CHAPTER / 13

Applications of Biotechnology

Topic Covered

Applications of Biotechnology in Agriculture and Medicine
- Applications in Agriculture and Forestry
- Applications in Medicine and Healthcare Management
- Gene Therapy
- Genetically Modified Organisms

Transgenic Animals and Ethical Issues
- Transgenic Animals
- Biosafety Issues
- Biopiracy

Biotechnology refers to an integrated applied branch of sciences which is useful to humans in producing various products by using microbes, plants, animals and their metabolic machinery. This is achieved by using recombinant DNA technology, in which valuable and useful manipulation of genes in various living species is done to obtain desirable products at a large scale.

TOPIC ~01 Applications of Biotechnology in Agriculture and Medicine

Applications in Agriculture and Forestry

Plant's genetic transformation has become an important biotechnology tool for the improvement of crops. A solid foundation for the rapid developement and implementation of biolechnology in agriculture has been laid by plant culture.

Plant Cell and Tissue Culture

Genetic engineering of plants is carried out by plant tissue culture which involves the manipulation of plant genome by introducing the gene of interest in it and then growing the transformed plant cell in an artificial culture media.

Plant tissue culture is used to generate novel economically important plants. Several applications of plant tissue culture are as follows

Drought Resistant Plants

Plant tissue culture technique is helpful in generating drought resistant plants by introducing those genes whose products enable the plants to retain water and withstand prolonged drought conditions.

Fungi and Bacteria Resistant Plants

The genes encoding certain enzymes and proteins which infer resistance abilities to a plant can be introduced into isolated plant cells in culture media to generate fungi and bacteria resistant plants.

Virus-Resistant Plants

Transgenic plants have been generated, which express the coat protein genes of infections plant viruses. The coat protein, thus expressed, turned on the plant version of the immune system.

Herbicide Resistant Plants

Weeds such as *Striga* which decrease crop yield and quality can be removed with the help of herbicide (weed killer). For example, **Roundup Ready** transgenic plant has been produced and commercialised as it is tolerant to the herbicide Roundup (Trade Name).

Glyphosate is most widely used broad-spectrum herbicide across world. *Petunia* plant contains a glyphosate resistant gene.

Frost (Ice) Protected Plants

Soil bacterium *Pseudomonas syringae* contains a gene that promotes ice nucleation. Using gene technology, this gene is deleted and a mutant *P. syringae* is engineered known as INA or ice minus strain and it prevents frost (ice) formation when sprayed on crops.

Bioplastic

An alternative to natural plastic called bioplastic, has been developed using biotechnology. Certain group of microorganisms synthesises biopolymers similar to natural plastic. The gene encoding the enzyme for biopolymer synthesis is isolated and transferred to corn plant cells in culture and transgenic corn plants are generated, which express the transgene and synthesise the biodegradable polymer which is used as bioplastic.

Novel Transgenic Plants

In *Petunia*, a gene that codes enzymes for flower colour was introduced in protoplasts and a transgenic plant with pink flowers was generated.

Another example of novel plant is the luminescent tobacco plant which is generated through gene manipulation.

A recombinant Ti plasmid with luciferase gene is used to develop a modified tobacco plant which glows in the dark in the presence of luciferin.

Florigen, a biotech company in 1966 released the first ever genetically manipulated flower into the market.

Protein Producing Plants

Transgenic plants also serve as bioreactors for the synthesis of many therapeutic proteins like enkephalin (a neuro-peptide) and human serum albumin.

Golden Rice

Transgenic golden rice has elevated level of β-carotene which is a precursor of vitamin-A. It has been produced by introducing the genes encoding the enzymes of β-carotene biosynthetic pathway into rice plant cells in culture.

Delayed Fruit Ripening (*Flavr Savr* Tomato)

By manipulating a gene for polygalacturonase (involved in softening and ripening of fruits), ripening can be delayed, e.g. *Flavr savr* tomatoes.

Nitrogen-Fixation

Nitrogen-Fixation Gene (*nif* gene) can be transferred from the genome of the bacterium *Rhizobium* into the chromosomes of non-leguminous plants, so that they can utilise atmospheric nitrogen.

Applications in Medicine and Healthcare Management

Monoclonal Antibodies

A single clone of β-lymphocytes is fused with a myeloma cell which results in hybridoma to produce monoclonal antibodies. These antibodies are used in the identification of blood groups, diagnosis of diseases and cancer, in vaccine production and immunotherapy.

Recombinant Therapeutic Molecules

Many therapeutic protein molecules are being produced commercially by the application of recombinant DNA technology, e.g. insulin, human growth hormone. coagulation factors (VIII and IX), hirudin, monoclonal antibodies, etc.

Clinical Investigation of Genetic Disorders

The genetic disorders caused by chromosomal aberrations and gene mutation disorders can be identified using the principles of molecular biology, immunology, biochemistry and genetic engineering.

