



Agricultural Biotechnology

(A Lot More than Just GM Crops)





All living organisms have the ability to improve themselves through natural means in order to adapt to changing environmental conditions. However, it takes hundreds of years before any detectable improvement is obtained. Man then learned how to domesticate and breed plants in order to develop crops to his own liking and needs using various means including biotechnology.

Biotechnology is defined as a set of tools that uses living organisms (or parts of organisms) to make or modify a product, improve plants, trees or animals, or develop microorganisms for specific uses. Agricultural biotechnology is the term used in crop and livestock improvement through biotechnology tools. This monograph will focus only on agricultural crop biotechnology. Biotechnology encompasses a number of tools and elements of conventional breeding techniques, bioinformatics, microbiology, molecular genetics, biochemistry, plant physiology, and molecular biology.

The biotechnology tools that are important for agricultural biotechnology include:

- Conventional plant breeding
- Tissue culture and micropropagation
- Molecular breeding or marker assisted selection
- Genetic engineering and GM crops
- Molecular Diagnostic Tools



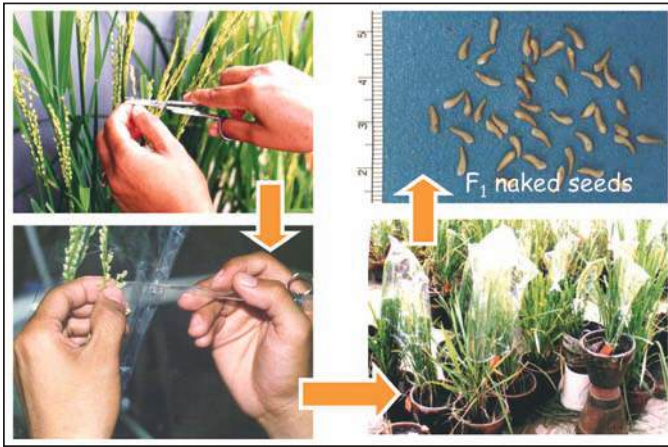
Conventional Plant Breeding

Since the beginning of agriculture eight to ten thousand years ago, farmers have been altering the genetic makeup of the crops they grow. Early farmers selected the best looking plants and seeds and saved them to plant for the next year. The selection for features such as faster growth, higher yields, pest and disease resistance, larger seeds, or sweeter fruits has dramatically changed domesticated plant species compared to their wild relatives. Plant breeding came into being when man learned that crop plants could be artificially mated or cross-pollinated to be able to improve the characters of the plant. Desirable characteristics from different parent plants could be combined in the offspring. When the science of plant breeding was further developed in the 20th century, plant breeders understood better how to select superior plants and breed them to create new and improved varieties of different crops. This has dramatically increased the productivity and quality of the plants we grow for food, feed and fiber.

Conventional plant breeding (Figure 1) has been the method used to develop new varieties of crops for hundreds of years. However, conventional plant breeding can no longer sustain the global demand with the increasing population, decline in agricultural resources such as land and water, and the apparent plateauing of the yield curve of the staple crops. Thus, new crop improvement technologies should be developed and utilized.



Figure 1. Conventional breeding entails sexual hybridization followed by careful selection



Source: Alfonso, A. 2007

Mutation breeding

The art of recognizing desirable traits and incorporating them into future generations is very important in plant breeding. Breeders inspect their fields and travel long distances in search of individual plants that exhibit desirable traits. A few of these traits occasionally arise spontaneously through a process called *mutation*, but the natural rate of mutation is very slow and unreliable to produce plants that breeders would like to see.

In the late 1920s, researchers discovered that they could greatly increase the number of these variations or mutations by exposing plants to X-rays and mutation-inducing chemicals. "Mutation breeding" accelerated after World War II, when the techniques of the nuclear age became widely available. Plants were exposed to gamma rays, protons, neutrons, alpha particles, and beta particles to see if these would induce useful mutations. Chemicals such as sodium azide and ethyl methanesulphonate, were also used to cause mutations. Mutation breeding efforts continue around the world today. In the 73 years of mutation breeding (1939-2013), a total of 3,218 varieties obtained through mutation breeding have been registered in the IAEA database. Staple crops such as rice has registered 824 varieties, barley (312), wheat (274), maize (96), common bean (57), tomato (20), potato (16), sugarcane (13), soybean (2), as well as other important crops that were improved to possess agronomically-desirable characteristics.

Pure line and hybrid seed technology

The end result of plant breeding is either an open-pollinated (OP for corn) or inbred (for rice) varieties or an F1 (first filial generation) hybrid variety. OP and inbred varieties, when maintained and properly selected and produced, retain the same characteristics when multiplied.

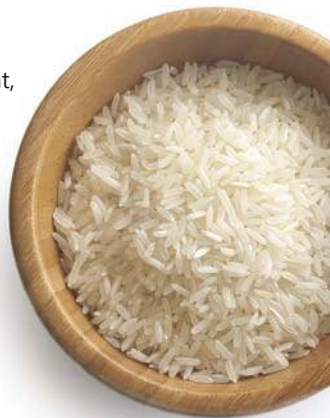
Hybrid seeds are an improvement over OP and inbred seeds in terms of yield, resistance to pests and diseases, and time to maturity.

Hybrid seeds are developed by the hybridization or crossing of diversely-related parent lines. Pure lines are offsprings of several cycles of repeated self-pollination that “breed true” or produce sexual offspring that closely resemble their parents.

Pure line development involves firstly, the selection of lines in the existing germplasm which express the desired characteristics such as resistance to pest and diseases, early maturity, yield, and others. These traits may not be present in only one line, thus selected lines are bred together by hand. In self-pollinated plants, flowers are emasculated by removing the anthers or the male part of the flower by hand, and are pollinated by pollen from another line. The female parent is usually the line that possesses the desired agronomic trait while the male parent is the donor of the new trait. F1 (first filial generation) offsprings are planted and selfed, as well as the F2 generation. Breeders then select in the F3 and F4 generation the lines which exhibit their desired agronomic characteristics and the added trait. Testing for resistances to pests and abiotic stresses are conducted also at this time. Lines with desired traits and are rated intermediate to resistant/tolerant to the pests and abiotic stresses are selected and selfed in two to three more generations. Lines which do not lose the new traits and are stable are termed pure lines.

In hybrid seed technology, two pure lines with complementing traits and are derived from diversely related parents are bred together by hand. F1 hybrids are tested for hybrid vigor in all agronomic and yield parameters and compared to both parents. The resulting offsprings will usually perform more vigorously than either parents.

Since the technology has been developed, it has brought tremendous impact in major crops including rice, corn, wheat, cotton, and other crops including many vegetables. In the USA, corn yield from 1866 to 1936 was only 26 bu/acre. Adoption of hybrid corn has increased corn yield by 0.8 bu/ac/yr from 1947-1955. With improved genetics, availability of N fertilizer, chemical pesticide and mechanization, corn grain yield has constantly increased by 1.9 bushels/acre/year to become 115 bushels in 1990's to an expected increase of 159 bu/acre in 2012. However, with the Great Drought in the US in 2012, grain yield was only 123.4 bu/acre. In 2013, an increase of 50 bu/acre of corn yield was obtained.



Hybrid rice technology helped China to increase production from 140 million tons in 1978 to 188 million tons in 1990. Since then, hybrid rice has helped increase rice production which yields 1.35 to 2 tons/hectare more than the ordinary rice, and hence an average yield of 7.2 to 7.5 tons/hectare. Hybrid rice production area is expected to increase by more than 6 million hectares in 2012. In September 2012, Yuan Long-pin, the father of hybrid rice has completed the

development of super rice DH2525 that sets a new record of hybrid rice yield at 926.6 kg/mu.

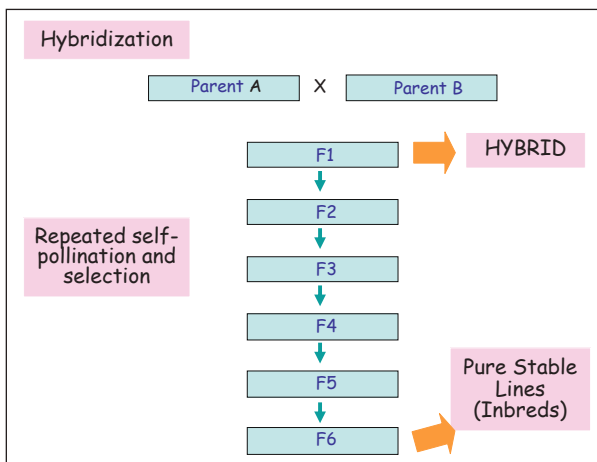
During the 6th Hybrid Rice Symposium in India in September 2012, Indian government and scientists realized the country's need to increase hectareage of hybrid rice from 2 to 5 million hectares, to be able to increase rice yield by 1.5 to 2 million tonnes of rice every year, and feed the teeming millions in the next 15 to 20 years. India has 59 hybrid rice varieties released form the public (31 varieties) and private (28 varieties) institutions.

With the proven impact of hybrid seed technology, new tools for hybrid breeding were discovered and utilized for self-pollinating crops including cytoplasmic male sterility (cms). Cytoplasmic male sterility is a condition where the plant is unable to produce functional pollen and would rely on other pollen source to produce seeds. This greatly facilitates large scale hybrid seed production, by-passing hand pollination.

