**Applications of Biotechnology in Transgenic Plants and Animals**

The applications of biotechnology includes: (i) therapeutics, (ii) diagnostics,

(iii) genetically modified crops for agriculture, (iv) processed food, (v) bioremediation, (vi) waste treatment and (vii) energy production.

Biotechnology mainly deals with industrial scale production of biopharmaceuticals and biological using genetically modified microbes, fungi, plants and animals.

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**[Image Courtesy : eplantscience.com/index/images/Biotechnology/chapter07/069\_large.jpg]**

**Research Areas of Biotechnology:**

Following are three research areas of biotechnology.

**(i) Catalyst:**

Providing the best catalyst in the form of improved organism; generally a microbe or pure enzyme.

**(ii) Optimum Conditions:**

Creating optimal conditions through engineering for a catalyst to act.

**(iii) Downstream Processing:**

Downstream processing technologies to purify the protein/organic compound.

**How biotechnology is being used to improve the quality of our life, mainly in the food production and health.**

**A. Biotechnological Applications in Agriculture:**

Options to Increase the Food Production:

There are three options to increase the food production.

1. Agrochemical based Agriculture:

The Green Revolution succeeded in increasing the yield of crops mainly due to

(i) Use of improved varieties of crops and

(ii) Use of agrochemicals (fertilizers and pesticides)

But it was not sufficient to feed the growing human population.

2. Organic Agriculture or Organic Farming:

In organic farming, farmers use ma¬nure, bio-fertilizers, bio-pesticides and bio-controls to increase the crop production instead of using artificial fertilizers and pesticides.

3. Genetically Engineered Crop-based Agriculture:

The organic farming cannot increase the yield of crop to appreciable degree. The solution of this problem is use of genetically modified crops. Plants, bacteria, fungi and animals whose genes have been changed by manipulations are called Genetically Modified Organisms (GMOs). Crops in which foreign genes have been introduced through genetic engineering are called genetically modified crops or GM Crops.

**Transgenic Plants:**

The plants in which foreign genes have been introduced through genetic engineering are called transgenic plants. There are two techniques for introducing foreign genes (transgenes) into the plant cell genome.

(i) The first, through a vector and

(ii) The second, through direct introduction of DNA.

**Production of Transgenic Plants:**

Here gene transfer through Ti plasmid vector is taken as an example: Interspecific gene transfer are now possible through genetic engineering. Ti plasmid (tumour inducing) from the soil bacterium Agrobacterium tumefaction’s is effectively used as vector for gene transfer to plant cells. This is, so called because in nature, it induces tumours in broad leaf plants such as tomato, tobacco and soybean.

For using Ti plasmid as a vector, researchers have eliminated its tumour causing properties while keeping its ability to transfer DNA into plant cells. This bacterium is called natural genetic engineer because genes carried by its plasmid produce effect in several parts of the plant. Ri plasmid of A. rhizogenes is also being used as vector.

 (i) This bacterium infects all broad-leaved agricultural crops such as tomato, soybean, sunflower and cotton etc. It does not infect cereals. It induces formation of cancerous growth called a crown gall tumour. This transformation of plant cells is due to the effect of Ti plasmid carried by the pathogenic bacterium. Hence, for genetic engineering purposes, Agrobacterium strains are developed in which tumour-forming genes are deleted. These trans¬formed bacteria can still infect plant cells,

(ii) The part of Ti plasmid transferred into plant cell DNA, is called the T-DNA. This T-DNA with desired DNA spliced into it, is inserted into the chromosomes of the host plant where it produces copies of itself, by migrating from one chromosomal position to another at random. But it no longer produces tumours,

(iii) Such plant cells are then cultured, induced to multiply and differentiate to form plantlets.

(iv) Transferred into soil, the plantlets grow into mature plants, carrying the foreign gene, expressed throughout the new plant.

**Insect Resistance in Transgenic Plants:**

**Bt Cotton:**

Soil bacterium Bacillus thuringiensis (Bt for short) produces proteins that kill certain insects like lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes). Bacillus thuringiensis forms some protein crystals. These crystals contain a toxic insecticidal protein. Why does this toxin not kill the Bacillus (bacte-rium)? The Bt toxin proteins exist as inactive protoxins but once an insect ingests the inactive toxin it is converted into an active form of toxin due to the alkaline pH of the alimentary canal that solublizes the crystals. The activated toxin binds to the surface of midgut epithelial cells and creates pores which cause cell swelling and lysis and finally cause death of the insect.

