

CHAPTER -12

BIOTECHNOLOGY AND ITS APPLICATIONS

- Biotechnology essentially deals with industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals.
- 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:
 - (i) Providing the best catalyst in the form of improved organism usually a microbe or pure enzyme.
 - (ii) Creating optimal conditions through engineering for a catalyst to act, and
 - (iii) Downstream processing technologies to purify the protein/organic compound.

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

There are three options that can be thought for increasing food production

- (i) agro-chemical based agriculture;
- (ii) organic agriculture; and
- (iii) Genetically engineered crop-based agriculture.

- We have succeeded in tripling the food supply by Green Revolution 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 (fertilisers 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.
- So there is a need to find alternative path that our understanding of genetics can show so that farmers may obtain maximum yield from their fields and to minimise the use of fertilisers and chemicals so that their harmful effects on the environment can be reduced. 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:

- (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 (this prevents early exhaustion of fertility of soil).
- (v) Enhanced nutritional value of food, e.g., Vitamin 'A' enriched rice.

In addition to these uses, GM has been used to create tailor-made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

- By applications of biotechnology in agriculture, pest resistant plants are produced, 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**.
- This toxin does not kill the Bacillus because this protein exists 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.
- Specific Bt toxin genes were isolated from *Bacillus thuringiensis* and incorporated into the several crop plants such as cotton. The choice of genes depends upon the crop and the targeted pest, as most Bt toxins are insect-group specific.
- 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 the cotton bollworms, that of *cryIAb* controls corn borer.

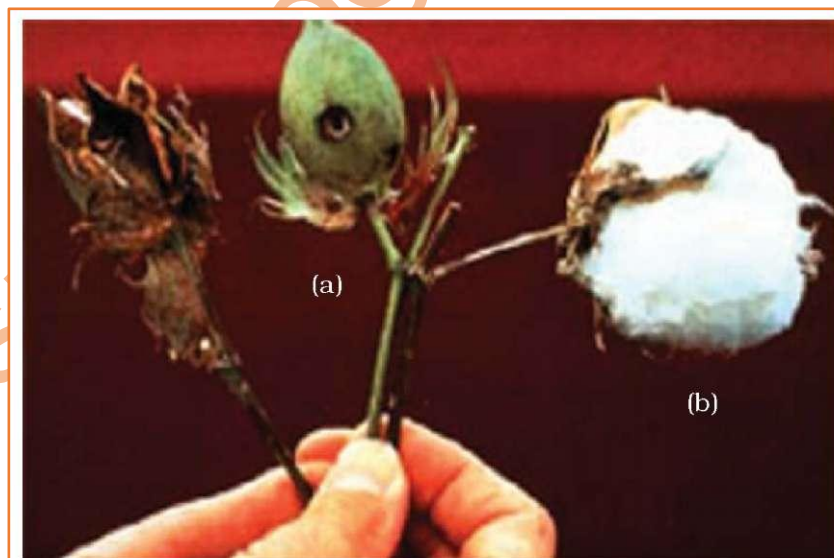


Fig: Cotton boll: (a) destroyed by bollworms; (b) a fully mature cotton boll

Pest Resistant Plants:

- Several nematodes parasitise a wide variety of plants and animals including human beings.
- A nematode *Meloidogyne incognita* 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.
- Using *Agrobacterium* vectors, nematode-specific genes were introduced into the host plant.
- The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells. These two RNA's being complementary to each other formed a double stranded (dsRNA) 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.

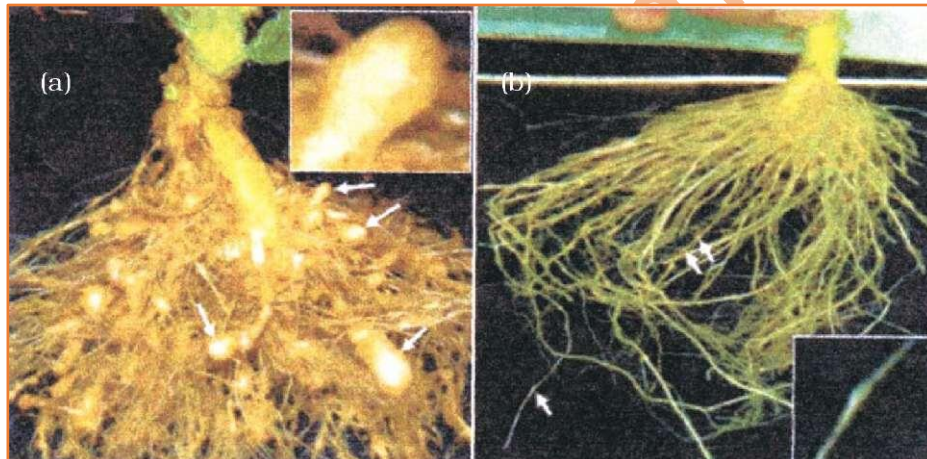


Fig: Host plant-generated dsRNA triggers protection against nematode infestation: (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism

BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

- By enabling mass production of safe and more effective therapeutic drugs.
- Further, the recombinant therapeutics do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources.
- 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

- Management of adult-onset diabetes is possible by taking insulin at regular time intervals.
- If enough human-insulin was not available, that one would have to isolate and use insulin from other animals.
- 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, which are linked together by disulphide bridges.
- In mammals, including humans, insulin is synthesised as a prohormone (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.

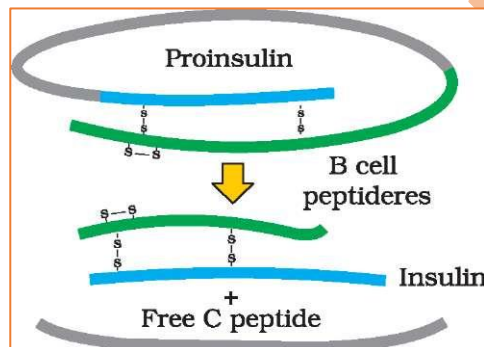


Fig: Maturation of pro-insulin into insulin after removal of C- peptide (to be simplified)

Gene Therapy

- Gene therapy is the corrective therapy for hereditary disease.
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.
- ADA deficiency can be cured by bone marrow transplantation or 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.
- In 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

- For effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important but 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.
- 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 technique to identify many other genetic disorders.
- **PCR –**
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.

TRANSGENIC ANIMALS

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

Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over 95 per cent of all existing transgenic animals are mice.

common reasons to produce transgenic animals:

(i) **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, e.g., study of complex factors involved in growth such as insulin-like growth factor.
- By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.

(ii) **Study of disease:**

- Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible.
- Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's.

(iii) **Biological products:**

- Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make.
- Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (α -1-antitrypsin) used to treat emphysema.
- Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis.
- In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow-milk.

(iv) **Vaccine safety:**

- Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans.
- Transgenic mice are being used to test the safety of the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.

(v) **Chemical safety testing:**

- This is known as toxicity/safety testing. The procedure is the same as that used for testing toxicity of drugs.
- 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. Toxicity testing in such animals will allow us to obtain results in less time.

ETHICAL ISSUES

The manipulation of living organisms by the human race cannot go on any further, without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms.

Going beyond the morality of such issues, the biological significance of such things is also important. Genetic modification of organisms can have unpredictable results when such organisms are introduced into the ecosystem.

Therefore, the Indian Government has set up organisations 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.

Bio-patent:

- The modification/usage of living organisms for public services (as food and medicine sources, for example) has also created problems with patents granted for the same.
- There is growing public anger that certain companies are being granted patents for products and technologies that make use of the genetic materials, plants and other biological resources that have long been identified, developed and used by farmers and indigenous people of a specific region/country.
- Rice is an important food grain, the presence of which goes back thousands of years in Asia's agricultural history. There are an estimated 200,000 varieties of rice in India alone. The diversity of rice in India is one of the richest in the world.

- Basmati rice is distinct for its unique aroma and flavour and 27 documented varieties of Basmati are grown in India. There is reference to Basmati in ancient texts, folklore and poetry, as it has been grown for centuries.
- 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, in the US and abroad.
- This 'new' variety of Basmati had actually been derived from Indian farmer's varieties. Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty.
- The patent extends to functional equivalents, implying that other people selling Basmati rice could be restricted by the patent.
- Several attempts have also been made to patent uses, products and processes based on Indian traditional herbal medicines, e.g., turmeric neem.
- If we are not vigilant and we do not immediately counter these patent applications, other countries/individuals may encash on our rich legacy and we may not be able to do anything about it.

Biopiracy

- It is the term used to refer to the use of bio-resources by multinational companies and other organisations without proper authorisation from the countries and people concerned without compensatory payment.
- Most of the industrialised nations are rich financially but poor in biodiversity and traditional knowledge. In contrast the developing and the underdeveloped world is rich in biodiversity and traditional knowledge related to bio-resources. Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used to save time, effort and expenditure during their commercialisation.
- There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore, some nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge.
- The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiative.