CHAPTER 14

Plant Breeding and Propagation

OVERVIEW

In this chapter, the processes that have produced and propagated domesticated plants are discussed. Proposed origins of our agricultural societies are presented along with the phenotypic changes that occurred during crop domestication. Plant breeding methods using conventional and nonconventional strategies are outlined. Then, propagation methods using seeds and asexual propagules are discussed.

Some Learning Goals

1. Provide explanations for the shift from hunter-gatherer socie-ties to agricultural ones.
2. Describe phenotypic changes that occurred in plant populations as a result of human selection.
3. Describe breeding methods used for self-pollinating crops.
4. Describe breeding methods used for cross-pollinating crops.
5. Explain the significance of germplasm banks to crop improvement programs.
6. Describe the method used to produce protoplast fusion hybrids.
7. Outline the major steps involved in creating a transgenic plant.
8. Outline the steps involved in growing a crop from seed.
9. Describe how cutting propagation methods produce genetically identical plants.
10. Explain the benefits of grafting and outline the steps involved in making a graft.
11. Provide examples of specialized roots and stems used for asexual propagation.
12. Explain the benefits of micropropagation and outline the steps involved in micropropagation.

CROP PLANT EVOLUTION

"It is clear that the human species is currently an eater of grass seeds. We have become 'canaries.' It is also clear that the world's food supply depends on 12 or 15 plant species. It probably was not always so, although wheat, barley, rice, and maize have been the foundations of our high civilizations. The current trend is for the major crops to become even more major and for the lesser ones to dwindle."[1]

n fact, although there are approximately 250,000 species of flowering plants, only six species—wheat, rice, corn, potato, sweet potato, and cassava—provide 80% of the calories consumed by humans worldwide (Fig. 14.1). An additional eight plants—sugar cane, sugar beet, bean, soybean, barley, sorghum, coconut, and banana—complete the list of major crops grown for human consumption.

In the 2 million years that we have inhabited this earth, we have cultivated plants for only the most recent 10,000 years, or less than 1% of our history. However, in that time, we have dramatically changed the plant landscape and our own lives. Hunter-gatherer societies have given way to agricultural societies and the development of cities and civilizations. Such concentrations of people are vulnerable to catastrophes such as drought and famine. Domesticated plants depend on us for their survival, but we have also become dependent on them for our survival.

We **domesticate** plants by altering them genetically to meet our needs. Strictly speaking, a domesticated plant is one whose reproductive success depends on human intervention. This is an ongoing evolutionary process, and plants are found in a continuum from purely wild to fully domesticated. Our current crop plants continue to evolve as a result of our breeding efforts. It is amazing that, although humans have eaten thousands of types of plants in the past, we currently rely on only a handful to supply almost all of our nutritional needs.

Origins of Agriculture

Domestication of crop plants, and the development of industrial societies that followed, is a recent event in our history. Of the 80 billion humans that have lived on this earth, 90% were hunter-gatherers, 6% were farmers, and only 4% were industrialized.[2]

We do not know why most humans shifted from a hunter-gatherer lifestyle to an agricultural one. Several hypotheses exist, but they are purely speculative. Hunter-gatherers may have had better lives than cultivators did. Their diet and health were better, their starvation rate was lower, and they worked far fewer hours. Their work week was only about 20 hours long! Because they had abundant free time, hunter-gatherers may have simply begun to grow plants in gardens as a hobby. They may have begun to cultivate a few plants to supplement lean times in the gathering schedule. Or, they may have had spiritual reasons for growing plants in a protected environment. Because agricultural practices arose independently in many parts of the world over thousands of years, each of these scenarios probably explains why humans began to domesticate plants.

People began to domesticate plants in the Near East around 10,000 years ago. Many agricultural sites from this time period have been located on the western edge of what is now Iran. Domesticated plants were developed in Asia and the New World 1,000 to 3,000 years later. Farming probably began in Africa and the New World about 4,000 years ago.

The first crops domesticated were cereal grains. Root crops and legumes, such as peas and beans, were domesticated 1,000 to 2,000 years later. These were followed by vegetables, and then oil, fiber, and fruit crops. Finally, plants used for forage, decoration, and drugs were first domesticated only about 2,000 years ago. Few crops have been brought into domestication in recent centuries. Plant breeders currently devote much more energy to improving existing crops than developing new ones. The map in Figure 14.2 shows where some of our major crops were domesticated. Sunflower is the only major crop that was domesticated in the present-day United States (Fig. 14.3). For further discussion on the origin of cultivated plants, see Chapter 24.

Evolution Under Domestication

"It is as if man had been appointed managing director of the biggest business of all, the business of evolution . . . whether he is conscious of what he is doing or not, he is in point of fact determining the future direction of evolution on this earth. That is his inescapable destiny, and the sooner he realizes it and starts believing in it, the better for all concerned."[3]

As soon as people began sowing seeds of selected plants, instead of simply harvesting wild plants, dramatic genetic changes occurred in those populations in response to selection pressure. The seed dispersal mechanism was probably the first trait altered during domestication. The seeds of wild plants are typically carried away by wind, water, or animals. This minimizes the competition for light and nutrients that results from overcrowding. People most likely gathered seeds from plants that retained them instead of dispersing them. Early domesticators would have focused also on plants with high seed numbers and large seed size (Fig. 14.4). Competition in crowded cultivated plots would favor high seedling vigor and fast germination rate. In addition, while seed dormancy mechanisms are important in wild plants, plants that survived in cultivated gardens were those that germinated immediately upon planting. These changes accumulated over many generations and created the cultivated plants we know today. Many of these plants have been so dramatically altered from their wild forms that they cannot survive without the aid of humans. For example, if a cornfield is abandoned, it will cease to produce any corn plants within just a year or two.

The first serious attempts to understand the geographic origins of domesticated plants were made by the Russian geneticist Nikolai Ivanovich Vavilov in the 1930s. Based on extensive collecting expeditions carried out worldwide, Vavilov proposed that each crop plant was first domesticated in the region of the world where its genetic diversity is greatest. Tragically, Vavilov's career and life were cut short when he was imprisoned by the Stalin government in 1940 on charges of espionage and, ironically, efforts to harm agriculture. He died in prison 3 years later. Although more recent studies indicate that patterns of crop domestication cannot be adequately explained by Vavilov's *Centers of Origin*, he can be credited with initiating studies that have led to our understanding of the relationships between cultivated plants and their wild relatives. As we will see later, wild relatives of crop plants are valuable sources of genes for modern crop improvement.

PLANT BREEDING

The systematic breeding of crop plants has been carried out only in the past 200 years. Until then, crop improvement was based on simply selecting and planting seeds from desirable plants. This method was moderately effective because plant populations were genetically diverse and could be improved gradually across generations (Fig. 14.5). However, it took an understanding of genetics and plant reproduction, and the training of scientists in these fields, to develop the sophisticated crop-breeding methods we know today.

