for food supplies. The authors point out that these results should be taken into account during environmental assessments, although it is still not known whether the hybrid salmon-trout variety could successfully breed in the wild. If GM salmon could escape, breed, and introduce transgenes into wild populations, there could be unknown negative downstream effects on fish ecosystems.


The AquAdvantage salmon grows twice as fast as a non-GM Atlantic salmon, reaching market size in half the time.
The most widely used GM insect-resistant crops are the Bt crops. Bacillus thuringiensis (Bt) is a group of soil-dwelling bacterial strains that produce crystal (Cry) proteins that are toxic to certain species of insects. These Cry proteins are encoded by the bacterial cry genes and form crystal structures during sporulation. The Cry proteins are toxic to Lepidoptera (moths and butterflies), Diptera (mosquitoes and flies), Coleoptera (beetles), and Hymenoptera (wasps and ants). Insects must ingest the bacterial spores or Cry proteins in order for the toxins to act. Within the high pH of the insect gut, the crystals dissolve and are cleaved by insect protease enzymes. The Cry proteins bind to receptors on the gut wall, leading to breakdown of the gut membranes and death of the insect.

Each insect species has specific types of gut receptors that will match only a few types of Bt Cry toxins. As there are more than 200 different Cry proteins, it is possible to select a Bt strain that will be specific to one pest type.

Bt spores have been used for decades as insecticides in both conventional and organic gardening, usually applied in liquid sprays. Sunlight and soil rapidly break down the Bt insecticides, which have not shown any adverse effects on groundwater, mammals, fish, or birds. Toxicity tests on humans and animals have shown that Bt causes few negative effects.

To create Bt crops, scientists introduce one or more cloned cry genes into plant cells using methods described in the next section. The GM crop plants will then manufacture their own Bt Cry proteins, which will kill the target pest species when it eats the plant tissues.

Although Bt crops have been successful in reducing crop damage, increasing yields, and reducing the amounts of insecticidal sprays used in agriculture, they are also controversial. Early studies suggested that Bt crops harmed Monarch butterfly populations, although more recent studies have drawn opposite conclusions (Box 2). Other concerns still exist and these will be discussed in subsequent sections of this chapter.

**GM Crops for Direct Consumption**

To date, most GM crops have been designed to help farmers increase yields. Also, most GM food crops are not consumed directly by humans, but are used as animal feed or as sources of processed food ingredients such as oils, starches, syrups, and sugars. For example, 98 percent of the U.S. soybean crop is used as livestock feed. The remainder is processed into a variety of food ingredients, such as lecithin, textured soy proteins, soybean oil, and soy flours. However, a few GM foods have been developed for direct consumption. Examples are rice, squash, and papaya (Box 3).

One of the most famous and controversial examples of GM foods is **Golden Rice**—a rice variety designed to synthesize beta-carotene (the precursor to vitamin A) in the rice grain endosperm.

Vitamin A deficiency is a serious health problem in more than 60 countries, particularly countries in Asia and Africa. The World Health Organization estimates that 190 million of the world’s children and 19 million pregnant women are vitamin A deficient. Between 250,000 and 500,000 children with vitamin A deficiencies become blind each year, and half of these will die within a year of losing their sight. As vitamin A is also necessary for immune system function, deficiencies lead to increases in many other conditions, including diarrhea and virus infections. The most seriously affected people live in the poorest countries and have a basic starch-centered diet, often mainly rice. Vitamin A is normally found in dairy products and can be synthesized in the body from beta-carotene found in orange-colored fruits and vegetables and in green leafy vegetables.

Several approaches are being taken to alleviate the vitamin A deficiency status of people in developing countries. These include supplying high-dose vitamin A supplements and growing fresh fruits and vegetables in home gardens. These initiatives have had partial success, but the expense of delivering education and supplementation has impeded the effectiveness of these programs.

In the 1990s, scientists began to apply recombinant DNA technology to help solve vitamin A deficiencies in people with rice-based diets. Although the rice plant naturally produces beta-carotene in its leaves, it does not produce it in the rice grain endosperm, which is the edible part of the rice. The beta-carotene precursor, geranylgeranyldiphosphate, is present in the endosperm, but the enzymes that convert it to beta-carotene are not synthesized (ST Figure 5–2).

