Biotechnology and Its Applications

1 INTRODUCTION

- **Biotechnology** : Essentially deals with industrial scale production of biopharmaceuticals and biologicals using GM microbes, fungi, plants and animals.
- **Applications of biotechnology include** :
  - Therapeutics
  - Diagnostics
  - Genetically modified crops for agriculture
  - Processed food
  - **Bioremediation**
  - Waste treatment
  - Energy production
- **Three critical research areas of biotechnology** :
  - **Providing best catalyst** in the form of improved microbes or pure enzymes
  - **Creating optimal conditions** through genetic engineering
  - **Downstream processing** technologies for purification

2 BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

- Food production could possibly be increased by three ways :
  - (i) Agrochemical based agriculture
  - (ii) Organic agriculture
  - (iii) Genetically engineered crop-based agriculture
- **Green revolution** resulted in **tripling** of food production :
  - **Reasons for success of green revolution :**
    - Improved crop varieties
    - Agrochemicals (fertilisers + pesticides)
    - Better management practices
  - **Problem Area and Hinderances :**
    - Enhancement in food production by green revolution was still **not enough** to feed growing population
    - **Agrochemicals** are often too **expensive** for farmers of **developing world**
    - **Increase in yield** with existing varieties is **not possible** using **conventional breeding**

3 INSECT RESISTANT PLANTS

- Provides resistance to insects without the need for insecticides **(bio-pesticide)**
- Examples of biopesticides are **Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.**

Bt cotton

Bacillus thuringiensis (A bacterium)
Source of the ***cry* gene**

↓

Codes for **Bt toxin**

- Produced during a particular phase of their growth
- Inactive protoxins (Protein crystals)
- Endotoxin

Sequence of events

cry gene from *Bacillus thuringiensis*
↓
Bt cotton (Cells express inactive protoxins)
↓
Ingested by cotton bollworms
↓
Alkaline pH in the midgut of insects solubilises protein crystals
↓
Active toxin
↓
Create pores in midgut epithelial cells
↓
Swelling of midgut epithelial cells and thereby lysis
↓
Death of insect

Genetically Modified Organisms

- **Organisms** including plants, bacteria, fungi and animals whose **genes have been altered by manipulation** are called genetically modified organisms **(GMO)**.
- **Applications of genetic modification :**
  - (i) Made **crops more tolerant** to abiotic stresses (cold, drought, salt, heat).
  - (ii) **Reduced reliance** on chemical pesticides (**pest-resistant crops**).
  - (iii) Helped to **reduce** post harvest **losses**.
  - (iv) Increased efficiency of mineral usage by plants (**prevents early exhaustion of fertility of soil**).
  - (v) Enhanced nutritional value of food, e.g., **golden rice**, i.e., **Vitamin 'A' enriched rice**.
    - **Tailor Made Plants :** Plants have been developed to supply alternative resources to industries in the form of starches, fuels and pharmaceuticals.

- **Choice of gene depends on :**
  - (i) Target pest (ii) Crop
  - *cry I Ac* and *cry II Ab* → Cotton bollworm
  - *cry I Ab* → Corn borer
- **Bt toxins are insect group specific :**
  - **Lepidoptera :** Tobacco budworm, armyworm, cotton bollworm
  - **Coleoptera :** Beetles
  - **Diptera :** Flies and mosquitoes

4 PEST RESISTANT PLANTS

- Method of cellular defense seen in **all eukaryotes** against pest infestation.
- **Technique responsible :** **RNA interference (RNAi)**

  - Based on **post transcriptional silencing of mRNA**
  - Translation of mRNA coded from pest specific genes is silenced/prevented due to formation of **complementary dsRNA**

↓ source

Viruses with RNA genome | Mobile genetic elements **Transposons** replicating via an RNA intermediate

The case of nematode resistant transgenic tobacco :

- Pest causing **roots knot** disease in tobacco plant : ***Meloidogyne incognita*** (Nematode/helminth)
- **Nematode specific gene** is introduced in **host plant (tobacco)**, by using Ti plasmid (vector) of *Agrobacterium tumefaciens*, in such a manner that it produces both **sense** and **antisense RNA** in the host cells.

↓

- Sense RNA and antisense RNA being complementary form **dsRNA** that **initiates RNAi**.

↓

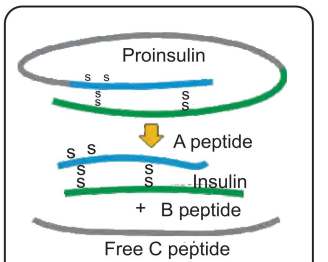
- Parasite could not survive in a **transgenic host** expressing **specific interfering** RNA.

↓

- **Host plant - generated dsRNA triggers protection against nematode infestation**.

