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Genetic Engineering: An Overview

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ABSTRACT *Genetic engineering is also known as genetic modification. Genetic engineering is the deliberate, controlled manipulation of the genes in an organism with the intent of making that organism better in some way. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms. New DNA may be inserted in the host genome by first isolating and copying the genetic material of interest using molecular cloning methods to generate a DNA sequence, or by synthesizing the DNA, and then inserting this construct into the host organism. This is usually done independently of the natural reproductive process. The result is a so-called genetically modified organism (GMO). Genetic engineering techniques have been applied in numerous fields including research, agriculture, industrial biotechnology, and medicine.*

Introduction

Genetic engineering is the alteration of genes and DNA in living organisms by scientists. DNA can now be added to animals, or plants with the technology that we have today. It is a new technology that combines genes from totally dissimilar species in combinations that are not achievable using conventional breeding methods.

Genetic engineering, also called genetic modification, is the direct manipulation of an organism's genome using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms.

Gene technology is the term given to a range of activities concerned with understanding gene expression, taking advantage of natural genetic variation, modifying genes and transferring genes to new hosts.

Identifying genes and their function is an important application of gene technology and can lead to more efficient conventional breeding processes. Marker-assisted breeding is one example.

With the improvement of genetic engineering techniques, the time for generating and evaluating new germplasm (a collection of genetic resources for an organism) can be drastically reduced. Genetic engineering may ultimately have their most significant effect on agriculture. Recent advances have raised possibility of development of new plant germplasm through introduction of any gene from any organism into plant.

History of Genetic Engineering

The first form of genetic engineering began with a man named Mendel, a monk in the late nineteenth century. He was the first to even formulate the concept of the gene from his experiments on pea plants. Since Mendel, other scientists have continued in agricultural engineering, a type of engineering that deals with the cross breeding of plants and cattle to maximize their productivity. Through time geneticists have continued to find better methods and strategies for improving the quantity of food from plants and improving the amount of milk and meat that cattle produce.

Until the mid 1990s, the organisms produced by genetic engineering were nearly all confined to laboratories or controlled factory setting. During this time, the main use of genetic engineering was to produce medically useful substances such as insulin.

Genetic engineering had its origins during the late 1960s in experiments with bacteria, viruses, and plasmids, small, free-floating rings of DNA found in bacteria. A key discovery was made by Swiss microbiologist Werner Arber, who in 1968 discovered restriction enzymes. These are naturally occurring enzymes that cut DNA into fragments during replication. A year later American biologist Hamilton O. Smith revealed that one type of restriction enzyme cut DNA at very specific points in the molecule. This enzyme was named type II restriction enzyme to distinguish it from type I and type III enzymes, which cut DNA in a different manner. In the early 1970s American biologist Daniel Nathans demonstrated that type II enzymes could be used to manipulate genes for research. For their efforts Smith, Nathans, and Arber were awarded the 1978 Nobel prize for physiology or medicine.

The true fathers of genetic engineering were American biochemists Stanley Cohen and Herbert Boyer, who were the first scientists to use restriction enzymes to produce a genetically modified organism. In 1973 they used type II enzymes to cut DNA into fragments. The new age of biotechnology had begun. The first field trials of genetically engineered plants occurred in France and the USA in 1986, tobacco plants were engineered to be resistant to herbicides.[1] The People's Republic of China was the first country to commercialize transgenic plants, introducing a virus-resistant tobacco in 1992.[2] In 1994 Calgene attained approval to commercially release the Flavr Savr tomato, a tomato engineered to have a longer shelf life.[3] In 1994, the European Union approved tobacco engineered to be resistant to the herbicidebromoxynil, making it the first genetically engineered crop commercialized in Europe.[4] In

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1995, Bt Potato was approved safe by the Environmental Protection Agency, after having been approved by the FDA, making it the first pesticide producing crop to be approved in the USA.[5] In 2009 11 transgenic crops were grown commercially in 25 countries, the largest of which by area grown were the USA, Brazil, Argentina, India, Canada, China, Paraguay and South Africa.[6]

In 2010, scientists at the J. Craig Venter Institute created the first synthetic life form by adding a synthetic genome to an empty bacterial cell. The resulting bacterium was namedSynthia.[7,8] In 2014, a bacterium was developed that replicated a plasmid containing a unique base pair, creating the first organism engineered to use an expanded genetic alphabet.[9,10]

Method of Genetic Engineering

Genetic modification involves an intended targeted change in a plant or animal gene sequence to effect a specific result through the use of r-DNA technology. A variety of genetic engineering techniques are described in the following text.