The location of the mutant gene causing abnormality in either chromosome number or structure is initially identified. Finally, remedial measures for the treatment of the disorder are selected.

Medical Diagnosis

Recombinant DNA technology, PCR (Polymerase Chain Reaction), DNA fingerprinting and Enzyme-Linked Immuno-Sorbent Assay (ELISA) are some of the techniques that are applied in several medical diagnostic procedures.

Antibiotics

The novel broad-spectrum antibiotics can be produced by using RDT. These antibiotics have increased activity, they do not have side effects and have a low cost.

Microbial Synthesis of Amino Acids

Metabolic engineering has been used in the biosynthesis of many amino acids by suppressing several steps in their complex biosynthetic pathways.

Vincristine and Vinblastine from Plants

Vincristine and Vinblastine, isolated from Madagascar periwinkle, *Catharanthus roseus*, are used as anticancer (chemotherapy) drugs.

Microbial Synthesis of Vitamin-C

Vitamin-C is synthesised by the process of microbial gene cloning, which reduces the cost of production.

Vitamin-E from Plants

Vitamin-E is synthesised from α-tokopherol (a hydrophobic compound) by combining gene and enzyme technology with metabolic engineering.

Microbial Synthesis of Indigo and Melanin

The microbial synthesis of the blue dye, **indigo** and the black pigment, **melanin** are two more examples of metabolic engineering. These two products are synthesised from tryptophan and tyrosine, respectively.

Human Insulin

Insulin is a small protein hormone that is synthesised and secreted by the pancreas. It regulates the blood glucose concentration by decreasing the excess glucose present in blood. However, hyposecretion of insulin causes a disorder called **diabetes mellitus** in which the blood glucose levels are abnormally high. This condition can be successfully treated by injecting insulin into the patient to equilibrate the blood glucose concentration.

Prior to the emergence of recombinant DNA technology, human insulin was recovered and purified from the pancreas of slaughtered animals like cattle and pigs. However, two problems were associated with this insulin. Firstly, the obtained insulin generated an immune response like allergy or other reactions in the recipient's body. Secondly, the production cost was high. Additionally, the purified insulin was contaminated by many pathogenic viruses. These problems have been overcome by the use of recombinant DNA technology. Insulin that is produced by recombinant DNA technology is known as recombinant human insulin.

Strategy for Insulin Synthesis

The main challenge for the production of insulin using *r*DNA technique was getting insulin assembled into a mature form and for this, two approaches have been developed:

(i) 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. These chains (A and B) were produced separately, extracted and combined by creating disulphide bonds to form human insulin called **humulin**.

Most of the biotech companies use this method only to produce insulin.

(ii) The second approach involves the use of yeast host cell for expression of cloned insulin gene. It is advantageous over *E.coli* host cell because yeast is on eukaryotic cell and it can express, cleave and join the two polypeptides correctly.

Human Insulin Manufacturing Companies

- Genentech
- Other noteworthy insulin manufacturing companies are:

(i) Novo Nordisk, Denmark.
(ii) Hoechst and Aventis, Germany
(iii) Pfizer, USA

Types of Recombinant Insulin

Insulin is marketed in two forms–injectable and inhalable. Injectable insulin is available in two forms-pen and vial.

Recombinant Vaccines

Vaccine refers to an antigenic agent, which when administered into an animal, generates an active acquired immune response.

In recombinant vaccines, a DNA insert encoding an antigen (like bacterial surface proteins) is introduced into a less virulent host. These elicit an immune response expressing the antigens but do cause infection. The expressed antigens are isolated and purified and injected into the human hosts as vaccines. These are called recombinant vaccines.

Strategies for Recombinant Vaccine Synthesis

Two main strategies of developing recombinant vaccines are as follows

(i) In the first strategy, the surface coat proteins of pathogenic viruses have been used. The gene expressing the surface coat protein of Hepatitis-B Virus (HBV) was identified, isolated, purified and cloned in a prokaryotic host cell with a high copy number. Later, it was expressed in a yeast host cell to produce surface antigen proteins (HBsAg). This protein was used in the development of hepatitis-B vaccine.

(ii) In another approach, naked DNA from the pathogenic microorganism which encodes the antigen is used as a vaccine. This DNA is inserted

into a suitable DNA vector forming a recombinant DNA which is directly introduced into the host.

In the host, the inserted DNA directs the synthesis of antigens, stimulating an active acquired immune response.

Recombinant Vaccine Manufacturing Companies

The common brands presently producing such vaccines for human use are– Recombivax (Merck); Energix B (Glaxo Smithkline); Elovac (Human Biologicals Institute, a division of Indian Immunological Limited); Genevac-B (Serum Institute) and Shanvac B. All these are hepatitis-B vaccines. Twinrix, manufactured by Glaxo Smithkline is the only combination vaccine used against hepatitis A and B.

