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Insect-Resistant Crops Through Genetic Engineering

Glenda D. Webber
Office of Biotechnology
Iowa State University

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Where the story began

For centuries, humans have searched for crop plants that can survive and produce in spite of insect pests. Knowingly or unknowingly, ancient farmers selected for pest resistance genes in their crops, sometimes by actions as simple as collecting seed from only the highest-yielding plants in their fields.

With the advent of genetic engineering, genes for insect resistance now can be moved into plants more quickly and deliberately. Bt technology is only one example of ways genetic engineering may be used to develop insect resistant crops now and in the future.

The Bt chapter

Bacillus thuringiensis, commonly known as Bt, is a bacterium that occurs naturally in the soil. For years, bacteriologists have known that some strains of Bt produce proteins that kill certain insects with alkaline digestive tracts. When these insects ingest the protein produced by Bt, the function of their digestive systems is disrupted, producing slow growth and, ultimately, death.

Bt is very selective — different strains of the bacterium kill different insects and only those insects. Strains of Bt are effective against European corn borers and cotton bollworms (Lepidoptera), Colorado potato beetles (Coleoptera), and certain flies and mosquitoes (Diptera). Bt is not harmful to humans, other mammals, birds, fish or beneficial insects.

Bt was first identified in 1911 when it was discovered that it killed the larvae of flour moths. Bt was registered as a biopesticide in the U.S. in 1961. Today it is used in insecticide sprays sold to home gardeners and others worldwide.

In 1983, the World Health Organization used Bt in West Africa to control disease-carrying blackflies. In the U.S., various strains of Bt are used to control spruce budworms and gypsy moths in forests, cabbage worms in broccoli and cauliflower, loopers or budworms in cotton and tobacco, and leaf rollers in fruits.

However, less than one percent of all pesticides used in the U.S. each year contain Bt (Monsanto). As an ingredient of commercial sprays, Bt is relatively expensive and has some drawbacks. Although some pesticides kill on contact, Bt must be eaten by insects to be effective. Sunlight breaks down Bt, and rain washes it from the plants. Therefore, Bt must be applied exactly where and when the target insects are feeding, and they must consume it quickly before it disappears.

Bt and biotechnology

Today, plants can be genetically engineered to produce their own Bt. Genetic (recombinant DNA) engineering is the modification of DNA molecules to produce changes in plants, animals or other organisms. DNA (deoxyribonucleic acid) is a double-stranded molecule that is present in every cell of an organism and contains the hereditary information that passes from parents to offspring. This hereditary information is contained in individual units or sections of DNA called genes. The genes that are passed from parent to offspring determine the traits that the offspring will have.

In the last twenty years, scientists made a surprising discovery — DNA is interchangeable among animals, plants, bacteria ... any organism! In addition to using traditional breeding methods of improving plants and animals through years of cross-breeding and selection, scientists can now isolate the gene or genes for the traits they want in one animal or plant and move them into another. Of course, when a trait is controlled by several genes, the transfer process is more difficult. The plants or animals modified in this way are called transgenic.

Out of Bt ... into the plants

DNA technology makes it possible to locate the gene that produces Bt proteins lethal to insects and transfer the gene into crop plants. The process is depicted in Figure 1.