Plant breeding is accelerated evolution guided by humans rather than nature. Breeders replace natural selection with human selection in order to modify plants genetically to meet our needs. Remember from Chapter 13 that, according to the

Hardy-Weinberg law, a population of plants will remain in genetic equilibrium unless an outside force changes its genetic makeup. Plant breeders act as that outside force, molding plant populations to contain higher proportions of alleles that are desirable to humans. Typically, the primary goal of plant-breeding programs is improved yield, with disease resistance, pest resistance, and stress tolerance contributing to yield. With new sources of agricultural land dwindling and the human population continuing to grow, efforts must concentrate on improving the amount of food harvested per acre and developing crops that can grow on low-quality land (Fig. 14.5).

Breeding Methods Using Sexually Compatible Germplasm

Strategies

A plant breeder tends to divide crop plant species into two groups: **self-pollinating** and **cross-pollinating.** A self--pollinating plant is capable of fertilizing itself, while a cross-pollinating one cannot. Breeding methods for self--pollinating plants are radically different from those for cross--pollinating ones. A self-pollinating plant tends to be highly **homozygous** because all of its genes came from the same parent. The plant's mother was also its father! As you might expect, these plants have undergone a significant amount of **inbreeding.** As the species has evolved, it has adapted to and thrives on inbreeding. On the other hand, a cross--pollinating plant is likely to be highly **heterozygous,** because its mother and father were different plants. Cross-pollinating species tend to require a high level of heterozygosity in order to be productive.

Self-pollinated crop plants include wheat, rice, oats, barley, peas, beans, tomatoes, and peppers. Some fruit trees, such as apricots, nectarines, peaches, and citrus fruits are also primarily self-pollinated. The most primitive form of breeding in this group of plants is called **pure-line selection.** This method simply involves collecting seed from each of several plants, growing all the seed from each plant in a row, and selecting the most desirable row. All the plants in a row will be related to each other, since they came from the same plant. The seeds from the best row can then be propagated as a new "pure-line" variety.

A breeder with an understanding of reproductive biology can make crosses between self-pollinating plants. In fact, today, most plant breeders create hybrid populations by crossing desirable parents. Then, as the hybrid plants self-pollinate, highly diverse groups of offspring will be created. These offspring of the self-pollinated hybrids provide the basis for selecting pure-line varieties. Crosses between normal wheat varieties and dwarf plants have been created to develop dwarf varieties. More of the energy in these plants is directed toward producing seeds rather than stalks, resulting in higher yields.

Norman Borlaug, who is known as the "Father of the Green Revolution," was awarded the Nobel Prize in 1970 for developing new strains of wheat in Mexico. The new high-yielding strains, which were produced from crosses with a dwarf variety from Japan, were widely planted. Before the project began in 1944, Mexico had imported wheat for many years, but the new varieties were so successful that, within 20 years, Mexico had quadrupled its wheat production and had enough to export to other countries. India and Pakistan experienced similar gains. It has been argued that, by increasing grain yields in developing countries, Borlaug has saved more lives than anyone in history (Fig. 14.6).

Although the Green Revolution has been a dramatic success, gains have not been unqualified. Irrigation and inorganic fertilizer are needed to support the rapid growth of these plants. Because many inorganic fertilizers are produced from fossil fuels, the cost of farming increases with fuel prices. On the other hand, the high-input system of agriculture has allowed food production to increase without a corresponding increase in the amount of land used for farming. This means that in areas where population growth is strong, high-yield farming prevents deforestation of wild areas.

Cross-pollinated species include corn, rye, alfalfa, and clover, as well as most fruits, nuts, and vegetables. The simplest form of selection in cross-pollinated crops is **mass selection.** With this method, many plants from a population are selected, and seeds from these plants are then used to create the next generation. Again, the seeds from the best plants are chosen and propagated, and so on, for many generations. This slowly molds the genetic makeup of the population to fit the breeder's preferences. For example, suppose variations in seed size are due to genetic differences and the breeder always collects from plants with the largest seeds. After several generations of selection, the breeder has genetically altered the population so that a larger proportion of plants carry the genes for large seed size.

Outcrossing (crossing between genetically different plants) in cross-pollinated crops often results in hybrid vigor, or **heterosis.** These plants are large, vigorous, fertile, and high yielding. Conversely, self-pollination of cross--pollinated plants will result in **inbreeding depression** in the form of small size, poor vigor, low reproductive capacity, and a high proportion of abnormal plants, due to the expression of deleterious recessive alleles. Breeders, however, do not avoid inbreeding of cross-pollinated species. In fact, a common breeding method involves forced self-pollination for several generations to create inbred lines in which deleterious alleles have been eliminated. Then, selected inbred lines are crossed to produce **hybrid seed.** Using this method, the most dramatic success story to date involves corn (maize), in which crosses between unrelated inbred lines often produce hybrids that dramatically outyield their parents. In 1908, in one of the earliest hybrid corn studies, plant breeder G. H. Shull crossed two inbred lines of corn, each of which produced 20 bushels per acre. The hybrid offspring yielded 80 bushels per acre, a quadruple increase in yield. Most of the corn in the United States is grown from hybrid seed (Fig. 14.7).

Some early American and European varieties, called **heirloom varieties,** are grown as open-pollinated populations of plants. Each variety is a mixture of genotypes, and all plants are allowed to pollinate each other, or open-pollinate, during seed production. These varieties are not as uniform as modern varieties, but their genetic variability allows them to produce a crop under many different environments. For example, a dry year and an insect pest might severely compromise all the plants in a hybrid variety if

the variety is not genetically capable of surviving these conditions. An open-pollinated variety is likely to have some plants that are adapted to drought and others that can resist insect damage. The Seed Savers Exchange in Decorah, Iowa, has been created to preserve and distribute heirloom varieties. Heirloom varieties are important for farm market producers and growers of organic crops. They also contribute genetic diversity to breeding programs.

Germplasm Collection and Gene Banks

Progress in plant breeding is absolutely dependent on genetic variability. It is impossible to improve a population if there is no genetic variability for the trait of interest. For example, a breeder who needs to develop rust fungus--resistant wheat must begin with a population containing at least one plant with some degree of resistance. Plant breeders are, therefore, concerned with the *germplasm* resources of crop plants. A crop plant's **germplasm** is the sum total of its genes. A breeder must have access to crop plant germplasm containing genes for traits that are important for current breeding efforts and in the future, new traits may become important. For example, in a decade or two, we may need to develop plants with resistance to a new pathogen.

