GeNET. REPORT 3

DESIGNER

SEEDS

FROM

National Academy of Sciences Web Site

QUICK FIND

This article was written by science writer Margie Patlak, with the assistance of Drs. Roger N. Beachy, Mary-Dell Chilton, Maarten J. Chrispeels, Nina Fedoroff, Robert Haselkorn, Ernest Jaworski, and Arthur Kelman for ***Beyond Discovery****^{TM}: **The Path from Research to Human Benefit**, a project of the National Academy of Sciences.*

Funding for this article was provided by the Pfizer Foundation, Inc., and the National Academy of Sciences, (www.nas.edu)

The National Academy of Sciences was created in 1863 by a congressional charter approved by President Abraham Lincoln. Under this charter, the National Academy of Engineering was established in 1964 and the Institute of Medicine in 1970. All three organizations are private, non-profit, self-governing membership bodies, and all three share the responsibility for advising the federal government, upon request and without fee, on questions of science and technology.

For millennia, farmers have battled insects, microorganisms, and weeds that destroy or compete with their crops--threatening their families with starvation. Indeed, many major events in history have resulted from devastating plant disease epidemics or insect infestations. The Irish potato famine of the mid-1800s, which was caused by the fungus Phytophthora infestans, killed more than a million people and prompted a massive Irish emigration to the United States.

In hopes of preventing crop-plant destruction by pests, ancient Romans made sacrifices to their various gods. Modern farmers use other techniques in their attempts to kill pests, including spraying pesticide and herbicides, and plowing under weeds. They also make use of improved management practices and benefit from traditional breeding techniques to strengthen their crops. Some of the newer methods, however, have substantial costs and disadvantages. Excessive plowing can cause soil erosion, for instance. And pesticides and herbicides can pollute both soil and water as well as contribute to species extinction.

Thanks to recent advances in the genetic engineering, or bioengineering, of plants, farmers are now beginning to have at their disposal crop seeds that are genetically endowed not only to resist damage from insects but also to be resistant to herbicides. These bioengineered seeds have the potential to revolutionize agriculture and improve environmental quality by making it possible to reduce the use of pesticides and keep plowing to a minimum.

Like most scientific innovations that have had significant effects on society, bioengineered seeds did not emerge solely from the efforts of researchers to improve pest or weed control. Rather they were the by-product of earlier researchers' curiosity about such basic science questions as: How do bacteria cause plant tumors? How do some viruses protect plants from other viruses? What enables some bacteria to kill insects? The following article explores the trail of research that ultimately led scientists to bioengineer the plants that are beginning to transform agriculture. This story provides a dramatic example of how science works and how basic research can lead to practical results that were unimaginable when the research began.

RETURN TO QUICK FIND

A New Breed of Seeds :

A farmer handles seeds of cotton while contemplating the freshly tilled fields that are ready to receive them. Although the seeds look just like the ones he planted last year, they are far from ordinary. These

seeds will yield nearly 10 percent more cotton and will need less than half as much insecticide as last year's standard seeds. This improvement stems from an additional gene that scientists have inserted in the seeds' genetic material, or genome. The gene, which originated in bacteria and is not found naturally in plants, encodes a toxic protein that kills two of the prime predators of cotton plants--bollworms and budworms. These caterpillars destroy millions of dollars worth of cotton each year, and are the main reason why more than half the insecticide used worldwide is sprayed on cotton plants.

Although the farmer might view the bioengineered seeds in his hands as merely the outcome of recent progress in plant-breeding efforts by the company that produced them, they are actually the result of more than 50 years of research by many scientists. Incrementally, these scientists paved the way for isolating the genes that protect a particular organism from pests and for transferring these genes into a wide variety of plants. They could not have known that the answers to their fundamental questions about living organisms would lead to practical results, such as these bioengineered crop seeds.