In the first version of Golden Rice, scientists introduced the genes phytoene synthase (psy) cloned from the daffodil plant and carotene desaturase (crtI) cloned from the bacterium Erwinia uredovora into rice plants. The bacterial crtI gene was chosen because the enzyme encoded by this gene can perform the functions of two of the missing rice enzymes, thereby simplifying the transformation process. The resulting plant produced rice grains that were a yellow color due to the presence of beta-carotene (ST Figure 5–3). This strain synthesized modest levels of beta-carotene—but only enough to potentially supply 15–20 percent of the recommended daily allowance of vitamin A. In the second version of the GM plant, called Golden Rice 2, the daffodil psy gene was replaced with the psy gene from maize. Golden Rice 2 produced beta-carotene levels that were more than 20-fold greater than those in Golden Rice. In the next section we describe the methods used to create Golden Rice 2.
In 1999, three years after the introduction of Bt corn in the United States, scientists published a report that ignited the anti-GM movement and triggered years of intensive research. The scientists had conducted a laboratory assay in which they fed milkweed leaves, coated with pollen from either non-GM or Bt corn, to Monarch butterfly larvae. They concluded that Bt corn pollen reduced larval survival by approximately 50 percent. Concerned about the possibility of unintended harm to nontarget organisms, the U.S. Department of Agriculture commissioned scientists from the United States and Canada to provide detailed follow-up research. In 2001, these studies culminated in a series of five published papers that examined Monarch butterfly biology and ecology and how these may be affected by different types of Bt corn and Bt proteins—in both laboratory and field conditions.

Data from these studies explained and contradicted the initial study. First, the authors of the original 1999 study had fed larvae on pollen contaminated with ground-up corn anthers, which contain 100-fold higher levels of the Cry1Ab protein than found in pollen. In the field, larvae do not eat anthers or other parts of corn plants, as larvae feed exclusively on milkweed leaves. Second, in laboratory tests, larvae were found to be sensitive to Cry1Ab and Cry1Ac proteins, but not to other Cry proteins. Third, the levels of Cry1Ab and Cry1Ac proteins present in Bt cornfields had little, if any, effects on Monarch butterfly larvae. The studies also examined the survival of larvae on Bt cornfields compared with non-Bt cornfields that are sprayed with a pyrethroid insecticide used to control cornfield pests. Larval survival on milkweed within pesticide-treated fields was between 0 and 10 percent, whereas survival within Bt cornfields was 80 to 93 percent.

Although these studies established a low risk for Monarch butterflies, a new twist in the tale may be emerging. Since 1993, the numbers of Monarch butterflies wintering in Mexico has dropped dramatically. Numbers have dropped significantly each year since 2007—including a 30 percent drop in 2011–2012 and a 59 percent drop in 2012–2013. The reasons for these declines appear to be complex. In Mexico, butterfly reserves have suffered from logging, water diversion, and drought. In the United States and Canada, where Monarch butterflies lay their eggs each year, milkweed habitat has suffered from drought, human development, and changes in agricultural practices. Tillage, mowing, and herbicide use have destroyed millions of acres of milkweed habitat. Along with the use of conventional herbicides that kill weeds, the growing of glyphosate-tolerant corn and soybean crops has resulted in efficient suppression of weeds in fields that used to contain small numbers of milkweed plants. By affecting milkweeds, herbicide-tolerant GM crops may be contributing to the serious decline in Monarch butterfly populations. Scientists are proposing a program to plant milkweed plants along north–south highways from Texas to Canada to provide food for Monarch butterfly larvae.

