



Biotechnology in Agriculture

A lot more than just GM crops



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Global Knowledge Center on Crop Biotechnology



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Biotechnology in Agriculture

A lot more than just GM crops

In many countries, the debate surrounding the use of biotechnology in agriculture is often solely associated with genetically modified (GM) crops. As a result, many believe that biotechnology is only about developing these products. What many do not realize is that there are many other important applications of biotechnology that have made (and will continue to make) a tremendous impact on agricultural productivity. Biotechnology encompasses a number of tools and elements of conventional breeding techniques, bioinformatics, microbiology, molecular genetics, biochemistry, plant physiology, and molecular biology.

The present applications of biotechnology that are important for agriculture and the environment include:

- Conventional plant breeding
- Tissue culture and micropropagation
- Molecular breeding or marker assisted selection
- Genetic engineering and GM crops
- The 'Omics' - Genomics, Proteomics, Metabolomics
- Plant disease diagnostics
- Microbial fermentation

**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.

The Global Knowledge Center on Crop Biotechnology is a science-based information network responding dynamically to the needs of developing countries on all aspects of crop biotechnology. Its activities include maintenance of an internet website, expert networking, continuous scanning of the agri-biotech environment, and multi-media communication.

Learn how technology has been used to improve the food we grow or eat. Follow the biotech timeline. (Source: <http://www.whybiotech.com>)

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. Then once the science of genetics became better understood, plant breeders used what they knew about the genes of a plant to select for specific desirable traits to develop improved varieties.



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. For example, when corn was first grown in North and South America thousands of years ago, the corn cobs farmers harvested were smaller than one's little finger. Today, there are hundreds of varieties of corn, some of which produce cobs as long as one's forearm.



Conventional plant breeding has been going on for hundreds of years and is still commonly used today. Early farmers discovered that some crop plants could be artificially mated or cross-pollinated to increase yields. Desirable characteristics from different parent plants could also 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.

The art of recognizing desirable traits and incorporating them into future generations is very important in plant breeding. Breeders scrutinize their

Conclusion

For thousands of years, human beings have been engaged in improving the crops they grow. And over the past 150 years, scientists have assisted their efforts by developing and refining the techniques of selection, breeding and crop protection through the application of biotechnology. As mentioned above, biotechnology in agriculture is not only about genetic modification but rather encompasses a number of tools and elements of conventional breeding techniques, bioinformatics, microbiology, molecular genetics, biochemistry, plant physiology, and molecular biology.

With the severe agricultural problems and challenges that developing countries face, scientists need all the tools available to ensure there is enough to eat for succeeding generations. Biotechnology is not a panacea for hunger and malnutrition but simply another set of tools to assist in developing better plant varieties and seeds and protecting them from devastating pests, diseases, and adverse environments.

2003

Farmers in 18 countries plant GM crops on 67.7 million hectares.

Inoculant Encyclopedia.

(http://www.inoculants.com/encyclopedia/encyclopedia_5.htm)

Integrated Pest Management Resource Centre. Biopesticides.

(<http://www.ipmrc.com/expert/biopesticides/index.shtml>)

Integrated Plant Protection Center. Database of Microbial Biopesticides.

(<http://www.ipppc.orst.edu/biocontrol/biopesticides/>)

International Biopesticide Consortium for Development. Biopesticides.

(<http://www.biopesticide.org/biopesticides.htm>)

UPLB Compendium of Mature and Developed Technologies.

(<http://www.uplb.edu.ph>)

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 all plants that breeders would like to see.

Mutation breeding

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 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, too, such as sodium azide and ethyl methanesulphonate, were used to cause mutations. Mutation breeding efforts continue around the world today. Of the 2,252 officially released mutation breeding varieties, 1,019 or almost half have been released during the last 15 years. Examples of plants that were produced via mutation breeding include wheat, barley, rice, potatoes, soybeans, and onions.



Hybrid seed technology

The end result of plant breeding is either an open-pollinated (OP) variety or an F1 (first filial generation) hybrid variety. OP varieties, when maintained and produced properly, retain the same characteristics when multiplied. The only technique used with OP varieties is selection of the seed-bearing plants.

Hybrid seeds are an improvement over open pollinated seeds in terms of qualities such as yield, resistance to pests and diseases, and time to maturity.

2002 The National Center for Food and Agricultural Policy (NCFAP) study found that

six GM crops planted in the United States - soybeans, corn, cotton, papaya, squash and canola- produced an additional 4 billion pounds of food and fiber on the same acreage, improved farm income by \$1.5 billion and reduced pesticide use by 46 million pounds.

10,000 - 9,000 BC

People start planting crops rather than relying on hunting and gathering for food.



Hybrid seeds are developed by the hybridization or crossing of parent lines that are 'pure lines' produced through inbreeding. Pure lines are plants that "breed true" or produce sexual offspring that closely resemble their parents. By crossing pure lines, a uniform population of F1 hybrid seed can be produced with predictable characteristics.

'The simplest way to explain how to develop an F1 hybrid is to take an example. Let us say a plant breeder observes a particularly good habit in a plant, but with poor flower color, and in another plant of the same type he sees good color but poor habit. The best plant of each type is then taken and self-pollinated (in isolation) each year and, each year, the seed is re-sown. Eventually, every time the seed is sown the same identical plants will appear. When they do, this is known as a 'pure line.'

If the breeder now takes the pure line of each of the two plants he originally selected and cross pollinates the two by hand the result is known as an F1 hybrid. Plants are grown from the seed produced and the result of this cross pollination should have the combined traits of the two parents.

This is the simplest form of hybridization, but there are complications, of course. A completely pure line can sometimes take seven or eight years to achieve. Sometimes, a pure line is made up of several previous crossings to build in desirable features. The resulting plant is then grown on until it is genetically pure before use in hybridization.