The plant varieties used in agriculture today have resulted from centuries of selection for specific traits. They are often genetically uniform and, therefore, may not be good sources of new genetic variability for further advances in breeding. In addition, their homogeneity makes them vulnerable to outbreaks of pests. The Irish potato famine in the mid-1800s occurred because people in that region relied on a few varieties of potatoes for most of their food supply. When a pathogen spread through the fields, all the plants succumbed, and the people lost their entire potato crop. As recently as 1970, 15% of the United States corn crop was wiped out by a fungal pathogen. Genetic uniformity in the corn varieties grown at that time resulted in this significant loss.

In order to meet current and future needs for plant genetic diversity, **gene banks** have been established worldwide. The International Plant Genetic Resources Institute is responsible for coordinating international efforts to collect and conserve crop plant germplasm. The Institute also assists regional and national gene banks. The collaborative efforts of state and federal government agencies, along with private industries, comprise the National Plant Germplasm System in the United States. Scientists regularly conduct collecting expeditions in regions where wild relatives of crop plants are found (Fig. 14.8A). In a similar fashion, botany students often learn how to identify and collect plant specimens for herbariums and museums worldwide (Fig. 14.8B). These skills help professional botanists to bring plant samples back to gene banks, where they are catalogued, propagated, and screened for desirable traits. Some seeds or other -**propagules** are put into long-term storage for future needs, while others are given to plant breeders for immediate use.

Breeding Methods Using Sexually Incompatible Germplasm

In recent years, powerful new tools have been added to the toolboxes of plant breeders. Most breeding progress today is made using crosses among crop plants and their relatives, as described in the previous section, "Breeding Methods Using Sexually Compatible Germplasm." However, in many breeding programs, species boundaries no longer exist. Through techniques such as *protoplast fusion* and *gene splicing* (discussed in sections that follow), genes from virtually any living organism can be incorporated into crop plants. Everything, therefore, from an antifreeze gene in North Atlantic fish to a pesticide gene in bacteria, is now accessible to breeding programs.

Protoplast Fusion

There are times when a breeder would like to tap into the genetic diversity of a plant species that is distantly related to a crop plant. However, it is impossible to make a cross between the two species and obtain viable seeds. An alternative strategy involves **protoplast fusion.** With this method, cells of each species are grown in a liquid nutrient solution. Then, their cell walls are chemically stripped off to produce protoplasts. The protoplasts of the two species are mixed together and stimulated, with the aid of an electric current or chemical solution, to fuse with each other. When screening the fusion products, a scientist must distinguish between *autofusions*, where two protoplasts of the same species have fused together, and *hybrid fusions*, in which the protoplast of one species has fused with that of the other species. One way of doing this involves dyeing the protoplasts of each species with a different color before the fusion event. Then, the scientist can examine the fusions with a microscope and identify protoplasts that show both colors. The selected protoplasts are then carried through a series of *tissue culture* events (see "Micropropagation" on page 261) that cause them to grow into whole plants. These new plants carry genes from two distantly related species. They are called **somatic hybrids** because they resulted from combining (hybridizing) *somatic*, or body, cells from two different plants. In contrast, plants usually reproduce when gamete cells (eggs and sperm) are combined, producing sexual hybrids.

Although protoplast fusion appears to have value for the incorporation of new genes into crop plants, few success stories are currently available. Somatic hybrids have been created between potato cultivars and nontuber-bearing wild relatives. Some are highly resistant to a number of bacterial and fungal diseases, but varieties have not yet been created from the hybrids or their offspring. Tobacco is probably the best example of a commercially successful somatic hybrid. A common variety produced in Canada is the result of a fusion between cultivated tobacco and a wild relative containing a novel form of disease resistance.

Gene Splicing and Transgenic Plants

Genes from virtually any organism, from viruses to humans, can now be inserted into plants, creating **transgenic plants.** The technologies that have been developed to support this work arose from basic research to understand DNA structure and function. **Recombinant DNA** technology is based on the knowledge that DNA behaves the same no matter what organism carries it. Because the DNA code is nearly universal, a plant cell can read a DNA sequence from almost any other organism and produce a foreign gene product.

The serendipitous discovery of **restriction enzymes** paved the way for molecular genetic engineering. These enzymes act as molecular scissors, chopping up DNA into pieces small enough for genetic analysis. In nature, bacteria produce restriction enzymes, protecting themselves from viral pathogens by destroying pieces of foreign DNA. A key feature of these enzymes is their ability to cut DNA at specific sites and create DNA fragments with "sticky" ends. When DNA is cut by certain restriction enzymes, one strand is slightly longer than the other. Each fragment then is a double-stranded DNA molecule with a single-stranded tail at each end. This tail is called a sticky end because it will base-pair with the tail of any other fragment cut with the same enzyme (Fig. 14.9).

How are transgenic plants made? As an example, let's say we would like to insert a bacterial insect resistance gene into a corn plant. First, we need to find the insect resistance gene in the bacterium. This is actually a very difficult task and beyond the scope of this book. As you might expect, finding a specific gene in the enormous genome of eukaryotes is even more difficult and is like looking for a needle in a haystack. Sometimes, if a protein gene product is known, then a machine called a **protein sequencer** can be used to determine the sequence of amino acids in the protein. Then, using information from the genetic code (see Chapter 13), a **DNA synthesizer** produces an artificial gene. An alternative approach is to find the gene in an organism's genome and then use a restriction enzyme to cut the bacterial chromosome on both sides of the gene, but not within the DNA sequence of the gene. After that, the bacterial DNA and a *cloning vector* are cut with the same restriction enzyme. A *cloning vector* is a piece of DNA that can copy itself in a living cell. A common example of a cloning vector is a **plasmid,** a small, circular piece of DNA naturally found in bacteria and capable of replicating independently of the bacterial chromosome. Dozens of custom-made plasmids containing restriction enzyme cutting sites have been created by genetic engineers. If the cut bacterial DNA is mixed with cut plasmids, then their sticky ends will join together and create recombinant DNA. In this case, the recombinant DNA is a plasmid with an insect resistance gene attached to it. *Escherichia coli*, the bacterial workhorse of molecular genetics, can be stimulated to take up the plasmid via a process called **transformation.** When the *E. coli* cells multiply in a flask of nutrient medium, they will replicate their recombinant plasmids. At this point, *E. coli* is cloning our insect resistance gene for us. A culture of the bacterium will contain millions of copies of the gene (Fig. 14.10).

After our insect resistance gene has been cloned, we have enough copies of it to manipulate and try to insert it into plant cells, again through transformation. We can remove the plasmids from a culture of *E. coli*, use the same restriction enzyme to cut out the resistance gene, and then purify it. Plant cells will not take up foreign DNA as readily as bacterial cells. Nevertheless, a number of techniques to transform plants with foreign DNA have been developed. We will focus on the two major ones.