Gene Therapy

It is a method of treatment which allows correction of a biochemical (like in phenylketonuria) or a genetic defect that has been diagnosed in a child or embryo. The defective mutant alleles of the gene are replaced by the normal gene insertion to take over the function and compensate for the non-functional gene. Gene therapy is widely used to treat

(i) Biochemical disorders, e.g. alkaptonuria, phenylketonuria, albinism, etc.

(ii) Chromosomal and gene disorders, e.g. Down's syndrome, Turner's, syndrome, Klinefelter's syndrome.

Types of Gene Therapy

(i) **Somatic Cell Gene Therapy**
It involves the introduction of normal genes into the somatic cells of affected individuals as to restore normal cellular activities. This change is confined to a generation only and successful for the genes that follow simple Mendelian inheritance.

Steps followed in enzyme replacement gene therapy are given below.

(a) In first step, lymphocytes from the blood of the patient are extracted and grown on a culture outside the body.

(b) A functional ADA *c*DNA (using a retroviral vector) is then introduced into these lymphocytes which are subsequently returned to the patient's body.

(c) As these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.

(ii) **Germ Cell Gene Therapy**
In this, the mutant gene is replaced with a normal gene in the gametes hence, the change can pass on to the next generation.

Methods of Gene Therapy

(i) ***Ex vivo* gene transfer** The transformed genetic material is incorporated directly into the cells of patient. The affected cells are isolated, transformed and cloned *in vitro*. These cells are reintroduced in the patient's body by transfusion or transplantation.

(ii) ***In vivo* gene transfer** The cells are removed from the patient's body and genetic material is modified transplanted back into the patient's body. However, this method is still in experimental phase.

- Severe Combined Immuno Deficiency (SCID) is caused due to a failure of synthesis of ADA enzyme.
- Familial Hypercholesterolemia (FH) is caused due to the absence of Low Density Lipoprotein receptors (LDL receptors) on the surface of hepatocytes.

Genetically Modified Organisms

The plants, bacteria, fungi and animals whose genes have been altered or manipulated are called Genetically Modified Organisms (GMOs).

GM Plants

Genetic modifications in plants allowed

(i) Crops to become more tolerant to abiotic stresses like cold, drought, salt and heat.

(ii) Formation of pest-resistant crops.

(iii) Reduction of post-harvest losses.

(iv) Efficiency of mineral usage to increase in plants.

(v) Enhanced nutritional value of food, e.g. vitamin-A enriched rice (golden rice).

Bt Crop

A major application of biotechnology in agriculture is the production of pest-resistant plants which could decrease the amount of pesticides being used.

Insecticidal Toxin of *Bacillus thuringiensis*

An alternative to toxic pesticidal chemicals is the use of bacterium with insecticidal property, i.e. a Gram positive soil bacterium *Bacillus thuringiensis,* which produces *Bt* toxin or δ-endotoxin or insecticidal crystalline protein.

Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to the insects without the need for insecticides and hence, it is named as biopesticide.

Bacillus thuringiensis forms protein crystals (Cry) during a particular phase of their growth. These crystals contain a toxic insecticidal protein. *Bt* protein in the presence of an alkali hydrolyses into prototoxins. These polypeptides are digested at an alkaline pH.

But, this toxin does not kill the *Bacillus* (bacterium), as it exists as inactive protoxins but once an insect ingests the inactive toxin, it is converted into its active form due to the alkaline pH of the gut which solubilises the crystals. The activated toxin binds to the surface of midgut epithelial cells and creates pores that cause cell swelling and lysis leading to the death of an insect. The toxin is encoded by the *cry* genes, which are insect group specific. For example,

- *cry* IAc and cry IIAb control cotton bollworms.
- *cry* IAb controls corn borer.
- *cry* IIIAb controls colorado potato beetle.
- *cry* IIIBb controls corn rot worm.

Strategy for Protection of Crops by *Bt* Protein

The two strategies which have been employed are discussed below:

(i) The spores of *B. thuringiensis* are sprayed with water on the crop plants. The target insect ingests these spores and are killed by the insecticidal action of *B. thuringiensis*.

(ii) **Genetic engineering** The cry gene that encodes for *Bt* protein can be used in two ways as follows:

(a) **Isolation of *cry* gene** followed by its introduction into other species like *E. coli* or *Pseudomonas fluorescens* to form transformed bacteria which are then sprayed on the crop plants as they are better suited for survival in the fields.

(b) **Isolation of *cry* gene** followed by its insertion into Ti plasmid (Tumour-inducing plasmid) of *Agrobacterium tumefaciens* to transform a plant protopast, generating insect resistant plants.

Bt Crops : A Ground Reality

Many crop plants have been genetically modified by the *Bt* protein gene (*cry*). Some of the examples are *Bt* cotton, *Bt* tobacco, *Bt* walnut, Bt coffee, etc.