In addition to qualities like good vigor, trueness to type, heavy yields and high uniformity which hybrid plants enjoy, other characteristics such as earliness, disease and insect resistance and good water holding ability have been incorporated into most F1 hybrids.



Examples of bioinsecticides and their mode of action.

Control agent	Mode of Action	Examples	Control against
Bacteria	Produce toxins that are detrimental to certain insect pests when ingested.	<i>Bacillus thuringiensis</i> <i>Bacillus popilliae</i> <i>Agrobacterium radiobacter</i>	Lepidopterans Japanese beetles Crown gall disease
Viruses	Kills insects when ingested. Insect's feeding behavior is disrupted thus it starves and dies.	Baculoviruses: Nuclear polyhedrosis virus (NPV) Baculoviruses: Granulosis virus (GV) Baculoviruses: Group C Entomopox	Lepidopteran and hymenopteran Lepidopteran Arthropods
Fungi	Controls insects by growing on them secreting enzymes that weaken the insect's outer coat, and then getting inside the insect and continuing to grow, eventually killing the infected pest.	<i>Entomophaga praecox</i> <i>Zoophthora radicans</i> <i>Neozygites floridana</i>	Grasshoppers Aphids Cassava Green Mite
Protozoa	Kills insects when ingested. Insect's feeding behavior is disrupted thus it starves and dies.	<i>Nosema</i> <i>Variimorpha Malanocha</i>	Grasshoppers Lepidoptera Locusts
Nematode	They kill their target organisms by entering natural body openings or by penetrating the insect cuticle directly.	<i>Heterorhabditis bacteriophora</i> <i>Phasmarhabditis hermaphrodita</i> <i>Stemernema carpocapsae</i>	Black vine weevil, Japanese beetles Various slugs and snails Black vine weevil, strawberry root weevil, cranberry girdler

6,000 BC

In Mesopotamia, Sumerians use yeast - a type of fungus - to make beer and wine.

2001

U.S. and Canadian scientists develop a transgenic tomato that thrives in salty conditions, a discovery with the potential to create tomatoes and other crops that can grow in marginal conditions.



bioinsecticides have become available in North America and Europe.

Inexpensive fermentation technology is used to mass produce fungi. Spores are harvested and packaged so they can be applied to insect-ridden fields. When the spores are applied, they use enzymes to break through the outer surface of the insects' bodies. Once inside, they begin to grow and eventually cause death.

Bioinsecticides based on Bb have many advantages. The fungus does not grow in warm-blooded organisms (such as people), nor does it survive long in water reservoirs or rivers. However, its spores can withstand long periods of dryness and other harsh environmental conditions. Studies to date have shown that the fungus also does not hurt plants and becomes inactivated by the sun's ultraviolet rays in one to eight weeks.

Fungal agents are viewed by some researchers as having the best potential for long-term insect control. This is because these bioinsecticides attack in a variety of ways at once, making it very difficult for insects to develop resistance.

Virus-Based Bioinsecticides

A group of virus-based insecticides that scientists are testing are the rod-shaped *baculoviruses*. Baculoviruses affect insect pests like corn borers, potato beetles, flea beetles and aphids. One particular strain is being used as a control agent for bertha army worms, which attack canola, flax, and vegetable crops. During the worst years of the infestation in the early 1980's in Canada, they cleaned out over one million hectares of prairie crops. In the past, farmers used chemical insecticides to control these pests. But Bertha army worms attack crops while in the larval (caterpillar) stage. Traditional insecticides do not affect the worm until after it has reached this stage and by then much of the damage has been done.

Unfortunately, these advantages come with a price. Because creating F1 hybrids involves many years of preparation to create pure lines and these pure lines have to be constantly maintained so that the F seed can be harvested each year, seed is more expensive. The problem is compounded because to ensure that no self pollination takes place, all the hybridization of the two pure lines sometimes has to be done by hand.

Another disadvantage is if the seeds of the F1 hybrids are used for growing the next crops, the resulting plants do not perform as well as the F1 material resulting in inferior yields and vigor. As a consequence, the farmer has to purchase new F1 seeds from the plant breeder each year. The farmer is, however, compensated by higher yields and better quality of the crop.



Though more expensive, hybrid seeds have had a tremendous impact on agricultural productivity. Today, nearly all corn and 50% of all rice are hybrids (DANIDA, 2002).

In the USA, the widespread use of corn hybrids, coupled with improved cultural practices by farmers, has more than tripled corn grain yields over the past 50 years from an average of 35 bushels per acre in the 1930s to 115 bushels per acre in the 1990s. No other major crop anywhere in the world even comes close to equating that sort of success story.

Hybrid rice technology helped China to increase its rice production from 140 million tons in 1978 to 188 million tons in 1990. Research at the International Rice Research Institute (IRRI) and in other countries indicates that hybrid rice technology offers opportunities for increasing rice varietal yields by 15-20% beyond those achievable with improved, semidwarf, inbred varieties.



2000

The first entire plant genome is sequenced, *Arabidopsis thaliana*, which provides researchers with greater insight into the genes that control specific traits in many other agricultural plants.

5,000 BC Farming communities in existence.

4,000 BC Egyptians use yeast to make bread rise.

Many cultivars of popular vegetable or ornamental plants are F1 hybrids. In terms of improved plant characteristics, tropical vegetable breeders can point to some rather clear achievements over the last two decades:



Yield improvement. Hybrids often outyield traditional OP selections by 50-100% thanks to improved vigor, improved genetic disease resistance, improved fruit setting under stress, and higher female/male flower ratios

Extended growing season. Hybrids often mature up to 15 days earlier than local OP varieties. For many crops, the hybrid's relative advantage over the OP is most pronounced under stress conditions

Quality improvement. Hybrids have helped stabilize product quality at a higher, more uniform level. This almost always means improved consumption quality (e.g. firm flesh of wax gourd, or crispy taste of watermelon).