The most common transformation technique relies on a natural genetic engineer, *Agrobacterium tumefaciens.* This bacterium infects plant cells and then inserts DNA (called **T-DNA,** or transfer DNA) from its plasmids into the plant's chromosomes. This causes the plant to produce cancerlike tumors that harbor and feed the bacterium (Fig. 14.11) Human genetic engineers, however, have removed the virulence (disease-causing) genes from the T-DNA region and replaced them with genes of human interest. In our example, we could insert the insect resistance gene into the T-DNA region of the *Agrobacterium* plasmid (Fig. 14.12). Then, we would allow the bacterium to infect corn cells and insert its T-DNA into the chromosomes of those cells. Transformed cells can be cultured to produce whole plants carrying and expressing the insect resistance gene. The gene has now been stably incorporated into a plant chromosome and will be passed on to subsequent generations (Fig. 14.13).

When the *Agrobacterium* transformation technique was first developed, it was not effective in monocots. As a result, an alternative strategy using a **particle gun** was developed especially for use with monocots. This technique actually first used a tiny modified shotgun, with DNA attached to the shot. Today's particle guns are much more refined (and expensive!) than that first prototype, but still rely on shooting DNA into plant tissue. With this technique, very small tungsten or gold pellets are coated with the cloned insect resistance gene. Then the DNA is shot into corn cells and, if enough plant cells are bombarded, some of them will have the gene incorporated into their chromosomes. Precisely how this process works is still a mystery, but through the use of this technique, foreign genes have been permanently inserted into crop plants. In fact, transgenic corn containing a bacterial insect resistance gene is now widely grown in the United States.

It is difficult to control the number of gene copies inserted during transformation. In addition, genes are inserted at random locations in plant chromosomes during this step. Sometimes, the foreign genes may be inserted in areas of the genome that are not expressed by the plant, or they may insert into a critical portion of a plant gene. In addition, considerable effort is often needed to get the foreign gene expressed at suitable levels in appropriate tissues. Transformation and gene expression, therefore, are the most challenging aspects of transgenic plant production.

Transgenic corn, soybean, cotton, potato, and canola varieties containing herbicide and insect resistance are grown extensively in North America (Fig. 14.14). Traits such as disease, insect, and herbicide resistance are important for the producers of those crops, but you would not notice them as a consumer. The new generation of transgenic crops focuses on traits that will be

more obvious. Transgenic plants can act as *bioreactors* to create pharmaceuticals. These plants would provide inexpensive access to vaccines and other medicines, especially in parts of the world where medical facilities are not readily available. People would simply take their medicine by eating potatoes or carrots. For example, transgenic plants are being tested for the production of vaccines against hepatitis B, rabies, cholera, tuberculosis, malaria, acute diarrhea, and even dental cavities. Transgenic plants have also been made to produce a protein that can prevent or delay the onset of insulin-dependent *diabetes mellitus.* At one time, all of our drugs were derived from plants, but we have learned to synthesize many of them in laboratories. Now, we are completing the circle by letting the plants once again create our drugs.

Nonedible transgenic plants are also showing potential in some novel ways. Plants are being created to produce biodegradable polymers in order to reduce our dependence on plastics made from nonrenewable resources. Plants are also being engineered to sequester heavy metals. These transgenics could be grown in contaminated soils where they will pick up wastes such as copper or mercury. Then, they could be harvested and disposed of properly.

Finally, transgenic technology is providing us with ornamental plants never before seen in nature. Most popular cut flowers do not produce blue hues, and efforts are presently being directed toward the development of blue carnations, chrysanthemums, and roses. For example, a violet carnation named Moonshadow contains a petunia gene for blue color (Fig. 14.15). Genetic engineers are also trying to extend the shelf life of cut flowers, mainly by blocking genes for ethylene synthesis. In addition, "flowering-time" genes are being used to develop plants that flower under day-length conditions that would normally prevent flowering; plant architecture genes are being used to produce either compact or vine-type plants; and fragrance genes have been identified in an effort to restore fragrance quality to roses and carnations.

Pros and Cons of Transgenic Plants

In October 2000, activists called the Green Streets destroyed a test plot of genetically engineered corn at a University of California test facility. In January 2001, a group called the Nighttime Gardeners destroyed a greenhouse containing genetically engineered wheat in Albany, California. And, in February 2001, a group called the Earth Liberation Front burned a research cotton gin in Visalia, California, to protest the development of genetically engineered cotton. In less than 2 years, over 40 antigenetic engineering acts of vandalism have occurred in North America. Why are people so concerned about transgenic crops?

There are approximately 109 million acres of transgenic crops grown worldwide, 68% of which are in the United States. The most common transgenic crops are soybean, corn, cotton, and canola. Most often, these plants contain a gene making them resistant to the herbicide glyphosate, commercially sold as Roundup or an insect resistance gene that produces a protein called Bt toxin (because it is derived from the bacterium *Bacillus thuringiensis*).

On the positive side, proponents of transgenic crops argue that transgenic crops are environmentally friendly because they allow farmers to use fewer and less noxious chemicals for crop production. For example, a 21% reduction in the use of insecticide has been reported on Bt cotton. In addition, when glyphosate is used to control weeds, then other, more-persistent herbicides do not need to be applied.

On the negative side, opponents of transgenic crops suggest that there are many questions that need to be answered before transgenic crops are grown on a large scale. One question deals with the effects that Bt plants have on "nontarget" organisms such as beneficial insects, worms, birds, and even humans who consume the genetically engineered crop. Monarch caterpillars feeding on milkweed plants near Bt cornfields will eat some corn pollen that has fallen on the milkweed leaves. Laboratory studies indicate that the caterpillars can die from eating Bt pollen. However, field tests indicate that Bt corn is not likely to harm monarchs. Remember, too, that application of pesticides (the alternative to growing Bt plants) has been demonstrated to cause widespread harm to nontarget organisms.

Another unanswered question is whether herbicide-resistance genes will move into populations of weeds. Crop plants are sometimes grown in areas where weedy relatives also live. This is especially true outside of the United States. (Remember that most of our crop plants did not originate in the United States, and they do not have wild relatives here.) If the crop plants hybridize with weedy relatives, then this herbicide-resistance gene will be perpetuated in the offspring. In this way, the resistance gene can make its way into the weed population. If this happens, a farmer can no longer use glyphosate, for example, to kill those weeds. This scenario is not likely to occur in many instances because there are no weedy relatives growing near the crop plant. However, in some cases, it may become a serious problem. For example, canola readily hybridizes with mustard weed species and could transfer its herbicide-resistance genes to those weeds. In addition, Canadian farmers have reported that herbicide-resistant canola has invaded wheat fields like a weed and, of course, cannot be killed with a major herbicide.

