APPLICATIONS OF BIOTECHNOLOGY

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

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

- There are three options to increase the food production :
  - Agrochemical based agriculture;
  - Organic agriculture ; and
  - Genetically engineered crop - based agriculture.
- The **Green Revolution** succeeded in increasing the food production but it was not sufficient to feed the growing human population. One solution of this problem is use of **genetically modified crops**. Genes of plants, bacteria, fungi and animals have been changed by manipulations, therefore, these organisms are called **Genetically Modified Organisms (GMO).**
- GM plants have been useful in many ways. Genetic modification has:
  - made crops more tolerant to **abiotic stresses** (cold, drought, salt, heat).
  - **reduced reliance** on chemical pesticides (pest-resistant crops).
  - helped to reduce **post harvest losses**.
  - increased efficiency of **mineral usage** by plants (this prevents early exhaustion of fertility of soil).
  - enhanced **nutritional value** of food, *e.g.*, vitamin 'A' enriched rice.

Production of transgenic plants

- Transgenic plants can be obtained by **combination of tissue culture** and **genetic engineering**. Transgenic plants have a **natural resistance** to **herbicides** and **pests**.
- The vector used to introduce new genes into plant cells is most often a plasmid from the soil bacterium Agrobacterium tumefaciens. This is the **Ti plasmid** (tumour inducing plasmid), so called because in nature, it induces tumours in broad leaf plants.
- For using Ti plasmid as a vector, researchers have eliminated its tumor causing properties while keeping its ability to transfer DNA into plant cells. Hence, for genetic engineering purposes, *Agrobacterium* strains are developed in which tumor-forming genes are deleted. These transformed bacteria can still infect plant cells.
- The part of Ti plasmid transferred into plant cell DNA, is called the **T-DNA**. This T-DNA with desired DNA spliced into it, is inserted into the chromosomes of the host plant where it produces copies of itself, by migrating from one chromosomal position to another at random. Such plant cells are then **cultured**, induced to multiply and differentiate to form **plantlets**. Transferred into soil, the plantlets grow into mature plants, carrying the foreign gene, expressed throughout the new plant.

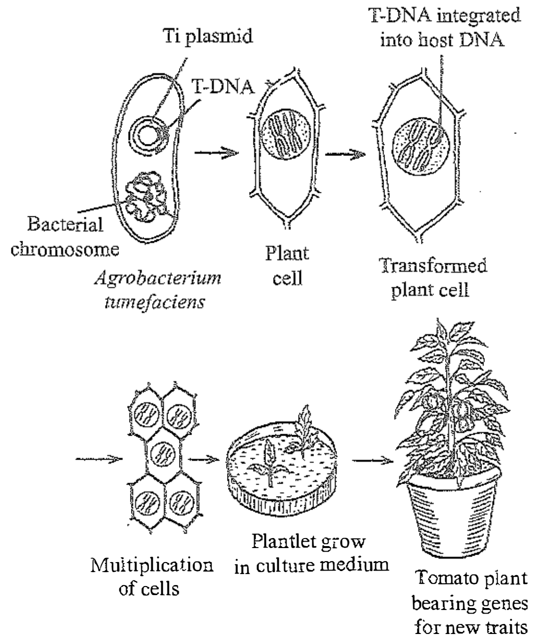

Fig.: *Agrobacterium* Ti plasmid-mediated genetic transformation in plants.

Bt Cotton (Insect resistant plant)

- Soil bacterium *Bacillus thuringiensis* produces proteins that kill certain insects like lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes). *B. thuringiensis* forms some protein crystals. These crystals contain a **toxic insecticidal protein**.
- The Bt toxin protein exists as inactive **protoxin** but once an insect ingests the inactive toxin it is converted into an active form of toxin due to the alkaline pH of the alimentary canal 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.
- 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 targeted pest, as most **Bt toxins are insect-group specific**. The toxin is coded by a gene named *cry*. There are numerous genes. Two *cry* genes ***cryIAc*** and ***cryIIAb*** have been **incorporated in cotton**. This genetically modified crop is called **Bt cotton** as it contains **Bt toxin** genes against **cotton bollworms**. Similarly, ***cryIAb*** has been introduced in Bt corn to protect the same from **corn borer**.