Monarch butterfly larvae feed exclusively on milkweed.
The Success of Hawaiian GM Papaya

In the mid-1990s, the papaya ringspot virus (PRSV) spread rapidly throughout Hawaii’s papaya fields and threatened to destroy the industry within a few years. To try to stop the destruction of Hawaiian papaya, a team of scientists from the University of Hawaii, the USDA Agricultural Research Center in Hawaii, and the Upjohn Company cloned the coat protein gene of PRSV and introduced it into cultured papaya cells using biolistic transformation. The goal was to create PRSV resistance using a mechanism known as pathogen-derived resistance. The presence of virus coat proteins within the plant is thought to interfere with the disassembly and movement of an infecting virus, slowing or preventing infection. Researchers tested resistance to PRSV in the transformed papaya plants and developed two GM varieties—SunUp and Rainbow. SunUp was homozygous for the PRSV coat protein gene, and Rainbow was an F₁ hybrid of SunUp and a non-GM variety Kapoho. After three years of field testing and two years of moving through federal regulatory processes, GM papaya was approved for use. Seeds were given for free to farmers who immediately planted them to replace their virus-devastated fields.

Within three years, papaya harvests in Hawaii doubled and consumer acceptance was positive. Virus-resistant GM papaya is credited with saving the Hawaiian papaya industry. An interesting side-effect of the presence of GM papaya in Hawaii was the recovery of non-GM and organically grown papaya. Because PRSV levels declined due to the presence of virus-resistant fields and the abandoning of infected fields, some growers can now produce non-GM papaya, albeit on a smaller scale than before the virus spread throughout Hawaii. At the present time, more than 70 percent of Hawaiian papaya is genetically modified. GM papaya is approved for sale in the United States, Canada, and Japan.

Since the development of GM papaya in Hawaii, efforts to develop similar varieties in other parts of the world have stalled because of increasing public resistance to GM foods. Since 2010, thousands of GM papaya trees in Hawaii have been cut down and destroyed by anonymous attackers. Efforts to introduce GM papaya in Thailand have failed, and the government recently banned GM foods. Japan has approved the sale of GM papaya, but only if it is labeled as genetically modified.

Clinical trials have shown that the beta-carotene in Golden Rice 2 is efficiently converted into vitamin A in humans and that about 150 grams of uncooked Golden Rice 2 (which is close to the normal daily rice consumption of children aged 4–8 years) would supply all of the childhood daily requirement for vitamin A.

At the present time, Golden Rice 2 is undergoing field, biosafety, and efficacy testing in preparation for approval by government regulators in Bangladesh and the Philippines. If Golden Rice 2 proves useful in alleviating vitamin A deficiencies and is approved for use, seed will be made available at the same price as non-GM seed and farmers will be allowed to keep and replant seed from their own crops.

Despite the promise of Golden Rice 2, controversies remain. Critics of GM foods suggest that Golden Rice could make farmers too dependent on one type of food or might have long-term health or environmental effects. These and other controversies surrounding GM foods are discussed in subsequent sections of this chapter.

Methods Used to Create GM Plants

Most GM plants are created using one of two approaches: the biolistic method or Agrobacterium tumefaciens-mediated transformation technology. Both methods...
target plant cells that are growing in vitro. Scientists can generate plant tissue cultures from various types of plant tissues, and these cultured cells will grow in either liquid cultures or on the surface of solid growth media. When grown in the presence of specific nutrients and hormones, these cultured cells will form clumps of cells called calluses, which, when transferred to other types of media, will form roots. When the rooted plantlets are mature, they are transferred to soil medium in greenhouses where they develop into normal plants.

The biolistic method is a physical method of introducing DNA into cells. Particles of heavy metals such as gold are coated with the DNA that will transform the cells; these particles are then fired at high speed into plant cells in vitro, using a device called a gene gun. Cells that survive the bombardment may take up the DNA-coated particles, and the DNA may migrate into the cell nucleus and integrate into a plant chromosome. Plants that grow from the bombarded cells are then selected for the desired phenotype.

Although biolistic methods are successful for a wide range of plant types, a much improved transformation rate is achieved using Agrobacterium-mediated technology. Agrobacterium tumefaciens (also called Rhizobium radiobacter) is a soil microbe that can infect plant cells and cause tumors.

These characteristics are conferred by a 200-kb tumor-inducing plasmid called a Ti plasmid. After infection with Agrobacterium, the Ti plasmid integrates a segment of its DNA known as transfer DNA (T-DNA) into random locations within the plant genome. (ST Figure 5–4). To use the Ti plasmid as a transformation vector, scientists remove the T-DNA segment and replace it with cloned DNA of the genes to be introduced into the plant cells.