We know that evolution will occur when transgenic plants are grown on a large scale over a period of time. Of special concern is the development of insect populations resistant to the Bt toxin. This pesticide has been applied to plants for decades without the development of insect--resistant populations. However, transgenic Bt plants express the toxin in all tissues throughout the growing season. Therefore, all insects carrying genes that make them susceptible to the toxin will die. That leaves only the genetically resistant insects to perpetuate the population. When these resistant insects mate, they will produce offspring capable of surviving in the presence of the Bt toxin. Farmers are attempting to slow the development of insect resistance in Bt crops by, for example, planting non-transgenic border rows to act as a refuge for susceptible insects. These insects may allow Bt susceptibility to remain in the population.

Perhaps the most serious concern about the transgenic crop plants currently in use is that they encourage farmers to head farther away from sustainable agricultural farming practices. Transgenics, at least superficially, simplify farming by reducing the choices made by the manager. The planting of a glyphosate-resistant crop encourages a farmer to use that herbicide for the season, probably to the exclusion of all other herbicides and other weed-control practices. In the long run, though, it may be in the best interest of the farmer and the land to use more integrated, sustainable weed--control approaches. Farmers who use Bt transgenics may not feel they need to follow through with integrated pest-management practices that use beneficial insects and timely application of pesticides to control insect pests. In fact, a farmer must decide whether to plant Bt corn even before he knows whether the European corn borer will be a problem during the growing season. In contrast, a more sustainable approach would be to plant non-transgenic corn, monitor the fields throughout the growing season, and then apply a pesticide only if and when it is needed.

The jury is still out on the long-term effects of transgenic plants on our agricultural and natural environments. The "Catch 22" is that we will not know whether transgenic crops cause serious negative consequences until we grow them on a large scale. However, if we do grow them on a large scale and find that the concerns are valid, then we cannot reverse what has been done. Transgenic pollen has been released, and populations of everything from weeds to corn borers have been altered.

In addition to questions regarding environmental safety, human health issues must be addressed. The major concern over the consumption of foods derived from transgenic crops is the potential for the transgene protein product to cause an allergic reaction. For example, a Brazil nut gene was added to soybean to increase its methionine content. Methionine is an amino acid commonly added to animal feed. However, this gene produced a protein that caused an allergic reaction in some people. Although the transgenic soybean was being developed as an animal feed, there was concern that it might find its way into the human food chain, and consequently, it was dropped from production. How likely is it that a crop designed for animal feed will end up in human food products? Recently, Starlink corn was found in taco shells, tortilla chips, and corn dogs. Starlink is a variety of transgenic corn containing a gene for Bt toxin and was developed as an animal feed. It has not been approved for human consumption because of concerns that it might cause an allergic reaction. So far, no antibodies to the Starlink protein have been found in people who have experienced a potential allergic reaction after eating these products. The Starlink incident is just the tip of an iceberg faced by the food industry as it tries to keep track of transgenic products.

PLANT PROPAGATION

After we create genetically superior clones or populations of plants through breeding efforts, we must have a method to perpetuate, or propagate, these plants. Plant propagation techniques allow us to grow thousands of acres of a superior corn variety or to have the same day lily variety growing in thousands of yards. Some plants are most easily propagated through sexual reproduction via seed propagation. With other plants, asexual reproduction methods are used more extensively.

Seed Propagation

Hybrid varieties are often grown from the seed produced by crosses between two inbred parents. A common summer job for teenagers in corn-belt regions is detassling corn (removing male flowers). Rows of one inbred parent are planted next to rows of a second inbred parent (Fig. 14.16). Then, the tassels of the first parent are removed to prevent self--pollination. Pollen from the second parent is carried by the wind to the female flowers (the ears) of the first parent and carry out fertilization. The mature ears of the detasseled parent are then collected, and the kernels are harvested as hybrid seed. That seed will be sold to farmers for planting their hybrid cornfields during the next growing season.

Inbred line varieties are typically grown from seed. It is especially easy to generate seed from these varieties. They are simply grown in a field and allowed to self-pollinate. Seeds collected from these plants will grow into a uniform population similar to the plants from which the seeds were collected. For example, if you collect seeds from an inbred green bean variety, you could grow plants that are nearly identical to that variety in appearance and fruit quality. In contrast, seeds collected from a hybrid plant will produce a highly variable population of plants.

Commercial seeds usually come from fields planted solely for seed production. The fields are isolated from other fields of the same crop in order to prevent contamination by foreign pollen and mixing of seeds by harvest equipment. Seed quality depends on the health of the plants that produce it. Growing conditions, consequently, are meticulously monitored. Often, the plants are grown in arid regions where irrigation can be controlled and seeds can dry out before harvest. Most grass and forage seed production is concentrated in the Pacific Northwest, while vegetable and flower seeds are generated in the coastal valleys of California. There is also a growing international seed production industry.

When seeds are mature, they are harvested and stored in a controlled environment. The **viability** of most seeds is best maintained in cool, dry storage conditions. Samples are periodically removed from a seed lot and tested for vigor and viability. You may sometimes see the results of these tests on packages of seeds you purchase for your garden. You will also see a date stamped on the seed package. Seeds of some species of plants retain their viability in storage much longer than others. Seeds of green beans, lettuce, onions, and peppers lose their viability after a year or two in storage, while those of beet and

tomato may be stored for 5 years or more.

Before planting seeds, steps may be taken to ensure the growth of a vigorous stand of seedlings. In preparation for planting, seeds may be dusted with a **protectant,** such as a fungicide. The red coating on seed corn is such a fungicide. Sometimes, seeds are dusted with beneficial bacteria before planting. Legumes such as peas and beans establish mutualistic associations with bacteria that *fix* nitrogen (convert nitrogen from the air into forms the plants can use). Dusting seeds with these bacteria will encourage that relationship to develop.

It is important to plant seeds in a suitable bed. The soil should be moist enough to allow the seeds to imbibe water, which is essential to begin germination. However, if the soil is soggy, the new roots will not have enough oxygen to keep up with the respiration needed for active growth. Seeds of different species are adapted for growth at different soil -temperatures. Celery, lettuce, and onion seeds will not germinate in hot summer soil, while tomato, bean, cucumber, and sweet-corn seeds germinate poorly in cool spring soils. Initially, seeds rely on stored food for growth. Fertilizer cannot be utilized until a root system has been established.

In their early stages of growth, seedlings are easily shaded and stunted by weed growth, so weed control is important until the plants can shade the spaces between rows. Seedlings may also compete with each other for light, water, and nutrients, making thinning necessary for production of a good crop.