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.

humans and animals compared to synthetic insecticides; they are very specific, often affecting only a single species of insect and have a very specific mode of action; slow in action and the timing of their application is relatively critical.

Some of these characteristics however, are seen by critics as a disadvantage. For example, because most of these bioinsecticide agents are living organisms, their success is affected by several factors like temperature, pH, moisture, UV, soil conditions, and other microbial competitors present in the environment. Slow in action means much longer time for it to eradicate pathogens compared to synthetic pesticides.

Bacteria-based bioinsecticides

One of the most widely used bioinsecticides is a naturally occurring soil bacterium called *Bacillus thuringiensis* or Bt. Bt produces a protein which is poisonous to insects. Often within 15 minutes of being eaten, the poisons begin to create ulcers in the insects' stomach lining. The insect stops eating and eventually dies.



Researchers have identified between 500 and 600 *strains*, or types of *Bacillus thuringiensis*. Bt is very selective — it affects only a specific species of insect pest and does not harm humans, birds, fish, or beneficial insects. In 1983, a strain of Bt was used in West Africa to wipe out disease-carrying black flies.

Fungi-based bioinsecticides

Fungi that cause disease in some 200 different insects are gaining prominence as bioinsecticides. One of the earliest to be discovered in the 1880s is *Beauveria bassiana* (Bb), a fungus found worldwide in soils and plants. Another half a dozen fungi are also known to have characteristics valuable for insect control. In China, over two million hectares are sprayed with Bb annually to control forestry pests. Since 1993, six new fungal

4,000 BC - 1,600 AD

Early farmers - like those in Egypt and the Americas - saved seeds from plants that produced the best crops and planted them the next year to grow even better crops.

1999

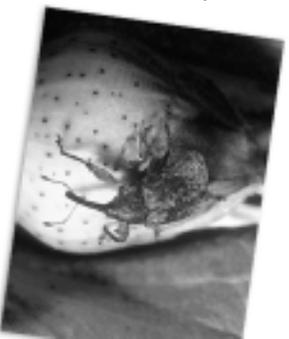
German and Swiss scientists develop golden rice, fortified with betacarotene, which stimulates production of Vitamin A that can prevent some forms of blindness.

to the environment compared to conventional herbicides and will not affect non-target organisms.

With the advances of genetic engineering, new generation bioherbicides are being developed that are more effective against weeds. Microorganisms are designed to effectively overcome the weed's defenses. Weeds have a waxy outer tissue coating the leaves that microorganisms have to penetrate in order to fully infect the weeds. Through biotechnology, these microorganisms will be able to produce the appropriate type and amount of enzymes to cut through the outer defenses. Streamlining of the microbe's plant host specificity will ensure that the weeds are taken out and not the crops. On the other hand, microbes can also be made to be effective against several host weeds and not only to one type of weed as this can be too expensive to produce for commercial use.

Bioinsecticides

The science of biotechnology can also help in developing alternative controls to synthetic insecticides to fight against insect pests. Research has found microorganisms in the soil that will attack fungi, viruses or bacteria which cause root diseases. Formulas for coatings on the seed (inoculants) which carry these beneficial organisms can be developed to protect the plant during the critical seedling stage.



While synthetic pesticides are an invaluable tool for agricultural productivity, some of them also have their drawbacks: they are expensive, they are often not foolproof; they can accumulate in our environment and pollute our water systems; and they are not species specific as they can also kill non-target organisms.

Bioinsecticides, on the other hand, do not persist long in the environment and have shorter shelf lives; they are effective in small quantities, safer to

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- International Atomic Energy Agency <http://www.infoctis.iaea.org/MVD/> and click first on "introduction" and then on "FAO/IAEA Mutant Variety Database."
- International Rice Research Institute. <http://www.iri.org>

1996

GM tomato paste approved in the UK, first GM herbicide tolerant soya beans and insect protected maize approved in the E.U. In total, farmers in six countries plant GM crops on 1.7 million hectares.

3,000 - 2,000 BC

Peruvians select potatoes (from around 160 wild species) with the lowest levels of poisons and grow them for food.

Tissue Culture (tc) and Micropropagation



Just as every person is different and unique, so is each plant. Some have traits like better color, yield, or pest resistance. For years, scientists have looked for methods to allow them to make exact copies of these superior individuals.

Plants usually reproduce by forming seeds through sexual reproduction. That is, egg cells in the flowers are fertilized by pollen from the stamens of the plants. Each of these sexual cells contains genetic material in the form of DNA. During sexual reproduction, DNA from both parents is combined in new and unpredictable ways, creating unique organisms.

This unpredictability is a problem for plant breeders as it can take several years of careful greenhouse work to breed a plant with desirable characteristics. Many of us think that all plants grow from seeds but now, researchers have developed several methods of growing exact copies of plants without seeds.

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. Tissue culture is seen as an important technology for developing countries for the production of disease-free, high quality planting material and the rapid production of many uniform plants. *Micropropagation*, which is a form of tissue culture, increases the amount of planting material to facilitate distribution and large scale

planting. In this way, thousands of copies of a plant can be produced in a short time. Micropropagated plants are observed to establish more quickly, grow more vigorously and taller, have a shorter and more uniform production cycle, and produce higher yields than conventional propagules.



The use of bioherbicides is another way of controlling weeds without environmental hazards posed by synthetic herbicides. Bioherbicides are made up of microorganisms (e.g. bacteria, viruses, fungi) and certain insects (e.g. parasitic wasps, painted lady butterfly) that can target very specific weeds. The microbes possess invasive genes that can attack the defense genes of the weeds, thereby killing it.

The better understanding of the genes of both microorganisms and plants has allowed scientists to isolate microbes (pathogens) whose genes match particular weeds and are effective in causing a fatal disease in those weeds. Bioherbicides deliver more of these pathogens to the fields. They are sent when the weeds are most susceptible to illness.

