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Methods and Techniques for Production of Genetically Modified Maize at Monsanto Global Seed

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ABSTRACT

The development of transgenic plants for agricultural purposes consumes much time and energy but is well worth the battle. From the laboratory perspective, hours are spent trying to force plant cells to uptake new DNA. Several methods are available for this practice, including Ti-plasmid mediated transformation, microprojectile bombardment, and electroporation. Each method depends primarily on the organism one wishes to transform. Once the gene of interest has been inserted, the seed from these transgenic plants goes to pilot stations to be grown in nurseries and yield production fields and tested for resistance against many elements. The final product is put through a series of government and health rules and regulations before finally allowed to be marketed.

THE LABORATORY EXPERIENCE

Making transgenic plants to cure a myriad of ailments in this world does not begin in the fields; but rather it begins in a laboratory and must undergo many processes. After endless hours of research and development, the final product is put through a series of governmental and health inspections before marketed for consumer use. There are a wide variety of transgenic and genetically altered plants on the market today. These plants range from cotton and corn to soybeans and potatoes. Each serves a vital part in this nation's economy, as well as the development of other countries.

Biotechnology has many uses: ethanol production, crop yields, disease prevention, insect protection, drought resistance, etc. The fundamental process behind biotechnology is genetic engineering. Through molecular biological techniques, such as Polymerase Chain Reaction (PCR), gel electrophoresis, cloning, transformation, and microprojectile bombardment, the insertion of bacterial genes encoding new traits into plants is possible. Monsanto Global Seed uses these molecular techniques to produce a wide variety of new traits in crop plants. New hybrids start in the laboratory with the insertion of genetic material, which can be accomplished using many methods. Transformation and microprojectile bombardment are just two of the common methods used in biotechnology today. Other methods include using electricity or chemicals to create pores in cells, which then allow the large DNA molecules to enter.

Agrobacterium tumefaciens is the bacteria responsible for producing tumors on crop plants. Injured plants produce phenolic compounds (acetosyringone, hydroxyacetosyringone) that attract *A. tumefaciens* to the plant. The phenolic compounds induce virulence (*vir*) genes that are encoded on the Ti (tumor inducing) plasmid carried

by *A. tumefaciens*. This plasmid transfers a segment called the T-DNA (12-24 kilobase

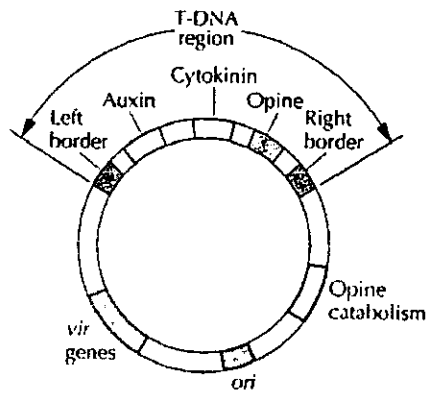


Figure 1. Ti Plasmid This is a condensed version of the Ti Plasmid as it is found in its natural host, *Agrobacterium tumefaciens* (3).

pairs) as a single-stranded linear molecule,

which then integrates into the plant genome

(3). This natural method used by *A.*

tumefaciens of inserting genetic material is

efficient and practical for biotechnology

purposes. Genes carrying new traits for crop

plants can be inserted into the T-DNA region

of the Ti plasmid by cutting around the gene

of interest with restriction enzymes, pasting

the segment into a plasmid with ligation

enzymes, and then transferring the plasmid into the plant cells. There are still many disadvantages to the uncut version of the Ti plasmid.

Because the Ti plasmid is only carried in *A. tumefaciens*, and this organism only infects a limited variety of plants (dicotyledons), a host bacterium is required to carry out a majority of the manipulation and cloning steps (6). *Escherichia coli* is often called the workhorse of molecular biology because it is the most widely studied and the most efficient host for cloning. The Ti plasmid, about 200-800 kbp, in its natural form, is too large to be readily taken up into a host (3). Some of the genes that are not required for cloning have to be removed. Also, since the Ti plasmid is normally found in *A. tumefaciens*, it contains an origin of replication (*ori*) specific for this bacterium. In order for the plasmid to replicate in the new host, a new *E. coli* origin of replication must be

inserted. In addition to the physical restraints of cloning from the natural Ti plasmid, there are several biochemical restraints as well.