RETURN TO QUICK FIND

Limits of Traditional Breeding :

Farmers have noticed for centuries that some individual plants in a given species manage to survive disease or epidemics of insects relatively unscathed, while their neighbors succumb to infection or insect predation. In 1905, Sir Roland Biffen of Cambridge, England, wondered whether healthy plants inherited pest resistance, just as they might inherit the tendency to be tall or short. His experiments on two varieties of wheat showed that the ability to resist infection by a rust fungus was indeed inherited, a discovery that intensified attempts by farmers and plant breeders to produce varieties of pest-resistant crop plants.

Similar attempts continue today and primarily involve screening a large number of plant varieties to identify those that are resistant to particular pests. Resistant varieties are then crossed with those desirable for other reasons--for example, because they produce more grain per acre. Careful selection and repetitive crossing of progeny can eventually generate varieties that are both high yielding and resistant to particular pests. But the process is extremely time consuming--it can take more than 15 years to bring a new variety to market.

One challenge encountered in traditional breeding is that generally only closely related species of plants can be cross-bred. If no varieties are naturally resistant to a particular fungus or insect, traditional breeders have no way to create resistance to that fungus or insect. Furthermore, breeders frequently face a situation in which a resistance gene is closely linked to a gene that adversely affects the quality of a crop, that is, where the two traits are always inherited together. For example, insect resistance in lettuce plants might be inherited along with a tendency for the lettuce to taste bitter. In the early 1990s, despite plant breeders' best attempts to improve the pest and disease resistance of cotton, corn, rice, and other crops, farmers worldwide lost about one-fourth of their crops to pests and diseases.

RETURN TO QUICK FIND

Taming the Crown Gall:

Some of the early steps that eventually led to a way around the limits of traditional breeding occurred in the first decade of the twentieth century with work on a disfiguring plant disease known as crown gall. Crown galls are bulbous tumor-like growths that, when well developed, protrude from the stems of many infected food plants, including fruit trees, grape vines, and berry canes. Crown gall disease makes plants grow poorly and can cause substantial crop losses. In 1907, Erwin Smith and C. O. Townsend, of the U.S. Department of Agriculture, discovered that the cause of crown galls was a rod-shaped soil bacterium, *Agrobacterium tumefaciens*. Other bacteria were known to cause plant tissue to die, wilt, or become discolored, but *A. tumefaciens* had the unusual ability to cause infected plant cells to proliferate and form of a tumor. Nothing came of this finding, however, until 40 years later, when plant pathologist Armin Braun, of the Rockefeller Institute for Medical Research, became curious about how a bacterium could cause such growths in plants.

One clue emerged in 1947, when Braun grew crown gall tissue that was free of the instigating bacteria. Braun found that crown galls, unlike normal plant tissue, were able to grow luxuriantly on a simple medium of salts and sugar; the plant cells didn't even need any growth-hormone supplements. Moreover, the cells continued to grow for many years. On the basis of his experiments, Braun surmised that the plant cells had been permanently transformed into tumor cells by some tumor-inducing factor introduced by *A. tumefaciens*.

During the 1950s and 1960s, scientists in other fields of biology were making groundbreaking discoveries about DNA and how it transmits genetic information in all living organisms. Braun's finding spurred several investigators to look for the tumor-inducing factor in the bacterium's DNA. Bacterial DNA is normally found on a single chromosome--a long molecule of DNA composed of many genes that encode information to construct an organism. A series of experiments, aided by the development of new research techniques, indicated that the tumor-inducing factor was genetic material carried on a smaller mobile DNA unit that was not part of the bacterium's single chromosome.

In 1974, Flemish scientists Jeff Schell and Marc Van Montagu isolated the tumor-inducing genes of the crown gall bacterium, and found that they were carried on a mobile unit of DNA in the bacterium known as a plasmid. The next step was to determine whether the genes on this bacterial plasmid were transferred into the chromosomes of plant cells when the bacteria infected the plants. This transfer was discovered in 1977 by microbiologists Eugene Nester, Milton Gordon, and Mary-Dell Chilton, who were then at the University of Washington.

