Transgenic Crops

Biotechnology has already created plants that withstand pests and fruits that resist spoilage. Recent advances confirm its environmental soundness and commercial viability

by Charles S. Gasser and Robert T. Fraley

Modification of crop plants to improve their suitability for cultivation has persisted for at least 10,000 years. Early farmers produced better crops simply by saving the seeds of desirable plants. During the past century, plant breeding has become more rigorous in its approach. Significant improvements in crops have resulted from the successful crossbreeding of different individuals of the same species. More recently, researchers have made advances in crossing sexually incompatible species of the same family. Now there exists a promising method of developing superior plants: genetic engineering. By using recombinant DNA techniques, biologists can direct the movement of specific and useful segments of genetic material between unrelated organisms.

That approach can add a significant degree of diversity to the total repertoire of traits from which the plant breeder can choose. In the laboratory, plants can now be made to withstand insects, viruses and herbicides. Fruits can be made to resist spoilage, and grains may become more nutritious and economical.

Biologists created the first transgenic plants less than 10 years ago. Since then, researchers have applied genetic engineering to more than 50 plant species. The technique has helped investigators gain critical insights into the fundamental processes that govern the development of plants, and the first commercial introductions of such genetically modified plants are now only a few years away.

Although genetic engineering is more complex than traditional plant-breeding practices, it is just as safe. In both methods, new DNA enters the plant's genome and is stably maintained and expressed. A recent National Academy of Sciences report concluded that "crops modified by molecular and cellular methods should pose risks no different from those modified by classical genetic methods for similar traits." This past February the White House stated that genetically engineered products should not be subject to additional federal regulations, because they do not pose any unreasonable risk.

In this article, we shall describe the methods used at present to engineer plants genetically. We shall also outline the rationale of and progress in the current applications.

The first practical—and still the most widely used—system for genetic engineering of plants relies on an innate ability of the plant pathogen *Agrobacterium tumefaciens*. This bacterium can transfer a portion of its DNA into plant cells. It does so by introducing a set of genes into one or more of its own DNA fragments. These fragments, called transferred DNA (T-DNA), then integrate into chromosomes of infected plant cells and induce the cells to produce elevated levels of plant hormones. These hormones cause the plant to form novel structures, such as tumors or prolific root masses, that provide a suitable environment and nutrient source for the *Agrobacterium* strain. This bacterial infection is called crown gall disease.

For the bacterium to be an effective vehicle for DNA transfer, its disease-causing genes had to be removed. This alteration is known as disarming. Researchers at the Monsanto Company and Washington University and groups directed by Jozef Schell of the Max Planck Institute for Plant Breeding in Cologne and by Marc van Montagu of the State University of Ghent in Belgium first accomplished the task in 1983. They relied on traditional DNA recombination to delete the genes that cause tumors. Disarming thus eliminates the bacterium's ability to cause disease but leaves the mechanism of DNA transfer intact [see "A Vector for Introducing New Genes into Plants," by Mary-Dell Chilton; SCIENTIFIC AMERICAN, June 1983].

The first engineered gene, constructed with *Agrobacterium* in the early 1980s by groups at the Max Planck Institute in Cologne and at Monsanto, made plant cells resistant to the antibiotic kanamycin, a compound that inhibits plant growth. The engineering of kanamycin resistance represented a breakthrough for two reasons. First, it showed that foreign genes and proteins could be expressed in plants. Second, it demonstrated that kanamycin resistance is useful as a "marker." Because only a small number of cells take up, integrate and express introduced DNA, marker genes help investigators to identify those cells into which genes have successfully been introduced.

Because plant cells are totipotent—that is, the undifferentiated cells can generate a whole organism—complete, reproductively competent plants can emerge from the transformed cells. Most methods today rely on the cells of explants, or small pieces of plant, for genetic engineering. Our colleague Robert B. Horsch of Monsanto popularized the use of a common paper hole punch to cut disks from leaves for *Agrobacterium*-mediated techniques. (He used to carry a punch in his coat pocket, always ready to give an impromptu demonstration of the leaf-disk transformation method.) *Agrobacterium*-mediated gene transfer is now routinely used in hundreds of industrial and aca-

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demic laboratories around the world. At Monsanto alone, more than 45,000 independent transgenic plant lines have been produced in this way.