The genes of disease-causing pathogens are very specific. The microbe's genes give it particular techniques to overcome the unique defenses of one type of plant. They instruct the microbe to attack only the one plant species it can successfully infect. The invasion genes of the pathogen have to match the defense genes of the plant. Then the microbe knows it can successfully begin its attack on this one particular type of plant. The matching gene requirement means that a pathogen is harmless to all plants except the one weed identified by the microbe's genetic code.

This selective response makes bioherbicides very useful because they kill only certain weed plants that interfere with crop productivity without damaging the crop itself. Bioherbicides can target one weed and leave the rest of the environment unharmed.

The benefit of using bioherbicides is that it can survive in the environment long enough for the next growing season where there will be more weeds to infect. It is cheaper compared to synthetic pesticides thus could essentially reduce farming expenses if managed properly. It is not harmful

1995-1996

GM soybeans and corn are approved for sale, and GM cotton is commercialized in the United States. GM crops become the most rapidly adopted technology in the history of agriculture.

the roots of plants prevent further infections by pathogens and make plants more tolerant to drought and heavy metals. BIO-Quick, a composting inoculum, helps hasten the decomposition of farm and agro-industrial wastes by as much as 80%.

Biopesticides

As we all know, there are also microorganisms found in the soil that are not so friendly to plants. These pathogens can cause extreme disease or damage to the plant. As with friendly microorganisms, scientists have developed biological “tools” which use these disease-causing microbes to control weeds and pests naturally.

Bioherbicides

Weeds are a constant problem for farmers. They not only compete with crops for water, nutrients, sunlight, and space but also harbor insect and disease pests; clog irrigation and drainage systems; undermine crop quality; and deposit weed seeds into crop harvests. If left uncontrolled, weeds can reduce crop yields significantly.



Farmers fight weeds with tillage, hand weeding, synthetic herbicides, or typically a combination of all techniques. Unfortunately, tillage leaves valuable topsoil exposed to wind and water erosion, a serious long-term consequence for the environment. For this reason, more and more farmers prefer reduced or no-till methods of farming.

Similarly, many have argued that the heavy use of synthetic herbicides has led to groundwater contaminations, death of several wildlife species and has also been attributed to various human and animal illnesses.



1994

Transgenic FlavrSavr® tomato is approved for sale in U.S. groceries. It was developed to have more flavor and to have a longer shelf-life than conventionally grown tomatoes.

Plant tissue culture is a straightforward technique and many developing countries have already mastered it. Its application only requires a sterile workplace, nursery, and green house, and trained manpower.

Unfortunately, tissue culture is labor intensive, time consuming, and can be costly. Plants important to developing countries that have been grown in tissue culture are oil palm, plantain, pine, banana, date, eggplant, jojoba, pineapple, rubber tree, cassava, yam, sweet potato, and tomato. This application is the most commonly applied form of biotechnology in Africa.



Examples of the use of tissue culture in crop improvement in Africa include:

1. A new rice plant type for West Africa (NERICA – New Rice for Africa) resulting from embryo rescue of wide crosses made between Asian rice (*Oryza sativa*) and African rice (*Oryza glaberrima*) followed by anther culture of the hybrids to stabilize breeding lines.

Benefits of TC technology for rice farmers in West Africa (Source: WARDA)

For years, scientists dreamed of combining the ruggedness of the African rice species (*Oryza glaberrima*) with the productivity of the Asian species (*Oryza sativa*). But the two are so different, attempts to cross them failed as the resulting offspring were all sterile. In the 1990s, rice breeders from the West Africa Rice Development Association (WARDA) turned to biotechnology in an attempt to overcome the infertility problems. Key to the effort were gene banks that hold seeds of 1500 African rices — which had faced extinction as farmers abandoned them for higher-yielding Asian varieties.

Advances in agricultural research helped scientists cross the two species — a breakthrough that is changing the lives of many rice farmers in West Africa. After cross-fertilization of the two species, embryos were removed and grown on artificial media in a process known as embryo-rescue.

Because the resultant plants are frequently almost sterile, they are re-crossed with the *sativa* parent wherever possible (known as back-crossing). Once the

1700 - 1720

Thomas Fairchild, the forgotten father of the flower garden, creates Europe's first hybrid plant.

fertility of the progeny was improved (often after several cycles of back-crossing), anther culture was used to double the gene complement of male sex cells (anthers) and thus produce true-breeding plants.

The first of the new rices dubbed ‘New Rice for Africa’ (or NERICA) was available for testing in 1994 and since then, the techniques have been refined and streamlined, so that many new lines are generated each year. The dream had come true. The new plants had the best of both worlds – some of them combined yield traits of the *sativa* parent with local adaptation traits from *glaberrima*.

The NERICAs inherited wide, droopy leaves from their African parent, which smother weeds in early growth. That reduces labor, and allows farmers to work the same land longer, rather than having constantly to clear new land.

The structure of the panicles, or grain heads, has also been changed.

Panicles of the African species produce only 75-100 grains. The new rices inherited, from their Asian parent, longer panicles with ‘forked’ branches, and hold up to 400 grains.

Like their Asian parent, the new rices hold grains tightly, not allowing them to shatter. They produce more tillers than either parent, with strong stems to support the heavy grain heads.

The new varieties outyield others with no inputs—but respond bountifully to even modest fertilization. During rice trials, yields as high as 2.5 tons per hectare at low inputs—and 5 tons or more with just minimum increase in fertilizer use, have been obtained, approximately 25% to 250% increase in production.

The new rices mature 30 to 50 days earlier than current varieties, allowing farmers to grow extra crops of vegetables or legumes. They are taller than most rices, which makes harvesting easier—especially for women with babies strapped to their backs. They resist pests and

A fungus called *Penicillium bilaii* is the roots’ key to unlock phosphate from the soil. It makes an organic acid which dissolves the phosphate in the soil so that the roots can use it. A biofertilizer made from this organism is applied either by coating seeds with the fungus (called inoculation), or putting it directly into the ground where the plant’s roots will live.