It became clear that some of the bacterium's genes are transferred into the chromosomes of plant cells, where they induce the cells to divide continually until galls develop. Several creative scientific minds next speculated on the following scenario: if bacteria can introduce foreign genes into plant chromosomes and those genes become stable and fully functional, perhaps scientists could manipulate the bacteria so that they no longer transferred the genes that cause tumors--but instead transferred genes that produce desirable traits, such as pest resistance.

By this time a number of elegant techniques had been developed that enabled researchers to cut and splice DNA to pre-selected specifications. (For more information about these techniques, see Human

Gene Testing in the *Beyond Discovery* series.) To convert the *A. tumefaciens* plasmid a useful tool (called a vector) for introducing desired genes into plants, researchers first had to locate and then remove the tumor-inducing genes. Scientists in several laboratories were able to accomplish this task in the late 1970s and early 1980s. By 1983, plant molecular biologists had developed the first plasmid vectors that promised to remove the limits of traditional plant breeding for plants naturally infected by *A. tumefaciens*.

In the 1980s and 1990s, scientists developed additional ways to introduce genes into plants. One was a "gene gun," a device that literally shoots DNA-covered particles through tough plant cell walls and membranes to the cell nucleus, where the DNA can combine with the plants own DNA. Other techniques involve electrical or chemical treatments that help introduced DNA molecules pass through the barriers of plant cell walls and membranes.

RETURN TO QUICK FIND

The Quest for Desirable Genes :

A tool for introducing genes into plants is useful only if scientists have found genes that they want to transfer. Part of the hunt for desirable genes began inadvertently in Japan in 1901, when bacteriologist Ishiwata Shigetane was asked to investigate the cause of a disease outbreak that was killing large numbers of silkworms. Shigetane discovered that the cause of the outbreak was a previously unidentified species of spore-forming bacteria, later named *Bacillus thuringiensis*, or *Bt*.

Researchers were quick to see the value of the insect-killing bacteria. By 1938 the first commercial insecticide containing *Bt* hit the market in France, where it was mainly used to kill flour moths. During the next 50 years, other insecticide sprays were developed that contained *Bt*. But the products had several limitations--rain washed away the insecticide, for example, and sunlight rapidly broke it down. Furthermore, many pests were not susceptible to the *Bt* spray, and some that were susceptible were able to avoid contact with it because they fed mainly on roots, inside the stems, or on other parts of crop plants that are inaccessible to sprays. Given those limitations and the availability of more effective chemical insecticides, *Bt* insecticides were used only by niche markets in agriculture and forestry.

That situation changed in the 1980s, as many insects grew increasingly resistant to the commonly used insecticides, and as scientists and the public became aware that many of these chemicals are harmful to the environment. *Bt* insecticides affect only specific insect pests and do not persist in the soil or on leaves. As a result, they are generally considered environmentally benign. Thus, a number of commercial, government, and academic research laboratories embarked on research aimed at increasing the effective uses of *Bt* insecticides.

A critical piece of missing information was how *Bt* kills insects. When the first *Bt* insecticides were commercialized in the 1930s and 1940s, researchers knew only that they killed insects, not what the mechanism was. By the 1950s, a series of experiments by several research groups revealed that proteins produced by *Bt* bacteria were lethal to particular insect species. Over the next 20 years several different strains of *Bt* bacteria were discovered, and each strain was found to produce specific proteins toxic to different groups of insects. By 1980, dozens of studies had made it clear that the different proteins

produced by different strains of *Bt* bacteria determined which groups of insects would be killed.

Researchers then zeroed in on identifying the genes associated with the production of *Bt* proteins. Information about the genes was gathered by a pair of microbiologists looking into why the *Bt* genes triggered production of their toxic protein only when *Bt* bacteria started to produce spores. In 1981, Helen Whiteley and Ernest Schnepf, then at the University of Washington, discovered that the insecticidal proteins were found in a crystal-like body that was produced by the bacteria. They used recombinant DNA techniques to isolate a gene that encodes for an insecticidal protein. By 1989, more than 40 *Bt* genes, each responsible for a protein toxic to specific groups of insects, had been pinpointed and cloned by various researchers.