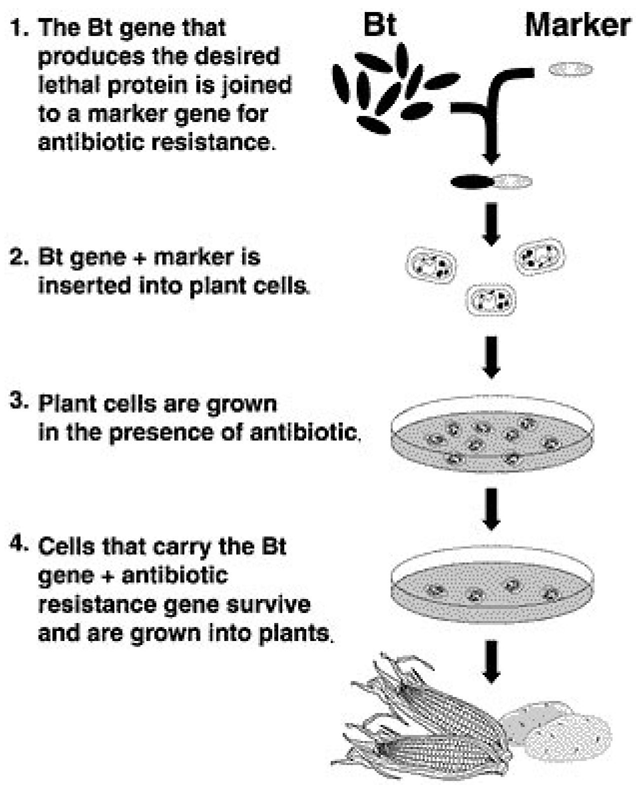

Figure 1
Bt technology.

First, scientists identify a strain of Bt that kills the targeted insect. Then they isolate the gene that produces the lethal protein. That gene is removed from the Bt bacterium, and a gene conferring resistance to a chemical (usually antibiotic or herbicide) is attached that will prove useful in a later step.

The Bt gene with the resistance gene attached is inserted into plant cells. At this point, scientists must determine which plant cells have successfully received the Bt gene and are now transformed. Any plant cell that has the Bt gene must also have the resistance gene that was attached to it. Researchers grow the plant cells in the presence of the antibiotic or herbicide and select the plant cells that are unaffected by it. These genetically transformed plant cells are then grown into whole plants by a process called tissue culture. The modified plants produce the same lethal Bt protein produced by Bt bacteria because the plants now have the same gene.

Research to transfer insect resistance genes from Bt to crop plants is well under way. Corn, cotton and potatoes are three of the many commercial crops targeted for Bt insect resistance.

Bt in corn confers resistance to European corn borers

Modern corn hybrids have some resistance to European corn borers (ECB), but they still sustain damage from moderate and high levels of infestation. Genetically engineered insect-resistant corn is providing a new defense against an old enemy.

The enemy

The European corn borer is a major pest of corn in North America and Europe. Researchers estimate that yield loss due to ECB averages 5 percent, but any one year or location can have much higher damage (Ciba Seeds). The number of larvae in the previous season, the type of winter weather, natural parasites and disease, and weather conditions during the growing season can affect ECB levels. For example, during the 1990 and 1991 growing seasons, ECB levels were very high across much of the U.S. Corn Belt and losses were heavy. But the cool, rainy weather and diseases during critical parts of the 1992 growing season reduced ECB populations and their damage in many areas.

ECB larvae attack corn two times during the growing season across most of the U.S. Corn Belt. First-brood larvae feed on plant leaves before flowering, injure leaf tissue, and eventually bore into the stalk. Second-brood larvae begin feeding in the leaf sheath and collar area of corn plants after flowering. Eventually, they bore into the stalk and ear shank where they can cause broken stalks and dropped ears.

The greatest source of ECB damage may not be visible. ECB disrupts the physiological processes of corn and can reduce yields even when stalk lodging (breaking) problems are not evident. Physiological damage can cause low test weight of harvested corn, small ears, or early death of the plant.

Chemical sprays must be applied before the borer tunnels into the stalk to be effective. Multiple applications may be required and, even then, complete control is not possible.

The Bt solution

Scientists know that a family of insecticidal proteins from Bt kills ECB. The bacteria produce the protein as an insoluble crystal. When a susceptible caterpillar, such as the corn borer, eats the crystal, part of it binds to, penetrates and collapses the cells lining its gut, causing death.

Although Bt genes have been introduced into tobacco, tomatoes, cotton and other broadleaf plants, gene transfer technology for corn is a recent achievement. The development of corn plants expressing Bt proteins requires substantial changes in the Bt genes, including the creation of synthetic versions of the genes, rather than the microbial Bt gene itself.

For example, one company has developed three versions of a synthetic Bt gene that "switches" on in pollen, in green tissue, or in other parts of the corn plant (Ciba Seeds). Along with a herbicide resistance gene, the three Bt versions were " shot" by a biolistic gene gun into immature corn embryos taken from developing seeds about 15 days after pollination. The gene gun bombarded the embryos with tiny gold particles coated with the genes.