Asexual Plant Propagation

In recent years, we have heard amazing stories about scientists who have cloned animals such as sheep and cows. Botanists are far ahead in that arena. They have been cloning plants for centuries! Many plants are easy to **propagate** asexually, using vegetative parts rather than seeds. For example, **crown division** is a simple technique in which a plant is separated into several pieces, each of which contains a portion of the crown and root system (Fig. 14.17). This is commonly used for many ornamental perennial plants. A breeder who has identified a superior plant of an asexually propagated species can perpetuate that genotype indefinitely. This avoids the genetic variability and consequent unpredictability that results from seed propagation.

Cuttings

We know that nothing lives forever. Or do we? If you are particularly fond of your grandmother's African violet plant, you can use a leaf to propagate a new plant. As that plant begins to age, you can propagate a new one again from a leaf and eventually pass the plant on to your grandchildren. This plant could be propagated indefinitely and would essentially live forever.

Propagation of plants from parts such as leaves is called propagation by **cuttings.** If a stem is used as a cutting, it needs to be coaxed into producing **adventitious** roots (roots produced on internodes or other parts of plant organs). Buds on the stem will grow into the shoot system. Leaf and root cuttings, though, must develop both adventitious roots and shoots (Fig. 14.18). The formation of adventitious structures requires plant cells near the wound to **dedifferentiate** (become less specialized) and create a new **meristematic** region.

After a cutting is made, it must be kept in an environment that will allow wound healing and development of new organs. The most critical step is prevention of water loss. Because a stem or leaf cutting has lost its root system, its tissues must be kept moist until roots can once again supply water. This is easily done by keeping the cuttings in an enclosed container to maintain high humidity and to reduce **transpiration.** In commercial production systems, frequent misting or constant fog is used to keep cuttings from drying out. Sometimes, rooting is stimulated by application of a rooting powder containing a growth regulator such as **auxin.** Rooting powders also often contain fungicides to prevent pathogens from entering wounds. The cuttings must be kept in a potting mix such as perlite or vermiculite, which holds water but also drains well. This is preferable to rooting cuttings in water, because oxygen levels in water are low and are quickly depleted as young cells in developing roots undergo respiration. Temperature is also a factor in successfully generating cuttings. Ideally, young roots should be warm enough to enhance growth, while the above-ground parts should be cool enough to minimize growth and transpiration. For this reason, cuttings are often grown on heating pads or in heated beds (Fig. 14.19). Once roots are established, fertilizer helps to stimulate growth.

Many house plants are easily propagated by cuttings. These include African violet (*Saintpaulia*), snake plant (*Sansevieria*), *Begonia*, *Coleus*, and *Kalanchoë.* Outdoor plants propagated by cuttings include *Forsythia*, *Geranium*, rose (*Rosa*), *Spiraea*, raspberry (*Rubus*), and juniper (*Juniperus*). The main advantage of propagation by cuttings is that identical copies of a valuable plant can be made. A major disadvantage is that diseases carried by the mother plant, including those caused by viruses, fungi, and bacteria, are also propagated.

Layering

Layering is a modified form of cutting propagation and works well for some plants that are not easy to propagate by cuttings. This procedure allows the adventitious root system to develop before the new plant part is severed from the parent plant.

Tip layering is used with blackberries, boysenberries, and other plants with flexible stems. The canes are bent over until the tips touch the ground; the tips are then covered with a small mound of soil. Roots form on the portion of buried stem, and eventually, shoots will also appear (Fig. 14.20). These new plants can then be separated from the parent stems. Variations of tip layering include forcing a stem to lie horizontally and covering it with small mounds of soil at intervals, or heaping soil around the

base of a plant so that the individual stems produce roots there. Once roots have been established, the individual plantlets or pieces of stem can be cut off from the original parent and grown independently.

Air layering is sometimes used to propagate tropical trees and shrubs. It is useful for producing a few large plants from a single plant with a rigid stem. A branch or main stem is wounded with a sterile knife and then may be dusted with a rooting powder. The wound is produced by gashing the stem or, alternatively, by *girdling.* **Girdling** is the removal of a ring of bark around the stem. Damp sphagnum moss is wrapped around the wound, and the area is covered with clear plastic film to retain moisture. Then, aluminum foil is placed over the plastic film to reflect sunlight and prevent overheating (Fig. 14.21). When roots are observed in the moss through the plastic film, the layer is removed from the parent plant and transplanted.

Grafting

Imagine walking out into your yard in the morning and picking a fresh grapefruit for your breakfast. Then, you go to the same tree at lunchtime and harvest an orange. Later in the day, you pick a lemon off the same tree to squeeze on your fish dinner. Sound impossible? It is not, if you are growing a tree that was created by grafting.

Grafting is a process by which segments of different plants are connected and induced to grow together as one plant. It has been performed as a horticultural art for thousands of years, dating back to around 1560 B.C. Historically, grafting has been performed mainly to clone plants that are difficult to propagate as cuttings. Today, many fruit and nut trees are grafted. Trees are bred for high-quality fruits or nuts. Then, they are grafted onto root systems selected for traits such as winter hardiness, dwarfing, and disease resistance. The top part of the graft is called the **scion.** The bottom portion, that forms the root system, is the **rootstock.** To take grafting a step farther, though, it is possible to graft several different but related plants onto the same rootstock. You could, for example, have grapefruit, orange, and lemon grafted onto the same *Citrus* rootstock. Almond, plum, and apricot can be grafted onto a peach rootstock. Many nurseries sell grafted apple trees containing two or more varieties. Brightly colored novelty cactus plants, which are not capable of photosynthesis, survive because they are grafted onto normal plants (Fig. 14.22).

To describe how grafting is performed, assume we have bred a new extra-juicy variety of apple and would like to graft it onto a young native tree with a hardy root system. In late spring, we would collect some young branches from the tree with the juicy apples (the scions) and keep them in a cooler to inhibit the buds from growing. Then, later in the spring when the rootstock begins to grow, we would bring the scion branches out of the cooler. Using sterilized instruments, we would cut the top of the rootstock diagonally several inches above the soil line. At the same time we would make a diagonal cut in a scion branch that is similar in diameter to that of the rootstock. Successful grafting depends on good contact between the **vascular cambium** of the scion and that of the rootstock. Therefore, we align the scion and rootstock so their cambia are in contact and hold them together with tape or string (Fig. 14.23). The graft union is often also sealed with wax to prevent the wounded tissues from drying out. If the graft is successful, several weeks later, the vascular cambia of the scion and rootstock will have grown together. The tree now consists of a single plant with one variety of rootstock below and a different fruiting variety above. An expanded and illustrated discussion of grafting is given in Appendix 4.