5 BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

- **Advantages of Recombinant Therapeutics :**
  - **30 recombinant therapeutics** have been approved for human use the **world** over. In **India**, **12** of these are presently being marketed.
  - Mass production of safe and effective drugs.
  - Do not induce unwanted immunological responses.
- **Genetically Engineered Human Insulin**
  - **Problem :** Insulin extracted from slaughtered cattle and pigs could cause **allergy**.
  - **Solution :** Production of **humulin**



Maturation of proinsulin to insulin

- **Recombiant insulin** manufactured by **Eli Lilly**, an American company, in **1983**
- The **main challenge** for production of insulin using rDNA techniques was **getting insulin assembled into a mature form**

Sequence of events :

Artificially synthesised

DNA sequence for A-chain → Plasmid → *E.coli* → Chain A

DNA sequence for B-chain → Plasmid → *E.coli* → Chain B

Chain A + Chain B → Produced separately, extracted and combined by creating **disulphide bonds**

→ Human insulin

Insulin is a peptide hormone and can be degraded by proteases in our gut

6 GENE THERAPY

- Insertion of genes into an individual's cells to treat diseases by
  (i) Replacing a defective mutant allele with a functional one
  (ii) Gene targeting which involves gene amplification.
- **First clinical gene therapy** was conducted in **1990** in a **4 year old** girl to treat **adenosine deaminase (ADA) deficiency**. ADA enzyme is crucial for **immune system** to function

Treatment for ADA Deficiency

(1) Enzyme replacement therapy
- Functional ADA is given by injection — Not completely curative

(2) Bone marrow transplantation in children

(3) **Gene therapy -** Could be a **permanent cure** if bone marrow transplantion is **done at early embryonic stages**.

- Steps in gene therapy

Lymphocytes of patient + ADA cDNA → Retrovirus mediated transformation → Genetically engineered lymphocytes **(not immortal)** → Infused in patient

7 MOLECULAR DIAGNOSIS METHODS

Parameters	Conventional	Modern
Early detection	Not possible	Possible
Examples	Serum and urine analysis	RDT, PCR, ELISA

- **PCR (Polymerase Chain Reaction) :**
  - **Basis :** Nucleic acid amplification
  - **Advantage :** Detection of very low concentration of a bacteria or virus
  - **Uses :** Detection of HIV infection, mutations in genes in cancer patients, genetic disorders
- **ELISA (Enzyme Linked Immuno-Sorbent Assay) :**
  - **Basis: Antigen - antibody interaction**
  - **Uses :** Detect the presence of antigens or antibodies synthesized against pathogens
- **Autoradiography**
  - **Probe :** Radioactive **ssDNA** or **ssRNA** that hybridises with complementary DNA
  - **Probe will not hybridise with mutated gene**, hence mutated gene will not appear on the photographic film due to lack of complementarity.

8 TRANSGENIC ANIMALS

- Possess manipulated DNA and express foreign gene
- Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced
- **95% of transgenic animals are mice**.

Uses of Transgenic Animals

- To study how genes are regulated and how they affect the normal functions of body, *e.g.* study of insulin - like growth factors
- Transgenic models exist for study of diseases like cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's
- Biological products
  - **α-1 antitrypsin** - To treat **emphysema.**
  - Similar attempts are made for treatment of PKU (Phenylketonuria) and cystic fibrosis.
  - **First** transgenic cow : **Rosie** developed in **1997** producing human protein enriched milk (**2.4 grams per litre**)
    - The milk contained **alpha-lactalbumin** : More balanced product for human babies than natural cow milk
- Vaccine Safety
  - **Transgenic mice** are being used to test the safety of **polio vaccine** to replace the use of monkeys.
- **Chemical safety testing**
  - Transgenic animals are made **more sensitive to toxic** substances to obtain results in less time.

9 ETHICAL ISSUES

- Genetic modification of organisms (GMO) can have unpredictable results when such organisms are introduced into the ecosystem.
- Genetic manipulation of living organisms by humans has to regulated for moral and biological significance.
- **GEAC (Genetic Engineering Approval Committee) :** Makes decisions regarding the validity of GM research and the safety of introducing GMO for public services
- Developing countries are rich in biodiversity and traditional knowledge related to bio-resources
- Biopiracy : Refers to the use of bio-resources by multinational companies and other organisations without proper authorisation from the countries and people concerned without compensatory payment.

Controversies regarding patents and biopiracy :

(i) **Basmatic rice :**
- **2,00,000 varieties of rice in India. 27 documented** varieties of Basmati rice in India
- In **1997**, an American company got patent rights on **Basmatic rice** through the **US patent and Trademark office**.

Basmati rice × Semi-dwarf variety of rice → New variety of Basmati rice

(ii) Turmeric

(iii) Neem

The Indian Parliament has recently cleared the **second amendment** of the Indian Patents Bill.