When transformed plant cells are grown in culture medium, they fail to regenerate into mature plants because of phytohormone production (3). Phytohormones regulate growth and development in plants containing the tumors. The T-DNA region of the Ti plasmid must be liberated of the auxin and cytokinin producing genes in order to obtain healthy and mature plants. In addition to auxin and cytokinin elimination, the gene for opine synthesis must be removed. Opines are condensation products of either an amino acid and a keto acid, or an amino acid and a sugar, which can ultimately be used as carbon sources for *A. tumefaciens* when the tumor is present (3). The biosynthesis of opines diverts plant energies and can lead to lower plant production yields.

Despite the many disadvantages of the natural Ti plasmid, the process of cloning and transforming the vector is still efficient and productive if the plasmid is engineered correctly. The natural plasmid must be engineered in such a way as to harbor the genes of interest and still maintain the properties that make the plasmid efficient and productive for recombinant DNA technology. The cloning vector must be constructed with the following components: marker gene, *E. coli* origin of replication, right border sequence of T-DNA (and most often left border sequence), and a multiple cloning site (3).

The marker gene most often used is neomycin phosphotransferase, which confers resistance into the plant cells to the antibiotic Kanamycin. The marker gene must be placed under the control of the plant to ensure that the gene of interest from the Ti plasmid was integrated into an appropriate place in the plant chromosome and is being expressed efficiently (3). The *E. coli* origin of replication must be added so *Escherichia*

coli can replicate the vector. The right border sequence of the T-DNA region in the plasmid is an absolute necessity because it is used as the cutting point for transfer and integration of the T-DNA region into the plant genome. The left border sequence is not a necessity, but is often still attached in the engineered plasmid. Finally, a multiple cloning site must be inserted into the T-DNA region. The multiple cloning site is used for the insertion of the gene of interest into the cloning vector (3).

The new and improved cloning vector has all of the needed genes to ensure proper positioning and integration of the gene of interest into the multiple cloning site of the plasmid, with the exception that the improved version has no way to transfer and integrate the T-DNA region into the plant cell genome because of its lack of virulence (*vir*) genes. There are two methods

to fix this problem: the binary cloning system and the cointegrate vector system.

In the binary cloning system

(See Figure 2.), *E. coli* and *A.*

tumefaciens origins of replication are

added, but the *vir* genes still remain

absent. The vector is put into *A.*

tumefaciens containing a separate

disarmed version of the Ti plasmid, which contains the *vir* genes. The virulence genes

are synthesized and the T-DNA region is transferred. The cointegrate vector system is

slightly different (See Figure 3.). The vector combines with the disarmed Ti plasmid

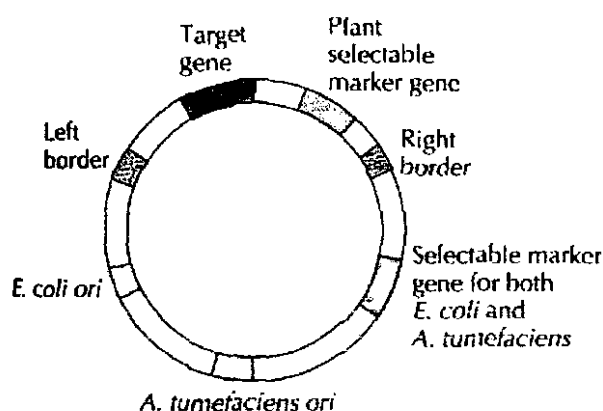


Figure 2. Binary Cloning Vector The engineered Ti Plasmid now contains origins of replication for *Agrobacterium tumefaciens* and *Escherichia coli*, selectable markers and the gene of interest (3).

lacking the virulence genes making a recombinant vector, which then expresses the virulence genes and the T-DNA region can then be transferred (3).