Propagation of Specialized Stems and Roots

If you would like to create a bed of tulips in your garden, you would not plant seeds or rooted cuttings. Instead, you would rely on **bulbs,** which are natural propagules produced by tulip plants. Herbaceous perennials produce shoots that die during the winter, but in the spring, they regrow from underground storage structures. The underground structures that survive over winter may be *bulbs, corms, tuberous roots,* or *rhizomes* (discussed in Chapter 6) (Fig. 14.24). These structures are typically fleshy, with abundant food-storage tissue, and contain buds that will sprout in the spring.

Micropropagation

One of the major disadvantages of most asexual propagation techniques is that they also propagate pathogens. Once a plant is diseased, there is no immune system to activate or antibiotic we can administer as a cure. The best way to eliminate the effects of plant disease is to prevent exposure to pathogens. This is nearly impossible in greenhouse and field environments. However, it is possible to maintain plants in a disease-free status if we grow them in sterile test tubes through **micropropagation.** Other advantages of micropropagation include the capacity to grow large numbers of plants in a small area, minimal maintenance required for established plants (they do not need to be watered), and rapid -multiplication.

Propagating plants through micropropagation is similar to growing them as cuttings. The major difference is that the plants are grown ***in vitro*** in a sterile **medium** and maintained in special controlled environment rooms. The medium includes a support matrix composed of agar, a gelatinous material extracted from red algae. Inorganic salts are added to the medium to provide macro- and micronutrients, such as nitrogen, phosphorous, calcium, and iron. Sucrose is added to supplement the sugars produced by the plant. In addition, vitamins such as thiamine, nicotinic acid, and ino-sitol are generally included in the growth medium. Commonly, growth regulators are also added. After the ingredients are combined and pH is adjusted, the mixture is poured into *test tubes.* The tubes are then capped and put in an **autoclave** (a large form of pressure cooker) to sterilize them (Fig. 14. 25). When the medium cools, it solidifies like Jell-O™. This acts as the “soil” in the micropropagation system, providing plants with support, nutrients, and water.

Micropropagation, like other forms of asexual propagation, relies on the property of **totipotency** (capacity of a cell to give rise to any structure of a mature organism) of plant cells. Each living cell has the genetic information and, therefore, the capacity to develop into any cell type. Micropropagation usually begins with an excised piece of leaf or stem tissue, or **explant,** and carries it through three steps.

The first step is establishment of explants in **tissue culture.** Micropropagation requires sterile plant material as well as growth media. Plant parts must be **disinfested** to remove surface contaminants without killing the plant tissue. Common disinfestants include bleach and ethanol. This procedure does not remove internal contaminants, including pathogens, so it is important to begin with disease-free plants. After plant parts are disinfested, they are inserted into the growth medium in test tubes under sterile conditions. Often, a special reach-in chamber, called a **laminar flow hood,** is used for this step (Fig. 14.26). Filtered air is blown across the work surface in the chamber to prevent the introduction of contaminants. Test tubes containing sterile plants are then placed in a clean room with artificial lighting and temperature control. The goal of this first step is to obtain sterile, viable plant tissue cultures (Fig. 14.27).

After cultures are established, they typically will be induced to develop multiple shoots in a multiplication medium. These **microshoots** can be separated and placed in a new medium by a process called **subculturing** (Fig. 14.28). This step is similar to propagation by cuttings, except it is carried out under sterile conditions. It is not unusual to subculture plants every four weeks, making approximately four new plants from every one in a test tube. At this rate, it is theoretically possible to produce a million plants from one plant in just 10 months. Although these multiplication rates are not realized in commercial systems, many tissue culture laboratories have the capacity to produce millions of plants per year.

The third step in micropropagation is root formation. Some explants, such as those from African violets, will spontaneously produce roots in multiplication medium. In other cases, plants are induced to form roots *in vitro* by transferring them to a rooting medium. Compared to the multiplication medium, the rooting medium usually contains reduced levels of cytokinins and increased auxin levels. When possible, the most economical approach is an ***ex vitro*** one, in which microshoots are rooted in potting mix and treated as cuttings.

The last step in micropropagation is the transfer of plants back to an outdoor environment. This is often the most difficult step. Because the humidity is high in test tubes, plants grown *in vitro* do not produce as much wax on their cuticles as do those grown outdoors. In addition, their stomata do not close as readily in response to water stress. Therefore, tissue culture plantlets must be acclimatized to an outdoor environment. First, the humidity in the growth chamber is reduced for a few weeks before the plants are brought out of their test tubes. Then, when they are transferred to soil in pots, they are maintained in a high-humidity environment for several weeks.

Commercial micropropagation has become a successful venture with a number of plants. Some plants that propagate slowly by other asexual means may be rapidly increased with micropropagation. These include orchids, Boston fern, African violet, and *Hosta*. In some cases, with the use of tissue culture techniques, as many plants can be produced in a month as would be produced in a year with other techniques. New cultivars can also be rapidly multiplied to meet high market demand. For example, new apple rootstocks are often propagated *in vitro* because conventional asexual reproduction methods cannot adequately supply market needs. Tissue culture protocols, using various combinations of nutrients and growth regulators, have been developed for a number of woody plants that are otherwise difficult to root.

Tissue culture is now being used to propagate endangered plant species. Small pieces of just a few plants can be used to establish tissue cultures. Then, nearly unlimited numbers of plantlets can be produced and returned to their natural habitat.

Summary

1. Most of our food is derived from just a handful of plant species.
2. We domesticate plants by genetically altering them to meet our needs.
3. The impetus for the shift from hunter-gatherer to agricultural societies is not clear. Current hypotheses are speculative.
4. Plant domestication began in the Near East approximately 10,000 years ago and spread to Asia, Africa, and the New World.
5. During domestication, plants were selected for nonshattering seeds, high yield, seedling vigor, and absence of seed dormancy.
6. Two methods for improvement of self-pollinated crops are pure-line selection and selection within self--pollinated offspring of hybrid plants.
7. Two methods for improvement of cross-pollinated crops include mass selection and creation of hybrids from inbred lines.
8. Germplasm banks are critical repositories of genetic diversity essential for plant-breeding progress.
9. Protoplast fusion combines entire genomes of related plant species that cannot mate with each other.
10. Transgenic plants contain DNA from foreign organisms. To make a transgenic plant, a gene is spliced out of the donor and inserted into a vector. The vector is inserted into a host, such as a bacterium, which clones the foreign donor DNA. The next

steps, transformation of the plant with the cloned foreign gene and expression of that gene, are the most difficult ones to perform.

11. Seed propagation requires production of high-quality seed, an adequate seed storage environment, and appropriate conditions for seedling growth.
12. Cuttings provide a method of asexual propagation for many plant species.
13. Grafting unites pieces of two different, but related, plants and can be used for cloning.
14. Another form of asexual reproduction utilizes natural propagules, such as bulbs, corms, and tubers.
15. Micropropagation is an asexual propagation technique carried out under sterile conditions.