Potential Risks

Bt technology has caused an increased fear of resistance of the pests to the *Bt* protein and appearance of resistant varieties of pests in the future.

PRACTICE QUESTIONS

Exams', Textbook's Other Imp. Questions

Exams' questions

- *Fill in the blanks with the correct answers*

Q.1 Geneticaly engineered rice rich in vitamin-A is known as ..... . **[2018]**

Ans Golden rice

Important Questions

- *Multiple choice questions*

Q.2 Which one of the following is/are applied in several medical diagnostic procedures?
(a) Recombinant DNA technology
(b) Polymerase chain
(c) ELISA
(d) All of the above

Ans (d) Recombinant DNA technology, polymerase chain reaction and ELISA are applied in several medical diagnostic procedures.

Q.3 Golden rice is produced by rice plant having a transgene encoding an enzyme in biosynthetic pathway of **[Textbook]**
(a) β-carotene (b) luciferin
(c) glyphosate (d) *Bt* protein

Ans (a) Golden rice is produced by rice plant having a transgene encoding an enzyme in biosynthetic pathway of β-carotene.

Q.4 Fruit ripening is delayed by preventing the expression of the enzyme **[Textbook]**
(a) luciferase (b) polygalacturonase
(c) nitrogenase (d) adenosine deaminase

Ans (b) Fruit ripening is delayed by preventing the expression of the enzyme polygalacturonase.

Q.5 Humulin is manufactured by **[Textbook]**
(a) Pfizer (b) Hoechst
(c) Eli Lilly (d) Aventis

Ans (c) Humulin is manufactured by Eli Lilly.

Q.6 Genetic correction of inflicted cells is made *in vitro* and then reimplanted into its natural environment. This therapy is known as **[Textbook]**

(a) *ex vivo* gene therapy (b) *in vivo* gene therapy
(c) *in vitro* gene therapy (d) *in toto* gene therapy

Ans (a) Genetic correction of inflicted cells is made *in vitro* and then reimplanted into its natural environment. This therapy is known as *ex vivo* gene therapy.

- *Fill in the blanks*

Q.7 Herbicide resistant plants are generated by plant tissue culture technique by transferring …… gene of a bacterium into a plant protoplast. **[Textbook]**

Ans Herbicide resistant plants are generated by plant tissue culture technique by transferring glyphosate gene of a bacterium into a plant protoplast.

Q.8 A bacterium species of …… genus is genetically engineered to prevent frost formation in plants. **[Textbook]**

Ans A bacterium species of *Pseudomonas* genus is genetically engineered to prevent frost formation in plants.

Q.9 Monoclonal antibody is synthesised and secreted by a cell known as …… . **[Textbook]**

Ans Monoclonal antibody is synthesized and secreted by a cell known as hydridoma.

Q.10 Severe Combined Immuno Deficiency (SCID) is expressed due to the absence of an enzyme, …… . **[Textbook]**

Ans Severe Combined Immuno Deficiency (SCID) is expressed due to the absence of an enzyme, adenosine deaminase.

Q.11 A bioluminescent plant is generated by transferring …… gene of a firefly into plant protoplasts. **[Textbook]**

Ans A bioluminescent plant is generated by transferring luciferase gene of a firefly into plant protoplasts.

Q.12 Golden rice producing plant is a transgenic plant, whose cells have a transgene encoding…… . **[Textbook]**

Ans Golden rice producing plant is a transgenic plant, whose cells have a transgene encoding β-carotene.

- *Express in one word or two words*

Q.13 An insecticidal protein, produced by *Bacillus thuringiensis*. **[Textbook]**

Ans Cry protein

Q.14 The tomato plant variety that bears tomatoes exhibiting delayed ripening. **[Textbook]**

Ans *Flavr savr*

Q.15 The somatic hybrid cell, which produces monoclonal antibodies. **[Textbook]**

Ans Hybridoma

Q.16 Name one plant used to create novel transgenic plants.

Ans *Petunia*

Exams' Questions

Q.17 What is humulin? **[2019]**

Ans Humulin became the first genetically engineered product approved for medical use. It was the synthetic insulin produced by genetic engineering technique by inserting a synthetic *c*DNA gene into the bacterium. *E. coli* and performing post-translational modifications.

Q.18 What is recombinant vaccine? **[2018]**

Ans A recombinant vaccine is produced by applying recombinant DNA technology. It involves the insertion of DNA encoding antigen (like bacterial surface protein) that can stimulate immune response in host cell and expresses the antigen. This antigen is then isolated, purified and cloned to high number and later processed.

Recombinant vaccines can be

- Subunit recombinant vaccine, i.e. components of pathogenic organisms.
- Attenuated recombinant vaccines, i.e. the genetically modified but weakened pathogenic agents.
- Vector recombinant vaccines, i.e. genetically modified viral vectors.