RETURN TO QUICK FIND

A. tumefaciens Meets Bt genes :

The stage was now set to develop plants that were resistant to insects. When both the *A. tumefaciens* vector for gene transfer and cloned *Bt* genes became widely available in the mid-1980s, a number of researchers realized that the two could be combined to modify crop plants so that they produce *Bt* proteins and thereby protect themselves from insect pests. Such plants would get around many of the limitations of *Bt* insecticides. Insects that had once hidden from *Bt* sprays would find the *Bt* toxins in whatever part of the plant they bit into, and sunlight or rain would not affect the persistence or potency of the toxins. Genetic engineers also could transfer genes for several different proteins, so that plants would be protected from several different predators.

RETURN TO QUICK FIND

Genetic Stumbling Block :

By 1987, three groups of scientists had spliced *Bt* genes into the genomes of cotton plants and exposed the altered plants to bollworms and budworms. Disappointingly, the bioengineered cotton plants showed the same degree of insect damage as the nonmodified cotton plants. The bioengineered plants did not produce enough *Bt* toxins to protect them from bollworms and budworms. Apparently something was lost in the transfer of *Bt* genes from bacteria to plants--another puzzle to be solved.

Fortunately, basic research provided a clue as to what had gone wrong. Just as in written language a group of letters in a particular order spells a specific word, in the language of DNA the order of the four bases "letters" known as nuclotide bases--adenine (A), guanine (G), cytosine (C), and thymine (T)--spells out the specific amino acids that make up a protein. Amino acids are the building blocks of proteins. To make proteins, cells must first copy their DNA into messenger RNA, or mRNA for short. The mRNA acts as a blueprint for a protein, helping to line up amino acids in the proper order. *Bt* genes contain much more A and T than normal plant genes, which makes the mRNA for the bacterial genes unstable in plants, leads to the production of incomplete *Bt* mRNA in the plant cells, and may slow reading of the mRNA blueprints. These problems result in reduced levels of *Bt* protein in plant cells.

To solve these problems, scientists altered the spelling of the *Bt* gene, changing some of the A and T to G and C. The changes they made to the *Bt* gene resulted in more stable and useable mRNA in the plants but did not alter the amino acid sequence of the protein. By 1990, *Bt* cotton plants had been genetically engineered to produce enough *Bt* toxin to be protective against insects, and a major milestone in plant bioengineering had been achieved.

RETURN TO QUICK FIND

Viral Resistance:

Bt cotton, which reached the market in 1996, was one of the first bioengineered crop seeds to become available commercially. Since then around a dozen other crop plants have been genetically modified to resist pests and diseases, many with genes derived from basic research findings reported decades earlier. Genetically engineered virus-resistant strains of squash and cantaloupe, for example, would never have made it to farmers' fields if plant breeders in the 1930s had not noticed that plants infected with a mild strain of a virus do not succumb to more destructive strains of the same virus. That finding led plant pathologist Roger Beachy, then at Washington University in Saint Louis, to wonder exactly how such "cross-protection" worked--did part of the virus prompt it?

In collaboration with researchers at Monsanto, Beachy used an *A. tumefaciens* vector to insert into tomato plants a gene that produces one of the proteins that makes up the protein coat of the tobacco mosaic virus. He then inoculated these plants with the virus and was pleased to discover, as reported in 1986, that the vast majority of plants did not succumb to the virus.

Eight years later, in 1994, virus-resistant squash seeds created with Beachy's method reached the market, to be followed soon by bioengineered virus-resistant seeds for cantaloupes, potatoes, and papayas. (Breeders had already created virus-resistant tomato seeds by using traditional techniques.)