Although the method is simple and precise, many plant species, including such critical grain crops as rice, corn and wheat, are not natural hosts for *Agrobacterium* and so are not readily transformed by the method. As a result, extensive efforts have been mounted to develop alternative systems.

One of the first was introducing free DNA into plant protoplasts. Protoplasts, plant cells that have had their cell walls removed by enzymes, must be used because the pores of cell walls are too small to allow the easy passage of DNA. The only barrier in protoplasts is the plasma membrane. Polyethylene glycol, a thick organic polymer, can penetrate the plasma membrane to transport DNA. It is the most commonly used chemical delivery agent. Electroporation can also carry DNA across the plasma membrane. In this process, short, high-voltage pulses briefly produce pores in the protoplast membrane. The DNA molecules can enter through these spaces.

Because these procedures do not rely on any special biological interaction, they are, in principle, general methods of transforming cells. But the regeneration of plants from isolated protoplasts has proved problematic in many species, especially the critical cereal grains. Corn and wheat respond very poorly, usually yielding infertile plants.

As a result, investigators have been searching for methods that introduce DNA into intact plant cells, those that still have their walls. A fairly obvious way is simply injecting the DNA. But microinjection has not been effective for several reasons. The fine needle tips break easily and clog frequently. Transforming cells one at a time is tedious, difficult work that would be inappropriate to a commercial operation. Furthermore, once DNA enters a cell, its incorporation into the genome of the recipient is by no means a certainty. A technician might have to inject DNA

GENETICALLY ENGINEERED RESISTANCE to the Colorado potato beetle *(Leptinotarsa decemlineata)* is shown in this false-color, infrared aerial image of test beds planted in a field recently irrigated by a center-pivot system at Hermiston, Ore. The beetles defoliated fields of ordinary potato plants, leaving behind wet ground (*green*), but avoided plants that were able to produce their own insecticide (*red*). The white patches are wheat plants kept dry for an unrelated experiment.

into at least 10,000 cells just to ensure that one of them will take up the new gene.

To increase the efficiency of gene delivery, John C. Sanford of Cornell University envisioned a way to bombard many plant cells with genetic material. He surmised that small metal particles, about one or two microns in diameter, could first be coated with DNA. Sufficiently accelerated, the particles could penetrate the walls of intact cells and thus deliver the DNA. Because small holes in cell walls and membranes rapidly close by themselves, the punctures are temporary and do not irreversibly compromise the integrity of the cells. Although the particles remain in the cytoplasm, they are too small to interfere with any cellular functions.

