Biotechnology and its Applications

Biotechnology has a wide range application such as biopharmaceuticals, therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment and energy production.

Biotechnology has 3 critical research areas:

a. Providing the best catalyst in the form of improved organism usually a microbe or pure enzyme.
b. Creating optimal conditions through engineering for a catalyst to act.
c. Downstream processing technologies to purify the protein/organic compound.

APPLICATIONS IN AGRICULTURE

3 options for increasing food production:

a. Agro-chemical based agriculture.
b. Organic agriculture.
c. Genetically engineered crop-based agriculture.

Genetically Modified Organisms (GMO) or **transgenic organisms** are the plants, bacteria, fungi & animals whose genes are altered by manipulation.

Advantages of genetic modification in plants:

It makes crops more tolerant to abiotic stresses (cold, drought, salt, heat etc).

Pest-resistant crops reduce the use of chemical pesticides.

It helps to reduce post-harvest losses.

It increases efficiency of mineral usage by plants (it prevents early exhaustion of fertility of soil).

It enhances nutritional value of food. E.g. Vitamin enriched rice.

To create tailor-made plants to supply alternative resources (starches, fuels, pharmaceuticals etc.) to industries.

Pest Resistant Plants

- They act as **bio-pesticide.**
- It reduces the need for insecticides.
- E.g. Bt cotton, Bt corn, rice, tomato, potato, soyabean etc.

Bt Cotton:

- Some strains of *Bacillus thuringiensis* have proteins that kill insects like coleopterans (beetles) lepidopterans (tobacco budworm, armyworm) & dipterans (flies, mosquitoes).
- *B. thuringiensis* forms a toxic insecticidal protein **(Bt toxin)** crystal during a particular phase of their growth. It does not kill the *Bacillus* as it exists as inactive *protoxins*.
- When an insect ingests the toxin, it becomes active due to the alkaline pH of the gut which solubilise the crystals. The toxin binds to the surface of mid-gut epithelial cells and creates pores. It causes cell swelling and lysis and death of the insect.
- Bt toxin genes were isolated from *B. thuringiensis* and incorporated into crop plants such as cotton.
- Most Bt toxins are insect-group specific. The toxin is coded by a gene named **cry**. E.g. proteins encoded by the genes *cryIAc* & *cryIIAb* control the cotton bollworms that of *cryIAb* controls corn borer.

Nematode resistance in tobacco plants:

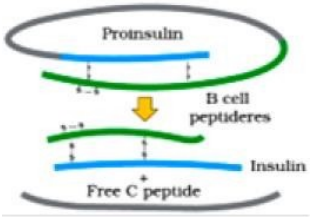
- A nematode *Meloidogyne incognitia* infects the roots of tobacco plants causing a reduction in yield.
- It can be prevented by **RNA interference** (RNAi) strategy.
- **RNAi** is a method of cellular defense in all eukaryotic organisms. It prevents translation of a specific mRNA (silencing) due to a complementary dsRNA molecule.
- The source of this complementary RNA is from an infection by RNA viruses or mobile genetic elements (transposons) that replicate via an RNA intermediate.
- Using *Agrobacterium* vectors, nematode-specific genes (DNA) are introduced into host plant. It produces both sense & anti-sense RNA in host cells. These RNAs are complementary. So they form double stranded (ds) RNA. It initiates RNAi and silences the specific mRNA of nematode. Thus the parasite cannot survive in a transgenic host expressing specific interfering RNA.

APPLICATIONS IN MEDICINE

- The recombinant DNA technology helps for the mass production of safe and more effective therapeutic drugs.
- The products from non-human sources induce unwanted immunological responses. But recombinant therapeutics does not have such problems.
- At present, about 30 recombinant therapeutics have been approved. Of these, 12 are being marketed in India.

1. Genetically Engineered Insulin

- Insulin is used to manage adult-onset diabetes.
- Insulin from the pancreas of animals (cattle & pigs) causes allergy or other types of reactions to the foreign protein.
- Now, it is possible to produce human insulin using bacteria.
- Insulin consists of two short polypeptide chains (chain A & chain B) that are linked by disulphide bridges.
- In mammals, insulin is synthesized as a **pro-hormone (pro-insulin).** It needs processing to become mature and functional hormone.



- The pro-hormone contains an extra stretch called **C peptide**. This is removed during maturation into insulin.
- In 1983, Eli Lilly (an American company) prepared two DNA sequences corresponding to A & B chains of human insulin and introduced them in plasmids of *E. coli* to produce insulin chains. Chains A & B were combined by creating disulfide bonds to form human insulin.