Ti plasmid-mediated transformation is very effective on most research plants with

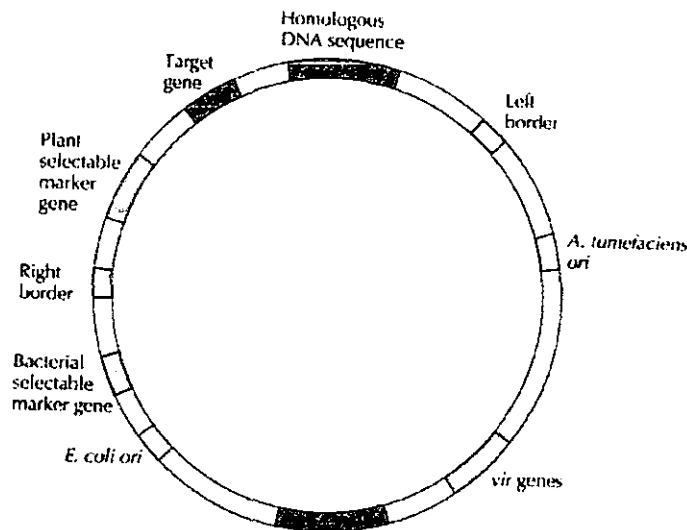


Figure 3. Cointegrate Cloning Vector The vector contains all of the necessary genes for cloning new desirable genes into different organisms. There are two homologous sequences due to the recombination of the cointegrate vector with the disarmed Ti Plasmid (3).

the exception of corn. Corn is considered a monocot and therefore, *A. tumefaciens* does not naturally infect the plants. Although it is possible to persuade the corn cells to uptake the plasmid, the method is difficult and inefficient

compared to other methods available specifically for difficult plant cells. A revised plan for transformation involves immersing

corn embryos in *A. tumefaciens* cells for several minutes and then incubating the embryos for several days at room temperature in the absence of selective pressure such as Kanamycin resistance (3). The embryos are then transferred to a selective medium (contains Kanamycin or another appropriate selective agent) and incubated in the dark for a few weeks. The selective agent prevents plants that are not integrated with the plasmid from growing. Finally, the plants are transferred to a growth medium, incubated in the light, and regenerated. This method takes much time and is somewhat inefficient due to

the amount of time consumed. There are other effective and efficient methods available for the transformation of genes of interest into difficult plants, such as corn.

Microprojectile bombardment, also referred to as biolistics, is a method that is better suited for difficult plants. DNA is dissolved in a buffer solution and then precipitated out with calcium chloride onto gold or tungsten particles about 0.4 to 1.2 μm in diameter (3). These particles are accelerated to high speeds (approximately 300-600 meters per second) and shot into plant cells using a specialized device called a particle gun. Microprojectile bombardment depends greatly on the vector, the presence of linear DNA, and the plasmid size. In order for this method to work, the DNA that is left behind from the particles must be integrated into the genome. If it is not integrated, when plant cell division occurs, the DNA is lost. Also, the plant cells naturally create certain endonucleases to combat against viruses and other predators as a defense mechanism. If the DNA does not integrate into the genome, the endonucleases cut up the foreign DNA in defense of a possible attack. Furthermore, if the plasmid used to transfer the DNA is large, it is often fragmented when accelerated and shot into the cell, leaving only pieces of the gene of interest, not the intact segment.

Regardless of the method used for transformation, the insertion of foreign DNA into plants that will ultimately be consumed in some form by humans or animals poses many concerns for consumers and regulatory agencies. Reporter genes are used to quantify the expression of the gene of interest after it has been integrated into the plant genome to be sure the gene is being expressed to its full potential. Antibiotic resistance genes cannot be used in crop plants because the production of the antibiotics often taints the final product of the plant. Marker genes, however, have not been shown to have any

ill effects on humans, animals, or the environment; but the products of some marker genes, or the gene of interest itself, might be allergenic or toxic to consumers (2). However, only segments of genes are transferred and humans are continuously exposed to organisms and their DNA in a random manner; therefore, it is difficult to say that genetic modification makes any difference as to the exposure level and health risks (2). In addition, concerns have been voiced about the possible transfer of antibiotic resistance from the genetically engineered plants into pathogenic soil bacteria. The pathogens would then be resistant to antibiotics and may potentially cause severe health issues (3). Health advisory committees recommend that antibiotic resistance marker genes be phased out of the plant genome as soon as possible to eliminate the risk of health problems (2).