The friendly fungus can wrap itself around the root, and prevent other less helpful organisms from living there. It has the first chance to use the plant’s byproducts. This will make the microbe stronger, and able to convert more phosphate for the roots to use. With additional phosphate, the plants will be stronger and more productive.

Another example of an organism that is used to make biofertilizers is the bacterium *Rhizobium*. This bacterium lives on the plant’s roots in cell collections called *nodules*.

The nodules are biological factories that can take nitrogen out of the air and convert it into an organic form that the plant can use. Because the bacteria live within the roots, it transfers the nutrient directly into the plant.

This fertilization method has been designed by nature. With a large population of the friendly bacteria on its roots, the legume can use naturally-occurring nitrogen instead of the expensive traditional nitrogen fertilizer.

Biofertilizers help plants use all of the food available in the soil and air thus allowing farmers to reduce the amount of chemical fertilizers they use. This helps preserve the environment for the generations to come.

NitroPlus, Bio-N and BIO-Fix are some Philippine examples of bio fertilizers that utilize the ability of microorganisms like rhizobia to fix free nitrogen. Other products like Mycogro and Mykovam help plants absorb water and phosphorus from the soil. The mycorrhizal fungi that colonizes



1750 - 1850

European farmers increase cultivation of legumes (to fix nitrogen in the soil) and rotate crops to increase yield.

Late 1980s/Early 1990s

China first to put GM crops on sale, namely VR tobacco and a tomato.

Microbial Fermentation



For many years, man has been taking advantage of the activities of millions of microorganisms found in the soil to improve agricultural productivity. With the large scale cultivation of microbes or other single cells, occurring with or without air – known as microbial fermentation -man has used naturally occurring organisms to develop *biofertilizers* and *biopesticides* to assist plant growth and control weeds, pests, and diseases, respectively.

Many of the microorganisms that live in the soil actually help plants absorb more nutrients than they would by themselves. Plants and these friendly microbes are involved in “*nutrient recycling*”. The microbes help the plant to “take up” essential energy sources. In return, plants donate their waste byproducts for the microbes to use for food. Because the microbes have helped plants digest more nutrients, plants develop stronger and bigger root systems. The larger the plants’ roots, the more living space and food there is for the microbes to enjoy.

Scientists use these friendly microorganisms to develop biofertilizers.

Biofertilizers

Phosphate and nitrogen are important for plant growth. These compounds exist naturally in the environment but plants have a limited ability to extract them. Phosphate is abundant in the soil but remains mostly bound, and nitrogen is abundant in the air. Phosphate plays an important role in crop stress tolerance, maturity, quality and directly or indirectly, in nitrogen fixation. If phosphate is not quickly used by the plant, it becomes locked into the soil through chemical reactions. This leaves only a small amount of this vital nutrient available to the plant. The plant cannot unlock phosphate by itself.

tolerate drought better than the Asian rices— vitally important for rainfed-rice farmers. The new rices grow better on infertile, acid soils—which comprise 70% of West Africa’s upland rice area.

They also have about 2% more body-building protein than their African or Asian parents.

Because of their success, NERICAs were quickly adopted by farmers. In 2000, it was estimated that the new rices covered some 8,000 ha in Guinea, of which 5000 ha grown by 20,000 farmers was under the supervision of the national extension agency. In 2002, WARDA projected that 330,000 ha would be planted to NERICAs, sufficient to meet the country’s own seed needs, with surplus for export to neighboring countries.

For more, please read “Farmers embrace African ‘miracle’ rice” (<http://www.in.org/ceosocher/gentinfo/afrec/vol17no4/174rice.htm>)

2. Bananas propagated from apical meristem in Kenya have been shown to have increased vigour and suffer lower yield loss from weevils, nematodes, and fungal diseases.

Benefits of TC technology for small-scale banana producers in Kenya (Source: ISAAA)

In Kenya, as in many parts of the tropical and subtropical developing world, banana is a highly important food crop. In last 20 years, however, there was a rapid decline in banana production due to widespread soil degradation and the infestation of banana orchards with pests and diseases. These problems were further aggravated by the common practice of propagating new banana plants using infected suckers. The situation was threatening food security, employment and incomes in banana-producing areas.

Tissue culture (tc) technology was considered an appropriate option to provide sufficient quality and quantity of such materials.

1866

Austrian monk Gregor Johann Mendel publishes important work on heredity that describes how plant characteristics are passed from generation to generation.

With proper management and field hygiene, yield losses caused by pests and diseases at farm level have been reduced substantially. Tissue culture technology has made it possible for farmers to have access to the following:

- large quantities of superior clean planting material that are early maturing (12-16 months compared to the conventional banana of 2-3 years)
- bigger bunch weights (30-45 kg compared to the 10-15 kg from conventional material)
- higher annual yield per unit of land (40-60 tons per hectare against 15-20 tons previously realized with conventional material)



Moreover, uniformity in orchard establishment and simultaneous plantation development has made marketing easier to coordinate with the possibility of transforming banana growing from merely subsistence to a commercial enterprise. An encouraging finding from a cost-benefit analysis of the project is that banana production is more remunerative as an enterprise than traditional banana production. The project has also benefited mainly women who tend the crop, thus helping to narrow the gender gap.

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West Africa Rice Development Association (WARDA)

<http://www.wardacgjar.org>

PCR

Polymerase Chain Reaction (PCR) also uses nucleic acid probes to detect the presence of a pathogen. This is a lot more sensitive compared to the other techniques as PCR can detect very small amounts of a pathogen's genetic material per sample and amplify certain sequences to a detectable level.

PCR can be used to detect the presence of pathogens in the air, soil, and water. Spores, especially those produced by fungi, are the primary source of infection to initiate epidemics. This can greatly help farmers in predicting possible diseases and the extent of the damage it can bring.