Pest resistant plants

- A nematode *Meloidogyne incognita* infests the **roots of tobacco plants** and causes a great **reduction in yield**. A novel strategy was adopted to prevent this infection that was based on the process of **RNA interference (RNAi)**. RNA interference is the phenomenon of **inhibiting activity of a gene through production of sense and antisense** RNA.

- This method involves a **specific *m*RNA silencing**. The result was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plants thus got itself protected from the parasite.
- Some other **agricultural applications** are :
  (i) The protein **hirudin** present in leech prevents blood clotting. Its gene was chemically synthesized and introduced in *Brassica napus*. The seeds of the latter came to have hirudin which could be extracted and purified.
  (ii) '**Flavr Savr**' tomato was the first transgenic variety to reach the market. Here inactivation of gene which produces **polygalactouronase enzyme** has been done. The **non-availability of this enzyme prevents over-ripening** because the enzyme is essential for degradation of cell walls.
  (iii) **Golden rice** is a transgenic variety of rice (*Oryza sativa*) which contains good quantities of β-carotene (provitamin A – inactive state of vitamin A).
  (iv) Production of value added products like nutrition supplements, pharmaceuticals, fuels etc. using transgenic crops **(molecular farming)**.

BIOTECHNOLOGICAL APPLICATIONS IN HEALTH

- The biotechnological processes have made great impact in the area of healthcare by mass production of safe and more effective **therapeutic drugs**. This is known as medical biotechnology or **red biotechnology**.
- In 1921, **Banting and Best** along with **Macleod** succeeded in preparing a pure extract of **insulin** from the **pancreatic** islets of a **dog**. Banting and Macleod won the 1923 Nobel Prize in Medicine or Physiology. They demonstrated that **administration of insulin could cure diabetes in human beings**. Later, insulin for curing diabetes used to be extracted from pancreas of slaughtered pigs and cattle. This insulin is slightly different from human insulin and brings about some undesirable side effects such as allergy.
- Insulin is made up of **51 amino acids arranged in** two **polypeptide chains**, *A* having 21 amino acids and *B* with 30 amino acids that are linked together by **disulphide bridges**. In mammals, including humans, insulin is synthesised as a **pro-hormone** (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, first prepared two DNA sequences corresponding to *A* and *B* chains of human insulin. It was introduced in plasmid of *Escherichia coli* to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating **disulfide bonds** to form human insulin (**humulin**).
- Biosynthesis of **somatotropin** was achieved through gene cloning procedures.
- **Bovine growth hormone** has veterinary uses *e g*., injecting a cow with this hormone can increase milk production by 25%.
- **Gene therapy** is the technique of genetic engineering to replace a faulty gene by a normal healthy functional gene. Gene therapy is being tried for sickle cell anaemia and severe combined immuno-deficiency (SCID).
- The first clinical gene therapy was given in 1990 to a 4-year old girl with **adenosine deaminase (ADA) deficiency**.
- This enzyme is very important for the immune system to function. SCID is caused due to defect in the gene for the enzyme adenosine deaminase. Because these patients do not have functional T-lymphocytes, they cannot provide immune responses against invading pathogens.
- As a first step towards gene therapy, lymphocytes are extracted from the bone marrow of the patient and are grown in a culture outside the body. A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are reinjected to the patient's bone marrow. But as these cells do not always remain alive, the patient requires periodic infusion of such genetically engineered lymphocytes. However, if the isolated gene from bone marrow cells producing ADA is introduced into cells at early embryonic stages, it can be a permanent cure.
- **Vaccines** represent an invaluable contribution of biotechnology as they provide protection against even such diseases for which effective cures are not yet available. The effectiveness of vaccines may be appreciated from the fact that **small pox**, once a dreaded disease the world over, has been completely eradicated from the world.
- Efforts to develop **vaccines** to control fertility have met with encouraging success. Basically, three approaches have been used:
  - vaccines against hCG (human chorionic gonadotrophin),
  - antibodies against follicle stimulating hormones (FSH) and
  - antibodies against vitamin carrier proteins.
- The various vaccines can be grouped under the following types
  - conventional vaccines (live vaccines, inactivated pathogens),
  - purified antigen and
  - recombinant vaccines (recombinant proteins/ polypeptides, DNA vaccines)
- Many new vaccines of great importance have been produced with the help of genetic engineering techniques. These are called '**second-generation vaccines**'. They are more uniform in quality and produce less side effects as compared to '**first-generation vaccines**' produced by conventional methods. Examples of second generation vaccines are the vaccines produced for hepatitis-B and herpes virus. Now-a-days even '**third generation vaccines**' called synthesized vaccines, have been produced. Due to the advances in the field of biotechnology, it is now possible to produce these vaccines into plants eaten as part of the normal diet, like banana and apple.
- Biotechnology has proved to be a **boon in solving crimes**, legal disputes, etc. Establishing the identity of victims (*e.g.*, of murder, accidents, etc.), criminals (*e.g.*, in cases of rape, murder, etc.), father (in cases of paternity dispute) etc. is critical to solving the problems of crimes/cases.