Current hybrid seed technology uses three lines in order to produce the hybrid seed: a) the A line which contains a defective mitochondrial genome in the cytoplasm and a suppressed restorer gene, b) the B line which is genetically similar to the A line but contains a normal cytoplasm and a suppressed restorer gene, and c) the restorer line, a distinctly unrelated line which contains normal cytoplasm and an active restorer gene (dominant).

The two line hybrid system, another hybrid seed technology relies on temperature and geographic location affecting the nuclear genome of the plant, manifested as male sterile. Hybrid seed technology assures hybrid vigor in the progenies but discovery and development of cms lines requires a lot of work and time.

Figure 2. Pure line (inbred line) development



Source: Alfonso, A. 2007

Conventional plant breeding resulting in open pollinated varieties or hybrid varieties has had a tremendous impact on agricultural productivity over the last decades. While an extremely important tool, conventional plant breeding also has its limitations. First, breeding can only be done between two plants that can sexually mate with each other.

This limits the new traits that can be added to those that already exist in that species. Second, when plants are crossed, many traits are transferred along with the trait of interest including traits with undesirable effects on yield potential. Agricultural biotechnology is an option for breeders to overcome these problems.



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Tissue Culture and Micropropagation

Plants usually reproduce through sexual means – they have flowers and seeds to create the next generation. Egg cells in the flowers are fertilized by pollen from the stamens (male part) of the flower of the same plant (self-pollination) or another plant (cross). Each of these sexual cells contains genetic material in the form of DNA. During sexual reproduction, DNA from both parents is combined creating offsprings similar to the parents (in self-pollinated crops), or in new and unpredictable ways, creating unique organisms (in cross-pollinated crops). Some plants and trees on the other hand need several years before they flower and set seeds, making plant improvement difficult. Plant scientists have developed the science and art of tissue culture to assist breeders in this task.

Tissue culture is the cultivation of plant cells, tissues, or organs on specially formulated nutrient media. Under the right conditions, an entire plant can be regenerated from a single cell. Plant tissue culture is a technique that has been around for more than 30 years. There are several types of tissue culture depending on the part of the plant (explant) used.

Anther culture (Figure 3) is a tissue culture method used to develop improved varieties in a short time. Pollen within an anther contains half dose of the genome (haploid) which spontaneously double (diploid) during culture. In some species however, colchicine treatment is necessary to induce doubling. Doubling of the genome will allow the expression of recessive traits which were suppressed, masked or undetected in routine plant breeding.



Figure 3. Anther Culture of Rice



Source: Desamero, NV. 2007

Anthers are placed in a special medium, and immature pollen within the anther divide and produce a mass of dividing cells termed as callus. Healthy calli (plural of callus) are picked and placed in another medium to produce shoots and roots (regeneration). Stable plantlets are allowed to grow and mature in the greenhouse. Plant breeders can then select the desired plants from among the regenerated plants.

Anther culture of F1 plants which are progenies in a specific breeding objective would allow many more different types of regenerants. This is because the genetic constitution of the pollen will be more varied than those from the inbreds, thus breeders will have a wider range of traits to choose from. This technology has been employed in the successful development of doubled haploid lines of rice, wheat, sorghum, barley, and other field crops.

Rice varieties developed through anther culture (AC) were released by the National Seed Industry Council of the Philippines since 1995. The first AC-derived, salt tolerant variety PSBRc50 (Bicol) was developed by IRRI and released in 1995.* The Philippine Rice Research Institute developed eight salt tolerant varieties and two rainfed varieties.**

Micropopagation is a tissue culture method developed for the production of disease-free, high quality planting material and for rapid production of many uniform plants. Actively-dividing young cells (meristem) are placed in a special medium and treated with plant hormones to produce many similar sister plantlets. Since the meristem divides faster than disease-causing virus, clean

materials are propagated and hundreds of uniform plantlets are produced in a short time.

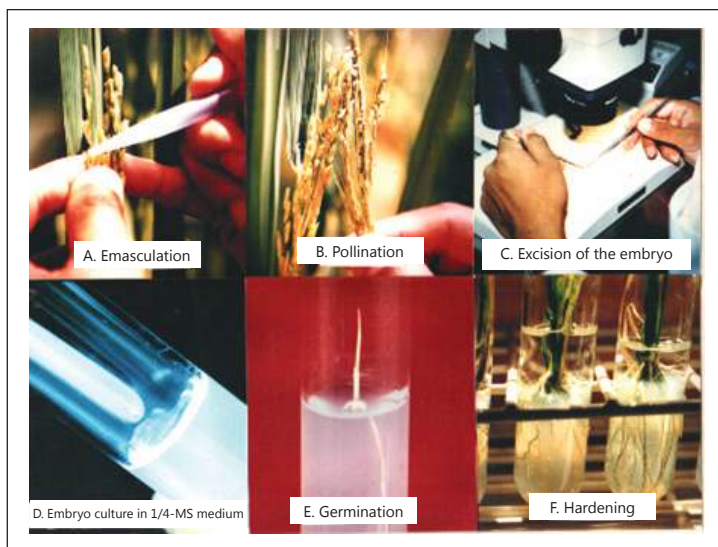


Through micropropagation, it is now possible to provide clean and uniform planting materials in plantations – oil palm, plantain, pine, banana, abaca, date, rubber tree; field crops – eggplant, jojoba, pineapple, tomato; root crops – cassava, yam, sweet potato; and many ornamental plants such as orchids and anthuriums. Micropropagated plants were found to establish more quickly, grow more vigorously and taller, have a shorter and more uniform production cycle, and produce higher yields than conventional propagules.

Embryo rescue involves the culture of immature embryos of plants in a special medium to prevent abortion of the young embryo and to support its germination (Figure 4). This is used routinely in breeding parental lines having different or incompatible genome such as in introducing important traits of wild relatives into cultivated crops.

The development of a new rice plant type for West Africa (NERICA – New Rice for Africa) was a result of wide crosses between the Asian *Oryza sativa* and the African rice *Oryza glaberrima*. It employs embryo rescue in the initial breeding and in the successive back crossing work followed by anther culture to stabilize the breeding lines. The new plants had combined yield traits of the *sativa* parent with local adaptation traits from *glaberrima*.

Figure 4. Embryo Rescue



Source: Alfonso, A. 2007

Wild rices are a rich source of traits for resistance to pests and abiotic stresses. At the International Rice Research Institute, embryo rescue is utilized and facilitated the transfer of bacterial blight resistance genes from wild rice *Oryza longistaminata* to variety IR24 resulting to a bacterial blight resistant line (IRBB21). *Oryza rufipogon* is a source of tungro resistance to a number of rice varieties. For a review of other wild rices, see <http://www.fao.org/docrep/015/i2554e/i2554e00.pdf>.

At IRRI, a new super salt tolerant rice was developed by saving the embryo produced in the cross between highly salt tolerant wild rice *Oryza coarctata* with cultivated rice variety IR56. The research team led by Dr. Kshirod Jena has been attempting to cross the two rices since mid 1990s and has only been successful fairly recently. Selected salt tolerant lines will be tested further by farmers in salt affected locations for a possible release within 4 to 5 years.***

Plant tissue culture belongs to the lower end of the agricultural biotechnology ladder. But the plant's ability to regenerate a new plant is an important requisite in the development of improved crops through agricultural biotechnology.

Plant tissue culture is a straightforward technique and many developing countries have already mastered it. Its application only requires a sterile workplace, nursery, green house, and trained manpower. Unfortunately, tissue culture is labor intensive, time consuming, and can be costly.

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***IRRI April 15, 2013 Wild Parent Spawns super salt tolerant rice. <http://irri.org/news/media-releases/wild-parent-spawns-super-salt-tolerant-rice>

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Molecular Breeding and Marker-Assisted Selection

The process of developing new crop varieties requires many steps and can take 10 to 25 years depending on the crop. Now, however, applications of agricultural biotechnology have considerably shortened the time it takes to bring them to market. It currently takes 7-10 years for new crop varieties to be developed. One of the tools, which make it easier and faster for scientists to select plant traits is called *marker-assisted selection* (MAS).

The different traits and physical features of plants are encoded in the plant's genetic material, the deoxyribonucleic acid (DNA). The DNA occurs in pairs of *chromosomes* (strands of genetic material), one coming from each parent. The genes, which control the plant's characteristics, are specific segments of each chromosome. All of the plant's genes together make up its genome.

Some traits, like flower color, may be controlled by only one gene. Other more complex characteristics, however, like crop yield or starch content, maybe influenced by many genes. Traditionally, plant breeders have selected plants based on their visible or measurable traits, called the *phenotype*. But, this process can be difficult, slow, influenced by the environment, and costly – not only in the development itself, but also for the economy, as farmers suffer crop losses.



As a shortcut, plant breeders now use *molecular marker-assisted selection*. To help identify specific genes, scientists use what are called molecular markers which are short strings or sequence of nucleic acid which makes up a segment of DNA. The markers are located near the DNA sequence of

the desired gene. Since the markers and the genes are close together on the same chromosome, they tend to stay together as each generation of plants is produced. This is called genetic linkage. This linkage helps scientists to predict whether a plant will have the desired gene. If researchers can find the marker for the gene, it means the gene itself is present.