In order to have the newly introduced gene expressed in the plant, the gene must be cloned next to an appropriate promoter sequence that will direct transcription in the required plant tissue. For example, the beta-carotene pathway genes introduced into Golden Rice were cloned next to a promoter that directs transcription of the genes in the rice endosperm. In addition, the transformed gene requires appropriate transcription termination signals and signal sequences that allow insertion of the encoded protein into the correct cell compartment.
interconversion of mannose 6-phosphate and fructose animals but is not found in most plants. It catalyzes the
This enzyme is common in phospho-
a selectable marker gene such as that encoding
the phenotype conferred by the introduced gene of interest. The cells are then tested for other characteristics, including
using gene-specific primers. Plants that express the gene of
This is often done by techniques such as PCR amplification
that the resistant cells also express the cotransformed gene. It is then necessary to verify
hygromycin—an antibiotic that also inhibits the growth of
The cells are then incubated in culture medium containing
introduced into plant cells along with the gene of interest.
Selectable Markers
The rates at which T-DNA successfully integrates into the
plant genome and becomes appropriately expressed are low. Often, only one cell in 1000 or more will be successfully
transformed. Before growing cultured plant cells into mature
plants to test their phenotypes, it is important to eliminate
the background of nontransformed cells. This can be done
using either positive or negative selection techniques.
An example of negative selection involves use of a
marker gene such as the hygromycin-resistance gene. This gene, together with an appropriate promoter, can be
introduced into plant cells along with the gene of interest.
The cells are then incubated in culture medium containing hygromycin—an antibiotic that also inhibits the growth of
eukaryotic cells. Only cells that express the hygromycin-
resistance gene will survive. It is then necessary to verify
that the resistant cells also express the cotransformed gene. This is often done by techniques such as PCR amplification
using gene-specific primers. Plants that express the gene of
interest are then tested for other characteristics, including
the phenotype conferred by the introduced gene of interest.
An example of positive selection involves the use of a
selectable marker gene such as that encoding phospho-
mannose isomerase (PMI). This enzyme is common in
animals but is not found in most plants. It catalyzes the
interconversion of mannose 6-phosphate and fructose
6-phosphate. Plant cells that express the pmi gene can
survive on synthetic culture medium that contains only
mannose as a carbon source. Cells that are cotransformed with the pmi gene under control of an appropriate pro-
motor and the gene of interest can be positively selected by
growing the plant cells on a mannose-containing medium.
This type of positive selection was used to create Golden
Rice 2. Studies have shown that purified PMI protein is
easily digested, nonallergenic, and nontoxic in mouse oral
toxicity tests. A variation in positive selection involves use
of a marker gene whose expression results in a visible phen-
type, such as deposition of a colored pigment.
The following descriptions illustrate the methods used
to engineer two GM crops: Roundup-Ready soybeans and
Golden Rice 2.
Roundup-Ready® Soybeans
The Roundup-Ready soybean GM variety received mar-
et approval in the United States in 1996. It is a GM plant
with resistance to the herbicide glyphosate, the active
ingredient in Roundup, a commercially available broad-
spectrum herbicide. Glyphosate interferes with the enzyme
5-enolpyruvylshikimate-3-phosphate synthase (EPSPS),
which is present in all plants and is necessary for plant syn-
thesis of the aromatic amino acids phenylalanine, tyrosine,
and tryptophan. EPSPS is not present in mammals, which
obtain aromatic amino acids from their diets.
To produce a glyphosate-resistant soybean plant,
researchers cloned an epsps gene from the Agrobacterium
strain CP4. This gene encodes an EPSPS enzyme that is
resistant to glyphosate. They then cloned the CP4 epsps
gene downstream of a constituatively expressed promoter
from the cytomegalovirus to allow gene expression in all
plant tissues. In addition, a short peptide known as a chlo-
roplast transit peptide (in this case from petunias) was
inserted into the plant genome.
The Roundup-Ready soybean GM variety as of 1996
was engineered by researchers at Pro馗ision Plant Sci,
a company formed by two of the inventors of the
 glyphosate resistance technology. They initially
engineered glyphosate-resistant tobacco plants,
which were used as a model for the soybean
transformation. The tobacco plants were
transformed with a single plasmid containing
the CP4 epsps gene and the petunia chlo-
roplast transit peptide. After a few
years, they also transformed maize
with the same plasmid. The project was
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GUS gene acted as a positive marker, as cells that expressed the plasmid after transformation could be detected by the presence of a blue precipitate. The final cell line chosen for production of Roundup-Ready soybeans did not contain the GUS gene.