TRANSGENIC ANIMALS

- A transgenic animal contains in its genome, a gene or genes introduced by one or the other technique of transfection. The gene introduced by transfection is known as **transgene**. In animals, transfection specifies the introduction of a DNA segment, either naked or integrated into a vector, into an animal cell. The same phenomenon is known as **transformation** in all other organisms.
- 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.
- Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease.
- 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.
- **Transgenic mice** are being developed for use in testing the safety of vaccine before they are used on humans.
- Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. **Toxicity testing** in such animals will allow us to obtain results in less time.
- Gene transfers have been successful in various **fish**, such as common carp, rainbow trout, Atlantic salmon, catfish, goldfish, zebra-fish etc. **Genetically modified salmon was the first transgenic animal obtained for food production.**
- **Rabbits** are quite promising for **gene farming or molecular farming**, which aims at the production of recoverable quantities of **pharmaceutically or biologically** important **proteins encoded by** the **transgenes**.
- Goats are being evaluated as **bioreactors**. Some human genes have been introduced in goats and their expression achieved in mammary tissues.
- **Transgenic sheep** have been produced to achieve better growth and meat production. For example, human genes for blood clotting factor IX and for a1-antitrypsin have been transferred in sheep and expressed in mammary tissue.
- **Dogie** is a transgenic dog with excellent smelling power.

INDUSTRIAL BIOTECHNOLOGY

- Use of microbes to obtain a product or service of economic value constitutes industrial biotechnology. It is also known as white or grey **biotechnology**.
- Industrial production of useful products began as early as 1875 with the production of alcohol. At present, several chemicals, such as, **lactic acid**, amylase, **glycerine**, **citric acid**, **gluconic acid**, **acetic acid**, **acetone**, **butanol**, a variety of **enzymes**, **vitamins**, aminoacids, and all the **antibiotics** are produced using microorganisms.

BIOSAFETY ISSUES

- **Measures taken to prevent any risk to plants, animals and microbes from transgenic organisms is known as biosafety.** It was feared that genetically engineered microorganisms (GEMs) may disturb the ecosystem and its processes, in which they might be released. They may rapidly multiply and outcompete the native microbes. They may also transfer genes related to virulence or pathogenesis into bacterial population and, thereby increase their virulence. Similarly, genetically modified plants could pose biological and ecological risk.
- The biosafety guidelines are developed to contribute to ensuring an adequate level of protection in the fields of safe transfer, handling and use of living modified organisms.
- A **patent** is the right granted by a government to an inventor to prevent others from commercial use of his invention. When patents are granted for biological entities and for products derived from them, these patents are called **biopatents**. Patents have been taken out on plants such as black pepper (*Piper nigrum*), basmati rice (*Oryza sativa*), Indian mustard (*Brassica campestris*), pomegranate (*Punica granatum*), turmeric and neem.
- Some organisations and multinational companies exploit and/or patent biological resources or bioresources of other nations without proper authorisation from the countries concerned, this is called **biopiracy**. For example, the Thai Ministry of Science and its Biotech Institute has accused the British University of Portsmouth of 'biopiracy' as they have refused to return up to **200 strains of marine fungi** that they collected in coastal waters and swamps around Thailand. Instead, Portsmouth University is reported to be in the process of selling the rights on 'their' Thai fungi to a drug company for screening, as the fungi are believed to contain compounds for treating everything from AIDS to cancer-worth millions of pounds. Thailand insists that keeping the fungi without permission is in breach of international agreements.
- **Bioethics** includes rules of conduct that may be used to regulate our activities in relation to the biological world. The main bioethical concerns pertaining to biotechnology are briefiy mentioned as follows:
  - Introduction of a transgene from one species into another species violates the 'integrity of species'.
  - Biotechnology may pose unforeseen risks to the environment, including risk to biodiversity.
  - Transfer of human genes into animals (and vice-versa) dilutes the concept of 'humanness'.
  - When animals are used for production of **pharmaceutical proteins**, they are virtually reduced to the status of a 'factory'.
  - Use of animals in biotechnology causes great suffering to them.
  - It is disrespectful to living beings, and only exploits them for the benefit of human beings.