Bt toxin genes were isolated from Bacillus thuringiensis and incorporated into several crop plants such as cotton. The choice of genes de¬pends upon the crop and targeted pest, as most Bt toxins are insect-group specific. The toxin is coded by a gene named cry. These are numerous genes. Two cry genes, cry lAc and cry II Ab have been incorporated in cotton. The genetically modified crop is called Bt cotton as it contains Bt toxin genes. The genes cry I Ac and cry II Ab control cotton bollworms. Similarly, cry I Ab has been introduced in Bt com to protect the same from corn borer.

Gene symbol usually has small letters and is invariably in italics, e.g., cry. The first letter of the protein symbol, on the other hand, is always capital and the symbol is always written in roman letters, e.g., Cry.

The Government has agreed to allow cultivation of genetically modified Bt Cotton.

Bt cotton farming has shown good results in Malwa region in Punjab. The government should encourage such farming. It will save water starved Malva region from turning into desert as cotton which needs much less water, will replace paddy.

**Pest Resistance in Transgenic Plants (Protection against Nemotodes):**

Many nematodes (Round worms) live in plants and animals including human beings. A nematode Meloidogyne incognitia infects the roots of tobacco plants and causes a great reduction in yield. A novel strategy was coined by Fire and Mello in 1998 to prevent this infestation that was based on the process of ***RNA interference (RNAi***). RANi takes place in all eukaryotic organisms as a method of cellular defence. This method involves silencing of a specific mRNA.

Using Agrobacterium vectors, nema¬tode specific genes are introduced into the host plant (tobacco plant). The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells. These two RNAs being complementary to each other formed a dsRNA (double stranded RNA) that initiated RNAi.

Different steps involved in making tobacco plant resistant to nematode are briefly described below:

1. Double-stranded RNAs are processed into approximately 21-23 nucleotide RNAs with two nucleotides. An RNase enzyme called Dicer cuts the dsRNA moelcules (from a virus, transposon, or through transformation) into ***small interfering RNAs (siRNAs).***

2. Each siRNA complexes with ribonucleases (distinct from Dicer) to form an ***RNA-induced silencing complex (RISC).***

3. The siRNA unwinds and RISC is activated.

4. The activated RISC targets complementary mRNA molecules. The siRNA strands act as guides where the RISCs cut the transcripts in an area where the siRNA binds to the mRNA. This destroys the mRNA.

5. When mRNA of the parasite is destroyed no protein was synthesized. It resulted the death of the parasite (nematode) in the transgenic host. Thus the transgenic plant got itself protected from the parasite.

**‘Flavr Sarv’ Transgenic Tomatoes:** (Post-Harvest Losses/Delayed Fruit Ripening):

In ‘Flavr Sarv’ transgenic tomato, expression of a native tomato gene has been blocked. This gene produces enzyme polygalacturonase which promotes softening of fruit. The production of this enzyme was reduced in the Flavr Sarv transgenic tomato. The non-availability of this enzyme prevents over-ripening because the enzyme is essential for degradation of cell walls. Thus fruit remains fresh for a longer period than the fruit of normal tomato variety. It retains flavour, has superior taste and higher quantity of total soluble solids.

**Golden Rice:**

Golden rice is a transgenic variety of rice (Oryza sativa) which contains good quantities of β-carotene (provitamin A – inactive state of vitamin A). β-carotene is a principal source of vitamin A. Since the grains (seeds) of the rice are yellow in colour due to P-carotene, the rice is commonly called golden rice.

β-carotene (provitamin A) is converted into vitamin A. Thus golden rice is rich in vitamin A. It is required by all individuals as it is present in retina of eyes. Deficiency of vitamin A causes night blindness and skin disorder.

Since the contents of vitamin A are very low in rice, vitamin A is synthesised from β- carotene which is precursor of vitamin A. Prof. Ingo Potrykus and Peter Beyer produced genetically engineered rice by introducing three genes associated with synthesis of carotene. The grains (seeds) of transgenic rice are rich in provitamin.

**Transgenic Tobacco Plants:**

 Brassica napus — Production of Hirudin (Fig. 12.6):

Hirudin is a protein that prevents blood clotting. Its gene was chemically synthesized and was transferred into Brassica napus where hirudin accumulates in seeds. The hirudin is extracted and purified and used as medicine.