Some plants received genes with a non-specific promoter (switch) that resulted in production of the Bt protein in many parts of the corn plant. Other plants received a mixture of genes with switches that resulted in Bt protein production only in the pollen and green tissue. The herbicide resistance gene allowed scientists to determine which plant cells had received a gold particle carrying DNA and successfully incorporated the gene. Subsequent screening for Bt production showed which cells received both herbicide resistance and the Bt gene(s).

When the cells were grown into plants and field tested for resistance, it was found that the Bt plants fared better than any of the control plants. In general, plants with both the pollen and green tissue Bt genes appeared more resistant to second-brood corn borer damage than plants with Bt genes that expressed themselves in many parts of the plant. Another advantage of the Bt genes with specific switches (pollen and green tissue) was that they produced insecticidal protein in those parts of the plant attacked by both first- and second-brood ECBs, while minimizing production in seed and other parts of the plant where protection is not critical.

Using Bt technology to control corn borers is an international effort. One U.S. company is using a biolistic gene gun to bombard Indonesian corn with Bt genes that produce proteins lethal to the Asian corn borer (Wilson). This pest is responsible for significant yield losses, up to 40 percent, in much of the corn-growing areas of Indonesia.

The warm Indonesian climate and the life cycle of the Asian corn borer allow 9 to 12 overlapping generations annually, making it a threat to corn virtually year-round. Introducing Bt insect resistance genes into commercial Indonesian corn seems to be the most feasible and effective way available to control the pest.

Comprehensive testing must be done, approval by relevant authorities obtained, and patent questions answered before any company can commercialize corn hybrids containing the Bt gene.

Bt resistance for the Colorado potato beetle

The potato is the most popular vegetable among North American consumers. It is estimated that potatoes, in some form, are consumed during one of every three meals (Monsanto). Despite their popularity, potatoes are not that easy to grow. Each year, potato growers lose a significant amount of their crops to an insect called the Colorado potato beetle. The beetle feeds on the growing plant leaves and stems during the growing season, stunting the plant and cutting yields. Repeated applications of chemical sprays are usually needed to control the pest.

The economic picture

According to the USDA, potato producers in the U.S. spend $20 million to $40 million each year for control of the Colorado potato beetle (Monsanto). In an average year, growers spray their fields with insecticides as many as five times at a cost of up to $150 per acre.

If insect-resistant plants were available, researchers estimate that the use of chemical insecticides in potatoes could be reduced as much as 30 percent to 40 percent. Translated into dollars, growers could save $6 million to $16 million annually, based on USDA estimates of current insecticide use.

Similar science, different target

The science being used to develop potatoes resistant to the Colorado potato beetle is similar to the European corn borer technology. One of the advantages of Bt is the diversity of strains that can be used to target different insect pests.

Like the corn researchers, potato scientists are using a specific type of the naturally occurring soil bacterium *Bacillus thuringiensis.* A single gene from the Bt is spliced into a chromosome of a potato cell. When the cell carrying the Bt gene is grown into a plant, the plant produces a protein that is toxic to the Colorado potato beetle. When the beetle feeds on the genetically improved plant, the toxic protein interferes with its digestive system and it dies.

The protein is highly selective, affecting only the Colorado potato beetle. It does not harm humans, animals or beneficial insects that help control other crop pests.

The outlook

One company conducting insect-resistant potato research expects farmers to be planting their beetle-resistant potatoes by the mid-1990s (Monsanto news release). This company began its development of insect-resistant potatoes with the Russet Burbank variety, since it comprises approximately 40 percent of U.S. potato acreage. However, company researchers are also working to improve other popular potato varieties such as Atlantic, Superior, Norchip and Shepody.

Like all genetically improved food crops, the exact timetable for commercialization of insect-resistant potatoes depends not only on the scientific tests that must be conducted, but also on approvals by the appropriate regulatory agencies. (See regulating Bt plants.)

Bt resistance for cotton

Cotton is another cash crop benefiting from Bt insect-resistance research. With a market value of $4.5 billion, cotton is the nation's fifth largest crop (Monsanto). Yet annually, cotton growers expect to lose up to 15 percent of each field due to insect damage.

Caterpillar insects, such as the tobacco budworm, cotton bollworm and pink bollworm, feed on cotton plants. Experts say that these and similar pests are responsible for millions of dollars in damage to the American cotton crop each year.