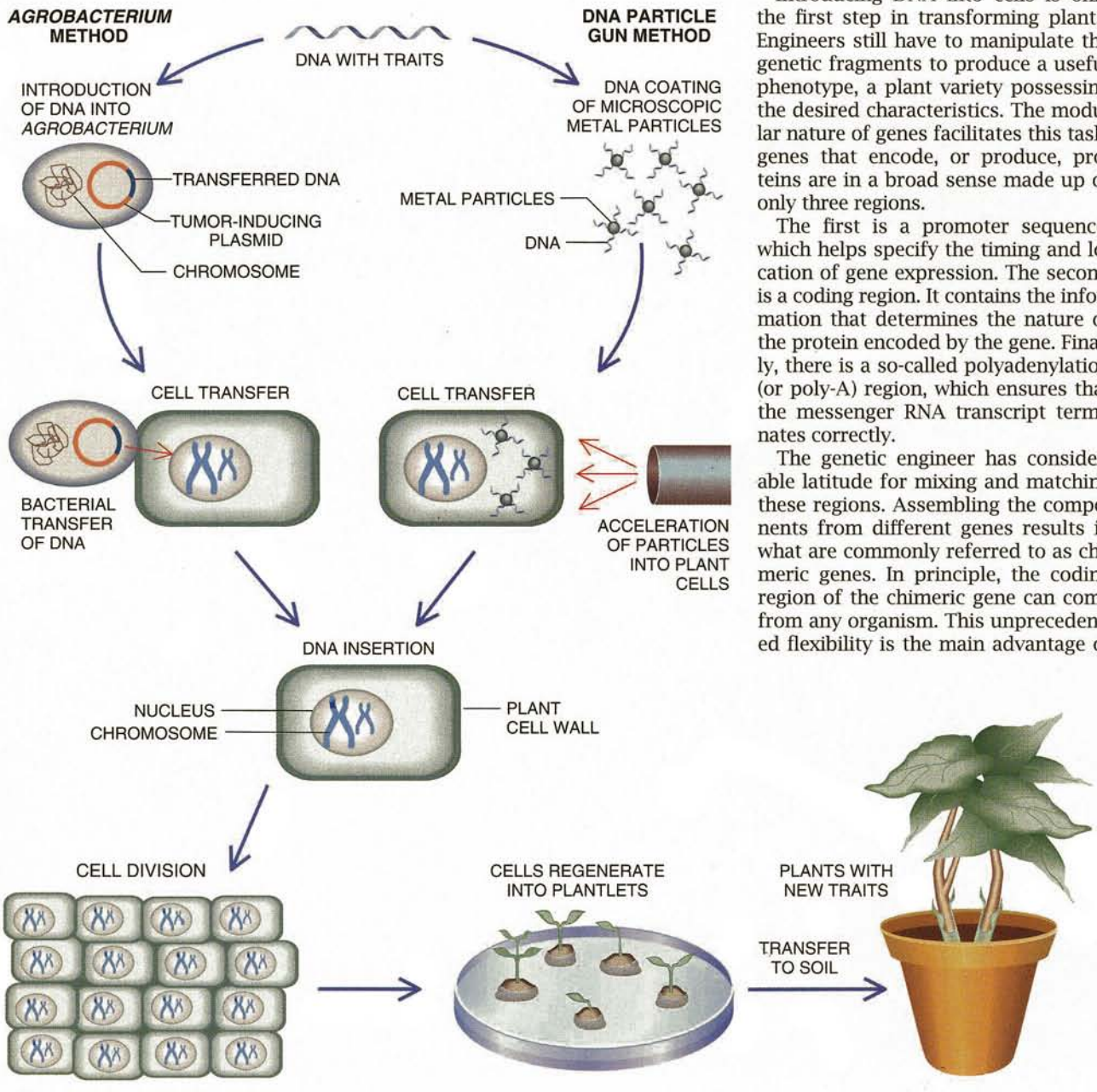
In 1987 Sanford and his co-worker Theodore M. Klein constructed a practical device that used tungsten particles to bombard plant cells. Their DNA particle gun, as it is called, uses a .22-caliber blank cartridge as the motive force. Researchers at Agracetus in Middleton, Wis., have developed a similar gun using gold particles propelled by the vaporization of a water droplet.

Both these particle guns have produced transgenic plants. Last year a group at DeKalb Plant Genetics in Groton, Conn., and a collaboration between Charles L. Armstrong of Monsanto and Michael E. Fromm, then at the U.S. Department of Agriculture in Albany, Calif., independently developed efficient, consistently functioning particle gun systems for the transformation of corn. Even more recently, we have collaborated with Indra Vasil's laboratory at the University of Florida in Gainesville to transform wheat plants.

Introducing DNA into cells is only the first step in transforming plants. Engineers still have to manipulate the genetic fragments to produce a useful phenotype, a plant variety possessing the desired characteristics. The modular nature of genes facilitates this task: genes that encode, or produce, proteins are in a broad sense made up of only three regions.

The first is a promoter sequence, which helps specify the timing and location of gene expression. The second is a coding region. It contains the information that determines the nature of the protein encoded by the gene. Finally, there is a so-called polyadenylation (or poly-A) region, which ensures that the messenger RNA transcript terminates correctly.