Q.19 Write a short note with 2-3 important points on *Bacillus thuringiensis*. **[2016, 14]**

Or Why do the toxic insecticide proteins secreted by *Bacillus thuringiensis* kill insects?

Ans *Bacillus thuringiensis* (*Bt*) is a soil borne, Gram positive bacterium. It is used to create transgenic plants having resistance to different pests. The genes having insecticidal properties in the bacterium are isolated and incorporated into plants by using advanced biotechnological methods to create *Bt* plants. During sporulation, many *Bt* strains produce crystal proteins (proteinaceous inclusions) called δ-endotoxins that have insecticidal action. When consumed by insect, these toxins bind

to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis, leading to the death of an insect, e.g. *Bt* cotton, *Bt* tomato, soyabean, coffee, etc.

Important Questions

Q.20 What is Golden rice? **[Textbook]**

Ans Golden rice is a transgenic variety of rice with an elevated level of β-carotene (provitamin-A), a precursor of vitamin-A. The genes encoding the enzymes of the β-carotene biosynthetic pathway are introduced into rice plant cells in culture. The transgenic rice plants generated produce rice with β-carotene.

Q.21 What is *Flavr Savr* tomato? **[Textbook]**

Ans Fruit ripening in tomato and other fruits and vegetables are delayed by manipulating a gene, involved in softening and ripening. A variety of tomato plant has been successfully engineered, which bears tomatoes, known as *Flavr savr* tomatoes. This variety exhibits delayed ripening of tomatoes.

Q.22 What do you understand by *ex vivo* gene therapy? **[Textbook]**

Ans ***Ex vivo* gene therapy** The affected cells are removed from the body and transformed by the remedial gene *in vitro*. The transformed cells are grown in a cell culture medium to a sufficient number and then returned to the body by transfusion or transplantation.

Q.23 What do you mean by a biopesticide? Give an example. **[Textbook]**

Ans Biopesticides are the type of pesticides produced from a living organism. They are equally potent but do not inflict a damage on the environment, e.g. a species of bacteria with insecticidal properties is *Bacillus thuringiensis*. It produces insecticidal Cry protein or *Bt* protein.

Q.24 What are frost protected plants?

Ans. Soil bacterium *Pseudomonas syringae* contains a gene that promotes ice nucleation. Using gene technology, this gene is deleted and a mutant *P. syringae* is engineered known as INA or ice minus strain and it prevents frost (ice) formation when sprayed on crops.

Q.25 How is a mature, functional insulin hormone different from its prohormone forms?

Ans In human, insulin is synthesised as a prohormone or proinsulin which contains three chains A, B and C (stretches of peptides). This C peptide is removed during maturation of insulin, while A and B chains are linked together by disulphide bridges.

Exams' Questions

Q.26 Differentiate between *ex vivo* gene therapy and *in vivo* gene therapy. **[2018]**

***Ex vivo* gene therapy**	***In vivo* gene therapy**
The cells are removed from the patient and genetic material is inserted in them *in vitro*, prior to transplantation of modified cells.	The genetic material is transferred directly into cells within a patient.
This approach is applicable to tissues that can be removed from the body and returned later and survive for longer period of time, e.g. hematopoietic cells.	It is only possible in tissues where the individual cells cannot be cultured *in vitro* in sufficient numbers or where cultured cells cannot be efficiently reimplanted, e.g. brain cells.

Q.27 Write a note on genetically modified organism. **[2018]**

Ans Plants, fungi, bacteria and animals whose DNA has been manipulated to possess and express a foreign DNA, are called, genetically modified organisms or transgenic organisms.

GMOs have wide ranging application in agricultural and animal husbandry, medicine and healthcare management and environmental monitoring and management.

Advantages of GMOs are as follows :

- Genetic modification has made the crops more tolerant to abiotic stresses like cold, heat, drought, etc.
- It has reduced the dependence of crops on chemical pesticides as they are made pest resistant.
- Post harvest losses are much reduced.
- These plants have increased efficiency of mineral usage and hence, the early exhaustion of soil fertility is prevented.
- Food produced from GM crops have enhanced nutritional value.

Important Questions

Q.28 Write a brief note on the herbicide resistant plants. **[Textbook]**

Ans Herbicide resistant transgenic plants are generated by transferring bacterial herbicide resistant genes into plant cells grown in culture. Glyphosate is the most widely used broad-spectrum herbicide world over. A glyphosate resistant gene from *Petunia* plant is transferred into isolated plant cells in culture and glyphosate resistant plants are generated.

7 MARK *Questions*

Important Questions

Q.29 Explain what is gene therapy. Give a detailed description of the two types of gene therapy.

Ans Refer to text on page no. 204.

Q.30 What are the various applications of biotechnology in the field of agriculture and forestry? Explain in detail.

Ans Refer to text on page no. 201 and 202.