Plasmid method

The plasmid method is the most common method used for altering microorganisms such as bacteria. In the plasmid method, a small ring of DNA called a plasmid found in bacteria is placed on a petri dish with special restriction enzymes that cut the DNA at certain base sequences. The restriction enzyme is also used to treat the DNA sequence to be engineered into the bacteria. The two separated DNA sequences are introduced into the same container, where the sticky ends fuse forming a ring of DNA with additional and new content. New enzymes are added to help stick the new linkages, and the culture is then separated by molecular weight. Those molecules that weigh the most have successfully incorporated the new DNA, and they are to be preserved. The newly formed plasmid is added to a culture of live bacteria with known genomes, some of which will take up the free-floating plasmids and begin to express them. The DNA introduced into the plasmid will include instructions for making a protein, and also antibiotic-resistance genes. The altered bacteria can then grow and reproduce and evolve on their own.

Microbial Vectors

Agrobacterium tumefaciens is a naturally occurring soil microbe best known for causing crown gall disease on susceptible plant species. It is an unusual pathogen because when it infects a host, it transfers a portion of its own DNA into the plant cell. The transferred DNA is stably integrated into the plant DNA, and the plant then reads and expresses the transferred genes as if they were its own. The transferred genes direct the production of several substances that mediate the development of a crown gall.

In the early 1980s strains of Agrobacterium were developed that lacked the disease-causing genes but maintained the ability to attach to susceptible plant cells and transfer DNA.

Agrobacterium is a naturally occurring genetic engineering agent and is responsible for the majority of GE plants in commercial production.

Microprojectile Bombardment

Klein and colleagues discovered that naked DNA could be delivered to plant cells by "shooting" them with microscopic pellets to which DNA had been adhered.[11] This is a crude but effective physical method of DNA delivery, especially in species such as corn, rice, and other cereal grains, which Agrobacterium does not naturally transform. Many GE plants in commercial production were initially transformed using microprojectile delivery.

Electroporation

In electroporation, plant protoplasts take up macromolecules from their surrounding fluid, facilitated by an electrical impulse. Cells growing in a culture medium are stripped of their protective walls, resulting in protoplasts. Supplying known DNA to the protoplast culture medium and then applying the electrical pulse temporarily destabilizes the cell membrane, allowing the DNA to enter the cell. Transformed cells can then regenerate their cell walls and grow to whole, fertile transgenic plants. Electroporation is limited by the poor efficiency of most plant species to regenerate from protoplasts.

Microinjection

DNA can be injected directly into anchored cells. Some proportion of these cells will survive and integrate the injected DNA. However, the process is labor intensive and inefficient compared with other methods.

Transposons/Transposable Elements

The genes of most plant and some animal (e.g., insects and fish) species carry transposons, which are short, naturally occurring pieces of DNA with the ability to move from one location to another in the genome. Barbara McClintock first described such transposable elements in corn plants during the 1950s.[12] Transposons have been investigated extensively in research laboratories, especially to study mutagenesis and the mechanics of DNA recombination. However, they have not yet been harnessed to deliver novel genetic information to improve commercial crops.

Non-transgenic Molecular Methods of Manipulation

Genetic features can be added to plants and animals without inserting them into the recipient organism's native genome. DNA of interest may be delivered to a plant cell, expressing a new protein—and thereby a new trait—without becoming integrated into the host-cell DNA. For example, virus strains may be modified to carry genetic material into a plant cell, replicate, and thrive without integrating into the host genome. Without integration, however, new genetic material may be lost during meiosis, so that seed progeny may not carry or express the new trait.