The genetic engineer has considerable latitude for mixing and matching these regions. Assembling the components from different genes results in what are commonly referred to as chimeric genes. In principle, the coding region of the chimeric gene can come from any organism. This unprecedented flexibility is the main advantage of



TRANSGENIC PLANTS are now commonly created by two methods. In the *Agrobacterium*-mediated technique, DNA with the desired trait is inserted into the tumor-inducing plasmid of the bacterium. The bacterium infects the plant cell and transfers the DNA. In the particle gun method, metal particles coated with DNA are fired into the plant cell. In either case, the plant cell incorporates the DNA into its chromosome and then divides and regenerates into full plants.

genetic engineering over more traditional methods, which can transfer genes only between closely related species. Furthermore, by choosing various promoters, researchers can target gene expression to specific organs such as leaves, roots, seeds and tubers and, in many cases, to specific cell types within these complex tissues.

One of the most promising traits gene transfer offers is resistance to diseases. Exciting results have been achieved in creating plants resistant to viruses, an important matter because currently no direct way to treat virus-infected crops exists. Most infections reduce crop yield, but occasionally some prove catastrophic. Good farming practices, such as rotating crops and removing weeds and crop litter, can contain viruses, but only partially. Insecticides are sometimes used to control the pests responsible for transmitting the virus.

Genetic work on virus resistance builds on previous basic research in plant biology. It had long been observed that infection of a plant with a mild strain of a virus protected it from subsequent infection of a more virulent strain. Apparently, the replication of the mild virus strain interferes with a virulent strain's ability to infect. Investigators have applied "cross-protection" to shield greenhouse-grown tomatoes against contagion by intentionally infecting them.

Roger N. Beachy and his co-workers at Washington University reasoned that a single component of the virus might be responsible for the protection. Collaborating with Stephen Rogers of Monsanto and one of us (Fraley), the investigators constructed a vector to introduce and express in tobacco and tomato plants the coat protein of the tobacco mosaic virus (TMV). Plants so modified were then inoculated with a heavy concentration of the virus. The plants were found to be strongly resistant to infection, thus confirming Beachy's hypothesis of viral protection.

Subsequent experiments have shown that the expression of the TMV coat protein confers resistance only to strains of TMV and a few other closely related viruses. Still, the mechanism appears to be generally applicable; expression of the coat protein gene of almost any plant virus, at a sufficiently high level, protects against infection by that virus. Workers have now engineered effective tolerance to more than a dozen different plant viruses in a broad range of crop species.

Resistance to insect predation is another important goal for genetic engineering, especially in cotton, potato and corn plants. During the past three decades, gardeners and farmers have relied on the bacterium *Bacillus thuringiensis* (*Bt*), which produces an insecticidal protein. Most commonly used preparations of *Bt* are highly specific to the caterpillar larvae of lepidopteran insects—moths and butterflies—which are major pests. The *Bt* proteins bind to specific receptors located on the gut membranes of the target insects. The binding interferes with ion transport in the epithelial cells of the gut, thus disrupting the insect's ability to feed. These natural insecticides have no toxicity to mammals or even to any other species of insects.

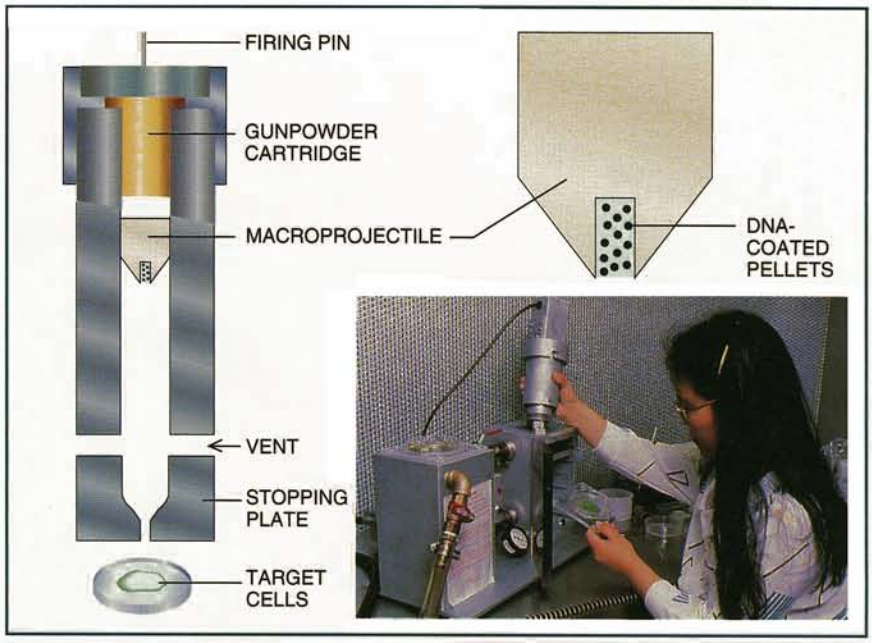
The usefulness of the *Bt*-based insecticides is often limited by the ease with which they are washed from plants. Furthermore, their effectiveness in the field lasts only briefly. In the mid-1980s genetic engineers at several companies, such as Plant Genetic Systems in Ghent, Belgium, Agrigenetics in Middleton, Wis., Agracetus and Monsanto, succeeded in isolating from the bacterium genes for the insecticidal proteins. They used the particle gun and *A. tumefaciens* to insert the genes into tomato, potato and cotton plants. At first, the genes expressed poorly; the *Bt* proteins the plant produced killed only the most sensitive laboratory insects.