As scientists learn where each of the markers occurs on a chromosome, and how close it is to a specific gene, they can create a map of the markers and genes on specific chromosomes. This genetic linkage map shows the location of markers and genes, and their distance from other known genes. Scientists can produce detailed maps in only one generation of plant breeding.

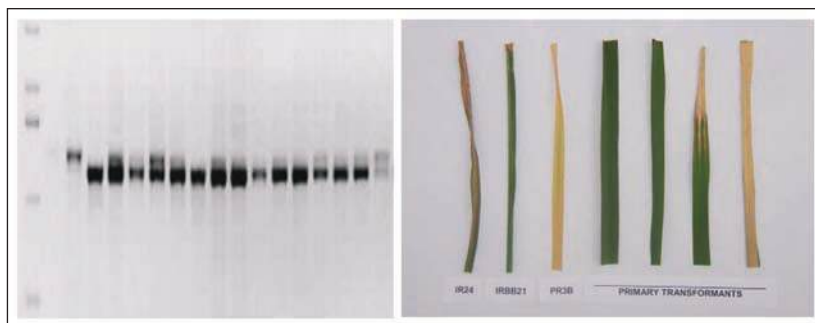
Previously, scientists produced very simple genetic maps using conventional techniques. It was observed long ago that as generations of plants were crossed, some traits consistently appeared together in the new generations (genetic linkage). However, since researchers could concentrate on only a few traits in each attempt at cross-breeding, it took many crosses to obtain even a very simple genetic map. Using very detailed genetic maps and better knowledge of the molecular structure of a plant's DNA, researchers can analyze a tiny bit of tissue from a newly germinated seedling. They don't have to wait for the seedling to grow into a mature plant to test for the presence of the specific trait. Once the tissue is analyzed through molecular techniques, scientists know whether that seedling contains the appropriate gene. If it doesn't, they can quickly move on and concentrate analysis on another seedling, eventually working only with the plants which contain the specific trait.

Currently, molecular marker-assisted breeding, an agricultural biotechnology tool is already a routine step in breeding of most crops where the gene and the markers for a specific trait are known. This technique is being used in the efficient introgression of important genes into various crops including bacterial blight resistance in rice, increased beta carotene content in rice, cassava, and banana, and submergence tolerance in rice, to name a few (Figure 5).



Molecular markers are also used to determine the genetic profile of a line or variety. Random primers are used to scan the genomic constitution of the plant through molecular methods. The information is fed to a computer program that will analyze the relatedness of one line to another. The information on genetic diversity of the lines is utilized in selecting for extremely unrelated parents useful for hybrid seed technology. The information will also provide details on the parentage of the line, the possible traits, and the unique identity of the plant useful for germplasm collection database.

Figure 5. Molecular marker-assisted breeding



Source: Alfonso, A. 2007

Increasing selection efficiency by selecting for markers associated/linked with the trait of interest

It should be noted, however, that molecular breeding through marker assisted selection is somewhat limited in scope compared to genetic engineering or modification because: 1) it only works for traits already present in a crop; 2) it cannot be used effectively to breed crops which have long generation time (e.g. citrus); and 3) it cannot be used effectively with crops which are clonally propagated because they are sterile or their offsprings does not resemble the parents. This includes many staples such as yams, bananas, plantain, sweet potato, and cassava.

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Genetic Engineering and GM Crops

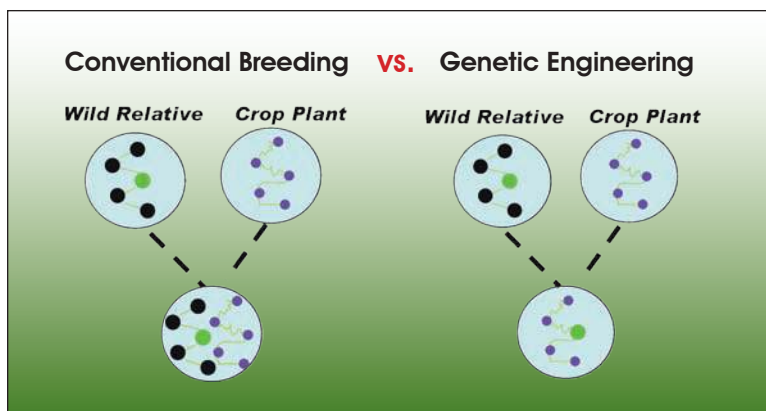
Over the last 30 years, the field of agricultural biotechnology has developed rapidly due to the greater understanding of DNA as the chemical double-helix code from which genes are made. Genetic engineering is one of the modern agricultural biotechnology tools that is based on recombinant DNA technology. The term *genetic engineering*, often interchanged with terms such as *gene technology*, *genetic modification*, or *gene manipulation*, is used to describe the process by which the genetic makeup of an organism can be altered using "*recombinant DNA technology*." This involves the use of laboratory tools and specific enzymes to cut out, insert, and alter pieces of DNA that contain one or more genes of interest. The ability to manipulate individual genes and to transfer genes between species that would not readily interbreed is what distinguishes genetic engineering from traditional plant breeding.

With conventional plant breeding, there is little or no guarantee of obtaining any particular gene combination from the millions of crosses generated. Undesirable genes can be transferred along with desirable genes or while one desirable gene is gained, another is lost because the genes of both parents are mixed together and re-assorted more or less randomly in the offspring. These problems limit the improvements that plant breeders can achieve, eating time and funds along the way (Figure 6).

In contrast, genetic engineering allows the direct transfer of one or just a few genes, between either closely or distantly related organisms. Not all genetic engineering techniques involve inserting DNA from other organisms. Plants may also be modified by removing or switching off particular genes and genetic controls (promoters).



Figure 6. Conventional vs. genetic engineering



Source: Biotech Mentor's Kit, 2003

Application of genetic engineering in crop production

Genetic engineering techniques are only used when all other techniques have been exhausted and when: 1) the trait to be introduced is not present in the germplasm of the crop; 2) the trait is very difficult to improve by conventional breeding methods; and 3) it will take a very long time to introduce and/or improve such trait in the crop by conventional breeding methods (see Figure 7).

Modern plant breeding is a multi-disciplinary and coordinated process where a large number of tools and elements of conventional breeding techniques, bioinformatics, biochemistry, molecular genetics, molecular biology and genetic engineering are utilized and integrated.

Development of transgenic crops

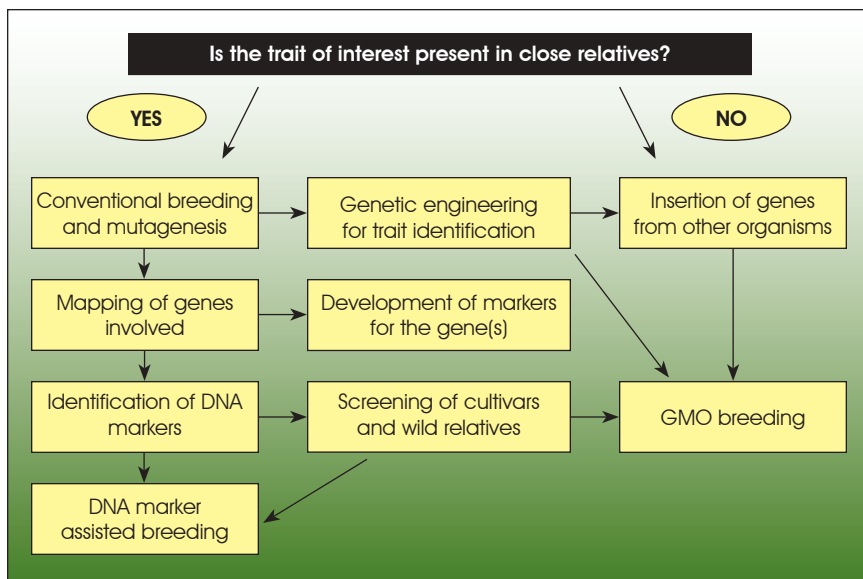
Although there are many diverse and complex techniques involved in genetic engineering, its basic principles are reasonably simple. It is however, very important to know the biochemical and physiological mechanisms of action, regulation of gene expression and safety of gene and gene product to be utilized.

The process of genetic engineering requires the successful completion of a series of six steps.

Step 1. Nucleic acid (DNA/RNA) Extraction

Nucleic acid extraction, either DNA or ribonucleic acid (RNA) is the first step in the genetic engineering process. It is therefore important that reliable methods are available for isolating these components from the cell. In any isolation procedure, the initial step is the disruption of the cell of the desired organism, which may be viral, bacterial or plant cells, in order to extract the nucleic acid. After a series of chemical and biochemical steps, the extracted nucleic acid can be precipitated to form thread-like pellets of DNA/RNA.