**Diagnostic and Therapeutic Proteins:**

Transgenic plants can produce a variety of proteins used in diagnostics for detecting and curing human and animal diseases in large scale with low cost. The monoclonal antibodies, peptide hormones, cytokinins and blood plasma proteins are being produced in transgenic plants and their parts such as tobacco (in leaves), potato (in tubers), sugarcane (in stems) and maize (in seed endosperm)

**Disease Resistance:**

There are many viruses, fungi and bacteria that cause plant diseases. Plant biologists are working to create plants with genetically engineered resistance to these diseases.

**Transgenic Plants for Floriculture:**

In 1990, the production of transgenic ornamental plants also gained momentum and transformation procedures became available for many ornamental plants, e.g., rose, tulip, lily, etc. Several of these cut flowers, many transgenic have novel aesthetic properties including new colours, longer life, etc. Some of these plants have commercial demand. Flower colour comes mainly from anthocyanin’s, a class of coloured flavonoids.

GM crops contain and express one or more useful foreign genes or transgenes. The technique of GM crops has two advantages.

(i) Any gene from any organism or a synthetic gene can be incorporated.

(ii) Change in genotype is precisely controlled. This technology is superior to breeding programmes because in breeding only the already present genes are reshuffled and that changes would occur in all traits for which the parents are different.

**Advantages of Transgenic Plants (= GM Plants):**

Due to genetic modification, GM plants have been useful in many ways:

1. Pest Resistance Crops:

Growing GM crops can help to reduce the use of chemical pesticides, e.g., Bt Cotton.

2. Tolerance:

GM crops have made more tolerant to abiotic stresses (cold, drought, salt, heat, etc.)

3. Reduction in Post-harvest Losses:

They have helped to reduce post harvest losses, e.g., Flavr Sarv transgenic tomato.

4. Prevention of Early Exhaustion of Fertility of Soil:

Increased efficiency of min¬eral usage by plants prevents early exhaustion of fertility of soil.

5. Increasing Nutritional Value of Food:

GM plants enhance nutritional value of food, e.g., golden rice is rich in vitamin A.

6. Herbicide Resistance:

Herbicides (weed killers) do not harm the GM crops.

7. Alternative Resources to Industries:

GM plants have been used to create alter¬native resources to industries in the form of starches, fuels and pharmaceuticals. Researchers are working to develop edible vaccines, edible antibodies and edible interferon’s.

8. Disease Resistance:

Many viruses, bacteria and fungi cause plant diseases. Scien¬tists are working to create genetically engineered plants having resistance to these diseases.

9. Phytoremediation:

Plants such as popular trees have been genetically engineered to clean up heavy metal pollution from contaminated soil.

**Disadvantages of Transgenic Plants (GM Plants):**

**1. Environmental hazards:**

These are as follows:

(i) Unintended harm to other organisms:

A laboratory study was published in ‘Nature’ showing that pollen from Bt corn caused high mortality rates in monarch butterfly caterpillars. Monarch caterpillars consume milkweed plants, not com, but the fear is that if pollen from Bt com is blown by the wind on to milkweed plants in neighbouring fields, the caterpillars could eat the pollen and perish. Although the ‘Nature’ study was not conducted under natural field conditions, the results seemed to support this viewpoint.

(ii) Reduced effectiveness of pesticides:

Just as some populations of mosquitoes developed resistance to the now-banned pesticide DDT, many people are concerned that insects will become resistant to Bt or other crops that have been genetically modified to produce their own pesticides.

(iii) Gene transfer to non-target species:

Another concern is that crop plants engi¬neered for herbicide tolerance and weeds will cross-breed, resulting in the transfer of the herbicide resistance genes from the crops into the weeds. These “super-weeds” would then be herbicide tolerant as well. Other introduced genes may cross over into non-modified crops planted next to GM crops.

**2. Human health risks:**

GM food can lead the following health problems.

(i) Allergies:

The transgenic food may cause toxicity and or produce allergies. The enzyme produced by the antibiotic resistance gene can cause allergies, because it is a foreign protein.

(ii) Effect on Bacteria of Alimentary canal:

The bacteria present in the human alimentary canal can take up the antibiotic resistance gene that is present in the GM food. These bacteria can become resistant to the concerned antibiotic and will be difficult to manage.