Monsanto scientists David A. Fischhoff and Frederick J. Perlak made improvements. They redesigned the original bacterial gene to mimic more closely the plant DNA sequences. The changes dramatically enhanced insect control. Two years of field testing have confirmed that the presence of these *Bt* genes within cotton plants effectively controlled all major caterpillar pests, including the bollworm. These genetically engineered plants should reduce the use of insecticides on cotton by about 40 to 60 percent.

Scientists have screened extensively for naturally occurring *B. thuringiensis* strains that are effective on insects other than caterpillars. One such strain has led to the redesign of a gene that is effective against the Colorado potato beetle. In the summer of 1991, Russet Burbank potato plants expressing a beetle-control gene were tested at several sites from Maine to Oregon. Researchers found the potato plants to be essentially immune to beetle damage.

Bt may continue to offer additional genes for the control of plant pests. Scientists at Mycogen Corporation in San Diego have now discovered *Bt* genes active against plant parasitic nematodes, and *Bt* genes active against mosquitoes have been identified. Some researchers are trying to produce the mosquitocidal protein in algae as a means to control malaria.

The target specificity of the *Bt* pro-



DNA PARTICLE GUN developed by John C. Sanford of Cornell University fires tungsten pellets coated with DNA into plant cells. The pellets are held by a plastic macroprojectile, which is accelerated by a gunpowder charge. The plate stops the macroprojectile; momentum sends the pellets into the target. The vents allow air in front of the projectile to escape. In the photograph, a technician readying the device holds the "gun barrel" in her right hand; the cells to be transformed are in her left.

tein and its localization within the tissues of the plant ensure that the protein is active only against attacking insects. In contrast to topical insecticides, proteins in the plant obviously cannot be washed off. Extensive toxicological testing of *Bt* proteins and experience gained from more than 30 years of using *Bt*-based products confirm their safety. In fact, many researchers refer to *Bt* as the world's safest insecticide. Furthermore, the *Bt* protein, which makes up less than 0.1 percent of the total protein in the modified plants, breaks down in exactly the same fashion as any other protein—both in the soil and the digestive tract.

Besides the threat from viruses and insects, crops face a challenge from weeds. Weeds that compete for moisture, nutrients and sunlight can reduce a field's potential yield by 70 percent. Moreover, weed material in the harvest significantly reduces the value of the crop, and weeds serve as a habitat for pests.

In most cases, a combination of herbicide and careful cultivation effectively controls weeds. But because a herbicide has a limited spectrum of activity, affecting only a small portion of the weeds, several kinds of chemicals are often used during the growing season.

Genetic engineering may offer a partial alternative to such weed control. The strategy is to create plants that can tolerate exposure to a single, broad-spectrum, environmentally safe herbicide. In contrast to views expressed by some critics of genetic engineering, the use of herbicide-tolerant plants will actually reduce the overall amount of herbicide applied.

There are two general approaches to engineering herbicide tolerance. Researchers at Monsanto and at Calgene in Davis, Calif., have been working to enable plants to tolerate glyphosate, the active ingredient of a herbicide called Roundup. Roundup is a broad-spectrum compound that can control broadleaf and grassy weeds. The compound kills plants by inhibiting the action of EPSP synthase. This enzyme is necessary for the production of the aromatic amino acids that a plant needs if it is to grow.