It can also help farmers detect the presence of pathogens that have long latent periods between infection and symptom development. Farmers can therefore keep track of the pathogen and apply the necessary control to prevent the spread of the disease.

PCR can also be used to detect if mutations are occurring in a given population of pathogens. These genetic mutations lead to the development of resistant strains.

The development of molecular test kits can be expensive but the returns are great. Further, they have short commercial timeframes, few regulatory barriers (because they are not consumed), and can be marketed widely, including directly to farmers.

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Biotechnology Information Series: Plant Disease Diagnostics
(http://www.biotech.iastate.edu/biotech_info_series/bio5.html)

1990

Genetic modifications used to make chymosin, an enzyme used in making hard cheese.

There are already numerous ELISA test kits available in the market to detect diseases of root crops (e.g. cassava, beet, potato), ornamentals (e.g. lilies, orchids), fruits (e.g. banana, apple, grapes), grains (e.g. wheat, rice), and vegetables. For example, these techniques can detect raouon stunting disease of sugarcane, tomato mosaic virus, Papaya ringspot virus, banana bract mosaic virus, banana bunchy top virus, and watermelon mosaic virus.



Direct tissue blotting

This technique also utilizes specific antibodies to detect the presence of plant pathogens. In this method, diseased tissue samples are pressed to draw out proteins onto a special paper and the antibodies are added to the sample. A color-inducing reagent is added afterwards to react with the antibody-pathogen complex. Color reaction indicates a positive result and pinpoints the location of the pathogen in the diseased tissue.

DNA/RNA probes

Another set of tools that can be used in plant disease diagnostics is nucleic acid (DNA/RNA) probes. These probes are fragments of nucleic acid arranged in a sequence complementary to that of the DNA or RNA of the pathogen. Because the sequences complement each other, the probes can be used to identify specific diseases.

Squash blot method

In the squash blot method, tissue from a plant that is suspected to be diseased is “squashed” onto a special piece of paper, called a *membrane*. This membrane is then treated with a probe that can bind with the DNA or RNA of the plant pathogen suspected to be in the tissue. Binding will occur when complementary sequences are present. After adding several more substances to the membrane, a color reaction indicates that the probe and the pathogen DNA/RNA have bound to each other and the disease is present. No color reaction means the test for the disease is negative.

Molecular Breeding and Marker-Assisted Selection



The process of developing new crop varieties requires many steps and can take almost 25 years. Now, however, applications of 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 makes it easier and faster for scientists to select plant traits is called *marker-assisted selection* (MAS).

Molecular shortcut

The differences which distinguish one plant from another are encoded in the plant’s genetic material, the 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, may be 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 *marker-assisted selection*. To help identify specific genes, scientists use what are called *molecular markers*. The

Plant Disease Diagnostics

Biotechnology has also allowed the development of diagnostics which has assisted farmers worldwide in managing different diseases affecting their crops.

To successfully manage a plant disease, it is critical to correctly identify the cause of the disease in its early stages. Delaying this can result in extensive crop damage and financial loss to farmers. Some diseases can be diagnosed quickly by visual examination although sometimes, visual detection at the plant level is usually only possible after major damage to the crop has been done, by which time, it is too late.

Other diseases require laboratory testing for diagnosis which may take days or even weeks to complete and are, in some cases, relatively insensitive. Delays are frustrating when a quick diagnosis is needed so that appropriate measures may be taken to prevent plant injury and loss.

Fortunately, new diagnostic techniques are now available that require minimal processing time and are more accurate in identifying pathogens. These diagnostics are based on rapid detection of proteins or DNA that are specific to each pathogen, disease or condition. Some procedures require laboratory equipment and training, while other procedures can be performed on site by a person with no special training.

Examples of existing diagnostic techniques:

ELISA diagnostic kits

ELISA (enzyme-linked immunosorbent assay) kits are based on the ability of an antibody to recognize a certain protein substance or antigen associated with a plant pathogen. The kits are very easy to use; some tests can be used in the field where a disease is suspected and can take only 5 minutes to perform. In addition, they do not require sophisticated laboratory equipment or training.



markers are a string 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 a 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. These genetic linkage maps show the location of markers and genes, and show 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 so that they can test for a specific characteristic. Once the tissue is analyzed, 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 a specific trait.



1870-1890

Plant researchers crossbreed cotton to develop hundreds of new varieties with superior traits.

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Meet the 'omics' 2003 Agbiotech Infosome. Saskatchewan Agricultural Biotechnology Information Centre, A service of Ag-west Biotech Inc.

Images and graphics used in this section ('Omics' Sciences: Genomics, Proteomics and Metabolomics) are courtesy of the US Department of Energy Human Genome Program, and US Department of Energy Genomics to Life Program. (<http://www.ornl.gov>; <http://www.doe.gov/esa/olife.org>)

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 do not breed true (this includes many staples such as yams, bananas, plantain, sweet potato, and cassava).

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Photo of chromosome and DNA strand on page 13 courtesy of the US Department of Energy Human Genome Program (<http://www.ornl.gov>).

1962

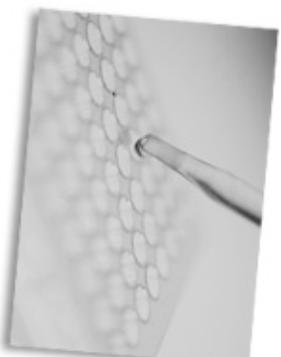
The first transgenic plant is produced - a tobacco plant resistant to an antibiotic. The breakthrough paved the way for beneficial traits, such as insect resistance, to be transferred to a plant.

1871 - Early 1900s

Researcher Luther Burbank developed the Russet Burbank Potato, and later went on to develop several new hybrid fruits, including plums, berries, prunbes and peaches.