The plasmids were introduced into cultured soybean cells using biolistic bombardment. Afterward, cells were treated with glyphosate to eliminate any nontransformed cells. (ST Figure 5–6). The resulting calluses were grown into plants, which were then field tested for glyphosate resistance and a large number of other parameters, including composition, toxicity, and allergenicity.

Golden Rice 2

To create Golden Rice 2, scientists cloned three genes into the T-DNA region of a Ti plasmid. The Ti plasmid, called pSYN12424, is shown in ST Figure 5–7. The first gene was the carotene desaturase (crtI) gene from Erwinia uredovora, fused between the rice glutelin gene promoter (Glu) and the nos gene terminator region (nos). The Glu promoter directs transcription of the fusion gene specifically in the rice endosperm. The nos terminator was cloned from the Agrobacterium tumefaciens nopaline synthase gene and supplies the transcription termination and polyadenylation sequences required at the 3′ end of plant genes. The second gene was the phytoene synthase (psy) gene cloned from maize. The maize psy gene has approximately 90 percent sequence similarity to the rice psy gene and is involved in carotenoid synthesis in maize endosperm. This gene was also fused to the Glu promoter and the nos terminator sequences in order to obtain proper transcription initiation and termination in rice endosperm. The third gene was the selectable marker gene, phosphomannose isomerase (pmi), cloned from E. coli. In the Golden Rice 2 Ti plasmid, the pmi gene was fused to the maize polyubiquitin gene promoter (Ubi1) and the nos terminator sequences. The Ubi1 promoter is a constitutive promoter, directing transcription of the pmi gene in all plant tissues.

To introduce the pSYN12424 plasmid into rice cells, researchers established embryonic rice cell cultures and infected them with Agrobacterium tumefaciens that contained pSYN12424 (ST Figure 5–8). The cells were then placed under selection, using culture medium containing only mannose as a carbon source. Surviving cells expressing the pmi gene were then stimulated to form calluses that were grown into plants. To confirm that all three genes were present in the transformed rice plants, samples were taken and analyzed by the polymerase chain reaction (PCR) using gene-specific primers. Plants that contained one integrated copy of the transgenic construct and synthesized betacarotene in their seeds were selected for further testing.

GM Foods Controversies

GM foods may be the most contentious of all products of modern biotechnology. Advocates of GM foods state that the technologies have increased farm productivity, reduced pesticide use, preserved soils, and have the potential to feed...
growing human populations. Critics claim that GM foods are unsafe for both humans and the environment; accordingly, they are applying pressure on regulatory agencies to ban or severely limit the extent of GM food use. These campaigns have affected regulators and politicians, resulting in a patchwork of regulations throughout the world. Often the debates surrounding GM foods are highly polarized and emotional, with both sides in the debate exaggerating their points of view and selectively presenting the data. So, what are the truths behind these controversies?

One point that is important to make as we try to answer this question is that it is not possible to make general statements about all “GM foods.” Each GM crop or organism contains different genes from different sources, attached to different expression sequences, accompanied by different marker or selection genes, inserted into the genome in different ways and in different locations. GM foods are created for different purposes and are used in ways that are both planned and unplanned. Each construction is unique and therefore needs to be assessed separately.

We will now examine two of the main GM foods controversies: those involving human health and safety, and environmental effects.