2. Gene Therapy

- It is a method to correct a gene defect in a child/embryo.
- Here, genes are inserted into a person's cells and tissues to treat a hereditary disease. It compensates for the non-functional gene.
- First clinical gene therapy (1990) was given to a 4-year old girl with **adenosine deaminase (ADA) deficiency.** The disorder is caused due to the deletion of the gene for ***adenosine deam inase*** (an enzyme crucial for the immune system to function).
- This can be cured by bone marrow transplantation or by enzyme replacement therapy (injection of functional ADA). But these approaches are not completely curative.
- In gene therapy, lymphocytes from the patient's blood are grown in a culture. Then, a functional ADA cDNA (using a retroviral vector) is introduced into these lymphocytes. Then, they are returned to the patient. This should be periodically repeated as these cells are not immortal. If the ADA gene (from marrow cells) is introduced into cells at early embryonic stages, it could be a permanent cure.

3. Molecular Diagnosis

- Early diagnosis of diseases using conventional methods (serum and urine analysis) are not possible.
- **Recombinant DNA technology, PCR & ELISA** are some techniques for early diagnosis.

PCR (Polymerase Chain Reaction):

- Presence of a pathogen is normally suspected only based on symptoms. By this time, the concentration of pathogen is already very high in the body.
- However, very low concentration of a bacteria or virus can be detected by amplification of their nucleic acid by PCR.
- **Uses of PCR:**
  - To detect HIV in suspected AIDS patients.
  - To detect gene mutations in suspected cancer patients.
  - To identify many other genetic disorders.
- A single stranded DNA or RNA, tagged with a radioactive molecule (probe) is hybridized to its complementary DNA in a clone of cells followed by detection using autoradiography. The clone having mutated gene will not appear on the photographic film, because the probe will not have complementarity with the mutated gene.

ELISA (Enzyme Linked Immuno-Sorbent Assay):

- It 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 synthesized against the pathogen.

TRANSGENIC ANIMALS

- These are the animals whose genome has been altered by introduction of an extra (foreign) gene by manipulation.
- E.g. Transgenic rats, rabbits, pigs, sheep, cows and fish.
- Over 95% of all existing transgenic animals are mice.

Benefits of transgenic animals

To study the regulation of genes and their action on normal physiology & development:
E.g. study of complex factors such as insulin-like growth factor. Genes (from other species) that alter the formation of this factor are introduced and the biological effects are studied. This gives information about the biological role of the factor in the body.

To Study the contribution of genes in the development of a disease and thereby new treatments: E.g. transgenic models for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis & Alzheimer's.

Biological products: Some medicines contain expensive biological products. Transgenic animals are used to produce useful biological products by introducing genes which codes for a particular product.
E.g. **human protein (a-1-antitrypsin)** used to treat emphysema, products for treatment of phenylketonuria (PKU) and cystic fibrosis etc.
In 1997, **Rosie** (first transgenic cow) produced human protein-enriched milk (2.4 gm per litre). It contains the human a **-lactalbumin** and is nutritionally more balanced product for human babies than natural cow-milk.

Vaccine safety testing: Transgenic mice are used to test the safety of the polio vaccine. If it is reliable, they can replace the use of monkeys to test the safety of vaccines.

Chemical safety testing (toxicity testing): Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are exposed to the toxic substances and the effects studied. It gives immediate results.

ETHICAL ISSUES

Problem of unpredictable results: Genetic modification may cause unpredictable results.
Indian Government has set up organizations like **GEAC** (Genetic Engineering Approval Committee) to make decisions about the validity of GM research and the safety of GM-organisms for public services.

Problems of patent: Certain companies have got patents for products and technologies that make use of the genetic materials, plants etc. that have been identified, developed and used by farmers and indigenous people of a country. E.g. Basmati rice, herbal medicines (turmeric, neem etc).
Basmati rice has unique aroma & flavour. India has 27 varieties of Basmati. In 1997, an American company got patent rights on Basmati rice through the US Patent and Trademark Office. This allowed the company to sell a 'new' variety of Basmati. This was actually derived from Indian farmer's varieties. Indian Basmati was crossed with semi-dwarf varieties and claimed as a novelty. Other people selling Basmati rice could be restricted by patent.

Biopiracy: It is the use of bio-resources by multinational companies and other organizations without proper authorization from the countries and people concerned. Most of the industrialized nations are poor in biodiversity and traditional knowledge. The developing and the underdeveloped world have rich biodiversity and traditional knowledge related to bio-resources.

It has to develop laws to prevent unauthorized exploitation of bio-resources and traditional knowledge.

Indian Parliament has cleared the second amendment of the **Indian Patents Bill** that has considered patent terms emergency provisions and research and development initiative.