Chapter 10: Biotechnology and its Application

(i) Biotechnological Applications in Agriculture; (ii) Biotechnological Applications in Medicine; (iii) Transgenic Animals; (iv) Ethical Issues.

The applications of biotechnology include **therapeutics**, **diagnostics** and genetically modified crops for **agriculture**, **processed food, bioremediation, waste treatment, and energy production**.

Three **critical research** areas of biotechnology are:

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

Biotechnological Applications in Agriculture:

The three options that can be thought for increasing food production are,

- Agro-chemical based agriculture
- Organic agriculture; and
- Genetically engineered crop-based agriculture.

The Green Revolution has succeeded in tripling the food supply but yet it was not enough to feed the growing human population. Scientists have decided that use of genetically modified crops is a possible solution.

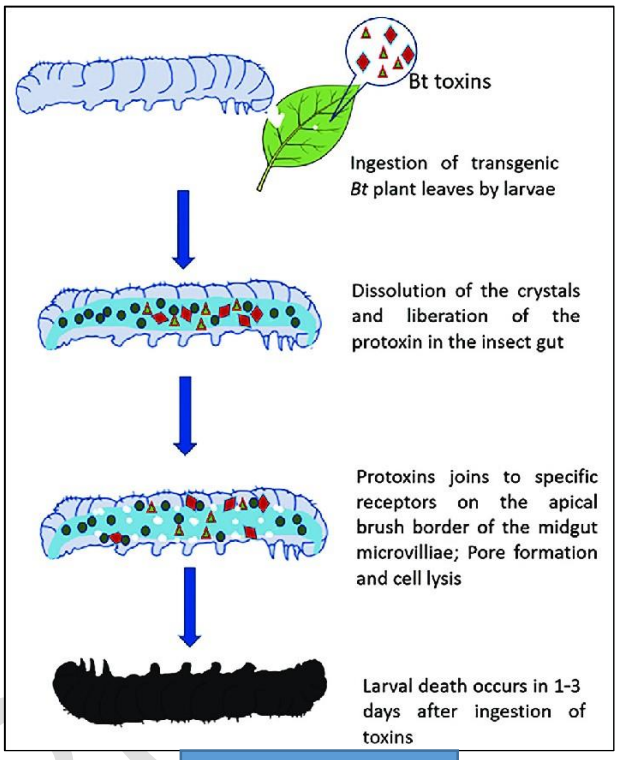
Plants, bacteria, fungi and **animals** whose genes have been altered by manipulation are called **Genetically Modified Organisms (GMO).** Genetic modification has;

- Made crops more tolerant to abiotic stresses
- Reduced reliance on chemical pesticides
- Helped to reduce post harvest losses
- Increased efficiency of mineral usage by plants
- Enhanced nutritional value of food, eg., **Vitamin 'A'** enriched rice.

Bt Cotton:

- Some strains of ***Bacillus thuringiensis*** produce 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 solubilize the crystals.

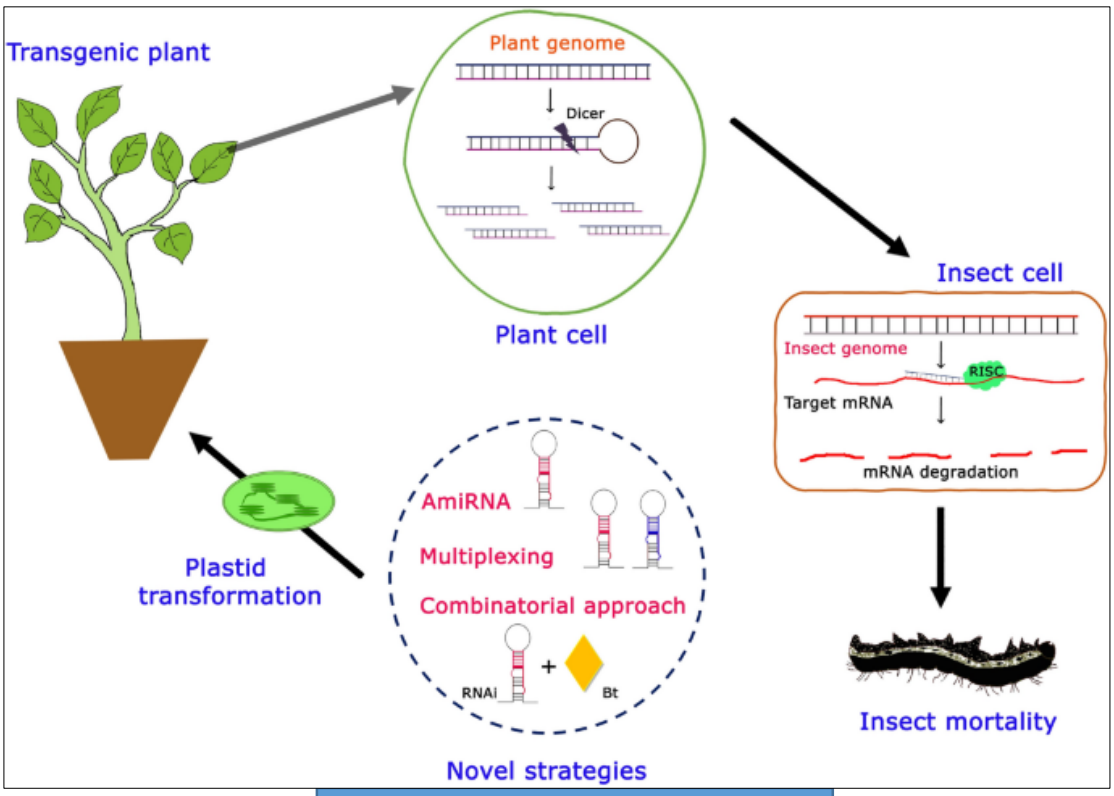
- The activated toxin binds to the surface of **midgut epithelial cells** and **creates pores that cause cell swelling and lysis** and eventually cause death of the insect.
- **Bt toxin genes** were isolated from ***B. thuringiensis*** and incorporated into the several crop plants such as cotton.
- The toxin is coded by a gene named 'cry'. There are a number of them, for example, the proteins encoded by the genes **cryIAc** and **cryIIAb** control **bollworms** and **cryIAb** controls **corn borer**.