**Health and Safety**

GM food advocates often state that there is no evidence that GM foods currently on the market have any adverse health effects, either from the presence of toxins or from potential allergens. These conclusions are based on two observations. First, humans have consumed several types of GM foods for more than 20 years now, and no reliable reports of adverse effects have emerged. Second, the vast majority of toxicity tests in animals, which are required by government regulators prior to approval, have shown no negative effects. A few negative studies have been published, but these have been criticized as poorly executed or nonreproducible.

Critics of GM foods counter the first observation in several ways. First, as described previously, few GM foods are eaten directly by consumers. Instead, most are used as livestock feed, and the remainder form the basis of purified food ingredients. Although no adverse effects of GM foods in livestock have been detected, the processing of many food ingredients removes most, if not all, plant proteins and DNA. Hence, ingestion of GM food-derived ingredients may not be a sufficient test for health and safety. Second, GM foods critics argue that there have been few human clinical trials to directly examine the health effects of most GM foods. One notable exception is Golden Rice 2, which has undergone two small clinical trials. They also say that the toxicity studies that have been completed are performed in animals—primarily rats and mice—and most of these are short-term toxicity studies.

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Supporters of GM foods answer these criticisms with several other arguments. The first argument is that short-term toxicity studies in animals are well-established methods for detecting toxins and allergens. The regulatory processes required prior to approval of any GM food demand data from animal toxicity studies. If any negative effects are detected, approval is not given. Supporters also note that several dozen long-term toxicity studies have been published that deal with GM crops such as glyphosate-resistant soybeans and Bt corn, and none of these has shown long-term negative effects on test animals. A few studies that report negative long-term effects have been criticized as poorly designed and unreliable. GM food advocates note that human clinical trials are not required for any other food derived from other genetic modification methods such as selective breeding. During standard breeding of plants and animals, genomes may be mutagenized with radiation or chemicals to enhance the possibilities of obtaining...
a desired phenotype. This type of manipulation has the potential to introduce mutations into genes other than the ones that are directly selected. Also, plants and animals naturally exchange and shuffle DNA in ways that cannot be anticipated. These include interspecies DNA transfers, transposon integrations, and chromosome modifications. These events may result in unintended changes to the physiology of organisms—changes that could potentially be as great as those arising in GM foods.

**Environmental Effects**

 Critics of GM foods point out that GMOs that are released into the environment have both documented and potential consequences for the environment—and hence may indirectly affect human health and safety. GM food advocates argue that these potential environmental consequences can be identified and managed. Here, we will describe two different aspects of GM foods as they may affect the natural environment and agriculture.

1. **Emerging herbicide and insecticide resistance.** Many published studies report that the planting of herbicide-tolerant and insect-resistant GM crops has reduced the quantities of herbicides and insecticides that are broadly applied to agricultural crops. As a result, the effects of GM crops on the environment have been assumed to be positive. However, these positive effects may be transient, as herbicide and insecticide resistance is beginning to emerge. (ST Figure 5-9).

   Since glyphosate-tolerant crops were introduced in the mid-1990s, more than 24 glyphosate-resistant weed species have appeared in the United States. Resistant weeds have been found in 18 other countries, and in some cases, the presence of these weeds is affecting crop yields. One reason for the rapid rise of resistant weeds is that farmers have abandoned other weed-management practices in favor of using a single broad-spectrum herbicide. This strong selection pressure has brought the rapid evolution of weed species bearing gene variants that confer herbicide resistance. In response, biotechnology companies are developing new GM crops with tolerance to multiple herbicides. However, scientists argue that weeds will also develop resistance to the use of multiple herbicides, unless farmers vary their weed management practices and incorporate tillage, rotation, and other herbicides along with using the GM crop. Scientists point out that herbicide resistance is not limited to the use of GM crops. Weed populations will evolve resistance to any herbicide used to control them, and the speed of evolution will be affected by the extent to which the herbicide is used.