RESISTANCE TESTING

The people that have dedicated their lives to agriculture and the production of crops understand the importance of innate resistance in their crops to a wide range of environmental forces. Fungus, bacteria, insects, weeds, and weather conditions destroy thousands of bushels of important crops each year. If plants somehow had intrinsic abilities to protect themselves against these anomalies, crop yields would increase, and agriculturists could eliminate the use of dangerous chemical herbicides and pesticides. Through biotechnology, insertion of new genetic material into the plant's genome to confer much needed abilities into those plants is possible. However, once the new traits have been instilled, testing the new abilities of each plant becomes a necessity. If the plant does not express the new gene correctly, the plant will not have the abilities described above. Field researchers spend their lives researching, testing, and

documenting plant behavior as the plants are intentionally exposed to normally harmful conditions.

Fungus resistance comes from the production of pathogenesis-related proteins, or PR proteins. These proteins are only produced by plants during invasion by pathogens or during environmental stresses. Some of these PR proteins destroy fungal cell walls or are protease inhibitors. For instance, chitinase (a PR protein) hydrolyzes the β -1,4 linkages in the N-acetyl-D-glucosamine polymers of the fungal cell walls (3). In the final stages (last seven growing seasons) of the new hybrids, the plants are sprayed continuously with an inoculum containing various fungal organisms. This testing selects for the plants containing the genes for resistance to the fungi and selects against the plants that have lost the genes during breeding or by another factor. Another fungus that is commonly tested for resistance against is called stem rot. An inoculum gun is filled with a mixture of two stem rot fungi and then stuck in the base of each corn plant during the late (after pollination) stages of plant development. The seeds from the plants that are resistant to these fungi are taken and planted again the following growing season. Another method for determining fungal resistance is using paddles with nails attached. The paddles are dipped into a fungal solution and tapped onto the plants. There are hundred of organisms that cause disease to corn plants. Specifically, *Diplodia maydis* causes ear rot, stem rot, seed rot, and seedling blight; however, all of these diseases can be caused from any number of particular fungal organisms (4). To check for Northern and Southern corn leaf blight, sorghum seeds were covered with the fungi and dropped into the whirl of each plant to be tested.

Intrinsic abilities against insects would eliminate the need to spray crops with health and environmentally harmful insecticides. This resistance is conferred from the introduction of protoxins from the bacterium *Bacillus thuringiensis* into the plant genome. The Bt toxin is naturally expressed in the form of crystals when *B. thuringiensis* sporulates to prevent death by ingestion to unsuspecting insects that try to consume the spores. Insect proteases inside the gut convert the crystallized protoxin into toxin, which then destroys epithelial cells (7). The plant expresses the functional protoxin and is therefore resistant against infestation by certain insects. Most *B. thuringiensis* protoxins destroy Lepidoptera (moths) and a few destroy Diptera (flies) (7). Expression of the protoxin by the plant is much more efficient and economic than spraying the fields with protoxin, because the protoxin is not environmentally stable and the timing of application is difficult to determine. These toxins can be amylase or protease inhibitors; however, protoxins are not well expressed by the plants once integrated. This is due to several differences between bacterial and plant DNA and protein structures.

In one *B. thuringiensis* subsp. *kurstaki*, several important insecticidal protoxin genes are not well expressed in plants. These include *cryIA(a)*, *cryIA(b)*, and *cryIA(c)* (3). Researchers working on this problem examined the sequences of each protoxin gene and determined which segments were conserved among the various strains of *B. thuringiensis*. The N-terminus portion of the molecule is about ninety-eight percent conserved, whereas the C-terminus portion is only about forty-five percent conserved (3). The C-terminus portion of the molecule was clipped off and a strong plant promoter was placed upstream (in front) of the N-terminus to ensure efficient production of the

protoxin once inside the plant genome. The result was an activated-protectant protein expressed diligently in plant systems.

Another problem associated with the introduction of Bt protoxins into plants is that primarily bacteria use more adenine and thymine (AT) base pairs in their DNA sequences (7). Plants only use about fifty percent AT pairs in their sequence; they are primarily rich in guanine and cytosine (GC) pairs (7). This difference in the DNA structures between higher eukaryotics and prokaryotics becomes important only when translation occurs. The bacterial translation system uses different codons for different amino acids than the plant system. When the plant expresses the bacterial gene rich in AT pairs, a non-functional protein results because of codon preference among different species. In addition, there are often long strings of thymines in bacterial DNA, which is perceived as a polyadenylation sequence in the plant system causing transcription of the segment to stop (7). The genes encoding the Bt toxin have been engineered with more GC pairs that still code for the same amino acid during translation; hence, a functional protoxin is produced by plants.