Mode of Bt toxin

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)**.
- This method involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA (silencing).
- Using ***Agrobacterium*** vectors, nematode-specific genes were introduced into the host plant. The introduction of DNA was such that it produced both sense and antisense RNA in the host cells.
- These two RNA's being complementary to each other formed a double stranded (ds DNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode.
- The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite.



Process of RNA interference (RNAi)

Biotechnological Application in Medicine:

- The **rDNA** technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutic drugs.
- At present, about **30 recombinant therapeutics** have been approved for human use the world over.
- In India, **12 of these** are presently being marketed.

Genetically Engineered Insulin:

- 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 prohormone, 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.
- In **1983, Eli Lilly an American company** prepared two DNA sequences corresponding to A and B, chains of human insulin and introduced hem 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**.

Gene Therapy:

- Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child / embryo.
- 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.
- 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** is then introduced into these lymphocytes, which are subsequently returned to the patient.
- 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:

- **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.

PCR:

- A very low concentration of a bacteria or virus can be detected by amplification of their nucleic acid by PCR.
- 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.

ELISA:

- It is based on the principle of antigen-antibody interaction. Infection by pathogen can be detected by **presence of antigens** or by detecting the **antibodies** synthesized against the pathogen.

Transgenic Animals:

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

Reasons for the production of transgenic animals:

a) **Normal physiology and development:** Transgenic animals can be specifically designed to allow the study of how genes are regulated and how they affect the normal functions of the body and its development.

b) **Study of disease:** Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease, so that investigation of new treatments for diseases is made possible.

c) **Biological products:** Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (gene) which codes for a particular product such as human protein (**alpha – 1-antitrypsin**) used to treat emphysema. The first transgenic cow, Rosie, produced human protein-enriched milk (**alpha-lactalbumin - 2.4 gm / litre**).

d) **Vaccine safety:** Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans (polio vaccine).

e) **Chemical safety testing:** Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic substances and the effects studied.

Ethical Issues:

The Indian Government has set up organizations such as **GEAC (Genetic Engineering Approval Committee)**, which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.

Biopatent:

A patent is the right granted by a government to an inventor to prevent others from making commercial use of his invention. Now, patents are granted for biological entities and for products derived from biological resources.

NCERT EXERCISES

1. Which part of the plant is best suited for making virus-free plants and why?

Ans: Meristem tissue, particularly the apical meristem, is ideal for producing virus-free plants because it is actively growing, minimally infected by viruses, capable of regeneration, and genetically stable.

2. What is the major advantage of producing plants by micropropagation?

Ans: The major advantage of producing plants by micropropagation is the rapid multiplication of a large number of genetically identical plants from a small amount of plant tissue. Spme crucial advantages are:

- **Uniformity:** Micropropagation ensures identical clones, guaranteeing consistency in traits.
- **Speed:** Rapid production compared to traditional methods meets high demand.
- **Year-round Production:** Independent of seasons due to controlled environment.
- **Pathogen Elimination:** Sterile conditions eliminate pathogens, ensuring disease-free plants.