Review Questions

1. Describe the traits that early humans selected during domestication of plants.
2 Explain why knowledge of a plant's reproductive system is important when choosing a breeding strategy.
3. Outline the steps you would use to create a petunia plant carrying a corn gene for red flower color.
4. Differentiate between a rootstock and a scion.
5. Explain how you would create an apple tree that produces both Granny Smith and Red Delicious apples.
6. Describe the four steps that are usually carried out in micropropagation.

Discussion Questions

1. Suppose you grow a hybrid tomato plant and save some of its seeds to plant in your garden next year. Would those seeds grow into plants that look like the hybrid plant from which you collected them? What if you collected seeds from an inbred line of tomato and planted them?
2. Describe advantages and disadvantages of micropropagation compared with making cuttings in a greenhouse.
3. What might be some advantages of having several varieties of apple or plum on the same tree?
4. Describe advantages and disadvantages of producing a crop of potato plants using asexual (tuber) rather than sexual (true seed) propagation.
5. Many people are opposed to the production of transgenic crop plants. Do you feel that position is justified?

Additional Reading

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A flower of butterfly pea (*Clitoria ternata*), a tropical vine whose flower construction ensures that both the anthers and pistil touch the backs of visiting insects. The seeds are believed to be toxic to livestock.

Figure 14.1 These six foods—corn, rice, wheat, potato, sweet potato, and cassava—meet most of the caloric needs of people worldwide.

1. J. P. Harlan. 1992. *Crops and Man,* 2d ed. Madison, WI: Amer. Soc. Agron.

2. See note 1.

Figure 14.2 Regions of domestication of some crop plants. *(Data from Jack R. Harlan.* Crops and Management, *2nd edition. Am. Soc. Agronomy.)*

3. J. S. Huxley. 1957. *New Bottles for New Wine.* New York: Harper and Sons.

Figure 14.3 The sunflower is the only major crop that was domesticated in the United States. *(© Stephen P. Lynch)*

Figure 14.4 Modern corn (*left*) was probably domesticated from teosinte (*right*). *(Teosinte photo courtesy John Doebley)*

Figure 14.5 Potato tuber diversity. Genetic variation provides the foundation for efforts to improve plants through breeding.

Figure 14.6 Norman Borlaug, the father of the Green Revolution. *(© Bill Meeks/AP Wide World Photos)*

Figure 14.7A Crosses between inbred lines (*top*) often produce high-yielding hybrids (*bottom*). Such hybrid vigor is called *heterosis*.

Figure 14.7B Hybrid corn plants.

Figure 14.8A Botanists on a plant-collecting expedition in Peru. *(Courtesy David Spooner)*

Figure 14.8B Botany students learning how to collect and identify plant specimens in the field.

Figure 14.9 Cutting of DNA by a restriction enzyme. Some restriction enzymes are capable of cutting DNA molecules into two strands. One strand, which is slightly longer than the other, has a *sticky end* that can pair with the sticky end of another similarly cut DNA strand. *(From Moore, Clark, and Vodopich,* Botany, *2nd edition. ©1998 The McGraw-Hill Companies. All rights reserved.)*

Figure 14.10 Gene cloning by bacteria. A plasmid from a bacterium and a gene of interest from foreign DNA are cut by restriction enzymes. The gene becomes inserted into the plasmid, which is taken up by bacteria. The bacteria multiply. *(From Moore, Clark, and Vodopich,* Botany, *2nd edition. © 1998 The McGraw-Hill Companies. All rights reserved.)*

Figure 14.11 A crown gall, caused by the bacterium *Agrobacterium tumefaciens,* on a tomato plant. *(Courtesy Terese Barta)*

Figure 14.12 Inserting foreign genes into a plant using an *Agrobacterium* T_i plasmid. *(From Moore, Clark, and Vodopich,* Botany, *2nd edition. © 1998 The McGraw-Hill Companies. All rights reserved.)*

Figure 14.13 Steps in making a transgenic plant. *(From Moore, Clark, and Vodopich,* Botany, *2nd edition. © 1998 The McGraw-Hill Companies. All rights reserved.)*

Figure 14.14 Transgenic potato plants expressing the Bt gene for insect toxin (*left*). The photo *below* is the same variety but has been defoliated by the Colorado potato beetle because it has not been transformed with the toxin gene. *(Courtesy Jeffrey Wyman)*

Figure 14.15 The Moonshadow variety of carnation contains a petunia gene for blue color.

Figure 14.16 A field planted for hybrid corn seed production. Rows that have been detassled are adjacent to rows with tassels. Pollen from the tassels will land on ears of detassled plants. Hybrid corn will be harvested from detassled plants.

Figure 14.17 Asexual reproduction of a daylily by crown division.

Figure 14.18 Asexual reproduction by cuttings. *Left:* Stem cuttings of an ornamental fig (*Ficus*). *Right:* Leaf cuttings of *Sansevieria.* Note the adventitious roots developing from both types of cuttings.

Figure 14.19 Cuttings are often grown on a heated bed to stimulate root development.

Figure 14.20 Tip layering. The tips of canes are bent to the ground and covered with a small mound of soil. When a new plant has developed at the tip, it can be cut from the parent plant and grown independently.

Figure 14.21 Steps in air layering. *A.* Cuts are made at an angle to the axis of a stem of a rooted plant. *B.* Damp sphagnum moss is wrapped around the cut area. *C.* Polyethylene film is wrapped around the moss, and the ends of the film are taped shut. *D.* Aluminum foil is wrapped around the film-covered moss. Adventitious roots develop and protrude through the moss. Then the rooted portion can be cut off and planted.

Figure 14.22 A brightly colored cactus stem has been grafted on to a green cactus plant.

Figure 14.23 A simple graft. The root portion (*stock*) and portion to be grafted onto the stock (*scion*) are cut so that the two parts will fit together with the cambium of both portions in close contact.

Figure 14.24A Propagation of specialized stems.

Figure 14.24B Some specific examples of specialized stems that are easily propagated asexually. *Left:* A tulip bulb. *Center: Crocus* corms. *Right:* A ginger rhizome.

Figure 14.25 An autoclave, in which pressurized steam is used to sterilize media, glassware, and instruments.

Figure 14.26 A laminar flow hood. A sterile work area is created by blowing sterile air from the back of the hood to the opening in front.

Figure 14.27 Flasks of sterile growing medium to which meristematic tissue has been added. The flasks are slowly rotated under lights. Roots and shoots appear within a few weeks.

Figure 14.28 Plantlets that have developed from cultured meristematic tissue are separated and further cultured to maturity.