Genetic engineers are especially interested in Roundup because it is one of the most environmentally attractive herbicides. It does not affect animals, because animals do not have an aromatic amino acid pathway. Furthermore, it degrades rapidly in the environment into harmless, natural compounds.

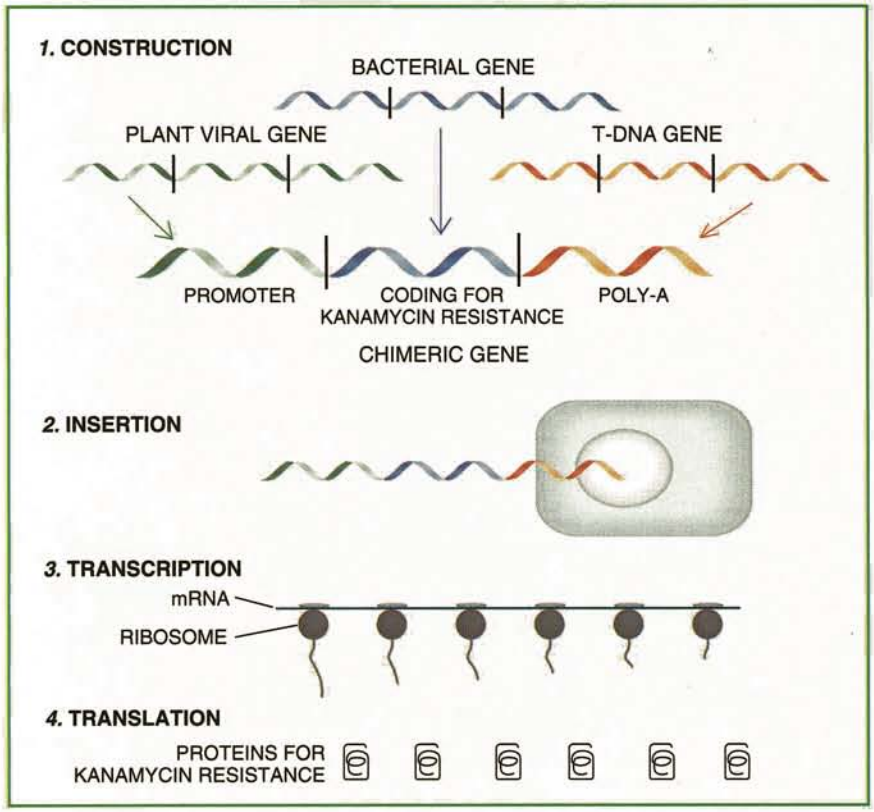
The first step in developing Roundup tolerance took place in 1983, when groups headed by Luca Comai and David M. Stalker of Calgene and Rogers and Ganesh Kishore of Monsanto isolated the genes for EPSP synthase from bacteria and plants. They also identified variants of the genes that produce proteins that have reduced sensitivity to Roundup. Later, investigators were able to construct genes that produced higher amounts of these proteins in plants. The genes were subsequently introduced into tomato, soybean, cotton, oilseed rape and other crops. As demonstrated by field tests performed during the past three years in the U.S., Canada and Europe, the crops were able to tolerate treatment with Roundup at levels that effectively controlled weeds. Researchers at Du Pont have used a technically similar approach to engineer plants that can tolerate certain kinds of sulfonylurea herbicides.

Scientists at Plant Genetic Systems and at the German company Hoechst took another approach to herbicide tolerance. From the microbe *Streptomyces hygroscopicus,* they isolated a gene for an enzyme that inactivates a herbicide called Basta, which affects the glutamine synthase pathway in weeds and thus interferes with their growth. But crop plants that have the gene inactivate Basta before damage can occur. Field tests performed on the Basta-tolerant plants demonstrate the effectiveness of the protection.

Engineered herbicide tolerance offers the farmer an alternative that is lower in cost and more effective than conventional weed-management measures. Careful selection of broad-spectrum herbicides should lead to an overall decrease in the use of weed-control chemicals and should enable farmers to replace existing herbicides with environmentally more attractive products.