Figure 7. Integration of conventional and modern biotechnology methods in crop breeding



Source: DANIDA, 2002

Step 2. Gene cloning

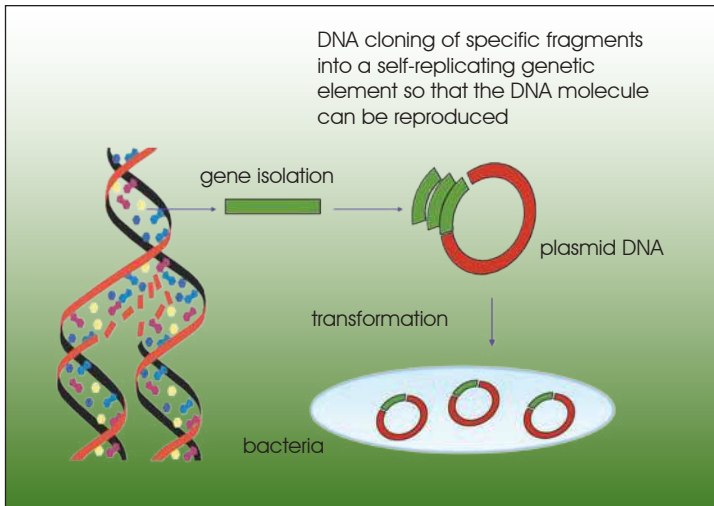
The second step is gene cloning. There are basically four stages in any cloning experiment: generation of DNA fragments, joining to a vector, propagation in a host cell, and selection of the required sequence. In DNA extraction, all DNA from the desired organism is extracted. This genomic DNA is treated with specific enzymes called restriction enzymes cutting it into smaller fragments with defined ends to allow it to be cloned into bacterial vectors. Copies of the vector will then harbor many different inserts of the genome. These vectors are transformed into bacterial cells and thousands of copies are produced (Figure 8).

Using information relating to specific molecular marker sequences and the desired phenotype, the vector harboring the desired sequence is detected, selected, isolated and clones are produced. Restriction enzymes are again utilized to determine if the desired gene insert was cloned completely and correctly.

Step 3. Gene Design and Packaging

Once the gene of interest has been cloned, it has to be linked to pieces of DNA that will control its expression inside the plant cell (Figure 9). These pieces of DNA will switch on (*promoter*) and off (*terminator*) the expression of the gene inserted. Gene designing/packageing can be done by replacing an existing promoter with a new one, incorporating a selectable marker gene and reporter gene, adding gene enhancer fragments, introns, and organelle-localizing sequences, among others.

Figure 8. Gene cloning



Source: Tabien, R. 2000

Promoters

Promoters allow differential expression of genes. For instance some promoters cause the inserted genes to be expressed all the time, in all parts of the plant (constitutive) whereas others allow expression only at certain stages of plant growth, in certain plant tissues, or in response to external environmental signals. The amount of the gene product to be expressed is also controlled by the promoter. Some promoters are weak, whereas others are strong. Controlling the gene expression is an advantage in developing GM plants.

Selectable Marker Genes

Selectable marker genes are usually linked to the gene of interest to facilitate its detection once inside the plant tissues. This enables the selection of cells that have been successfully incorporated with the gene of interest, thus saving considerable expense and effort.

Genetic engineers used antibiotic resistance and herbicide resistance marker genes to detect cells that contain the inserted gene.

Cells that survive the addition of marker agents

to the growth medium indicate the presence of the inserted gene. Although increase in antibiotic resistance in humans and animals is unlikely to occur using antibiotic resistance marker, genes coding for resistance to non-medically important antibiotics are preferred. In addition, alternative types of marker genes have been developed which are related to plant metabolism such as phosphomannose isomerase, xylose isomerase and others.

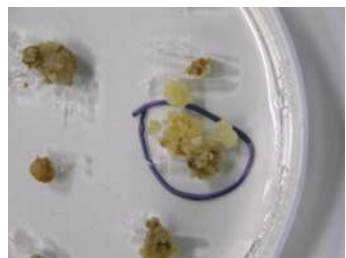
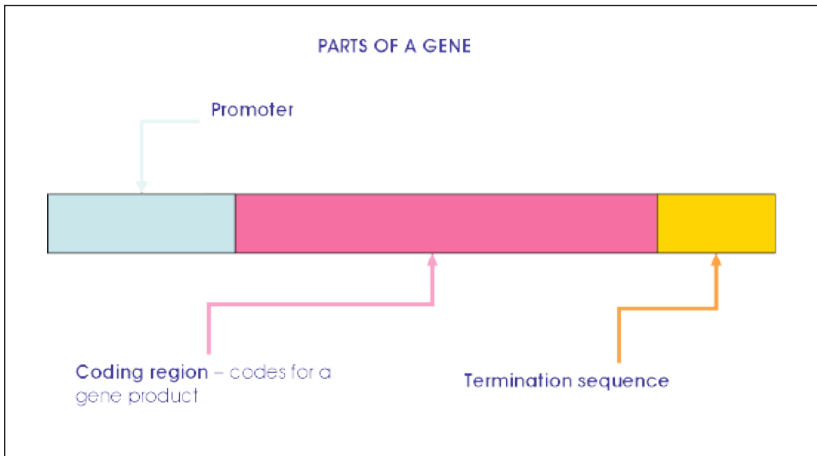


Figure 9. Parts of a gene



Source: Alfonso, A. 2007

Reporter Genes

Reporter genes are cloned into the vector in close proximity to the gene of interest, to facilitate the identification of transformed cells as well as to determine the correct expression of the inserted gene. Reporter genes that have been used include: the beta glucuronidase gene (*gusA* gene) which acts on a particular substrate producing a blue product, hence making the transformed cells blue; the green fluorescent protein (*gfp*) which allows transformed cells to glow under a green light; and luciferase gene that allows cells to glow in the dark, among others.

Enhancers

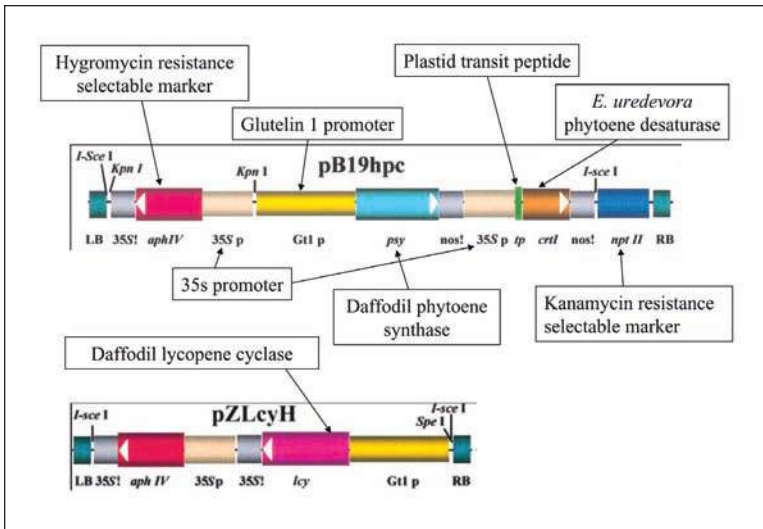
Several genetic sequences can also be cloned in front of the promoter sequences (enhancers) or within the genetic sequence itself (introns, or non-coding sequences) to promote gene expression. An example is the cloning of the cauliflower mosaic virus promoter enhancers in front of the plant promoter.

Once the gene of interest is packaged together with the promoter, reporter and the marker gene (Figure 10), it is then introduced into a bacterium to allow for the creation of many copies of the gene package. The DNA isolated from the bacterial clones can then be used for plant cell transformation using particle bombardment. If however the use of bacteria *Agrobacterium tumefaciens* is preferred in the plant transformation, the whole gene package should be cloned in between two border sequences (left and right border) of a binary vector plasmid. This will allow processing of the *Agrobacterium* so that only the transfer DNA (T-DNA) will be incorporated into the plant genome.

Step 4. Transformation

The most common methods used to introduce the gene package into the

Figure 10. Components of a gene construct used in developing Golden Rice



Source: Ye et al, 2000

plant cells in a process called *transformation* or *gene insertion*, include biolistic transformation using the gene gun and *Agrobacterium*-mediated transformation (Figure 11).