TOPIC TEST 1

1. The first genetic disorder treated by gene replacement therapy is **[Textbook]**
   (a) Familial Hypercholesterolemia (FH)
   (b) Cystic Fibrosis (CF)
   (c) Duchenne Muscular Dystrophy (DMD)
   (d) Severe Combined Immuno Deficiency (SCID)
   [**Ans.** (d)]
2. Delayed ripening in tomato is due to the inhibition of expression of an enzyme ..... . **[Textbook]**
   [**Ans.** polygalacturonase]
3. The first recombinant human vaccine produced and marketed is ..... vaccine. **[Textbook]**
   [**Ans.** humulin]
4. Recombinant insulin in the trade name of humulin is manufactured by ..... [**Ans.** Eli Lilly company]
5. Name the drugs isolated from *Catharanthus roseus* for cancer treatment. [**Ans.** Vincristine and Vinblastine]
6. Given the answer in one word only.
   The corn was genetically engineered by transferring *Bt* protein gene into plant protoplasts. The brand was marketed and later was withdrawn due to safety reasons. **[Textbook]**
   [**Ans.** Starlink corn]
7. What does the recombinant hepatitis-B vaccine contain?
8. Write any three ways on how genetically modified plants are found to be useful.
9. Name the genes responsible for making *Bt* cotton plants resistant to bolloworm attacks. How do such plants attain this resistance? Explain.
10. What is ADA deficiency? Mention its cause. Also, describe three methods to cure it.

TOPIC ~02 Transgenic Animals and Ethical Issues

Transgenic Animals

Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as **transgenic animals**, e.g. transgenic rats, rabbits, pigs, sheep, cows and fish. Over 95% of all the existing transgenic animals are mice. The gene that is being transferred is called **transgene**.

It is done by using several methods but in animal, microinjection technique is most suitable for DNA manipulation.

The possible benefits and reasons of producing transgenic animals are as follows:

(i) Study of physiology and development
(ii) Study of diseases
(iii) Vaccine safety
(iv) Chemical safety testing
(v) Biological products

Transgenic Mouse

The first transgenic mouse was developed by **RL Brinster** and **R Palmiter** in 1982. They transferred growth hormone gene isolated from a rat into a fertilised mouse egg by microinjection *in vitro*. Then, this fertilised egg was implanted in the uterus of pseudopregnant mouse. The resulting progenies were called supermouse because of their larger size and abnormal growth.

Pharming

It is the commercial use of transgenic animals as a source of important pharmaceutical products.

For example, the supermouse was genetically altered to produce **Tissue Plasminogen Activator** (TPA), an agent that dissolves blood clot.

Animal Cloning and Making of Dolly

Ian Wilmut of Roslin Institute in Scotland came up with a cloned sheep, named Dolly in February, 1997. He used nuclear transplantation technique to create Dolly, a clone of a sheep. This was the first ever clone of an animal. Following this, two other sheep, named, Polly and Molly were also created in the same manner.

Biosafety Issues

Genetic engineering holds great promise for improving crop plants and domestic animals, for correcting genetic disorders in human beings, for improving species and evolving new and beneficial varieties. However, it also creates new threats like

(i) Non-pathogenic bacteria like *Escherichia coli* may get transformed into harmful disease-causing or cancer-causing form.

(ii) New harmful strains of bacteria and viruses may be developed by scientists which if used as warfare can cause havoc.

(iii) Some 'superviruses' may develop bychance or by intention, for which we might have no defence.

(iv) Drug-resistant pathogens may leak intentionally or bychance in the environment and create harmful effects on living beings.

Thus, Genetically Modified Organisms (GMOs) are introduced in a planned way.

Biosafety in broad sense refers to the preservation of biological integrity of an organism or biological process and products from being lost. It helps to protect the rights of the inventor as they put in their knowledge, skill and money to successfully execute a complete process. Regulatory authorities where matters of biosafety are addressed include United Nation Convention on Biological Diversity (CBD) and Cartagena Protocol on Biosafety issues.

Convention on Biological Diversity (CBD)

The convention on biological diversity is dedicated in the promotion of sustainable development, raising a concern on the potential hazards of various biotechnological processes applied for the development of plant and animal transgenics.

Cartagena Protocol on Biosafety

It is an international agreement established by CBD as a draft protocol (1995) on biosafety. It aims to ensure the safe handling, transport and use of living modified organisms developed using techniques of modern biotechnology.

Patent

It refers to a set of exclusive legal rights granted by a government to the inventors or their assignee for a limited period of time to prevent others from commercial use of their invention. When patent is granted for biological entities and for products derived from them, they are called biopatents.

Biopatent Laws

Biopatents or Intellectual Property Rights (IPRs) are under the domain of national laws. Different countries have framed different IPR laws to maintain balance between industry's desire to capitalise on investments in technological development and the right of the society to get benefit from the resources.