   Since 1996, more than eight different species of insect pests have evolved some level of resistance to Bt insecticidal proteins. For example, in 2011 scientists reported the first cases of resistance of the western corn rootworm to Bt maize expressing the cry3Bb1 gene, in maize fields in Iowa. In 2010, scientists from Monsanto detected large numbers of pink bollworms with resistance to the toxin expressed from the cry1Ac gene in one variety of Bt cotton. In order to slow down the development of Bt resistance, several strategies are being followed. The first is to develop varieties of GM crops that express two Bt toxins simultaneously. Several of these varieties are already on the market and are replacing varieties that express only one Bt cry gene. The second strategy involves the use of “refuges” surrounding fields that grow Bt crops. These refuges contain non-GM crops. Insect pests grow easily within the refuges, which place no evolutionary pressure on the insects for resistance to Bt toxins. The idea is for these nonselected insects to mate with any resistant insects that appear in the Bt crop region of the field. The resulting hybrid offspring will be heterozygous for any resistance gene variant. As long as the resistance gene variant is recessive, the hybrids will be killed by eating the Bt crop. In fields that use refuges and plant GM crops containing two Bt genes, resistance to Bt toxins has been delayed or is absent. As with emerging herbicide resistance, farmers are also encouraged to combine the use of Bt crops with conventional pest control methods.

2. **The spread of GM crops into non-GM crops.** There have been several documented cases of GM crop plants appearing in uncultivated areas in the United States, Canada, Australia, Japan, and Europe. For example, GM sugar beet plants have been found growing in commercial top
soils. GM canola plants have been found growing in ditches and along roadways, railway tracks, and in fill soils, far from the fields in which they were grown. A 2011 study found “feral” GM canola plants growing in 288 of 634 sample sites along roadways in North Dakota. Of these plants, 41 percent contained the CP4 EPSPS protein (conferring glyphosate resistance), and 39 percent contained the PAT protein (conferring resistance to the herbicide glufosinate). In addition, two of the plants (0.7 percent of the sample) expressed both proteins (resistant to both herbicides). GM plants that express both proteins have not been created by genetic modification and were assumed to have arisen by cross-fertilization of the other two GM crops. The researchers who conducted this survey were not surprised to find GM canola along transportation routes, as seeds are often spilled during shipping. More surprising was the extent of the distribution and the presence of hybridized GM canola plants.

One of the major concerns about the escape of GM crop plants from cultivation is the possibility of out-crossing or gene flow—the transfer of transgenes from GM crops into sexually compatible non-GM crops or wild plants, conferring undesired phenotypes to the other plants. Gene flow between GM crops and adjacent non-GM crops is of particular concern for farmers who want to market their crops as “GM-free” or “organic” and for farmers who grow seed for planting.

Gene flow of GM transgenes has been documented in GM and non-GM canola as well as sugar beets, and in experiments using rice, wheat, and maize. GM critics often refer to controversial studies about GM outcrossing in Oaxaca, Mexico. In the first study in 2001, it was reported that the local maize crops contained transgenes from Monsanto’s Roundup-Ready and Bt insect-resistant maize. As GM crops were not approved for use in Mexico, it was thought that the transgenes came from maize that had been imported from the United States as a foodstuff, and then had been planted by farmers who were not aware that the seeds were transgenic. Over the next ten years, subsequent studies reported mixed results. In some studies, the transgenes were not detected, and in others, the same transgenes were detected. There is still no consensus about whether gene flow has occurred between the GM and non-GM maize in Mexico.

It is thought that the presence of glyphosate-resistant transgenes in wild plant populations is not likely to be an environmental risk and would confer no positive fitness benefits to the hybrids. The presence of glyphosate-resistant genes in wild populations would, however, make it more difficult to eradicate the plants. This is illustrated in a case of escaped GM bentgrass in Oregon, where it has been difficult to get rid of the plants because it is no longer possible to use the relatively safe herbicide glyphosate. The potential for environmental damage may be greater if the GM transgenes did confer an advantage—such as insect resistance or tolerance to drought or flooding.

In an attempt to limit the spread of transgenes from GM crops to non-GM crops, regulators are considering a requirement to separate the crops so that pollen would be less likely to travel between them. Each crop plant would require different isolation distances to take into account the dynamics of pollen spreading. Several other methods are being considered. For example, one proposal is to make all GM plants sterile using RNAi technology. Another is to introduce the transgenes into chloroplasts. As chloroplasts are inherited maternally, their genomes would not be transferred via pollen. All of these containment methods are in development stages and may take years to reach the market.