Caterpillar economics

Caterpillar insects are responsible for 60 to 70 percent of all insect damage to cotton plants (Monsanto). Efforts to control them chemically account for about 60 to 70 percent of a cotton grower's pesticide costs. In the Mississippi Delta growing region, a cotton grower's pesticide costs can average $40 to $45 per acre each season. In Arizona, pest control costs are usually higher, perhaps double that of the Mississippi Delta.

Chemical sprays containing Bt are seldom practical in cotton, since Bt breaks down in sunlight, washes away in rain, and is nearly impossible to apply to the plant parts where insects feed. However, other chemical insecticides are used that may need to be applied as many as 10 times in one season. By planting insect-resistant cotton, researchers hope farmers can reduce the number of insecticide applications they must pay for.

Same song, third verse

Creating insect resistance in cotton by using Bt technology is a procedure similar to that used in corn or potatoes. Researchers find one or more strains of Bt that produce a protein fatal to some of the most damaging cotton insect pests. The gene that regulates the protein's production is inserted into the genetic structure of cells from a cotton plant. The cells that successfully receive the Bt genes are grown into whole plants through tissue culture and enter a long period of laboratory and field testing. When certain caterpillar pests feed on the new cotton plants, the protein damages their digestive systems, and they die.

Commercialization timetable

Current estimates are that it will be several years before insect-resistant cotton plants are commercially marketed. They must meet the regulatory requirements administered by the USDA and the Environmental Protection Agency (EPA). (See regulating Bt plants.)

The insect resistance problem

Transgenic Bt plants such as corn, potatoes and cotton have a possible problem — what if insects build up a resistance to the lethal proteins?

The potential of pests to develop resistance against the defense mechanisms of crops is well-known and is not unique to genetically engineered plants. Insects may develop resistance to a crop defense no matter how it was developed. The crop defense might be a chemical or biological agent, a gene already in the crop species and transferred to commercial plants by conventional plant-breeding methods, or a gene introduced by recombinant DNA technology. Because more than 500 insects and mites already have acquired resistance to a number of insecticides, there is concern that similar resistance to Bt toxins could develop (McGaughey and Whalon).

Several major pests, including the tobacco budworm, Colorado potato beetle, Indianmeal moth and diamondback moth, have demonstrated the ability to adapt to Bt in the laboratory. It has been reported that the diamondback moth evolved high levels of resistance in the field as a result of repeated use of Bt (McGaughey and Whalon). As Bt use increases on more acres, some scientists predict that insect resistance to Bt will be a major problem. Considerable controversy exists about how Bt should be managed to prolong its usefulness.

Bt durability in non-transgenic crops

The crystal proteins of Bt that are lethal to insects have been widely used as microbial insecticides in traditional corn and vegetable crops for years. In recent years, field populations of insects tolerant to these Bt proteins have been reported. To date, these cases are limited and have been associated with frequent and prolonged use of Bt on geographically isolated insect populations (Ciba Seeds). Scientists have been able to induce higher levels of resistance in these field populations by taking them into the laboratory and exposing them to specific Bt insecticidal crystal proteins.

A laboratory population of tobacco budworm that is resistant to more than one class of crystal proteins has been reported. However, the resistance of this population is not high, and its ability to survive is significantly reduced when the proteins are absent.

In the case of ECB-resistant corn, it is thought that the development of insect resistance may be delayed by the availability of non-Bt corn fields (refuges) that are still susceptible to the borers. Borers that could not survive ECB-resistant fields may do well in the refuges. There they could breed with resistant borers, diluting the resistance trait in the gene pool.

Bt durability in transgenic crops

About 13 U.S. and European companies that fund academic Bt research have formed a Bt Management Working Group to support research on the interaction of transgenic plants with target pests.

The plant industry is employing several approaches to deal with insect resistance to transgenic insecticidal plants, including the following:

Very high levels of insecticidal crystal proteins (ICPs) are being produced in new genetically modified plants. The only insects that can survive feeding on such plants will be those that already possess a high-level resistance gene as part of their genetic code. Such genes are expected to be extremely rare in the insect population. Insects with more common genes for partial, or low-level, resistance will not survive the very high levels of ICPs.