Additional advances in the simplicity and breadth of genetic engineering techniques and increasing knowledge of plant biology promise to extend greatly the beneficial changes that gene transfer can confer. For example, researchers have already identified and isolated several genes that play a role in the biosynthesis of ethylene, the signal molecule that triggers the ripening of fruits. Delayed spoilage would allow harvesting at a later stage than is currently practical, which may improve the flavor and even the nutritional value.

To increase the shelf life of fruit, re-



CHIMERIC GENES can be constructed from the genes of different organisms. Here the chimeric gene for kanamycin resistance is assembled from diverse sources: the promoter region of a plant virus, the coding region of an *E. coli* bacterium and the poly-A site from the transferred DNA (T-DNA) of *Agrobacterium* (*1*). After the chimeric gene is inserted into a plant cell (*2*), it is transcribed into messenger RNA (mRNA) (*3*). The ribosomes translate the mRNA to produce the proteins (*4*).

GENETICALLY ENGINEERED SPECIES

ALFALFA	CRANBERRY	PAPAYA	SPRUCE
APPLE	CUCUMBER	PEA	STRAWBERRY
ASPARAGUS	EGGPLANT	PEPPER	SUGARBEET
BROCCOLI	FLAX	PLUM	SUGARCANE
CABBAGE	GRAPE	POPLAR	SUNFLOWER
CARROT	HORSERADISH	POTATO	SWEET POTATO
CAULIFLOWER	KIWI	RASPBERRY	TOBACCO
CELERY	LETTUCE	RICE	TOMATO
CORN	MUSKMELON	RYE	WALNUT
COTTON	OILSEED RAPE	SOYBEAN	WHEAT

GENETICALLY TRANSFORMED CROPS, shown to the left of their ordinary counterparts in each photograph, include herbicide-tolerant cotton plants (*a*), insect-resistant tobacco plants (*b*) and tomato plants whose fruits resist spoilage (*c*). The list identifies familiar plant life in which genetic engineering has successfully been demonstrated.

searchers developed two genetic methods. The first is inserting so-called antisense versions of the ripening genes. Antisense molecules bind with specific messenger RNA to turn off the genes. Athanasios Theologis of the USDA in Albany, Calif., and Don Grierson of the University of Nottingham have shown that fruits of tomato plants with the antisense genes resist softening. In a different approach, Monsanto scientists Kishore and Harry Klee have introduced a gene into tomato plants that induces them to manufacture an enzyme. This enzyme degrades the precursor compounds that form ethylene, thus retarding spoilage.

Genetic engineers may also be able to fashion healthier foods: genes for proteins that have superior nutritional properties have been isolated. It should be possible to insert these genes into crops. Plants could also be tailored to produce specialty chemicals such as starches, industrial oils, enzymes and even pharmaceuticals. Preliminary trials are now under way.

More than 400 field tests of engineered plants have now been conducted in the U.S. and Europe. The tests confirm the inherent safety and commercial validity of these approaches, and crops containing these traits should be available to farmers during the mid-1990s. Still, there are some limitations. In practical terms, genetic engineers can only modify traits expressed by no more than three to five genes. Furthermore, some crops do not respond to current gene-transfer methods, and isolating useful genes is sometimes difficult.

Yet to many in plant biotechnology, these challenges seem less likely to delay commercialization than are nontechnical issues. Genetically modified crops are being developed at a time when both public and political support for agricultural research is in general tepid. Concerns about food safety, the environmental impact of agriculture and a rapidly changing farm infrastructure have combined with a lack of understanding of new technologies to overshadow the long-term need for economical, high-quality food products. World food production will have to increase threefold during the next 40 years to meet the needs of an estimated nine billion people. Biotechnology is one of the few new solutions to this problem.

Another important advantage of the genetic engineering of plants is that it provides the very latest technology to farmers in a very traditional package—the seed. Even the most impoverished nations will thus have access to the benefits without the need for high-technology supplies or costly materials. Although not a panacea, biotechnology promises to become an important component of agriculture around the world.

FURTHER READING

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