Many food plants are perennials or are propagated by vegetative means, such as grafting or from cuttings. In these cases the virus and new genes would be maintained in subsequent, nonsexually generated populations. Technically such plants are not products of rDNA because there is no recombination or insertion of introduced DNA into the host genome. Although these plants are not GE, they do carry new DNA and new traits. No such products are known to be currently on the market in the United States or elsewhere.[13]

How Genetic Engineering Works

The action of restriction enzymes—also called restriction endonucleases—is the crux of genetic engineering. These enzymes are found only in bacteria, where they protect the host genome against invading foreign DNA, such as a virus. Each restriction enzyme recognizes a short, specific sequence of nucleotide bases in the DNA molecule. These regions, called recognition sequences, are randomly dis-

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tributed throughout the DNA molecule. Different bacterial species make restriction enzymes that recognize different nucleotide sequences. By convention, restriction enzymes are named for the genus, species, and strain designations of the bacteria that produce them and for the order in which they were first identified. For example, the enzyme *Eco*RI was the first restriction enzyme isolated from the Escherichia coli (*E. coli*) strain RY13.

Of the three types of restriction enzymes, type II is the most useful in genetic engineering. Today more than 3,600 type II restriction enzymes are known, forming a molecular tool kit that allows scientists to cut chromosomes into various desired lengths, depending on how many different restriction enzymes are mixed with the chromosome under investigation.

At the cleavage site, different restriction enzymes cut DNA in one of two ways.Some enzymes make incisions in each strand at a point immediately opposite the another, producing "blunt end" DNA fragments. Most enzymes cut the two strands at a point not directly opposite each other, producing an overhang in each strand. These are called "sticky ends" because they readily pair with complementary bases on another fragment. Genetic engineers use restriction enzymes to remove a gene from a donor organism's chromosome and insert it into a vector, a molecule of DNA that will function as a carrier. Plasmids are the most common vectors used in genetic engineering. These are circular DNA molecules found in some bacteria; they are extrachromosomal molecules, meaning that they replicate independently of the bacterial chromosome. The first step in the process involves mixing the donor organism's DNA with a set of restriction enzymes that will isolate the gene of interest by cutting it from its chromosome. In a separate step, a plasmid is cut with the same restriction enzymes. The donor gene DNA is then spliced into the plasmid, producing a recombinant DNA (rDNA) molecule that will function as a vector, which is introduced into bacterial cells. Inside the host cells, the plasmids replicate when the bacteria replicate. Because this produces many copies of the recombinant DNA molecule, recombinant DNA technology is often called gene cloning. In addition, when the bacteria's DNA initiates protein synthesis, the protein coded for by the inserted gene is produced.

Role of Genetic Engineering in Human Life

Genetic engineering in Agriculture

With genetic engineering, you can change plants and animals in many ways that are beneficial to our health and way of life. A genetically engineered vegetable can withhold ice cold conditions and contain more vitamins than a natural vegetable. Genetic engineering is also a way to improve food production and could possibly even result in the solution to world hunger. Another reason why genetic engineering recently became so popular is because its developers claim that it will reduce the use of pesticides and herbicides. However, there are many bad things that come along with these good aspects of genetic engineering. enetic manipulation results in our food becoming healthier.

Genetic engineering, promises to have an enormous impact on the improvement of crop species. Genetic transformation can boost plant breeding efforts for developing disease resistant varieties. Scientists are using Agrobacterium gene transfer system to produce tobacco plants with increased resistance to Tobacco Mosaic Virus (TMV). Insect resistant plants are also developed, using biotechnological applications. Several biopesticides are developed e.g. Bt cotton, Bt corn, rice, tomato, potato, and soybeans etc.

Genetic engineering in industry

Genetic engineering has been especially valuable for producing recombinant microorganisms that have a wide variety of industrial uses. Among the most important achievements have been the production of modified bacteria that devour hydrocarbons. These microbes are used to destroy oil slicks and to clean up sites contaminated with toxic wastes. Genetically engineered microbes are used to produce enzymes used in laundry detergents and contact lens solutions. Recombinant microbes also are used to make substances that can be converted to polymers such as polyester for use in bedding and other products.

Genetic engineering and Health

Medicine was the first area to benefit from genetic engineering. Using recombinant DNA technology, scientists can produce large quantities of many medically useful substances, including hormones, immune-system proteins, and proteins involved in blood clotting and blood-cell production.

Another benefit of genetic engineering is realized in production of valuable proteins. Recombinant DNA made possible the use of bacteria to produce proteins of medical importance. One such example is that of genetically engineered human insulin which is of great importance and now marketed throughout the world.