Since 1992, researchers have pinpointed and cloned several of the genes that make selected plants resistant to certain bacterial and fungal infections; some of these genes have been successfully inserted into crop plants that lack them. Many more infection-resistant crops are expected in the near future, as scientists find more plant genes in nature that make plants resistant to pests. Plant genes, however, are just a portion of the arsenal; microorganisms other than *Bt* also are being mined for genes that could help plants fend off invaders that cause crop damage.

RETURN TO QUICK FIND

Herbicide Resistance :

If pests and disease can be deadly to crop plants, weeds are a constant scourge. Weeds compete for nutrients, water, and sunlight and can reduce potential yield by as much as 70 percent. A standard way for farmers to deal with weeds is to spray their fields with several herbicides, each targeted to a specific type of weed. But many herbicides can damage crops as well as weeds and they can also cause air and water pollution. Farmers also use tilling to kill weeds before planting, or they spray fields with more

environmentally benign broad-spectrum herbicides before the emergence of a new crop; but these practices can subject fields to erosion by wind and water.

Bioengineering has recently offered farmers an alternative for weed control. The approach--the brainchild, in part, of Ernest Jaworski, a biochemist working at Monsanto--involves spraying fields with broad-spectrum herbicides after herbicide-resistant crops have sprouted and are holding down the soil. In the late 1960s, Jaworski wanted to know why a newly developed herbicide made from the simple chemical compound glyphosate was remarkably effective against many kinds of plants. Most herbicides were able to kill only a select few weeds. What made glyphosate so deadly to so many types of weeds?

Jaworski spent three years trying to decipher how the unique herbicide worked. In 1972, he published a paper showing that glyphosate does its damage by inhibiting a critical biochemical pathway in plants. A few years later German scientists showed that glyphosate specifically disrupted the function of an enzyme known as EPSP synthase, which is vital to all plants.

That information might not have gotten much play were it not for bioengineering which offered the possibility of genetically altering crop plants to withstand treatment with glyphosate. In 1983, researchers at Calgene and Monsanto succeeded in isolating and cloning the genes that produce EPSP synthase. Scientists at Monsanto then modified the gene so that the enzyme it produced was no longer sensitive to glyphosate and used the *A. tumefaciens* vector to introduce the modified gene into crop plants. The new tomatoes and other crop plants produced an EPSP synthase that made them resistant to damage from glyphosate, as reported in 1985. In 1996, the first glyphosate-resistant soybean, cotton, canola, and corn seeds were made available to farmers. Plants have also been engineered to tolerate other types of herbicides, many of which are "friendly" to the environment. These herbicide-resistant crops can be used to support farming practices that protect soil and water quality. Once such crops have sprouted and are holding down the soil, farmers can spray their fields with glyphosate to kill weeds.

RETURN TO QUICK FIND

New Possibilities on the Horizon :

Not content with just making crops resistant to pests and weeds, plant bioengineers are beginning to cut and paste genes to make crop plants more salt tolerant or drought tolerant--or to produce foods that are tastier or more nutritious. They are also exploring ways to bioengineer plants to produce specific compounds, such as industrial oils, plastics, enzymes, and even drugs and vaccines. These "biorenewable resources" also would have the advantage of being biodegradable.

The possibilities are many, and the number of bioengineered plants is likely to grow enormously as we enter the next century. Between 1996 and 1997 alone, the acreage in industrial countries planted with bioengineered crops increased nearly 20-fold.
As illustrated here, many of the scientific breakthroughs that led to bioengineered seeds stemmed from findings in unrelated basic research, much of it publicly funded, whose practical applications were completely unexpected by their discoverers--curious people who just wanted to understand how nature works.