Particle Bombardment

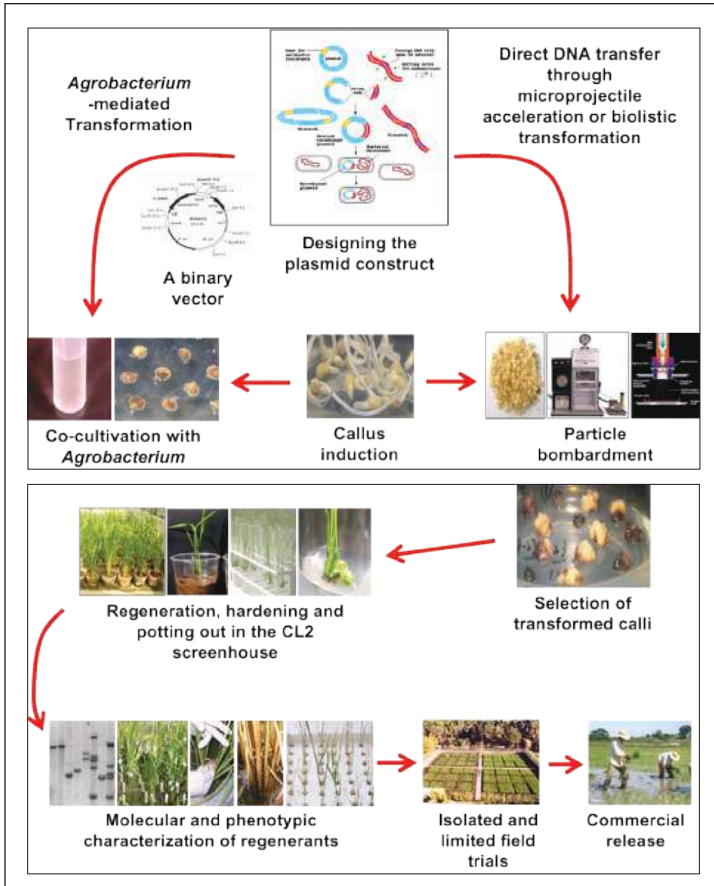
Particle bombardment is a mechanical method of introducing the desired gene. The desired genetic sequence is cloned into a plant DNA vector and introduced into the plant using the gene gun or particle gun. As in the common gun, the gene gun uses minute particles of tungsten or gold as the bullet. These particles are coated with the DNA solution and fired to the plant cells through the force of the Helium gas inside a vacuum-filled chamber. The DNA and the tungsten/ gold particles get inside the cell, and within 12 hours, the inserted DNA gets inside the nucleus and integrated with the plant DNA. The tungsten/ gold particles are sequestered to the vacuole and eliminated later.

Transformed cells are cultured *in vitro* and induced to form small plants (regeneration) that express the inserted gene.

Agrobacterium tumefaciens-mediated transformation

The "sharing" of DNA among living forms is well documented as a natural phenomenon. For thousands of years, genes have moved from one organism to another. For example, *Agrobacterium tumefaciens*, a soil bacterium known as 'nature's own genetic engineer', has the natural ability to genetically engineer plants. It causes crown gall disease in a wide range of broad-leaved plants, such

Figure 11. Genetic transformation methods (Biolistics or Gene Gun and *Agrobacterium tumefaciens*-mediated transformation methods)



Source: Alfonso, A. 2007

as apple, pear, peach, cherry, almond, raspberry and roses. The disease gains its name from the large tumor-like swellings (galls) that typically occur at the crown of the plant, just above soil level. Basically, the bacterium transfers part of its DNA to the plant, and this DNA integrates into the plant's genome, causing the production of tumors and associated changes in plant metabolism.

Molecular biologists have utilized this biological mechanism to improve crops. The genes that cause the galls are removed and replaced with genes coding for desirable traits. Plant cells infected with the bacterium will not form galls but produce cells containing the desired gene, which when cultured in a special medium will regenerate into plants and manifest the desired trait.



The main goal in any transformation procedure is to introduce the gene of interest into the nucleus of the cell without affecting the cell's ability to survive. If the introduced gene is functional, and the gene product is synthesized, then the plant is said to be *transformed*. Once the inserted gene is stable, inherited and expressed in subsequent generations, then the plant is considered a transgenic.

Step 5. Detection of Inserted Genes

Molecular detection methods have been developed to determine the integrity of the transgene (introduced gene) into the plant cell.

Polymerase chain reaction or PCR is a quick test to determine if the regenerated transgenic cells or plants contain the gene. It uses a set of primers (DNA fragments) – forward and backward primers, whose nucleotide sequences are based on the sequence of the inserted gene. The primers and single nucleotides are incubated with the single stranded genomic DNA and several cycles of DNA



amplification is conducted in a PCR machine. Analysis of the PCR products in agarose gel will show if the plants are really transformed when DNA fragments equivalent in size with the inserted gene is present and amplified.

Southern blot analysis determines the integrity of the inserted gene: whether the gene is complete and not fragmented, at the correct orientation, and with one copy number. The DNA coding sequence is the probe binding to the single stranded genomic DNA of the transgenic plant which is implanted on a nitrocellulose paper. Autoradiography will reveal the transgenic status of the plant. One copy of the transgene is desired for optimum expression.

Northern blot analysis determines whether the transcript or the messenger RNA (mRNA) of the introduced DNA is present and is correctly transcribed in the transgenic plant. The messenger RNA of the transgenic plants are isolated and processed to bind to the nitrocellulose membrane. Labeled DNA is used to bind to the mRNA and can be visualized through autoradiography.

Western blot analysis or protein immuno blotting is an analytical technique used to detect whether the transgenic plants produce the specific protein product of the introduced gene. Protein samples are extracted from the transgenic plants, processed into denatured proteins and transferred to a nitrocellulose membrane.

The protein is then probed or detected using the antibodies specific to the target protein.

Step 6. Backcross Breeding (if needed)

Genetic transformation is usually conducted in elite or commercial varieties which already possess the desired agronomic traits but lacks the important trait of the transgene. Thus, once successfully conducted, the genetically modified plant will be easily recommended for commercialization if it shows stability in several generations and upon successfully passing and fulfilling varietal registration requirements.

However, some plant transformations may have been performed in plant varieties which are amenable to genetic transformation but are not adapted nor important in the target country. There may also be sterility problems in the transgenic plant. In such cases, conventional plant breeding is performed where the transgenic plant becomes the pollen source in the breeding program and the elite lines or commercial varieties as the recurrent parent. Backcross breeding enables the combination of the desired traits of the recurrent parent and the transgenic line in the offsprings.

The length of time in developing transgenic plant depends upon the gene, crop species, available resources and regulatory approval. It varies from 6 to 15 years before a new transgenic plant or hybrid is ready for commercial release.

Commercially available crops improved through genetic engineering

There has been a consistent increase in the global area planted to transgenic or GM crops or biotech crops from 1996 up to the present. ISAAA's Annual Global Status Report downloadable at the ISAAA website: <http://www.isaaa.org> presents an up to date record of the number of countries planting GM crops, the hectareage planted, the benefits derived from the biotech crops, farmer accounts of planting biotech crops as well as future prospects and directions of the technology. So far, 27 transgenic crops which are planted commercially: alfalfa, Argentine canola, bean, carnation, chicory, cotton, creeping bentgrass, eggplant, flax, maize, melon, papaya, petunia, plum, Polish canola, poplar, potato, rice, rose, soybean, squash, sugar beet, sugarcane, sweet pepper, tobacco, tomato, and wheat.

With genetic engineering, more than one trait can be incorporated into a plant and are called stacked traits. These are currently corn, cotton, and soybean crops with both herbicide and insect tolerance traits. Transgenic crops with combined traits are also available commercially such as the herbicide tolerant and insect resistant maize and cotton. Stacking different genes for one trait makes the crop more durable to resist the pest/disease and tolerate more herbicides.



Another strategy to improve sustainability of the technology is the use of the refuge. Technology developers have studied effective refuge systems for specific transformation event. These are discussed to farmers extensively for proper implementation, and are monitored regularly to observe any resistant insects or weeds.

New and future initiatives in crop genetic engineering

To date, commercial GM crops have delivered benefits in crop production, but there are also a number of products in the pipeline which will make more direct contributions to food quality, clean environment, pharmaceutical production, and livestock feeds. Examples of these products include: rice with higher levels of iron and beta carotene (an important micronutrient which is converted to vitamin A in the body); long life banana that ripens faster on the tree and can therefore be harvested earlier; maize with improved feed value; delayed ripening papaya; papaya ringspot virus resistant papaya; tomatoes with high levels of flavonols, which are powerful antioxidants; drought tolerant maize and wheat; maize with improved phosphorus availability; arsenic-tolerant plants; insect resistant eggplant and rice; edible vaccines from fruit and vegetables; low lignin trees for paper making among others.

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Answers to Frequently Asked Questions

FOOD SAFETY

1. *Is the safety of genetically-engineered food assessed?*



Before GE foods and products made from GE crops are approved for use, they have undergone safety testing by the companies or institutions that developed them. Data were reviewed by government regulatory agencies and scientific reviewers based on internationally-accepted protocols. Frequently,

GE foods are also tested by outside groups and the results published in peer-reviewed journals. The process is comparable to safety assessments for pharmaceutical drugs and biomarkers; pharmaceutical companies provide safety data that are subsequently reviewed by the U.S. Food and Drug Administration (FDA) scientists. To date all GE products in the marketplace have undergone full reviews by regulatory agencies regarding safety and content relative to unmodified forms. Submitting the safety data is in the developer's best interests given the legal liabilities incurred should a problem with the food arise following market introductions.

2. *What happens to DNA when it is eaten?*

DNA is chemically identical regardless of its source and is mostly degraded during industrial processing and in the digestive tract. Small fragments can be detected in certain body tissues, such as leukocytes, liver, and spleen. The daily human intake of DNA in food is estimated at 0.1-1g. Estimates of the total daily transgene DNA intake can be calculated assuming 50% of the diet is from GE foods and transgenes represent an estimated 0.0005% of total DNA in food, as 0.5-5ug/day.