The Future of GM Foods

Over the last 20 years, GM foods have revealed both promise and problems. GM advocates are confident that the next generation of GM foods will show even more promising prospects—and may also address many of the problems.

Research is continuing on ways to fortify staple crops with nutrients to address diet problems in poor countries. For example, Australian scientists are adding genes to bananas that will not only provide resistance to Panama disease—a serious fungal disease that can destroy crops—but also increase the levels of beta-carotene and other nutrients, including iron. Other GM crops in the pipeline include plants engineered to resist drought, high salinity, nitrogen starvation, and low temperatures.

Scientists hope that new genome information and more precise technologies will allow them to accurately edit a plant’s endogenous genes—decreasing, increasing, or eliminating expression of one or more of the plant’s genes in order to create a desirable phenotype. These approaches avoid the use of transgenes and address some of the concerns about GM foods. The current techniques that researchers use to introduce genes into plant cells result in random insertions into the genome. New techniques are being devised that will allow genes to be inserted into precise locations in the genome, avoiding some of the potential unknown effects of disrupting a plant’s normal genome with random integrations.

Researchers are also devising more creative ways to protect plants from insects and diseases. One intriguing project involves introducing into wheat a gene that encodes a pheromone that acts as a chemical alarm signal to aphids. If successful, this approach could protect the wheat plants from aphids without using toxins. Another project involves cassava, which is a staple crop for many Africans and is afflicted by two viral diseases—cassava mosaic virus and brown streak virus—that stunt growth and cause root rot (ST Figure 5–10). Although some varieties of cassava are resistant to these viruses, the life cycle of cassava is so long that it would be difficult to introduce resistance into other varieties using conventional breeding techniques. Scientists plan to transform plants with genes from resistant cassava. This type of cisgenic gene transfer is more comparable to traditional breeding than transgenic techniques.

In the future, GM foods will likely include additional GM animals. As described in Box 1, a transgenic Atlantic salmon variety is likely to receive marketing approval in the near future. In another project, scientists have introduced a DNA sequence into chickens that protects the birds from spreading avian influenza. The sequence encodes a hairpin RNA molecule with similarity to a normal viral RNA that binds to the viral polymerase. The presence of the hairpin RNA inhibits the activity of the viral polymerase and interferes with viral propagation. If this strategy proves useful in vivo, the use of these GM chickens would not only reduce the incidence of avian influenza in poultry production, but also reduce the transmissibility of avian influenza viruses to humans.

Although these and other GM foods show promise for increasing agricultural productivity and decreasing disease, the political pressure from anti-GM critics remains a powerful force. An understanding of the science behind these technologies will help us all to evaluate the future of GM foods.

Selected Readings and Resources

**Journal Articles**


**Web Sites**

Review Questions

1. How do genetically modified organisms compare with organisms created through selective breeding?
2. Can current GM crops be considered as transgenic or cisgenic? Why?
3. Of the approximately 200 GM crop varieties that have been developed, only a few are widely used. What are these varieties, and how prevalent are they?
4. How does glyphosate work, and how has it been used with GM crops to increase agricultural yields?
5. Describe the mechanisms by which the Cry proteins from Bacillus thuringiensis act as insecticides.
6. What measures have been taken to alleviate vitamin A deficiencies in developing countries? To date, how successful have these strategies been?
7. What is Golden Rice 2, and how was it created?
8. Describe how plants can be transformed using biolistic methods. How does this method compare with Agrobacterium tumefaciens-mediated transformation?
9. How do positive and negative selection techniques contribute to the development of GM crops?
10. Describe how the Roundup-Ready soybean variety was developed, and what genes were used to transform the soybean plants.

Discussion Questions

1. What are the laws regulating the development, approval, and use of GM foods in your region and nationally?
2. Do you think that foods containing GM ingredients should be labeled as such? What would be the advantages and disadvantages to such a strategy?
3. One of the major objections to GM foods is that they may be harmful to human health. Do you agree or disagree, and why?