Now, patent rights have been internationalised. However, General Agreement on Tariffs and Trade (GATT) under the auspices of World Trade Organisation (WTO), established in January 1995, the rule that all member countries have to frame their national IPR laws in agreement with Trade Related Intellectual Property Rights (TRIPRs).

Patent Act of India

India enacted the Patent Act in 1970. Later, this act has undergone amendments in 1999, 2002, 2005 and 2006. Its headquarter is in Kolkata, West Bengal.

The nodal centre for Indian biosafety network is the Department of Biotechnology, Government of India.

Prerequisite for a Patent Grant

An invention can be submitted for patent grant when it fulfils the following set of conditions laid by the Indian Patent Act of 1970.

(i) The invention (like a product, process, concept, model, etc.) must be non-obvious and inventive, i.e. it should not be available to the public earlier.

(ii) This invention should have industrial utility.

(iii) Sufficient details should be provided for the invention before filling the patent. This will help an moderately skilled individual to work the invention without further experimentation.

Patent Related Case Studies

(i) **Diamond *vs* Chakraborty Case** Dr. Anand Mohan Chakraborty, an Indian born American scientist created a superbug species of the bacterium *Pseudomonas* genus that could eat the oil and consequently clear oil spill.

His superbug could not be patented on the plea that it was a living organism.

(ii) **Neem Patent Case** A patent was granted to the the multinational agribusiness company, WR Grace of New York and United States Department of Agriculture, Washington DC for the fungicidal use of neem oil.

Dr. Vandana Shiva of Research Foundation for Science and Technology and Natural Resource Policy, New Delhi and others filed a legal opposition to the grant of patent.

A case was admitted and after several rounds of hearing the patent granted to WR Grace was withdrawn.

(iii) **Turmeric Patent Case** In 1995, the USA Lab granted a patent to the Medical Centre, University of Missisippi (USA) for the use of turmeric (haldi) powder as a wound healing agent. CSIR, New Delhi (India) objected this patent and the patent was cancelled in 1997.

Biopiracy

It refers to the use of bioresources by multinational companies and other organisations without proper authorisation from the countries and the people concerned without compensatory payment. The majority of industrialised nations are financially rich but poor in biodiversity and traditional knowledge, in comparison to developing and underdeveloped countries. Another cause of biopiracy is bioprospecting which means a thorough survey of a source material to expand the knowledge and applications in biotechnology. During the course of bioprospecting, scientists may transfer any biological resource which they may consider as novel.

Controversies in India Regarding Patent and Biopiracy

Following are the plants on which controversies occurred in India

Basmati Rice

An American company **Rice Tec** won a patent on Basmati rice lines and grains in 1997 through the US Patent and Trademark Office (USPTO) and was allowed to sell a 'new variety' in US and Abroad. This new variety of Basmati was actually derived from the Indian farmer's varieties. Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty by the American company.

Turmeric and Neem

In May, 1995 the US Patent Office granted to the University of Missisippi Medical Centre (a patent for 'Use of Turmeric in Wound Healing').

In 1996, another patent was granted to the firm of WR Grace and Company, by the European Patent Office, Munich for 'fungicidal uses of neem oil'.

Thus, if we do not pay attention or counter these patent application, our rich legacy will be encashed by other countries and individuals.

PRACTICE QUESTIONS

Exams', Textbook's Other Imp. Questions

1 MARK *Questions*

Exams' Questions

- *Give the answer in one word only*

Q.1 What is the name of the scientist who cloned a sheep named Dolly? **[2019]**

Ans Keith Campbell and lan Wilmut

Q.2 Which department of the Government of India is the nodal centre for India biosafety network? **[2018]**

Ans Department of Biotechnology

- *Fill in the blanks with correct answer*

Q.3 The transgenic mouse is called as ..... . **[2018]**

Ans The transgenic mouse is called as supermouse.

Important Questions

- *Choose the correct answer*

Q.4 Which of the following patent cases, India is not directly or indirectly connected with? **[Textbook]**

(a) Soyabean patent case (b) Neem patent case
(b) Turmeric patent case (d) Chakraborty patent case

Ans (a) India is not directly connected with soyabean patent case.

Q.5 The supermouse is a genetically modified animal with **[Textbook]**

(a) insulin transgene
(b) lipid biosynthesis transgene
(c) growth hormone transgene
(d) steroid hormone transgene

Ans (c) The supermouse is a genetically modified animal with growth hormone transgene.

- *Fill in the blanks with correct words*

Q.6 A legal right, privilege and authority granted to a person for a limited period for an invention is known as ..... . **[Textbook]**

Ans A legal right, privilege and authority granted to a person for a limited period for an invention is known as patent.

Q.7 The use of novel biological resource of a sovereign country without its due permission is known as ..... . **[Textbook]**

Ans The use of novel biological resource of a sovereign country without its due permission is known as biopiracy.