3. Economic concerns:

Bringing a GM food to market is a lengthy and costly process, and of course agro-biotech companies wish to ensure a profitable return on their investment.

Some other transgenic plants have been produced. These are sunflower, cauliflower, cabbage, banana, pea, lotus, cucumber, carrot, strawberry, papaya, grape, popular, apple, pear, neem, rye, etc.

**Transgenic Microorganisms:**

Various microorganisms, particularly bacteria have been modified through the techniques of genetic engineering to meet specific needs.

1**. Crop Production and Protection:**

Several bacteria have been modified by introduc¬tion of foreign genes to control, (i) insects by production of endotoxins, (ii) fungal disease by production of chitinases, which suppress fungal flora in the soil and (iii) by production of antibiotic which will degrade the toxin produced by pathogen.

There are also positive measures where the N2 fixing efficiency of bacteria Rhizobia can be increased by transfer of useful nif genes, nif means nitrogen fixation.

2. **Biodegradation of Xenobiotic and Toxic Wastes:**

Bacteria can be modified geneti¬cally for degradation of xenobiotic (Wastes from non-biological systems) and other waste material. Bacterial genes for this purpose are isolated from bacteria found at waste sites. For example bacteria Pseudomonas are not very efficient degraders but multiple genes may sometimes be needed for efficient biodegradation. Therefore, for efficient biodegradation, efficient degraders have to be prepared through genetic engineering.

3. **Production of Chemicals and Fuels:**

Genetic engineering also has an important impact on microbial production of chemicals and fuels. Examples: (i) genetically engineered strains of Bacillus amyloliquefaciens and Lactobacillus casei have been prepared for pro¬duction of amino acids on a large scale (ii) E. coli and Klebsiella planticola carrying genes from Z. mobilis could utilize glucose and xylose to give maximum yield of ethanol.

4. **Living Factory for the Production of Proteins:**

In bacteria, genetic engineering turns the bacterium into a living factory for the production of proteins. Examples: The transfer of genes for human insulin, human growth hormone (hGH) and bovine growth hormone.

**Transgenic Animals:**

The animals which carry foreign genes are called transgenic animals.

Production of Transgenic Animals:

The foreign genes are inserted into the genome of the animal using recombinant DNA technology. The production of transgenic animals includes

(i) Location, identification and separation of desired gene,

(ii) Selection of proper vector (generally a virus) or direct transmission,

(iii) Combining the desired gene with the vector,

(iv) Introduction of transferred vector in cells, tissues, embryo or mature individual,

(v) Demonstration of integration and expression of foreign gene in transgenic tissue or animal.

**Advantages of Transgenic Animals:**

(i) Biological Products:

Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make. Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (a-1-antitrypsin) used to treat emphysema, tissue plasmogen activator (goat), blood clotting factors VIII and IX (sheep) and lactoferrin (cow).

Attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis. In 1997, the first transgenic cow, Rosie, produced human protein- enriched milk (2.4 gms per litre). The milk contained the human alpha-lactalbumin. It is a more balanced product for human babies than natural cow-milk.

(ii) Vaccine Safety:

Transgenic mice are being formed for use in testing the safety of vaccines before they are used on human beings. Transgenic mice are being used to test the safety of the polio vaccine.

(iii) Chemical Safety Testing:

It is called as toxicity/safety testing. Transgenic animals are developed that carry genes exposed to the toxic substance and their effects are studied.

(iv) Normal Physiology and Development:

Transgenic animals are specifically developed to study how genes are regulated, and how they affect the normal functions of the body and its development, e.g., study of complex factors involved in growth such as insulin-like growth factor.

(v) Study of Diseases:

Many transgenic animals are developed to increase our understanding of how genes contribute to the development of disease so that investigation of new treatments for diseases is made possible. Now transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis, Alzheimer’s disease, haemophilia, thalessaemia, etc.

(vi) Growing of Spare Parts:

Spare parts (e.g., heart, pancreas) of pig for human use can be grown through the formation of transgenic animals.

(vii) Replacement of Defective Parts:

Replacement of defective parts with freshly grown part from own cells can be done.

(viii) Production of Clones:

Clones of some animals can be produced. Even human clones may be formed if ethics allow the same.

**Examples of Transgenic Animals:**

Some important examples of transgenic animals are as follows:

1. Transgenic Fish:

Gene transfers have been successful in various fish, such as common carp, rainbow trout, Atlantic salmon, catfish, goldfish, zebra-fish, etc.