Additional insecticidal genes are being sought. The idea is to develop transgenic plants that express several insecticidal genes targeting different sites within the insect. In order to survive plants with such multiple defenses, an insect would probably have to possess multiple resistance genes. Some scientists believe it is highly unlikely that insects can acquire such genes. In any case, these scientists anticipate that plants expressing additional insecticidal genes will be available before insect resistance to a single, highly expressed ICP from Bt develops.

Regulating Bt plants

Insect-resistant crops developed through Bt technology are subject to the same regulations as other genetically engineered plants. It should be noted that U.S. regulatory policy for genetically engineered plants has evolved over time and additional changes in the requirements discussed below may occur. Three government agencies share responsibility for administering plant biotechnology regulations in the United States.

U.S. Department of Agriculture (USDA)

The USDA administers the Federal Plant Pest Act (FPPA), which regulates interstate movement, importation, and field testing of genetically engineered plants. A special permit from the USDA and approval by the individual state departments of agriculture are required to move any genetically engineered organism into the U.S. or between states. Applicants for a permit must provide details about the nature of the organism, its origin, and its intended use. If granted, the permit certifies that the applicant's facility and procedures meet certain operating standards, such as appropriate levels of containment to prevent accidental escape of the organism. Guidelines set by the National Institutes of Health for laboratory research involving recombinant DNA molecules are included in these standards and must be followed by the applicant.

The USDA oversees field testing of genetically engineered crops. The applicant must provide complete information about the plant, including all new genes and new gene products, their origin, the purpose of the test, the experimental design (how the test will be conducted), and precautions to be taken to prevent the escape of pollen, plants or plant parts from the field test site. The approval process can take a maximum of four months.

Before a genetically engineered crop can be sold commercially, a petition must be filed for USDA exemption. This petition requires more information than a field test permit, including environmental product safety information.

Recently, the USDA streamlined the permit process for movement and field testing of certain genetically engineered crops such as corn, soybeans, cotton, potatoes, tomatoes and tobacco. The streamlining was based on an extensive history of safe use in field trials in the United States. In the streamlined process, an applicant can field test specific crops if the crop and genetic material meet certain USDA performance standards. For a field test that does not meet these performance standards, the current 120-day field test permit process would be followed.

Environmental protection agency (EPA)

The EPA recently proposed new policies for review of applications for field trials of genetically engineered plants. It proposed that substances introduced into crops by genetic engineering to provide disease or insect resistance (Bt crops are in this category) fall under the Federal Insecticide, Fungicide, and Rodenticide Act and the Federal Food, Drug, and Cosmetic Act.

To date, the USDA has consulted with EPA when reviewing applications to test such crops on a small scale. The EPA will most likely formally review a product when a company decides to move into large-scale testing. As with traditional pesticides, an Experimental Use Permit (EUP) will be required for tests that exceed 10 acres total in the U.S.

After receiving the EUP, a company follows a process that involves extensive testing of the crop to ensure food and environmental safety before the crop is commercialized. In addition, it is likely that some applicants also will consult the Food and Drug Administration (FDA) before a pest-resistant food crop is commercialized, even though a recent FDA policy proposal emphasized that such products fall under the EPA's jurisdiction and are not subject to FDA pre-market approval.

Food and Drug Administration (FDA)

The FDA also has very broad authority under the Federal Food, Drug, and Cosmetic Act to regulate the introduction of new food crops, whether they were conventionally produced or created by genetic engineering. FDA policy states that any new food developed through genetic modification may require testing and/or labeling if the genetic material introduced into the host plant comes from outside the traditional food supply, is a known or suspected cause of allergic reactions, or significantly alters the nutritional composition of the food (*Federal Register*). Every company or individual that produces food or food products through recombinant DNA technology is legally required to assure its safety and quality before it enters the food supply — the same assurance required of traditional food products.

The Bt outlook

Crops that have been genetically engineered for Bt resistance could dramatically lower production costs and provide farmers with new insect control options within the next few years. The success of their commercialization depends on several factors, including the regulatory climate, patent issues, and the ability of scientists to deal with targeted insects that develop resistance to the lethal proteins. Several companies currently working on Bt insect resistance in crops foresee marketing their products in the mid-1990s.

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