In July 2007, the European Food Safety Authority released statements on the fate of genes and proteins in food and feed: "After ingestion, a rapid degradation into short DNA or peptide fragments is observed in the gastrointestinal tract of animals and humans" and "To date, a large number of experimental studies with livestock have shown that recombinant DNA fragments or proteins derived from GM plants have not been detected in tissues, fluids or edible products of farm animals"

3. *Are there changes in the nutritional content of genetically-engineered food?*

GE foods are tested in comparison with conventional counterparts in terms of the nutritional composition: levels of protein, carbohydrate, fat, vitamin, mineral, fiber, moisture, and



phytochemicals, and analyzed if the composition is substantially equivalent. GE crops and conventional crops should have been grown in comparable conditions to eliminate the effect of the environment in the nutritional composition.

There are also GE crops which are developed to change the nutritional profiles of the foods such as those with increased B-carotene, flavonoids, calcium, folate, and iron availability. According to US-FDA policy, GE foods with altered nutritional traits must be labeled to indicate nutritional differences; one example is Vistive™, a low-linoleic oil from GE soybeans that can be used instead of trans fat-containing oils. Such crops should be tested for substantial equivalence to compounds unrelated to the introduced trait.

4. Does the Bt protein affect humans?

Bt proteins are naturally occurring insecticides produced by the soil bacterium, *Bacillus thuringiensis*, used to control crop pests such as larvae of butterflies and moths, beetles, and mosquitoes since the 1920s. The crystalline, inactive insecticidal Bt proteins, form bodies inside the bacterium and become active when they are eaten by the target insect larva and cleaved. The active peptides bind to specialized receptors in the midgut of the insect, creating holes in the gut membrane that cause contents to leak and kill the larvae. The precision of different Bt proteins for their targets resides in the specificity of their tight binding to companion receptors in the insect gut. In recent years, a variety of safety studies were conducted specifically on native Bt proteins to show that they do not have characteristics of food allergens or toxins. Data on Cry1Ab in maize and cotton and Cry1Ac in tomato, maize and cotton have been carefully reviewed by regulatory agencies in numerous countries, including the U.S., Canada, Japan, UK, EU, Russia, and South Africa.



Mycotoxin are toxic and carcinogenic chemicals produced by fungi that gain entry into the holes produced by the larva in corn. The reduction of mycotoxin incidence in Bt corn results in a positive impact in the improvement of corn yield, human and animal health.

5. Do genetically engineered foods cause food allergies?

No food is 100% safe, be it conventional, GE, or organic. Allergies are present in the “big eight” which is composed of milk, eggs, fish, shellfish, tree nuts, soybeans, wheat, and peanuts. Since food safety testing conducted on GE foods focuses on the introduced gene and its protein product, it seems unlikely that allergenicity issues related to a commercialized GE food that has undergone strict government health regulatory scrutiny will be greater than that of conventional foods, created by classical breeding and mutation that have not undergone such scrutiny.

6. Can the viral genetic sequences inserted in the genetically engineered crops create a human risk?

One of the important concerns is the use of the virus-derived promoter which are introduced sequences in the transgenic plants that regulates how much, where, and when the encoded protein is expressed. This includes the cauliflower mosaic virus 35S which was used in some commercial GE crops, eg. Bt 11, Bt 176, Mon 810 maize, and Roundup Ready soybean. Speculations that the "35S promoter could affect the stomach and colonic lining and cause a growth factor effect with the unproven possibility of hastening cancer formation in those organs" were forwarded earlier without any scientific experimentation. These speculations have been extensively rebutted by the scientific community because the 35S promoter can be found everywhere in nature. For instance, an estimated 14-25% of oilseed rape in the field is infected with CaMV; similar numbers have been estimated for cauliflower and cabbage. Because of its prevalence in foods, humans have consumed CaMV and its promoters at high levels for decades with no observable effects. The presence of the CaMV promoter in GE plants does not in principle present a different situation. Additionally, DNA in food is rapidly broken down during digestion, giving it little time to interact with the stomach and colonic linings.



7. Can the antibiotic resistance genes in genetically engineered foods increase antibiotic resistance in humans and animal intestinal flora?



To develop antibiotic resistance in microorganisms present in the human and animal digestive tract, there should be a functional transfer of the antibiotic resistance gene, its controlling elements, and its integration in the bacterial chromosome. This is next to impossible, since during chewing, cells in food are broken down. In raw

food, as the cells are destroyed, DNA is released and highly active enzymes in the saliva and in the plant start degrading the DNA. This process continues in the digestive tract where other enzymes further breaks down DNA and proteins. In humans, food remains in the stomach for approximately 2 hours, where the remaining DNA is fragmented into small pieces. The antibiotic resistance gene from GE maize was shown not to transfer to gut bacteria in chickens fed with GE maize.

To refrain from using the controversial antibiotic resistance or herbicide tolerance genes as selectable markers, new selection strategies for identifying engineered plants have been developed. These include genes such as phosphomannose and xylose isomerase that facilitate selection by

giving transgenic cells a metabolic advantage over non transgenic cells, as well as other means to excise the marker genes in the commercial product.

8. Can genetic engineering be used to make pharmaceuticals? Could genetically engineered crops contaminate the food supply?

Plant-derived pharmaceuticals and vaccines for common diseases such as hepatitis B, pneumonic and bubonic plague, as well as against allergy sufferers, asthma, seasonal allergies and atopic dermatitis have been developed since the early 1990s. Plant vaccines have the advantage of being readily consumed with limited or no processing without the need for cold storage. However, these GE crops may enter the food supply if not properly handled and monitored. In the USA, where such pharmaceutical crops are cultivated, government regulations are in place. APHIS



which regulates the movement and field testing of GE plants requires special steps to prevent plants that produce drugs or industrial enzymes from contaminating food crops: 1. labeling, packaging, and segregating regulated plant materials; 2. reproductive isolation to prevent GE pollen from fertilizing conventional plants; 3. postharvest monitoring to remove volunteer plants; and 4. proper disposal of the transgenic materials. This regulation was further strengthened in 2005 to include the following: 1. exclude field growth without a permit; 2. include crop inspections seven times a year, twice after harvest; 3. increase field isolation distances; and 4. use dedicated farm equipment.

The National Corn Growers Association proposed safeguards such as 1. Using plants that are male-sterile or that produce non GE pollen, 2. dedicated production systems that isolate pharma crops, 3. third party verification and 4. grower training programs. In Sept. 2002, the FDA released a guidance document that recommends multiple strategies to prevent pharma crops from contaminating human or animal feed. This documents suggests that those who are growing drug-producing plants that cross pollinate, such as corn and canola, strengthen containment procedures by growing plants in geographical regions where little or none of those crops are grown for food.

9. Why labeling of genetically engineered foods is not required by the FDA?

Government policy on labeling has been developed differently in many countries. In the USA, the FDA's labeling policy for GE foods is the same as for conventional foods and it assures that consumers are given information about nutritional, health safety or food quality changes in the end product. FDA mandated labels are not used to provide information about the process by which the food is made. If a GE food is significantly different from its



conventional counterpart, the food must be labeled to indicate the difference. Instances where the nutritional profile changes are included, for example if the GE food is created using genetic information from a previously recognized allergenic source, such as peanut, soy, or wheat, or if the new proteins has characteristics of known allergens. For example, oils made from GE soybeans and canola varieties with changes in fatty acid composition must be labeled; foods containing those oils must be labeled and companies producing that oil must use

a new name. For example, Monsanto is using the name Vistive™, to market its low-linoleic acid product from GE soybean oils. If a food contains a new potentially allergy-causing introduced protein, the label must state that the product contains the allergen and name its source.

10. What are organic foods?

Organic farming is a method of agricultural production that does not allow the use of synthetic pesticides, fertilizers or growth enhancers. Food grown under organic certification differ from conventionally-produced food by the manner in which they are grown, handled, and processed, but an “organic” label does not guarantee the nature of the product, the food, or ingredient, only its production method. The important factors for many people who consume organic foods relate to the perceptions that they are healthier, taste better, are better for the environment, have lower pesticide levels and fewer food additives, and are better for animal welfare. However, organic certification does not imply that foods produced using organic methods are more nutritious or safer than those produced without organic methods.

Differences reported in nutrient composition between organically and conventionally produced foods are interesting but it is very difficult to control all variables that might affect nutritional quality and ensure that the observed variations are significant and reproducible. In addition, there are many important nutrients for which no significant differences have been found. Much more research is needed to determine whether the nutritional differences observed between organic and conventional food products are reproducible and have a significant impact on human health.

Strictly from a nutritional perspective, not enough data exist at present to show nutritional benefits from conventionally or organically produced foods that favor consuming either for health benefits. However, if the goal is to promote healthy eating, it is more important for consumers to focus on eating a healthy, balanced diet, rich in fruits and vegetables, than focusing on foods that are produced by particular methods. Convincing epidemiological evidence shows that diets rich in fresh fruits and vegetables, regardless of the methods used to produce them, improve health and are associated with reduced frequency and severity of a number of health conditions.