Transgenic Salmon:

Genetically modified salmon was the first transgenic animal for food production. The genetically modified sperms were fused with normal ova (eggs) of the same species. The zygotes which developed into embryos gave rise to much bigger adults than either parent. The transgenic salmon possesses an additional gene that codes for the growth hormone that allows the fish to grow larger more rapidly than the non-transgenic salmon.

2. Transgenic Chicken:

Avian leukosis virus (ALV) is a serious viral pathogen of chickens. D.W. Salter and L.B. Crittenden (1988) have produced an ALV-resistant strain of the chicken by introduc¬ing a defective genome of this virus into the genome of the chicken. This principle is also applied to evolve transgenic fish that can resist viral infections.

3. Transgenic Mice:

Mouse is the most preferred mammal for studies on gene transfers due to its many favourable features like short oestrous cycle and gestation period, relatively short generation time, production of several offspring per pregnancy (i.e., litter), convenient in vitro fertilization, successful culture of embryos in vitro, etc. As a result, the techniques for gene transfer and transgenic produc¬tion have been developed using mice as models in other animals. Recently, rats and rabbits are being used for research work on gene transfer.

 4. Transgenic Rabbits:

Rabbits are quite promising for gene farming or molecular farming, which aims at the production of recoverable quantities of pharmaceutically or biologically important proteins encoded by the transgenes.

The following human genes encoding valuable proteins have been transferred into rabbits: interleukin 2, growth hormone, tissue plasmi¬nogen activator, α1 antitrypsin, etc. These genes were expressed in the mammary tissues and their proteins were harvested from milk.

5. Transgenic Goats:

Goats are being evaluated as bioreactors. Some human genes have been introduced in goats and their expression achieved in mammary tissues. The initial results are encouraging.

6. Transgenic Sheep:

Transgenic sheep have been produced to achieve better growth and meat production. For example, human genes for blood clotting factor IX and for α1-antitryspin have been trans-ferred in sheep and expressed in mammary tissue. This was achieved by fusing the genes with the mammary tissue-specific promoter of the bovine β-lactoglobulin gene. Human growth hormone gene has also been introduced in sheep in order to promote growth and meat production. However, they also showed several undesirable effects like joint pathology, skeletal defects, gastric ulcers, infertility, etc.

In 1990 Tracy, the transgenic ewe was born in Scotland.

7. Transgenic Pigs:

The rate of transgenic production in pigs, sheep, cattle and goats is much lower (usually < 1%) than that in mice (usually between 3-6%). The objectives in transgenic swine (pi. same, meaning pig), production are (i) increased growth and meat production and (ii) to serve as bioreactors. Transgenic pigs expressing human growth hormone do show improved growth and meat production, but they also show several health problems.

In January 2002, an Edinburgh based therapeutics company announced the birth of a litter of transgenic pig clones.

8. Transgenic Cows:

The only successful transfection technique in cows is microinjection of fertilized ova, which may either be recovered surgically or may be obtained from ovaries extracted from slaughtered cows and cultured in vitro. The two chief objectives of transgenic production are as follows: (i) increased milk or meat production and (ii) molecular farming. Several human genes have been successfully transferred in cows and expressed the mammary tissue; the protein is secreted in milk from where it is easily harvested. The name of first transgenic cow is Rosie.

9. Transgenic Dogs:

Dogie is a transgenic dog with excellent smelling power. It was used during attack on World Trade Centre (WTC) of the USA in 2001 to recover injured people from heaps of devastated building.

10. ANDI:

DNA of a fluorescent jelly fish was introduced into an unfertilized egg of a Rhesus monkey in the test tube. The diploid egg underwent cleavage and the early embryo was implanted in a surrogate mother. ANDI, the first transgenic monkey was born on Oct 2, 2000. It has been named ANDI, the acronym of “inserted DNA”.

The credit for production of ANDI goes to Dr. Gerald Schatten of Oregon Health Sciences University, USA.

This work would be helpful for curing diseases such as breast cancer, Alzheimer’s disease, diabetes and AIDS.

i. Recently rats and rabbits are being used for research work on genetic transfer.

ii. The first transgenic farm animals were rabbits, pigs and sheep which were produced in 1985.

iii. The first transgenic animal was mouse which was produced in 1981/82.

iv. In plants gene transfer is often described by the term “transformation”. However in animals this term has been replaced by the term “transfection”.

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