The uses of Bt protoxins are environmentally friendly and do not have adverse effects on humans or other mammals. However, insertion of these protectant proteins in plants could have possible serious implications in the future. For instance, transgenic plants can act as selecting agents for insects that are naturally resistant against the Bt protoxins. Also, protease inhibitors are often introduced into plants to combat against some insects. There is a possibility that plants might express potentially harmful protease inhibitors in edible plants for humans and mammals. The results could be unfavorable.

Despite the possibilities that Bt protoxins could have adverse effects on humans and animals, the reported advantages greatly outweigh the unconfirmed disadvantages. Corn borers constitute about a two percent loss in crops each year. Monsanto tests for resistance of corn borers by applying a certain amount of larvae to the whirls of the plants during their early stages of development.

The process of "making up" the corn borer mixture is tedious. Monsanto actually grows and harvests its own corn borers. Egg masses are ordered from the parent company and dropped into large plastic bags at the various research stations. A mist of water ensures that the eggs are spread out in the bag and not clumped together. The eggs are incubated at about 80°F for forty-eight hours, at which time the masses hatch into millions of tiny larvae with black heads. A measured amount of finely ground corncob is added to the bags and the borers are scrapped from the sides and carefully mixed in with the cob mixture. The corn borer-cob mixture is placed into bottles and corked, then taken into the research nursery. Special dispensers are screwed on to the top of the bottles. Workers walk through each row in each plot of the nursery that requires testing and dispense a certain amount of the corn borer-cob mixture into the whirl of young plants. Once the larvae are inside the protection of the whirl, they bore into the plant and can cause destructive damage in unmodified plants. The borers bore through the stalk and eventually the ear. The ear often falls to the ground and the stalk becomes weak. If there is no intrinsic ability conferred into the plant, the insects grow to be about an inch in length and will eventually, through metamorphosis, become moths.

The effects of the corn borers are not known until later stages of plant development (after pollination), at which case a plant is broken from its stalk and the

layers are slowly stripped away and examined for young borers, bite marks on the corn tissue, or fecal material. If the plant's genetic material has been altered correctly, there should only be a few bite marks (one or two from each borer) and several dead borers. The plant expresses and produces the Bt protoxin. When the borer bites into the tissue, the protoxin is ingested, converted to a toxin, which then destroys epithelial cells and may inhibit proteases or amylases. The basic mechanism is that the borer starves to death.

The destruction of crop plants due to fungi, bacteria, or insects is a major concern for farmers; but if the weeds are allowed to gain control in the field, the destruction can be just as severe if not more destruction than an infestation from organisms. Each year, ten percent of global crops are lost due to weeds (3). For this reason, chemical resistance to herbicides is an important trait to integrate into the plant. Chemical herbicides kill anything that is green and often have to be applied to the field before the crops are planted, which means they are environmentally stable and could pose some health risks for the environment, humans, and animals. Several methods of plant engineering can be used to produce chemical herbicide-resistant plants. The plants could be engineered to inactivate the herbicide, the target protein of the herbicide in the plants could be altered so that it no longer binds to the herbicide causing destruction, or the plant could overproduce the target protein so that not all of the protein is bound to and affected by the chemical (3). The latter method is the preferred method for resistance to glyphosate, a herbicide produced by Monsanto Global Seed in the form of RoundUp that is not environmentally stable.

The target protein for glyphosate is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is an enzyme that helps plants and bacteria synthesize aromatic amino acids via the shikimate pathway (3). Glyphosate resistant crop plants are engineered by taking the gene encoding EPSPS in a glyphosate-resistant *E. coli* strain and cloned into plant cells. The cells express the bacterial enzyme enough to replace the original plant EPSPS that was inhibited by the herbicide. The weeds in the crop fields, however, do not contain the bacterial gene encoding EPSPS and are therefore sensitive to glyphosate-containing herbicides. The method for testing for resistance is obvious and straightforward. The plants are sprayed with the herbicide; if they contain the gene conferring resistance, then they live.

