BIOTECHNOLOGY AND ITS APPLICATIONS

The critical areas of biotechnology are:

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

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE:

- Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called **Genetically Modified Organisms (GMO).**
- **Advantages of Genetic Modification in plants.**
  - Made crops more tolerant to abiotic stresses (cold, drought, salt, heat)
  - Reduce reliance on chemical pesticides (pest resistant crop)
  - Helped to reduce post harvest losses.
  - Increased efficiency of mineral usage by plants.
  - Enhanced nutritional values of food e.g. vitamin A enriched rice.

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.**
- These proteins are present in inactive ***protoxin*** form, but become active toxin in the alkaline pH of insect gut.
- 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 insect
- Specific Bt toxin genes were isolated form B. thuringiensis and genetically transferred to several plants such as cotton.
- **Crystal proteins** are produced by a gene called **cry** in *B. thuringiensis*.
- The protein coded by genes **cryIAc** and **cryIIAb** control the cotton **bollworms.**
- The protein coded by gene **cryIAb** controls **corn borer.**

Pest resistant plants:

- Several **nematodes** parasitize a wide variety of plants and animals including human beings.
- A nematode ***Meloidegyne incognitia*** infects the root of **tobacco plants** and causes a great reduction in yield.
- Strategy based on **RNA interference (RNAi)** prevents this infestation.
- Process by which double-stranded RNA (dsRNA) directs sequence-specific degradation of mRNA

Steps of RNA interference:

- Double stranded RNA is produced endogenously or exogenously.
- Using Agrobacterium vectors nematode specific genes were introduced into the host plant (tobacco plant).
- Introduction of DNA produces both sense and antisense RNA in the host.
- These two RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi.
- The dsRNA injected into the host plant from outside called exogenous dsRNA.
- The dsRNAs are cleaved into 21-23 nt segments **("small interfering RNAs",** or **siRNAs)** by an enzyme called **Dicer**.
- siRNAs are incorporated into **RNA-induced silencing complex (RISC)**
- Guided by base complementarity of the siRNA, the RISC targets mRNA for degradation.
- The consequence was that the parasite could not survive in a transgenic host.

BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE:

- Biotechnology enables **mass production** of safe and more effective therapeutic drugs.
- Recombinant therapeutics does not induce **unwanted immunological responses** as is common in case of similar products isolated from non-human sources.
- At present around 30 recombinant therapeutics, approved for human-use.

Genetically Engineered Insulin:

- Taking insulin at regular interval of time is required for adult-onset diabetes.
- Previously the source of insulin was the slaughtered cattle and pigs.
- This insulin caused allergy in some patients.
- Each insulin made of two short polypeptide chains; chain A and chain B that are linked together by disulphide linkage.
- Insulin synthesized in pancreas as pro-hormone which is a single polypeptide with an extra stretch called **C-peptide.**
- C-peptide is removed during matured insulin.
- 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.
- Chain A and chain B produced separately, extracted and combined by creating disulfide bonds to form mature human insulin.

Gene therapy:

- Gene therapy is an attempt to cure hereditary or genetic diseases.
- Genes are inserted into a person's cells and tissue to treat the disease.
- The first clinical gene therapy was given in 1990 to a 4-yr old girl with adenosine deaminase (ADA) deficiency.
- This enzyme is required for breakdown of **deoxyadenosine** into uric acids.

- In the absence of ADA toxic deoxyadenosine is accumulated and destroy the infection fighting immune cells called T-cells and B-cells.
- This disorder is caused due to the deletion of the gene for adenosine deaminase in chromosome 20.

Treatment:

- Treated by bone marrow transplantation.
- Enzyme replacement therapy, involving repeated injections of the ADA enzyme
- Lymphocytes from the blood of the patient are grown in a culture. A functional ADA cDNA is then introduced into these lymphocytes and returned into the body.
- The patient required periodic infusion of genetically engineered lymphocytes because these cells are not immortal.
- Functional ADA cDNA introduced into cells at early embryonic stages, could be the permanent cure.

Molecular diagnosis:

- Early detection of disease is not possible by conventional methods (serum and urine analysis)

Molecular diagnosis techniques:

- Recombinant DNA technology.
- Polymerase chain reaction (PCR)
- Enzyme linked Immuno-sorbent Assay (ELISA)
- Very low concentration of a bacteria or virus can be amplified and detected by PCR.
- It used to detect genetic disorders.
- PCR is use full to mutation in genes in suspected cancerous patient:
    - A single stranded DNA or RNA tagged with radioactive molecule (probe) is allowed to hybridize to its complementary DNA in a clone of cells followed by detection using autoradiography.
    - The clone having mutated gene unable make complementary bonding of probe, hence not appears in photographic film.

TRANSGENIC ANIMALS:

- Animals that have an **alien DNA** which able to express in it is called **transgenic animals.**

Reasons for creation of transgenic animals:

- **Normal physiology and development:**
    - Transgenic animals are specifically designed to allow study of:
        - How the genes are regulated.
        - How the gene affects normal functioning of body
        - How it affects growth and development. E.g. insulin like growth factor.

    - The animals made transgenic to know the biological effect and result.
- **Study of disease:**
    - Transgenic animals are designed to understand how genes contribute to the development of disease like cancers, cystic fibrosis, rheumatoid arthritis and Alzheimer's.
- **Biological products:**
    - Transgenic animals are used to produce biological product of human interest:
        - **α-1-antitrypsin** used to treat emphysema.
        - Proteins for treatment for PKU and cystic fibrosis.
        - Transgenic cow Rosie, produce human protein enriched milk (2.4 gm/lit. **human α-lactalbumin**)
- **Vaccine safety:**
    - Transgenic mice are being developed and use in testing the safety of vaccines before they are used for humans.
    - Polio vaccine is tested in mice.
- **Chemical safety testing:**
    - This is also known as toxicity/safety testing
    - Transgenic animals are made to known the effect of toxic chemicals.

ETHICAL ISSUES:

- GEAC (Genetic Engineering Approval Committee) set up by Indian Govt, which will make decisions regarding validity of GM research and safety of introducing GM-organisms for public services.
- A **patent** is the right granted by a government to an inventor to prevent others from commercial use of his invention
- Patents granted for biological entities and for products derived from them; these patents are called **biopatents**.
- 27 documented varieties of Basmati are grown in India.
- **Biopiracy** is the term used to refer to the use/exploit or patent, of biological resources by multinational companies and other organizations without proper authorization from the countries and people concerned without compensatory payment.