Some important genetically engineered proteins include:

Human Insulin- Human insulin or Humulin has great importance. Earlier, patients could not tolerate pig insulin, as it has slightly different amino acid sequence as compared to human. Humulin eventually became cheaper than that extracted from animal pancreas and is now available.

Interferon- The interferons also were among the first recombinant proteins produced for therapeutics. Interferons belong to a class of immune-system proteins called cytokines and are used to treat viral infections and some cancers, notably the virulent form of Kaposi's sarcoma common in patients with AIDS. Genetic engineering enables the cost-effective production of vast quantities of very pure recombinant interferon.[14]

Recombinant technology is used to produce a wide range of therapeutic substances. These include cytokines, interleukins, and monoclonal antibodies, all of which are used to fight certain viruses and cancers.

Critical blood factors are now mass-produced through recombinant technology; these include clotting proteins such as factor VIII, used to treat bleeding disorders such as hemophilia; erythropoietin, which stimulates red blood cell production and is needed to combat anemia; and tissue plasminogen activator, a protein that helps dissolve the blood clots that block arteries during a heart attack or certain types of stroke.

Growth hormone- In humans, growth hormone helps in treatment of hypopituitary dwarfs. Genetically engineered growth hormones may prove useful in the treatment of bone fractures, skin burns and bleeding ulcers of digestive tract. The human hormone is marketed in United States and bovine hormone is expected to yield bigger cattle and thus more beef. Hence growth hormones are commercially very demanding.

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Genetic Engineering and Vaccine production
Genetic engineering has also provided a means to produce safer vaccines.

The first step is to identify the gene in a disease-causing virus that stimulates protective immunity. That gene is isolated and inserted into a vector molecule such as a harmless virus. The recombinant virus is used as a vaccine, producing immunity without exposing people to the disease-causing virus. Vaccines produced by genetic engineering offer an advantage that the microbial strains from which the proteins are extracted do not contain complete viruses. And thus, there are no risks of accidental inoculation with live virus.

Crop plants can bear cheaper bioreactors to produce antigens to be utilized as Edible vaccines. These edible vaccines are said to be a cheap alternative as compared to recombinant vaccines.

The transgenic plants are treated as edible vaccines and consumption of these transgenic plants viz. transgenic banana and tomato cure diseases like Cholera and Hepatitis-B. Foot and mouth diseases can be cured by feeding them transgenic sugar beet. In the near future, these vaccines can be used as conventional vaccines.

Recently a genetically engineered malarial vaccine SPF – 66 has been produced.

In gene therapy, scientists use vector molecules to insert a functional gene into the cells of individuals suffering from a disorder caused by a defective gene. Vector molecules containing a functional gene are inserted into a culture of the patient's own cells, which then deliver the inserted genes to the targeted diseased organs or tissues. The most commonly used vectors in gene therapy are viruses. In the target (human host) cell, the virus "unloads" the inserted gene, which then begins functioning, restoring the cell to a healthy state. Another method is to take a cell from the patient, use recombinant technology to remove the nonfunctional gene and replace it with a functional one, allow the cell to replicate, and then infuse the engineered cells directly into the patient. For example, to treat the life-threatening deficiency of the immune system protein adenosine deaminase (ADA), scientists infuse cells from the patient's own blood into which researchers have inserted copies of the gene that directs production of ADA. Although there are still a number of challenges to overcome in developing gene therapy, it remains a research area of great promise.

Genetic Engineering and Environment
It is possible to improve the environment with genetic manipulation. For instance by cloning endangered species or by creating organisms that prevent pollution.Plastics are bad for the environment in general; the environment is unable to destroy them. However, it turns out that certain bacteria can create a biodegradable plastic. Feeding these bacteria however is very expensive. It would be possible to implant the gene responsible for the production of the plastic from the bacteria into a plant, this plant could become a producer of this plastic. This plastic would then be cheap and biodegradable.

Disadvantages of Genetic Engineering
Of course there are two sides to the coin; here are some possible eventualities and disadvantages.

Nobody knows the effects on the long term- Nature is an extremely complex inter-related chain consisting of many species linked in the food chain. Some scientists believe that introducing genetically modified genes may have an irreversible effect with consequences yet unknown. Genetic manipulation is still in its infancy and nobody knows from practical experiences what the consequences could be.