Plants are exposed to many elements during the course of their development. Having the intrinsic ability to combat against these elements enables the plants to survive, which consequently produces more yield and a better economy for this country and other. All of the new strains of plants are truly put to the test against the elements in the nursery and the other fields for yield research. Monsanto tries everything possible to kill the new strains for the benefit of society and for a better tomorrow.

SEVEN GROWING SEASONS IN THE FIELD

Once a new trait has been introduced into a crop plant, years of careful breeding must follow to ensure the final product always carries that gene of interest. Approximately seven growing seasons are needed for this process. Often, Monsanto uses the summer growing seasons in the Midwestern United States, and then in the Midwestern winter season, sends the crops to Hawaii, Florida, Central and South

America because each has about two growing seasons, which cuts the amount of time for producing homologous plants in half.

The very first plants to enter the field are called the S0's. These plants are potential new strains and require more development. They are often very tall because of their heterogeneous state due to the crosses from several different kinds of corn plants. For example, corn grown in the Amazon rainforest is taller than Midwestern American corn and often researchers use this particular plant because of a desirable trait that it contains. Once the plant breeding begins, usually the height of the plant diminishes. Some plots in this section require breeding in the utmost care. Usually this is because there is a gene that Monsanto is looking for and labels each plant carefully. Tissue cultures from selected plants are sent to the main headquarters. Researchers at the headquarters examine the tissue using molecular techniques to decide which plants have the gene of interest, and therefore will be self-pollinated with the utmost care. The S0 section of the field is sprayed with inoculum when the plants are young, and tested for stem rot after pollination. Enough plants must be self-pollinated to ensure there are plenty of resistant seeds for the second generation, the S1's.

The second generation of plants is also subjected to the same tests as the S0's to select only for plants containing resistance to inoculum and stem rot. In addition to the fungal tests, the plants are also subjected to corn borers. Enough plants, as with the S0's, are self-pollinated to have enough seed for the S2's. This process of resistance testing and self-pollination continues through each generation up to the S45's. The height of these plants is much smaller than the original S0's. Through careful breeding, these plants have maintained their resistance to various outside forces and will most likely

become the new hybrids on the agriculture market. All of the S45's are self-pollinated and then eventually go on to field trials. A few of the plants with exceptional characteristics may be cross-pollinated with another S45 hybrid with noteworthy traits to produce future strains. The new strains will start at the beginning, the S0's, and through a series of selecting agents, will eventually end up in the S45's.

The S45 plants that are promising hybrids are taken from the nursery and sent to fields to test the yield. The crops are planted in sections fourteen rows wide, each section contains seven blocks, and each block contains seven plots. The number of plants in each block is key to determining the amount of seed produced for that particular hybrid. Workers walk down the length of the field in between two rows and count the number of plants. A team leader writes the amount on a diagram and each row is thinned to a certain number. In this way, the amount of seed produced for each hybrid can be quantitatively measured, which is important for the marketing of the final product.

In addition to the S-type plants grown in the nursery, there are Inbred Cross-Breeding (ICB) plots, the Crossing Blocks, and the show plots. The ICB field is not actually part of the nursery, rather it is a separate field. The field is designed in such a way as to have only male and female rows. There are four female rows in between two rows of males. In the center of the male rows is a marker row to indicate that the rows are actually designated male and will not be confused with the female rows. The difference between male and female plants is that the female plants have been de-tasseled so they cannot self-pollinate. The male's tassels are left intact so that the females can be pollinated. The purpose of this is to eliminate self-pollinating, create more yield of a specific hybrid, and maintain the corn plant genetic diversity. If the plants are self-

pollinated for more than the required time, the plants lose some of their genetic diversity. Since only the female rows are harvested, the male rows of the ICB are eventually plowed under after pollination of the females is successfully accomplished.

The crossing blocks are also an important area within the nursery. The hybrids are set up in short rows and paired with another hybrid. Each hybrid in the pair lacks a trait that the other one has. In the early stages of development, the young ear shoots are bagged before silks appear to prevent pollination by another plant not in the pairs. The pollen from each row is crossed over to the silked-out shoots on the other row. No self-pollination takes place in the crossing blocks. The end result is a new hybrid, hopefully containing a trait that the other cross contained.