Genetic Engineering and GM Crops

Over the last 30 years, the field of genetic engineering has developed rapidly due to the greater understanding of deoxyribonucleic acid (DNA) as the chemical double-helix code from which genes are made. 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 using laboratory tools to insert, alter, or cut out 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. 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.

Nature's own genetic engineer

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 of a wide range of broad-leaved plants, such as apple, pear, peach, cherry, almond,

Proteomics can also be applied to map protein modification to determine the difference between a wild type and a genetically modified organism. It is also used to study protein-protein interactions involved in plant defense reactions.

For example, proteomics research at Iowa State University includes:

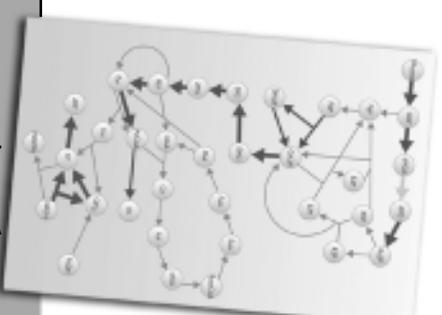
- An examination of changes of protein in the corn proteome during low temperatures which is a major problem for young corn seedlings
- Analysis of the differences that occur in the genome expression in developing soybean stressed by high temperatures
- Identifying the proteins expressed in response to diseases like soybean cyst nematode.

Metabolomics

Metabolomics is one of the newest ‘omics’ sciences. The *metabolome* refers to the complete set of low molecular weight compounds in a sample. These compounds are the substrates and by-products of enzymatic reactions and have a direct effect on the phenotype of the cell. Thus, metabolomics aims at determining a sample’s profile of these compounds at a specified time under specific environmental conditions.

Genomics and proteomics have provided extensive information regarding the genotype but convey limited information about phenotype. Low molecular weight compounds are the closest link to phenotype.

Metabolomics can be used to determine differences between the levels of thousands of molecules between a healthy and diseased plant. The technology can also be used to determine the nutritional difference between traditional and genetically modified crops, and in identifying plant defense metabolites.



1973 Cohen and Boyer successfully splice a gene from one organism and move it into another, launching the modern biotechnology era.

1978 Boyer’s lab created a synthetic version of the human insulin gene.

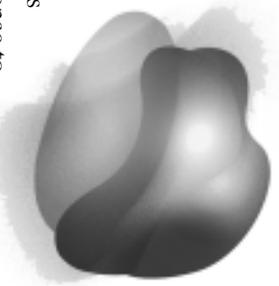
Genomics is an entry point for looking at the other ‘omics’ sciences. The information in the genes of an organism, its genotype, is largely responsible for the final physical makeup of the organism, referred to as the phenotype. However, the environment also has some influence on the phenotype.

DNA is the genome is only one aspect of the complex mechanism that keeps an organism running – so decoding the DNA is one step towards understanding, but by itself it does not specify everything that happens within the organism.

The basic flow of genetic information in a cell is as follows. The DNA is transcribed or copied into a form known as RNA. The complete set of RNA (also known as its *transcriptome*) is subject to some editing (cutting and pasting) to become messenger-RNA, which carries information to the ribosome, the protein factory of the cell, which then translates the message into protein.

Proteomics

Proteins are responsible for an endless number of tasks within the cell. The complete set of proteins in a cell can be referred to as its *proteome* and the study of protein structure and function and what every protein in the cell is up to is known as proteomics. The proteome is highly dynamic and it changes from time to time in response to different environmental stimuli. The goal of *proteomics* is to understand how the structure and function of proteins allow them to do what they do, who or what they interact with, and how they contribute to life processes.



An application of proteomics is known as protein expression profiling where proteins are identified at a certain time in organism as a result of the exposure to a stimulus. Proteomics can also be used to develop a protein-network map where interaction among proteins can be determined for a particular living system.

1960s Work on creating high yield varieties of major grains, especially wheat, corn, millet, and rice massively increase production of these crops in many countries - launching the Green Revolution. The creation of dwarf wheat increases yields by 70%.

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.

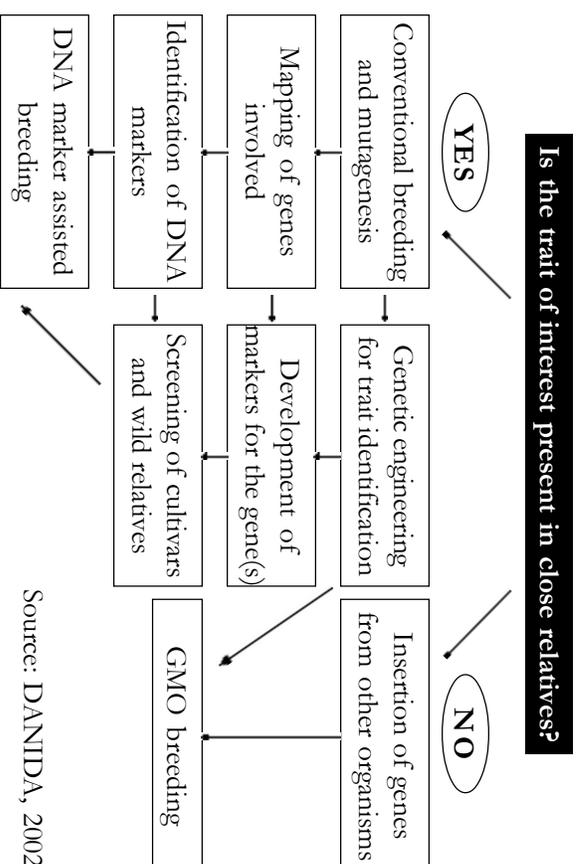
Application of genetic engineering in crop production

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



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

Figure 1.



Source: DANIDA, 2002.

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 five steps.



Step 1. Nucleic acid (DNA/RNA) Extraction

Nucleic acid extraction, either DNA or 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 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 a thread-like pellets referring to the DNA/RNA.