3. Find out what the various components of the medium used for propagation of an explant *in vitro* are?

Ans: The components of the medium used for propagation of an explant in vitro typically include:

1. **Basal Medium:** Provides essential nutrients and growth factors for plant tissue culture. Examples include Murashige and Skoog (MS) medium, Gamborg's B5 medium, or Woody Plant Medium (WPM).
2. **Plant Growth Regulators (PGRs):**

   Auxin: Stimulates root formation and enhances callus growth. Common auxins include Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA).

   Cytokinin: Promotes shoot proliferation and differentiation. Examples include Benzyladenine (BA), Kinetin, and Zeatin.
3. **Carbon Source:** Usually sucrose or glucose, providing energy for growth and metabolism.
4. **Vitamins:** Essential for various metabolic processes in tissue culture. These can include thiamine (B1), pyridoxine (B6), nicotinic acid (B3), and others.
5. **Micronutrients:** Inorganic elements like iron, magnesium, calcium, and others, required in small amounts for plant growth.

6. **Agar or Gelrite:** Solidifying agents that provide a supportive matrix for plant tissue culture.
7. **pH Adjusters:** Typically acids (e.g., HCl) or bases (e.g., KOH) to adjust the pH of the medium to the optimal range for plant growth.
8. **Antibiotics/Antifungal Agents**: Added to prevent contamination from bacteria, fungi, or other microorganisms.

4. Crystals of Bt toxin produced by some bacteria do not kill the bacteria themselves because –

(a) bacteria are resistant to the toxin

(b) toxin is immature;

(c) toxin is inactive;

(d) bacteria encloses toxin in a special sac.

Ans: Toxin is inactive:

In bacteria, the toxin is present in an inactive form, called prototoxin, which gets converted into active form when it enters the body of an insect.

5. What are transgenic bacteria? Illustrate using any one example.

Ans: Transgenic bacteria contain foreign gene that is intentionally introduced into its genome. They are manipulated to express the desirable gene for the production of various commercially important products.

An example of transgenic bacteria is *E.coli*. In the plasmid of *E.coli*, the two DNA sequences corresponding to A and B chain of human insulin are inserted, so as to produce the respective human insulin chains. Hence, after the insertion of insulin gene into the bacterium, it becomes transgenic and starts producing chains of human insulin. After that, these chains are extracted from *E.coli* and combined to form human insulin.

6. Compare and contrast the advantages and disadvantages of production of genetically modified crops.

Ans:

Advantages	Disadvantages
1. **Increased Crop Yields**	1. **Environmental Concerns**
2. **Pest Resistance**	2. **Potential Health Risks**
3. **Herbicide Tolerance**	3. **Contamination Risks**
4. **Drought Tolerance**	4. **Economic Concerns:** Dominance of GM seed companies may lead to increased costs for farmers, limiting access for small-scale farmers in developing countries.
5. **Nutritional Enhancement:** GM crops can be engineered to contain higher levels of essential nutrients, addressing malnutrition and nutritional deficiencies.	5. **Ethical and Social Implications:** Ethical concerns related to corporate control of agriculture, farmers' rights, and food sovereignty.

7. What are Cry proteins? Name an organism that produce it. How has man exploited this protein to his benefit?

Ans: Cry proteins are encoded by cry genes. These are toxins, which are produced by ***Bacillus thuringiensis*** bacteria. This bacterium contains these proteins in their inactive form. When the inactive toxin protein is ingested by the insect, it gets activated by the alkaline pH of the gut of the insects. This results in the lysis of epithelial cell and gradually the death of the insect. Thus, man has exploited this protein to develop certain transgenic crops with insect resistance.

For example- Bt cotton, Bt corn, etc.