The show plots are perhaps the most important areas in the nursery due to advertisements. The new crosses, after much research and development, are lined up next to the road and shown-off to the public. Each individual segment within the plot contains only one hybrid. The segments are labeled with the correct hybrid number, which is an easy way for Monsanto to advertise and publicize its new lines.

The nursery is hand-harvested to ensure that the seed from each new hybrid is correctly labeled and processed at the production plant. The remaining plants that do not get pollinated are machine-harvested and sold to grain elevators, with the exception of a new line containing the Bt protoxin against the Monarch butterfly larvae. This corn is also hand-harvested and the seeds are dumped into a large hole dug into the ground. Grain elevators refuse to accept this hybrid because Europe is not entirely convinced the product is safe and the elevators lose money on the product. Also, governmental

regulations make key decisions in the production of the new hybrid, as well as Monarch butterfly enthusiasts.

The amount of time spent in the field, the energy of the hardworking people, and the total cost of the entire project all play a major part in the production of new, valuable hybrids in agriculture plants. Even as each hybrid is tested and cycled through the nursery, governmental policies and regulations play an important role in the outcome of the entire project.

MARKETING AND REGULATION

After many years of experimentation, self-breeding, and careful planning, the final products must continue through a series of regulations before being marketed to consumers. Many regulatory organizations oversee the engineering, production, and testing of the various hybrids. The United States Department of Agriculture (USDA) consists of a conglomerate of regulatory and marketing agencies each playing important roles in the production of new transgenic plants and animals. Animal and Plant Health Inspection Service (APHIS) regulates the transportation, importation, and field testing of seeds and plants involved in the production of new strains (8). The Agricultural Research Service (ARS) researches genetically altered organisms to ensure each is safe for consumers and animals, whereas the Economic Research Service (ERS) researches the economic impact of the new various hybrid plants (10). The Foreign Agricultural Service (FAS) monitors foreign regulation on genetically engineered organisms (10). Some countries do not agree with genetic engineering or are not convinced that the products of genetic engineering are safe for consumption by humans or animals. This agency

monitors the concerns of the various countries. Funding for the research, risk assessment for genetically altered organisms, gene mapping, and sequencing comes from the Cooperative State Research, Education, and Extension Service (CSREES) (10). Finally, the Agricultural Marketing Service (AMS) administers new organic labels on products of genetic engineering for consumer benefit (8).

Other organizations such as the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) play key roles in the marketing and production of genetically modified organisms. The EPA regulates herbicide and pesticide use and determines the environmental impact of such uses. The FDA regulates products of genetic manipulation for human consumption, as well as animal consumption and food additives for transgenic animals.

Once the final product passes through the regulatory system, it may be marketed to consumers. Farmers produce mass amounts of seed that is eventually sold to companies for further processing or is sold to grain elevators as animal feed or future field plants.

Archer Daniel Midland Company (ADM), as well as other companies, uses corn seed for the production of ethanol, which is then added to automobiles as a low cost and low air pollutant fuel additive. In addition, beer companies, such as Anheuser-Busch, use the corn for ethanol production in the form of human consumption. The corn is fermented by yeast or bacteria to produce the final product, ethanol. Other companies use corn for the production of biodegradable plastics.

Whatever the uses of corn production, genetic engineering certainly plays the most important role in keeping costs low, consumers happy, and continual economic

growth within the United States. Without genetic engineering, many crops would be lost due to infestation by fungus and insects, and also from chemical herbicides.

Agriculturists would need to spend more money on herbicides and plant more acres for yield, which all costs money and consumes valuable space. The idea is to create more, using less. The insertion of genetic material from bacteria enables costs to remain low by giving plants their own power to fight against insects and fungal infestation, and remain resistant against chemical herbicides. Biotechnology involves many principles and can be used to add nutritional values to many foods, combat animal diseases, fight hunger by increasing crop yields, produce antibiotics, and help cut down on the use of chemical herbicides and pesticides. From the tiny Ti plasmid contained in *Agrobacterium tumefaciens*, in the laboratory, through the various resistance tests and fields, the final marketable product emerges as a safe and effective means for supplying America and many other countries with food and other corn-derived products.

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