BIOTECHNOLOGY AND ITS APPLICATIONS

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE –

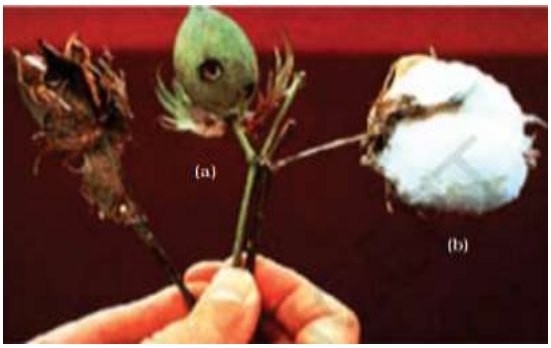
The Green Revolution succeeded in tripling the food supply but yet it was not enough to feed the growing human population. Increased yields have partly been due to the use of improved crop varieties, but mainly due to the use of better management practices and use of agrochemicals (fertilizers and pesticides). However, for farmers in the developing world, agrochemicals are often too expensive, and further increases in yield with existing varieties are not possible using conventional breeding.

Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called **Genetically Modified Organisms (GMO).** GM plants have been useful in many ways. Genetic modification has:

1) made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
2) reduced reliance on chemical pesticides (pest-resistant crops).
3) helped to reduce post harvest losses.
4) increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).
5) enhanced nutritional value of food, e.g., golden rice, i.e., Vitamin 'A' enriched rice.

Some of the applications of biotechnology in agriculture that you will study in detail are the production of pest resistant plants, which could decrease the amount of pesticide used. Bt toxin is produced by a bacterium called Bacillus thuringiensis (Bt for short). Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the need for insecticides; in effect created a bio-pesticide. Examples are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.

Bt Cotton: Some strains of Bacillus thuringiensis produce proteins that kill certain insects such as lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes). B. thuringiensis forms protein crystals during a particular phase of their growth. These crystals contain a toxic **insecticidal protein.** The Bt toxin protein exist as inactive protoxins but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilise the crystals. The activated toxin binds to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis and eventually cause death of the insect.



Cotton boll: (a) destroyed by bollworms; (b) a fully mature cotton boll

Pest Resistant Plants: A nematode Meloidegyne incognitia infects the roots of tobacco plants and causes a great reduction in yield. A novel strategy was adopted to prevent this infestation which was based on the process of **RNA interference** (RNAi). RNAi takes place in all eukaryotic organisms as a method of cellular defense. This method involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA (silencing). The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

The transgenic plant therefore got itself protected from the parasite.

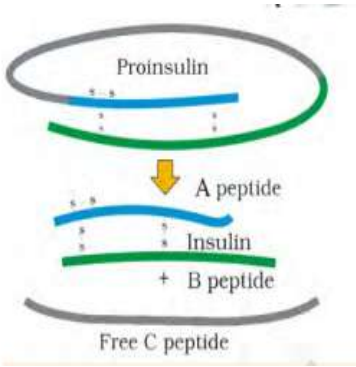
Host plant-generated dsRNA triggers protection against nematode infestation: (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

At present, about 30 recombinant therapeutics have been approved for human-use the world over. In India, 12 of these are presently being marketed.

(1) Genetically Engineered Insulin – Management of adult-onset diabetes is possible by taking insulin at regular time intervals.

Insulin used for diabetes was earlier extracted from pancreas of slaughtered cattle and pigs. Insulin from an animal source, though caused some patients to develop allergy or other types of reactions to the foreign protein. Insulin consists of two short polypeptide chains: chain A and chain B, that are linked together by disulphide bridges. In mammals, including humans, insulin is synthesized as a pro-hormone (like a pro-enzyme, the pro-hormone also needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the C peptide. This C peptide is not present in the mature insulin and is removed during maturation into insulin. The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form. In 1983, Eli Lilly an American company prepared two DNA sequences corresponding to A and B, chains of human insulin and introduced them in plasmids of E. coli to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin.



Maturation of pro-insulin into insulin (simplified)

Gene Therapy

If a person is born with a hereditary disease, can a corrective therapy be taken for such a disease? Gene therapy is an attempt to do this. Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo. Here genes are inserted into a person's cells and tissues to treat a disease. Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.

The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency. This enzyme is crucial for the immune system to function. The disorder is caused due to the deletion of the gene for adenosine deaminase. In some children ADA deficiency can be cured by bone marrow transplantation; in others it can be treated by enzyme replacement therapy, in which functional ADA is given to the patient by injection. But the problem with both of these approaches that they are not completely curative. As a first step towards gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body. A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are subsequently returned to the patient. However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. However, if the gene isolate from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

Molecular Diagnosis

You know that for effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important. Using conventional methods of diagnosis (serum and urine analysis, etc.) early detection is not possible. Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.

Presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom. By this time the concentration of pathogen is already very high in the body. However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR. Can you explain how PCR can detect very low amounts of DNA? PCR is now routinely used to detect HIV in suspected AIDS patients. It is being used to detect mutations in genes in suspected cancer patients too. It is a powerful techqnique to identify many other genetic disorders.

A single stranded DNA or RNA, tagged with a radioactive molecule (probe) is allowed to hybridise to its complementary DNA in a clone of cells followed by detection using autoradiography. The clone having the mutated gene will hence not appear on the photographic film, because the probe will not have complementarity with the mutated gene.