Unknown Consequences of Viral Genes in the Human Body-Considering that genetic engineering employs viral vector that carries functional gene inside the human body; the consequences are still unknown. There are no clues as to where functional genes are being placed. They may even substitute the important genes, instead of mutated genes. Thus, this leads to another health condition or disease to human.

Genetic Diversity: Now a Thing of the Past-As defective genes are replaced with functional gene, then it is expected that genetic diversity will now be a thing of the past. And if human beings will have identical genomes, the population as a whole will be susceptible to virus or disease. This may lead to human extinction in the earth.

It Borderlines on the Issues Pertaining on Morality-With many people who religiously believe in God, or who are born and baptized as Christians, genetic engineering may not be acceptable. Somehow, people can be questioned of what gives them the right to manipulate divine laws. It also questions the theory of Darwin, "the survival of the fittest".

Uncertain Effects That May be Brought by Genetically Modified Life Form-One of the clear disadvantages of genetic engineering is that it may bring uncertain effects to the environment and human health.

No Long-Term Safety Testing—Genetic engineering uses material from organisms that have never been part of the human food supply to change the fundamental nature of the food we eat. Without long-term testing no one knows if these foods are safe.

Toxins—Genetic engineering can cause unexpected mutations in an organism, which can create new and higher levels of toxins in foods.

Allergic Reactions—Genetic engineering can also produce unforeseen and unknown allergens in foods.

Decreased Nutritional Value—Transgenic foods may mislead consumers with counterfeit freshness. A luscious-looking, bright red genetically engineered tomato could be several weeks old and of little nutritional worth.

Antibiotic Resistant Bacteria—Genetic engineers use antibiotic-resistance genes to mark genetically engineered cells. This means that genetically engineered crops contain genes which confer resistance to antibiotics. These genes may be picked up by bacteria which may infect us. (New Scientist 1999)

Problems Cannot Be Traced—Without labels, our public health agencies are powerless to trace problems of any kind back to their source.

Side Effects—Genetic engineering is like performing heart surgery with a shovel. Scientists do not yet understand liv-

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ing systems completely enough to perform DNA surgery without creating mutations which could be harmful to the environment and our health. They are experimenting with very delicate, yet powerful forces of nature, without full knowledge of the repercussions.

Widespread Crop Failure—Genetic engineers intend to profit by patenting genetically engineered seeds. This means that, when a farmer plants genetically engineered seeds, all the seeds have identical genetic structure. As a result, if a fungus, a virus, or a pest develops which can attack this particular crop, there could be widespread crop failure.

Threatens Our Entire Food Supply—Insects, birds, and wind can carry genetically altered seeds into neighboring fields and beyond. Pollen from transgenic plants can cross-pollinate with genetically natural crops and wild relatives. All crops, organic and non-organic, are vulnerable to contamination from cross-pollinatation.

Increased use of Herbicides—Scientists estimate that plants genetically engineered to be herbicide-resistant will greatly increase the amount of herbicide use. (Benbrook 1999) Farmers, knowing that their crops can tolerate the herbicides, will use them more liberally.

More Pesticides—GE crops often manufacture their own pesticides and may be classified as pesticides by the EPA. This strategy will put more pesticides into our food and fields than ever before.

Ecology may be damaged—The influence of a genetically engineered organism on the food chain may damage the local ecology. The new organism may compete successfully with wild relatives, causing unforeseen changes in the environment.

Gene Pollution Cannot Be Cleaned Up—Once genetically engineered organisms, bacteria and viruses are released into the environment it is impossible to contain or recall them. Unlike chemical or nuclear contamination, negative effects are irreversible.

Conclusion

Genetic engineering is a radical new technology that has the potential to change our society. Genetic engineering allows people to insert specific genes into a plants and animals to alter the species. Currently over 70% of food that Americans eat is genetically altered in some way. The main crops that are altered are canola, cotton, potatoes and soy. However, there are many other items that are being genetically engineered as well, including animals and trees. Although genetic engineering has a lot of good points, there are many bad points as well. Nevertheless, genetic engineering is still a rather new technology and there is a lot of time and room for improvement

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