- *Answer in one word*

Q.8 The biosafety protocol that was drafted in 1995 and adopted in 2000. **[Textbook]**

Ans Cartagena Protocol

2½ MARK *Questions*

Exams' Questions

Q.9 What is patent? **[2018]**

Ans A patent is the set of exclusive rights granted by a government to an inventor in order to prevent others from commercially using this invention. When a patent is granted for a biological invention, it is called as biopatent.

A patent is granted for

(i) an invention
(ii) improvement in an earlier invention
(iii) a process
(iv) a concept or a design

Important Questions

Q.10 Write a note on transgenic animals. **[Textbook]**

Ans Transgenic animals are those animals that carry foreign genes or animals that have had their DNA manipulated to possess and express an extra foreign gene. The potential of the technology has also made it possible to consider employing animals like rats, rabbits, cattle, swine, etc., in manufacture of biomolecules or organ donors.

Some benefits of transgenetic animals are :

(a) Vaccine safety
(b) Chemical safety testing
(c) Study of diseases
(d) Normal physiology and development

3½ MARK *Questions*

Important Questions

Q.11 Enumerate and explain in brief two biosafety issues, biotechnology is confronted with. **[Textbook]**

Ans Biosafety in a broad sense, refers to the prevention of loss of biological integrity of biological processes and products, harvested by using living organisms. For example, recombinant insulin was manufactured in a complex biological process putting in thought, knowledge, skill and execution method of the inventor. Secondly, a lot of energy and money was spent in the successful execution of the process. Therefore, the right of the inventor needs to be protected by law considering it as a property. Insulin that is manufactured needs a prescribed trial process to prove that it is suitable for human use.

Another potential hazard was the release of Genetically Modified Organisms (GMOs) into the environment. There was a threat that it might exchange genes, consequently changing the gene pool.

Q.12 Write a detailed note on biopiracy. **[Textbook]**

Ans Refer to text on page no. 210.

TOPIC TEST 2

1. Patent is not granted for **[Textbook]**
(a) a novel invention
(b) an invention having an industrial application
(c) a discovery made by previously existing knowledge
(d) an invention having an inventive step
[**Ans**. (c)]

2. Which of the following is not related to biosafety? **[Textbook]**
(a) Convention on Biological Diversity
(b) Cartagena Protocol
(c) World Trade Organisation
(d) UNICEF [**Ans**. (d)]

3. The transgenic protein that is used to treat emphysema. [**Ans.** α-1 antitrypsin]

4. ..... was the first transgenic cow. [**Ans.** Rosie]

5. ..... plants are those plants which possess genes from other organisms or plants. [**Ans.** Transgenic]

6. What is a supermouse?

7. What do you mean by biopatent?

8. Differentiate between animal pharming and animal cloning.

9. Describe the evolution of Indian Patent Act. **[Textbook]**

10. Describe the three controversial case regarding patent and biopiracy in India.

Chapter Test

1 MARK *Questions*

- *Choose the correct options*

1 A biotech company that released first ever genetically manipulated flower into the market is
(a) Cryobank (b) Eli Lilly
(c) Florigene (d) Genentech

2 Which of the following is produced by genetically engineered bacteria?
(a) Thyroxine (b) Insulin
(c) Glucagon (d) ADH

3 Cry II Ab and Cry I Ab produce toxins that control
(a) cotton bollworms and corn borers, respectively
(b) corn borers and cotton bollworms, respectively
(c) tobacco budworms and nematodes, respectively
(d) nematodes and tobacco budworms, respectively

4 First genetically modified plant commercially released in India is
(a) Golden rice (b) slow ripening tomatoes
(c) *Bt* brinjal (d) *Bt* cotton
[**Ans.** 1. (c), 2. (b), 3. (a), 4. (d)]

- *Correct the statements, if required, by changing the underlined word(s)*

5 Soil bacterium *Nitrosomonas syringae* promotes ice nucleation in plants.

6 Antitrypsin is an agent that dissolves blood clot.

7 The first transgenic sheep was Lilly.
[**Ans.** 5. *Pseudomonas syringae*, 6. Tissue Plasminogen Activator (TPA), 7. Dolly]

- *Express in one or more words*

8 State the number of polypeptides found in mature human insulin.

9 Which bacterium species is involved in Diamonds *vs* Chakraborty case?
[**Ans.** 8. 2, 9. *Pseudomonas* species]

- *Fill in the blanks*

10 ..... is a mammalian protein that have been successfully expressed in plants.

11 ..... is an infant nutrition formula that has been harvested using transgenic animals as bioreactors.
[**Ans.** 10. Enkephalin, 11. Lactoferrin]

2½ MARK *Questions*

12 What do you mean by the term transgene?

13 Mention the steps involved in insulin production.

3½ MARK *Questions*

14 How recombinant DNA technology is useful is detecting the presence of mutated genes?

15 State why biopiracy should be prevented and how?

16 Write a short note on convention on biological diversity.