Step 2. Gene cloning

The second step in the genetic engineering process is gene cloning. Upon DNA extraction, all DNA from the desired organism is extracted at once. Through gene cloning, the desired gene/s can be isolated from the rest of the DNA extracted, which is then mass-produced in a host cell to make thousands of copies of the desired gene.

There are basically four stages in any cloning experiment involving generation of DNA fragments, joining to a vector, propagation in a host cell, and selection of the required sequence.



1908 First U.S. hybrid corn produced through self-pollination.

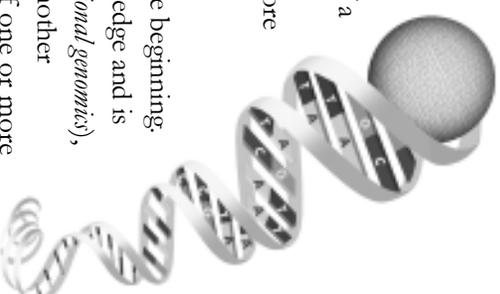
1919 Word 'biotechnology' coined by Hungarian immigrant Karl Ereky.

'Omics' Sciences:

Genomics, Proteomics, and Metabolomics

Genomics

Genomics is the new science that deals with the discovery and noting of all the sequences in the entire genome of a particular organism. The genome can be defined as the complete set of genes inside a cell. Genomics, is therefore the study of the genetic make-up of organisms.



Determining the genomic sequence, however, is only the beginning. Once this is done, the data is translated into new knowledge and is used to study the function of the numerous genes (*functional genomics*), to compare the genes in one organism with those of another (*comparative genomics*), or to generate the 3-D structure of one or more proteins from each protein family, thus offering clues to their function (*structural genomics*).

In crop agriculture, the main purpose of the application of genomics is to gain a better understanding of the whole genome of plants. Agronomically important genes may be identified and targeted to produce more nutritious and safe food while at the same time preserving the environment.

An important current genomic research is the International Rice Genome Sequencing Project which is a collaborative effort of several laboratories worldwide. This project aims to completely sequence the entire rice genome (12 rice chromosomes) and subsequently apply the knowledge to improve rice production.

In 2002, the draft genome sequences of two agriculturally important subspecies of rice, *indica* and *japonica*, were published. Once completed, the rice genome sequence will serve as a model system for other cereal grasses and will assist in the identification of important genes in maize, wheat, oats, sorghum, millet, etc.

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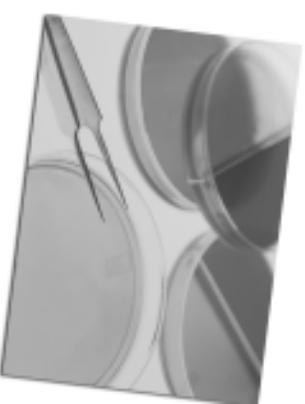
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Step 3. Gene Design and Packaging

Once the gene of interest has been cloned, it has to be linked to pieces of DNA that will control how the gene of interest will work once it is inside the plant genome. These pieces of DNA will switch on (*promoter*) and off the expression of the gene inserted. Gene designing/packaging is done by replacing an existing promoter with a new one and incorporating a selectable marker gene.

Promoters allow differential expression of genes. For instance some promoters cause the genes inserted to be expressed all the time, 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.



Selectable marker genes are also usually linked to the gene of interest to facilitate its detection once inside the plant tissues. This enables to select the cells that have been successfully incorporated with the gene of interest, thus saving considerable expense and effort. Currently, genetic engineers use antibiotic resistance marker gene to screen plant tissues with the insert. Those cells that survive the addition of antibiotics to the growth medium indicate the presence of the inserted gene. Because of some concern that the use of antibiotic resistance marker genes will increase antibiotic resistance in humans and animals, genes coding for resistance to non-medically important antibiotics are preferred. In addition, alternative types of marker genes are being developed.

Once the gene of interest is packaged together with the promoter and the marker gene, it is then inserted into a bacterium to allow for the creation of many copies of the gene package.

1928 Impact of X-rays and radium on barley mutation described.

1933 Hybrid corn becomes available commercially in the United States, causing corn yields to triple over the past 50 years.

1950s/1960s

Understanding of the structure of genes, and how they work deepens.

Step 4. Transformation

Once the gene package is ready, it can then be introduced into the cells of plant being modified through the process called *transformation* or *gene insertion*.

The most common methods used to introduce the gene package into the plant cells include biolistic transformation using the gene gun or *Agrobacterium*-mediated transformation. 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 gene inserted is stable, inherited and expressed in subsequent generations, then the plant is considered a *transgenic*.



Step 5. Backcross Breeding

Backcross breeding is the final step in producing genetically engineered crops. This is done by crossing the transgenic plant with elite lines using conventional plant breeding methods. This enables the combination of the desired traits of the elite parents and the transgenic into a single line. The offspring are repeatedly crossed back to the elite line to obtain a high yielding transgenic line.

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 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 from 1996 to 2003. Close to 68 million hectares was planted in 2003 with high market value transgenic crops such as herbicide tolerant soybean, maize, canola, cotton; insect resistant maize and cotton; and virus resistant squash and papaya.



With genetic engineering, more than one trait can be incorporated into a plant. Transgenic crops with combined traits are also available commercially. These are the herbicide tolerant and insect resistant maize and cotton.

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, environmental benefit, pharmaceutical production, and non-food crops.

Examples of these products include: rice with higher levels of iron and b-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; tomatoes with high levels of flavonols, which are powerful antioxidants; drought tolerant maize; maize with improved phosphorus availability; arsenic-tolerant plants; edible vaccines from fruit and vegetables; and low lignin trees for paper making.



1941 Discovery that chemicals can cause mutations.

1944 Discovery that DNA is genetic molecule - in other words, it is the way genetic information is passed between generations.

1953

Watson and Crick describe the double helix structure of DNA, providing more insight into how DNA carries genetic information.