- Scientists cannot rule out the possibility of other biological damage.
- It can accidentally create new infectious agents.

- All these aspects indicate that biotechnology is focussed on exploiting the biological world. Therefore, we have to decide which activities are ethical and which are not.
- 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.

CONCEPT MAP

Applications of Biotechnology

Ethical issues

Biopiracy

- It is exploitation of bioresources of a country by organisations and multinationals for commercial exploitation with or without patent but without any **Access and Benefit Sharing Agreement** (ABA).
- Some nations are developing laws to prevent such unauthorised exploitation of their bioresources and traditional knowledge.

Biopatent

- It is an official licence to use a particular biological material for commercial exploitation like (i) A strain of microorganism. (ii) GM plant/animal. (iii) DNA sequence. (iv) Biotechnological procedure. (v) Production process. (vi) Product and (vii) Product application.
- Biopatents are going to give not only increased economic benefits to holders, but will also provide a spurt in research.

Biotechnological applications in agriculture

- **Genetically modified crops (GM crops)** or **Transgenic plants :** GM crops contain and express one or more useful foreign genes or transgenes (*e.g.*, Bt cotton, golden rice, Flavr Savr tomato).
- GM plants have been useful in many ways:
  - Growing GM crops can help to reduce the use of **chemical pesticides**.
  - GM crops are **more tolerant** to abiotic stresses (cold, drought, salt, heat, etc.).
  - They have helped to reduce **post-harvest losses**.
  - Increased efficiency of mineral usage by plants prevents **early exhaustion of fertility of soil.**
  - GM plants enhance **nutritional value** of food. *e.g.*, vitamin A enriched rice.
  - Herbicides (weed killers) do not harm the GM crops.
  - Plant biologists are working to create genetically engineered plants having resistance to these diseases.
  - GM plants have been used to create **alternative resources** to industries in the form of starches, fuels and pharmaceuticals **(molecular farming).**

Biotechnological applications in medicine

- Recombinant DNA technology is engaged in production of newer and safer therapeutic drugs, called **recombinant drugs**. These drugs do not induce immunological reactions as some other drugs obtained from nonhuman sources (*e.g.*, insulin from animals).
- A large number of human genes encoding **pharmaceutically valuable proteins** have been cloned and expressed in microorganisms.
- **Gene therapy** is a collection of methods that allows correction of a gene defect that has been diagnosed in a

Production of transgenic animals

- These are animals which have been genetically modified by incorporating foreign and other specific genes through recombinant DNA technology. A number of animals have been genetically modified, *e.g.*, rats, rabbits, pigs, sheep, cows, fish. **95%** of them are transgenic mice.
- Some of the important uses of transgenic animals are as follows :
  - Transgenic animals enable scientists to study the working of genes, their regulation, effect on normal body functions and development.
  - Transgenic animals are used to study how genes take part in development of diseases.
  - The important biological products being harvested by this method are **protein α-1 antitrypsin** for treating emphysema, **tissue, plasminogen activator** (goat), blood clotting factors VIII and IX (sheep) and lactoferrin (cow).
  - Transgenic animals are the best for checking the **safety of vaccines** before their use in humans.

Biotechnological applications in industry

Mostly microorganisms are used in many industries to produce alcohol, enzymes, vaccines, vitamins, antibiotics, organic acids, etc.