8. What is gene therapy? Illustrate using the example of adenosine deaminase (ADA) deficiency.

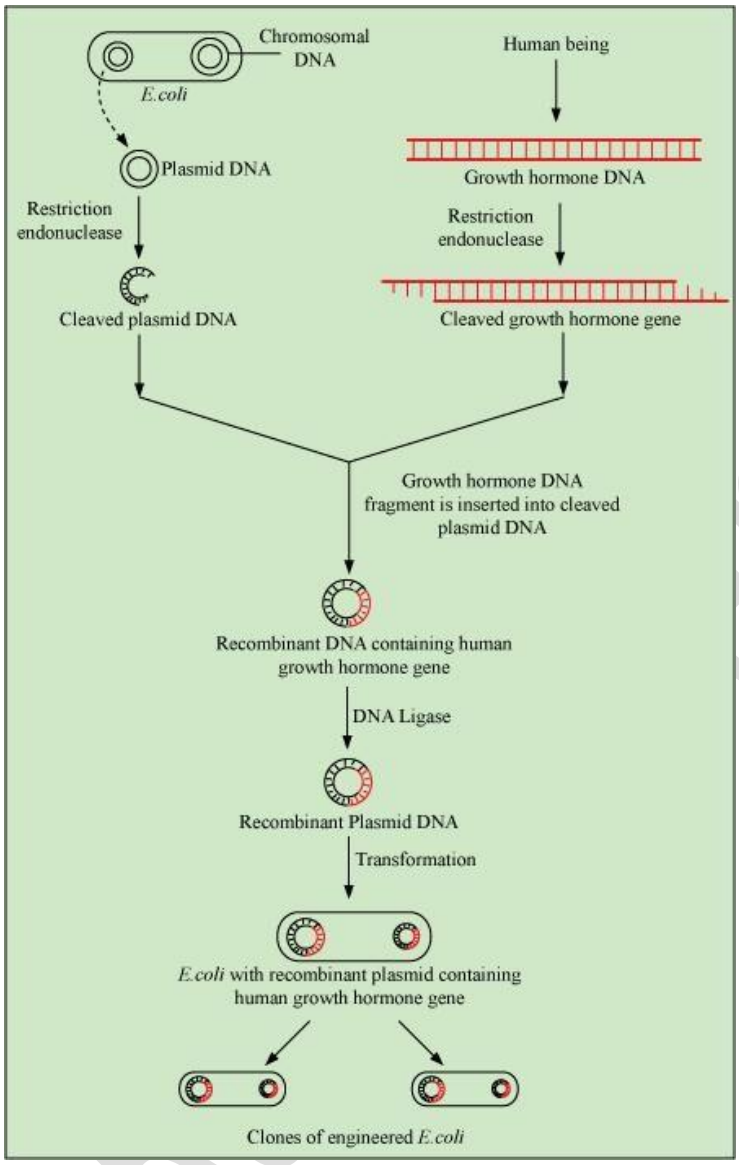
Ans: Gene therapy is a medical approach that corrects genetic defects by introducing functional genes into cells. In the case of **adenosine deaminase** (ADA) deficiency:

- **Identification of Defective Gene:** Scientists identify the faulty ADA gene causing immune system dysfunction.
- **Gene Therapy Vector Development:** A harmless virus is engineered to carry a functional ADA gene.
- **Administration:** The virus delivers the therapeutic gene into the patient's cells either ex vivo (outside the body) or in vivo (inside the body).
- **Expression of Functional ADA:** The introduced gene produces ADA enzyme, restoring immune function and treating ADA deficiency.

Gene therapy offers a promising treatment for ADA deficiency, addressing the genetic cause of the disorder and potentially providing a cure.

9. Digrammatically represent the experimental steps in cloning and expressing an human gene (say the gene for growth hormone) into a bacterium like *E. coli* ?

Ans: DNA cloning is a method of producing multiple identical copies of specific template DNA. It involves the use of a vector to carry the specific foreign DNA fragment into the host cell. The mechanism of cloning and transfer of gene for growth hormone into *E.coli* is represented below.



10. Can you suggest a method to remove oil (hydrocarbon) from seeds based on your understanding of rDNA technology and chemistry of oil?

Ans: Recombinant DNA technology (rDNA) is a technique used for manipulating the genetic material of an organism to obtain the desired result.

For example, this technology is used for removing oil from seeds. The costituentsf oil are glycerol and fatty acids. Using rDNA, one can obtain oilless seeds by stoping the synthesis of either glycerol or fatty acids. This is done by removing the specific gene responsible for the synthesis.

11. Find out from internet what is golden rice?

Ans:

- Golden rice is a genetically modified variety of rice, ***Oryza sativa***, which has been developed as a fortified food for areas where there is a shortage of dietary vitamin A.

- It contains a precursor of pro-vitamin A, called beta-carotene, which has been introduced into the rice through genetic engineering.
- The rice plant naturally produces beta-carotene pigment in its leaves. However, it is absent in the endosperm of the seed. This is because beta-carotene pigment helps in the process of photosynthesis while photosynthesis does not occur in endosperm.
- Since beta-carotene is a precursor of pro-vitamin A, it is introduced into the rice variety to fulfill the shortage of dietary vitamin A. It is simple and a less expensive alternative to vitamin supplements.

However, this variety of rice has faced a significant opposition from environment activites. Therefore, they are still not available in market for human consumption.

12. Does our blood have proteases and nucleases?

Ans: No, human blood does not include the enzymes, nucleases and proteases. In human beings, blood serum contains different types of protease inhibitors, which protect the blood proteins from being broken down by the action of proteases. The enzyme, nucleases, catalyses the hydrolysis of nucleic acids that is absent in blood.

13. Consult internet and find out how to make orally active protein pharmaceutical. What is the major problem to be encountered?

Ans: Protein pharmaceutical cannot be taken orally because they can be degraded by the proteases of our alimentary canal.

Thus, major problem to be encountered is the action of digestive enzymes. It has to be made digestible for the digestive system and also protect it from the degradation of HCl present in stomach so it is coated by a film that is resistant to protein degrading enzymes.