SUMMARY POINTS

1. Foods consumed today are derived from plants and animals whose genetic make up has been modified by sexual crosses and mutations. Recombinant DNA provides a new tool to make genetic modifications, and this technology is termed genetic engineering or biotechnology.
2. Technically, researchers are now able to transfer genes using recombinant DNA methods, not only within a species, but also from one kingdom to another, which can lead to significant changes in various attributes of agricultural crops.
3. The safety of genetically-engineered crops and foods, just as those created by classical breeding and mutation and grown conventionally or organically, needs to be evaluated on a case-by-case basis so that informed decisions can be made about their utility, safety and appropriateness.
4. Data and information from peer-reviewed science on the safety of these products should be a part of the information considered when growing and consuming foods from these crops.
5. Factors beyond the technical, science-based facts should also be considered during the decision-making process.
6. Although scientific testing and governmental regulation can reduce the safety risks of conventionally and organically produced and genetically engineered crops and food, 100% safety is not achievable.
7. To date, no scientifically valid demonstrations have shown that food safety issues of foods containing genetically engineered (GE) ingredients are greater than those from conventionally or organically produced foods.
8. In commercial fields, only a few crops have been modified using rDNA technologies (canola, corn, cotton, papaya, squash, and soy), but many others are being developed.

ENVIRONMENTAL ISSUES

1. Will insect resistance to Bt be developed with the widespread use of Bt crops?



Resistance of insects against synthetic insecticides and Bt toxins in sprays occur and this will be true for GE crops. To slow this development in GE crops, several strategies have been developed. First generation GE crops produced only one Bt toxin in each plant. Planting refuges of non-Bt crops near Bt crops in the field is the primary strategy

of delaying insect resistance. This is based on the idea that insects feeding on plants in the refuge are not selected for resistance. Insect resistance to Bt toxins is recessive. The heterozygous offsprings produced when

homozygous resistant insects mate with susceptible insects are killed by the Bt crops. This high-dose/refuge strategy creates plants that produce Bt toxin concentrations high enough to kill heterozygous insects, making resistance functionally recessive. Insect resistance to Bt toxins can thus be postponed substantially.

Another approach is called the pyramid or stacking strategy that combines two or more toxins in a single plant, each with different modes of action. An example is Bollgard II cotton producing Cry1Ac and Cry2b, which targets the same pest in two different ways.

Other approaches to delaying insect development are:

1. Mixing seeds of Bt and non-Bt varieties are under small scale experiments.
2. The use of inducible promoter to drive Bt gene expression only during insect attack.
3. Use of modified toxins to kill resistant insects, as exemplified by the use of modified Bt toxin that will not be affected by the mutations in the midgut cadherins. Cadherins promote toxin oligomerization of Cry1A protein which has alpha helix in the binding site. Modified Cry1A which does not contain the alpha helix are independent of the cadherins and can thus be effective with insects which has developed resistance due to mutated or silenced cadherins.

To date, the elapsed time before the first cases of field resistance of insects to Bt crops were reported has been longer than what was predicted under worst-case scenarios, suggesting that management strategies may have delayed resistance development. Despite documented cases of resistance, Bt crops remain useful against most target pests in most regions. As insect resistance to Cry toxins currently deployed in Bt crops increases, other strategies to create GE crops resistant to insects are being developed.

2. *Can genetically engineered crops cause adverse effects on non target organisms? Have there been adverse effects on non-target organisms caused by GE crops?*

Effects on GE crops on non target organisms have been studied with focus on:

- a. Monarch butterflies and black swallow tails. USA Environmental Protection Agency have concluded based on two studies that Bt corn was not a significant factor in field deaths of monarch larvae, particularly relative to factors such as the widespread use of pesticides and destruction of the butterfly's winter habits.
- b. Non target soil microorganisms. Studies on four maize varieties with two different Bt proteins (Cry1Ab



and Cry3Bb1) versus near isogenic non-Bt varieties reveal that although numbers and types of microbes and enzyme activities differ from season to season among varieties, no statistically significant differences were seen in number of different microbes, enzyme activities, or pH. Similar results were found comparing Bt and non-Bt cotton, and no Cry2Ab protein was detected in the rhizosphere in the field grown with Bt cotton.

- c. Non-target arthropods. Studies on foliage-dwelling arthropods on Bt maize expressing Cry3Bb1 compared with those of conventional insecticide treated maize show that there is no adverse impacts on abundance of any non target arthropods. Insecticide treated arthropods however reduced the number of non target insects: ladybird beetles, lacewings, and damsel bugs.
- d. Microbes and non target water insects. Water sediments and surface water after labeling genomic DNA of GE Bt corn revealed that sediments had more DNA than surface water. In addition, the Cry1Ab protein was not detectable in both samples.

3. Could the use of genetically engineered crops result in the population decline of other organisms?



Population decline of other organisms has been an ongoing phenomenon since man learned how to domesticate crops. The introduction of modern agricultural technologies including new varieties; competition between local and introduced varieties led to a displacement of local varieties; and displacing local varieties eroded genetic variability of regional crop populations. Extensive plant breeding in the early 1960s to feed the tremendous increase in the population produced high-yielding varieties of major food crops, resulting in yield increases but also significant displacement of traditional varieties and a concomitant loss in genetic diversity, particularly landraces of cereals and

legumes. Recognition of this fact led to establishment of genebanks across the globe with focus on specific crops.

One issue on diversity is the gene flow from GE crops to wild and weedy relatives which could render selective advantage of recipients in certain environments. Gene flow can also happen naturally in conventionally bred and commercialized crops. This is addressed by the adoption of measures needed in cultivating GE crops near centers of origin depending on the nature of the trait and the frequency of its introduction into an ecosystem. Currently, studies on impact assessment of transgenes moving into wild relatives and the potential to change ecosystem dynamics are requested in environmental impact statements before any GE plant is released. It provides insights into the possible outcomes on the environment. Certain impact assessments of some GE crops are also monitored even after deregulation.

4. Can herbicide-tolerant (HT) crops lead to superweeds?

Development of herbicide-tolerant weeds has occurred with both traditionally-bred and GE crops. This phenomenon reduces the effectiveness of certain weed control strategies and decreases weed management options. Strategies have been developed to minimize the development of herbicide tolerant weeds, such as:



- a. Use of HT cultivars with resistance genes for herbicides with alternative modes of action that can be used in rotation.
- b. Use of restriction technologies to prevent gene passage to the next generation through the pollen, i.e. transgenes can be targeted to the cytoplasmic organelles, not in the pollen.
- c. Rotate the use of HT crops with different modes of action or with non HT crops.

A few points to consider in using HT crops are: Weeds can also escape herbicide treatment on the basis of application rate, weed age and size, spray volume adjuvants used, water quality and interactions with other herbicides that affect efficacy. Late germination of weeds can also escape herbicide application, thus a second pass of sprays can be done.

5. What is the effect of using GE crops in pesticide use?

Having crops tolerant to herbicides and pest attack increases pest management options and can also reduce the number and strength of pesticide applications. Growth of GE HT crops also allows topical application of herbicide to crops and weeds, which replaces spraying between crop rows and mechanical removal of weeds, both of which can damage crops and result in environmental damage. Reducing mechanical tillage lowers fuel consumption and helps conserve soils prone to erosion and compaction. HT crops can also lead to more flexible herbicide treatment regimes.

The National Center for Food and Agricultural Policy published surveys on U.S. pesticide usage on GE crops. In 2004, HT canola, cotton, maize and soybean as well as Bt cotton and maize showed reductions in herbicide active ingredient (AI) of 25 to 30%. In a 2006 publication, the USDA National Statistics Service found that from 1996 to 2002, AI use rates for HT cotton and corn, and Bt corn declined as adoption of Bt and HT cotton, corn, and soybeans increased and concurrent shifts occurred towards less environmentally persistent herbicides such as pendimethalin, trifluralin, and metolachlor.

The Environmental Impact Quotient (IEQ) assessment which takes into account the pesticide AI and the environmental impact (EI) of GE crops resulted in significant reductions in the global EI of production agriculture; such that since 1996, the overall EI associated with pesticide use on HT soybean, corn, cotton, canola, and Bt cotton decreased by 15.3%.



Cultivation of GE HT crops has also had other positive effects on the environment, i.e. increases in low-or no-till practices and use in combination with integrated pest management schemes, which were made possible because early season pesticide sprays could be eliminated, allowing beneficial insects to establish. Most reports indicate pesticide use and cost decrease following adoption of Bt varieties. In Argentina, numbers of herbicide applications increased with HT soybean but use shifted to more environmentally friendly herbicides. Reduction in pesticide use can also be achieved by using the best methods and tools available, including integrated pest management, biocontrol, organic production methods, and GE organisms to reduce EI while achieving adequate production levels.

6. *Would Bt crops need additional insecticide applications?*

Bt or Cry toxins are toxic to susceptible larvae when cleaved to generate their active form, which then binds to specific receptors in the midgut and creates holes that cause lepidopteran larvae to die. The first BT GE crops introduced into corn and cotton were targeted to control European corn borer, corn rootworm and cotton armyworm. Some pests belong to groups insensitive to Bt have to be sprayed to prevent crop damage. With the commercial introduction of corn and cotton varieties with two stacked Bt genes, i.e. Cry1Ac and Cry2Ab in cotton, bollworms and secondary armyworm pests were controlled.