RETURN TO QUICK FIND

Meeting the Challenges Posed by Bioengineered Plants :

Among the problems that researchers and regulators face with regard to bioengineered seeds is that continual exposure to *Bt* proteins in bioengineered crops will result in selection for new strains of insects that can withstand the bacteria's toxic proteins. To counteract this problem, seed companies are asking farmers to plant a small portion of their fields--as little as 4 percent--with standard seeds to create a refuge for *Bt*-susceptible insects. The hope is that the rare *Bt*-resistant insects that survive feeding on *Bt* crops will mate with *Bt*-susceptible insects in the refuge and that the *Bt* susceptibility trait will predominate in their offspring. Computer models predict that, without such refuges, *Bt* resistance will be widespread within 10 years. If refuges are used, however, more than 50 years might pass before *Bt* resistance becomes a major problem.

Another issue is that genes for *Bt* or for herbicide resistance could be passed, via cross-pollination, to related weed species. Such cross-pollination is a normal mechanism in plant evolution. Many plant species produce fertile hybrids, and genes have regularly moved between crops and their wild and weedy relatives. Thus resistance to a particular herbicide may appear in some strains of weeds. Even so, researchers consider the environmental benefits to far outweigh the possible negative consequences.

Finally, there is concern that, through bioengineering, genes that produce an allergy-inducing protein in one food plant might be introduced into another plant, which might then be eaten by an unsuspecting allergic individual. For that reason, the U.S. Food and Drug Administration (FDA) requires that all genetically engineered foods with genes from a known allergenic organism be extensively tested to ensure that the modified food does not induce allergies.

Studies show that introduced genes sometimes produce unexpected consequences in crop plants. However, the same can be said for traditional breeding techniques. Indeed, both genetic engineering and traditional breeding require extensive testing of their final products to demonstrate their properties and to ensure food safety. The National Research Council, U.S. Department of Agriculture, and FDA have all declared that bioengineered crops pose no greater threat to human health than do crops created by more traditional means. The regulatory constraints that apply to the introduction of other new crop varieties should therefore also be used here.

RETURN TO QUICK FIND

Important Events in the Development of Designer Seeds:

This timeline shows the chain of basic research that led to the development of seeds bioengineered to resist insects, viruses, and herbicides.

1901

Ishiwata Shigetane discovers that the cause of a disease outbreak in silkworms is a new species of bacteria, later called *Bacillus thuringiensis*, or *Bt.*

1905
Sir Roland Biffen shows that the ability of wheat to resist infection with a fungus is genetically inherited.

1907
Erwin Smith and C. O. Townsend discover that the cause of crown galls is a bacterium called *Agrobacterium tumefaciens*.

1930s
Plant breeders notice that plants infected with a mild strain of a virus are protected from infection with a more destructive strain.

1938
The first commercial insecticide that contains *Bt* hits the market.

1947
Armin Braun shows that *A. tumefaciens* introduces a factor into plant cells that permanently transforms them into tumor cells.

1950s
Studies show that proteins produced by *Bt* bacteria kill insects.

1972
Ernest Jaworski reports that glyphosate herbicides work by inhibiting a critical biochemical pathway in plants.

1974
Jeff Schell and Marc Van Montagu discover that a circular strand of DNA (a plasmid) carried by *A. tumefaciens* transforms plant cells into tumor cells.

1977
Eugene Nester, Milton Gordon, and Mary-Dell Chilton show that genes on the *A. tumefaciens* plasmid are transferred into infected plant cells.

1981
Helen Whiteley and Ernest Schnepf, at the University of Washington, clone a *Bt* toxin gene.

1983
Jeff Schell and Marc Van Montagu, Mary-Dell Chilton and colleagues, and scientists at Monsanto introduce genes into plants by using *A. tumefaciens* plasmid vectors.

1986
Roger Beachy shows that plants bioengineered to produce a viral coat protein are protected from infection with the virus.

1990
Field trials show that *Bt* cotton strains resist bollworm and budworm.

1994
Genetically engineered virus-resistant squash seeds hit the market.

1996
Herbicide-resistant strains of soybeans, cotton, canola, and corn reach the market.

RETURN TO QUICK FIND

RETURN TO QUICK FIND