ELISA is based on the principle of antigen-antibody interaction. Infection by pathogen can be detected by the presence of antigens (proteins, glycoproteins, etc.) or by detecting the antibodies synthesised against the pathogen.

(2) TRANSGENIC ANIMALS

Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as **transgenic animals**.

(3) Biotechnological Application in industry –

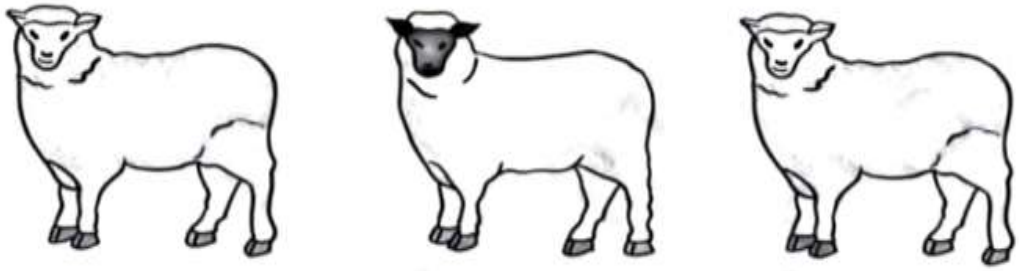
- This is generally also known as 'White Technology'.
- It is used to develop microorganism which can increase the rate of fermentation of organic matter in order to convert it into alcohol, acid and Biomass.
- Eg – **(1) Oil Zapper –** Mixture of 5 types of Bacteria which are used to clean oil from surface of water.
- **(2) Phytomining –** It is an approach in which mining is done with the help of plants which absorb minerals in roots which is burned and the ash received is full of that compound.

(4) Biotechnology application in Animal Husbandry –

(a) Cloning –

Story of Dolly, the Clone

Cloning is the production of an exact copy of a cell, any other living part, or a complete organism. Cloning of an animal was successfully performed for the first time by Ian Wilmut and his colleagues at the Roslin Institute in Edinburgh. Scotland. They successfully cloned a sheep named Dolly. Dolly was born on 5th July 1996 and was the first mammal to be cloned.



(a) Finn Dorsett sheep *(b) Scottish blackface ewe* *(c) Dolly*

During the process of cloning Dolly, a cell was collected from the mammary gland of a female Finn Dorsett sheep. Simultaneously, an egg was obtained from a Scottish blackface ewe. The nucleus was removed from the egg. Then, the nucleus of the mammary gland cell from the Finn Dorsett sheep was inserted into the egg of the Scottish blackface ewe whose nucleus had been removed. The egg thus produced was implanted into the Scottish blackface ewe. Development of this egg followed normally and finally Dolly was born. Though Dolly was given birth by the Scottish blackface ewe, it was found to be absolutely identical to the Finn Dorsett sheep from which the nucleus was taken. Since the nucleus from the egg of the Scottish blackface ewe was removed. Dolly did not show any character of the

Scottish blackface ewe. Dolly was a healthy clone of the Finn Dorsett sheep and produced several offspring of her own through normal Sexual means. Unfortunately, Dolly died on 14th February 2003 due to a certain lung disease.

Since Dolly, several attempts have been made to produce cloned mammals. However, many die before birth or die soon after birth. The cloned animals are many-a-times found to be born with severe abnormalities.

(5) Biotechnology in Environment –

- Issue related to environment are worked upon.
- Eg:- Pollution
- Biomass use
- Bioremediation

Biotechnology –

Biotechnology can be defined as **the industrial** application of living organisms and their biological processes such as biochemistry, microbiology, and genetic engineering. The overall objective is to make the best use of microorganisms for the benefit of mankind.

Different types of biotechnology –

- Green biotechnology
- Red biotechnology
- White biotechnology
- Blue biotechnology
- Yellow biotechnology

Green Biotechnology

- It incorporates the application of biological techniques to plants to enhance the quantity, nutritional quality, and production economics.
- This is performed to plant economically valuable plants by implanting foreign genes.
- **This consists of three main areas:**
  - plant tissue culture
  - plant genetic engineering
  - plant molecular marker-assisted breeding

Red Biotechnology

- Red biotechnology is mainly concerned with the discovery, innovation and development of new drugs and treatments.

- A critical prerequisite was an enhanced understanding of how proteins function, their role in communication between & within cells, and the diseases caused by malfunctioning of these proteins.
- This includes Gene Therapy, Stem Cells, Genetic Testing, etc.

White Biotechnology

- This field of biotechnology is associated with the industry.
- White biotech uses yeasts, molds, enzymes, and bacteria to produce goods and services or parts of products.
- It offers a wide range of bio-products like detergents, vitamins, antibiotics, etc.
- As compared to the traditional methods, most of the white biotech processes result in the saving of energy, water, chemicals and the reduction of waste.

Blue Biotechnology

- Blue biotechnology is associated with the application of molecular biological methods to freshwater and marine organisms.
- It involves the use of marine & freshwater organisms, and their derivatives, for various purposes, the most remarkable one is the identification and development of new active ingredients from marine origin.

Yellow Biotechnology

- Yellow biotechnology' refers to biotechnology with insects- analogous to the green (plants) and red (animals) biotechnology.