New developments to target different insect pests are: corn with six insect resistant genes against lepidopteran (Cry1F, Cry1A.105, Cry2Ab2) and rootworm (Cry34Ab1 + Cry 35Ab1, modified by Cry3Bb1) pests; the use of a hybrid Cry protein with two binding domains to target lepidopteran and coleopteran pests of potato; use of plant defense proteins such as alpha amylase inhibitors from legumes; use of insecticidal compounds from nematodes, bacterial cholesterol oxidase, avidin, volatile communication compounds, and RNAi approaches targeted to specific insect proteins. Even with GE approaches, other methods of insect control will be needed, e.g., chemical pesticides, biocontrol, integrated pest management, or organic approaches, because insects are plentiful and ever changing.

7. Would the introduction of virus-resistant genetically engineered plants lead to novel viruses?



Development of GE crops with resistance to viral diseases has been conducted in squash and papaya using a viral coat protein gene. The USDA APHIS has already deregulated the GE squash allowing commercial production after the virus was shown not to infect wild squash varieties; the resistance gene gave no advantage to wild squash varieties, and the presence of the coat protein gene did not increase viral competitiveness. For GE papaya with the viral coat protein, concerns on viral recombination became a concern since from analyses of viruses, homologous and non homologous recombination could occur between viruses and between viral genomes and plant genes. Experimental results indicate

however that most recombinant viruses are not fully virulent because the new gene combinations are not fully compatible, leaving new hybrids at a competitive disadvantage. To compete effectively, recombinant viruses must have functional recombinatorial ability, capacity to establish systemic infection, and ability to compete with their progenitors during replication. These requirements place powerful negative selection pressure on newly evolved viruses. Reduced viral replication capacity could also negatively affect recombination frequency in transgenic plants.

Large-scale field releases of plants engineered with viral genes are necessary to obtain realistic assessments of the types and recombination frequencies that might occur. Currently, no novel viruses have been reported resulting from GE plants in the field, but likely they would be detected only if their appearance had adverse effects. At present, the only commercially propagated plants engineered with viral coat protein genes, GE squash, and papaya are grown on small acreages.

To minimize the possibility for gene exchange among the viruses, strategies such as RNAi-mediated viral resistance is employed. There is no protein introduced, and the RNAi construct is used to silence a gene from bean golden mosaic virus in *Phaseolus vulgaris* leading to virus-resistant plants.



8. Can genes from genetically engineered plants move to bacteria in the field?

Horizontal gene transfer is the process of transferring genes among non-sexually related organisms such as from plants to bacteria. Sequence analyses of genes and proteins show that some genes have transferred from plants to bacteria over a very long evolutionary time frame. This transfer can

only be simulated in the laboratory using optimized conditions – situations which are difficult to replicate in natural settings. If, however it were to happen in the field, it would be at very low frequencies and the gene would need to provide a selective advantage to survive.



An experiment to determine the persistence of kanamycin resistant bacteria in the soil by increasing the levels of kanamycin in the soil concludes that natural soil conditions rarely would have the selective pressure necessary to keep *nptII* in the bacterium. Data from this and other studies indicate that homologous recombination and integration of plant genes into

competent soil bacteria could occur, but at very low frequencies, and the environmental significance would depend on selective pressure for the trait.

9. *What happens when pollen moves from genetically engineered crops to wild relatives or non-genetically engineered varieties? In areas of genetic diversity?*

Gene flow or the movement of pollen from one plant to another is made possible when the parental plants (a) flowers at the same time; (b) close enough to allow a vector (insect, wind, or animal) to transfer pollen to receptive females; and (c) produce pollen that can result in embryos developing into viable seeds and germinating. Successful pollination also depends on



the longevity of pollen viability, pollen travel distance and the mode of pollination the plant has, whether self or cross-pollinated.

Gene flow may present significant economic or environmental risks for either conventionally bred or GE crops on a case-by-case evaluation. Crop-to-wild relative gene flow could result if the plants grow in overlapping regions resulting in new combinations of genes that can improve, harm, or have no effect on the fitness of recipient plants. Genes can also flow from wild relatives to cultivated crops, introducing new traits into the next generation seeds, but only affect the crop if it is replanted.

Planting of GE varieties in areas of genetic diversity of plants needs additional precautions to reduce possible impacts of introgression of GE traits and the potential significant environmental consequences. To minimize this occurrence, planting of GE crops near wild species should be avoided or

other technologies could be used to prevent gene(s) from moving to wild varieties.

Gene flow could also occur when compatible plants are present within the vicinity. GE varieties like conventional plants can also persist in the environment. Organic farmers should be aware of these occurrences to be able to adopt the necessary precautions of spatial and temporal isolation.

10. Can organic, conventional and genetically engineered cropping systems coexist?

Farmers are used to planting different varieties and planting strategies in order to develop farm products that meet the requirements of the consumers. They are used to planting white and yellow maize, hot and sweet peppers, high and zero erucic acid rapeseed, and still achieve purity standards dictated by certified seed specification.



Coexistence strategies must be devised to allow neighbor farmers to farm in an economically viable manner. This can involve alerting each other to their plans and modifying them to accommodate each others' needs. When GE crops are grown next to organic farming operations, certain practices that minimize synthetic pesticide drift can also limit GE gene flow, such as spatial separation of fields, staggered planting dates, and planting varieties with different maturity dates and those that are not sexually compatible. Other crops-specific methods have been devised to aid coexistence strategies. Gene flow is not only the means for GE to commingle with conventional or organic crops; crops must also be segregated during harvest, shipping and processing. Methods limiting such commingling have in some cases been implemented.

With the use of various production methods comes the mixing of permissible inputs and methods, whether with their own farms with products from neighboring farms, or during harvest and processing. The commingling or adventitious presence (AP) is the unintended occurrence of materials other than specific crops and can include weed seeds, seeds from other crops, dirt, insects, and other foreign materials such as stones or plastics. Different countries have set rules on the degree of AP. In the U.S., for seed crops, rules for AP are specified by the Association of Official Seed Certifying Agencies (AOSCA), where a level of 0.5% seed of other varieties and 2% AP of inert materials is permitted in "pure seed" of hybrid corn.

11. Can use of genetically engineered crops or organic farming lead to more sustainable agricultural production systems?

Sustainable agricultural systems should meet the basic needs of the

population while preserving the resources for future generations. The United Nation's Millennium Development Goals to "Ensure environmental sustainability by integrating principles of sustainable development into a country's policies, and programs to reverse the loss of environmental resources." This need has been widely accepted and the manner to fulfill this may vary.

Conventional farming has led to impressive gains of between 70 and 90% of increases in food production in the last few decades. Unfortunately, these were accompanying environmental impacts as well as sizeable consumption of fossil fuels, unsustainable rates of water use and topsoil loss, and contributions to environmental degradation, air pollution, soil erosion, reduced biodiversity, pest resistance, pollution of lakes and streams, and overuse of surface and ground water.

Achieving agricultural sustainability can be addressed through numerous agricultural practices such as: integrated pest management (IPM), biological control, organic methods, and use of GE plants, coupled with selected conventional agricultural methods, can play important roles in future sustainable agricultural



practices. Biological control can be a part of an IPM strategy and neither biological control nor IPM specifically excludes the use of GE organisms. Organic production relies on practices, such as cultural and biological pest management, that can include IPM and biological control but excludes the use of synthetic chemicals and GE organisms. The use of GE organisms can also contribute to sustainable practices by augmenting and replacing certain conventional practices. For example, plants can be created that increase water use, and fertilizer efficiencies, that remediate soil contaminants, increase no-till or low-till practices to help reduce greenhouse gases and produce higher yields without increasing land usage, particularly in developing countries. To achieve true sustainability agriculture must use the best of all practices.

SUMMARY POINTS

1. The environmental safety of products of agricultural biotechnology, just as with those created by classical breeding and mutation and grown conventionally or organically, must be evaluated on a case-by-case basis to perform meaningful risk assessments.
2. Information from the peer-reviewed literature on the safety of these products should be considered when growing and consuming foods from these crops.

Factors beyond the technical, science-based facts should also be part of the decision-making process.

3. Although scientific testing and governmental regulation can reduce the safety risks of conventionally and organically produced and GE crops and food, 100% safety is not achievable.
4. Robust efforts should be made to conserve and enlarge global genebanks and collections created to preserve precious landraces and wild relatives, which are the foundation for future classical breeding, marker-assisted selection, and genetic engineering approaches.
5. On the basis of the bulk of data from field tests and farm surveys, pesticide use for GE crop adopters is lower than for conventional variety users. More importantly, extensive data confirm that the environmental impact is substantially lower.
6. Generalizations about whether gene flow causes significant environmental or economic risks for conventional, organic or GE crops require case-by-case evaluation.
7. Adequate methods for the coexistence of differing varieties and production methods in agriculture are available and being encouraged worldwide; however, minimum standards, not zero tolerance, for GE presence need to be established for this approach to be attainable.
8. Farmers worldwide have adopted GE crops because of the realized economic benefits (which have been demonstrated in numerous studies), time savings, and ease of agricultural practices. Reluctance to adopt mainly relates to apprehensions about rejection in the export market.

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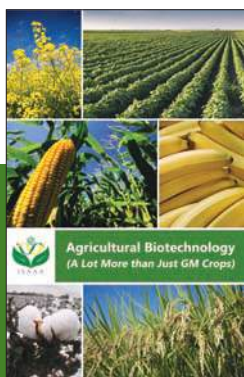
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