BIOLOGY

BIOTECHNOLOGY AND ITS APPLICATIONS

Biotechnology:

The use of biology to develop technologies and products for the welfare of human beings is known as biotechnology.

It has various applications in different fields such as therapeutics, diagnostics, processed food, waste management, energy production, genetically modified crops, etc.

Biotechnological Application in Agriculture:

- Biotechnology has different applications in agriculture.
- It can be used in agrochemicals, organic agriculture, and genetically engineered crop-based agriculture.
- To produce genetically modified organisms it can be used. Genetically modified organisms (GMO) can be obtained by alteration in their genetic material.

A different application of genetically modified organisms is:

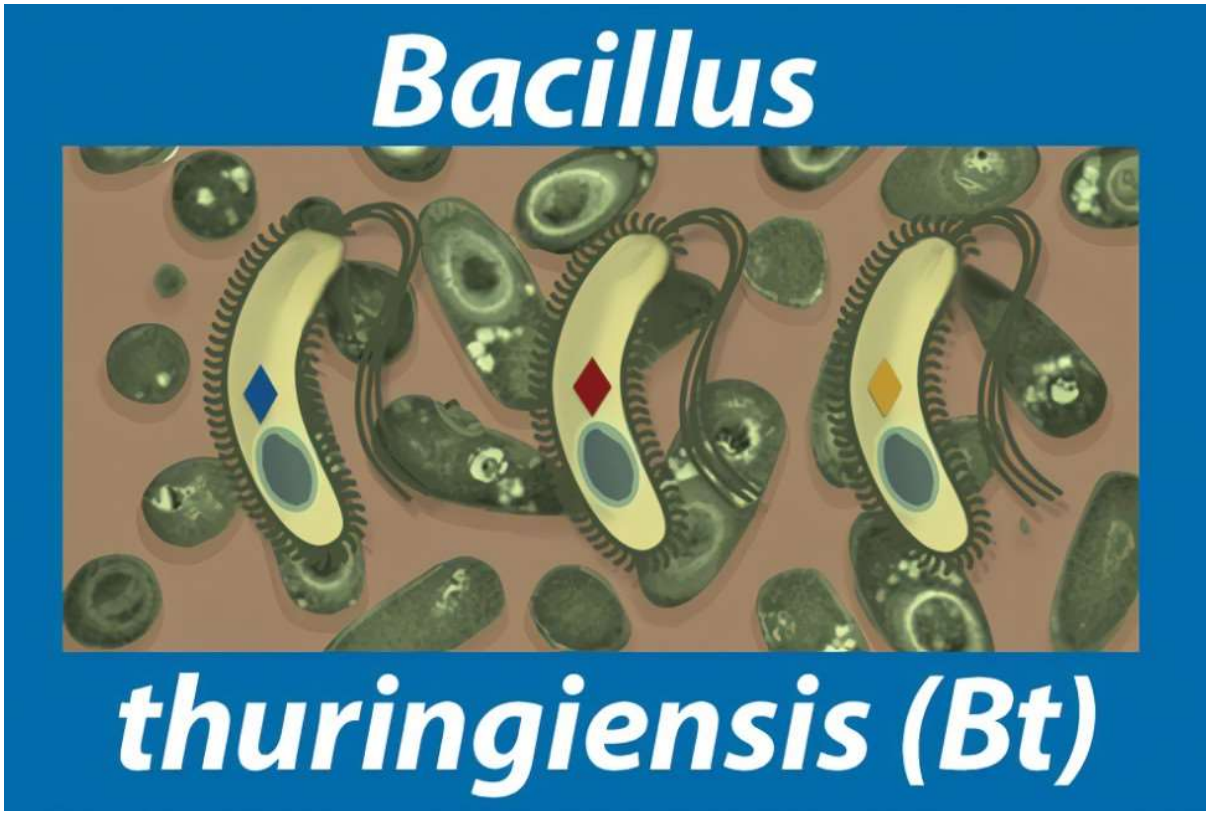
- Production of crops that are resistant to abiotic factors such as heat, cold, etc.
- Pest-resistant crops.
- Help to reduce post-harvest losses.
- Minerals can be used by the plants efficiently.
- Food with enhanced nutritional values.

Green Revolution:

The Green Revolution succeeded in tripling the food supply 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). 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 Use of genetically modified crops is a possible solution.

Bacillus thuringiensis (Bt):

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 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. Bt toxin protein exist as inactive protoxins once the insect ingest the inactive toxin it is converted into an active form of toxin due to alkaline pH of gut which solubilise crystals activated toxin binds to the surface of midgut epithelial cells create pores that cause cell swelling and lysis and eventually cause death of the insect.

Cry:

Specific Bt toxin genes were isolated from Bacillus thuringiensis and incorporated into the several crop plants such as cotton as most Bt toxins are insect-group specific. The toxin is coded by a gene named cry. for example, the proteins encoded by the genes cryIAc and cryIIAb control the cotton bollworms, that of cryIAb controls corn borer.

Pest Resistant Plants:

Various pests affect the plants which cause loss as well as a decrease in the yield of the plant.

A nematode Meloidogyne incognita infects the roots of tobacco plants and causes a decrease in the yield of the plant. To prevent this, RNA interference technology was used. This method involves silencing a specific mRNA due to a complementary dsRNA molecule. This inhibits the

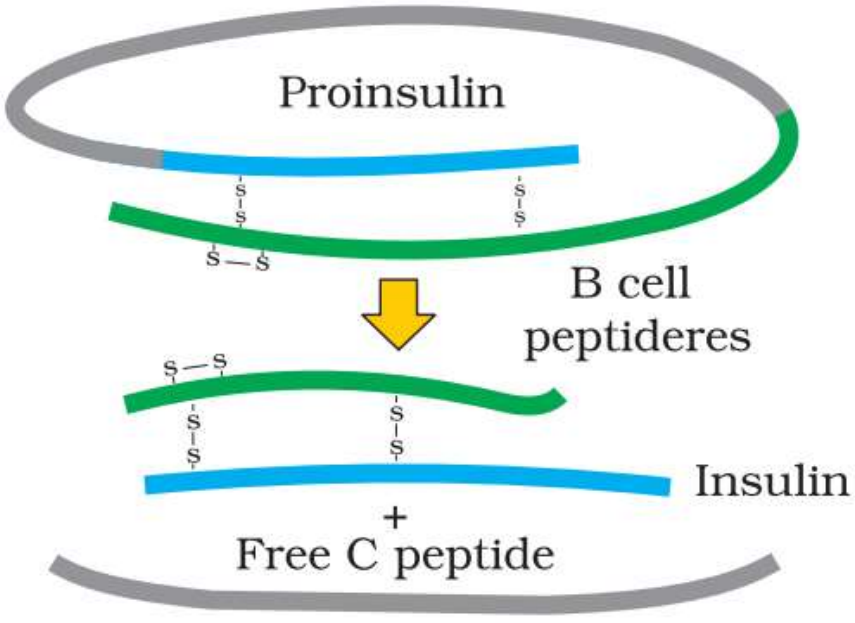
translation of the mRNA.

Biotechnological Applications 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.

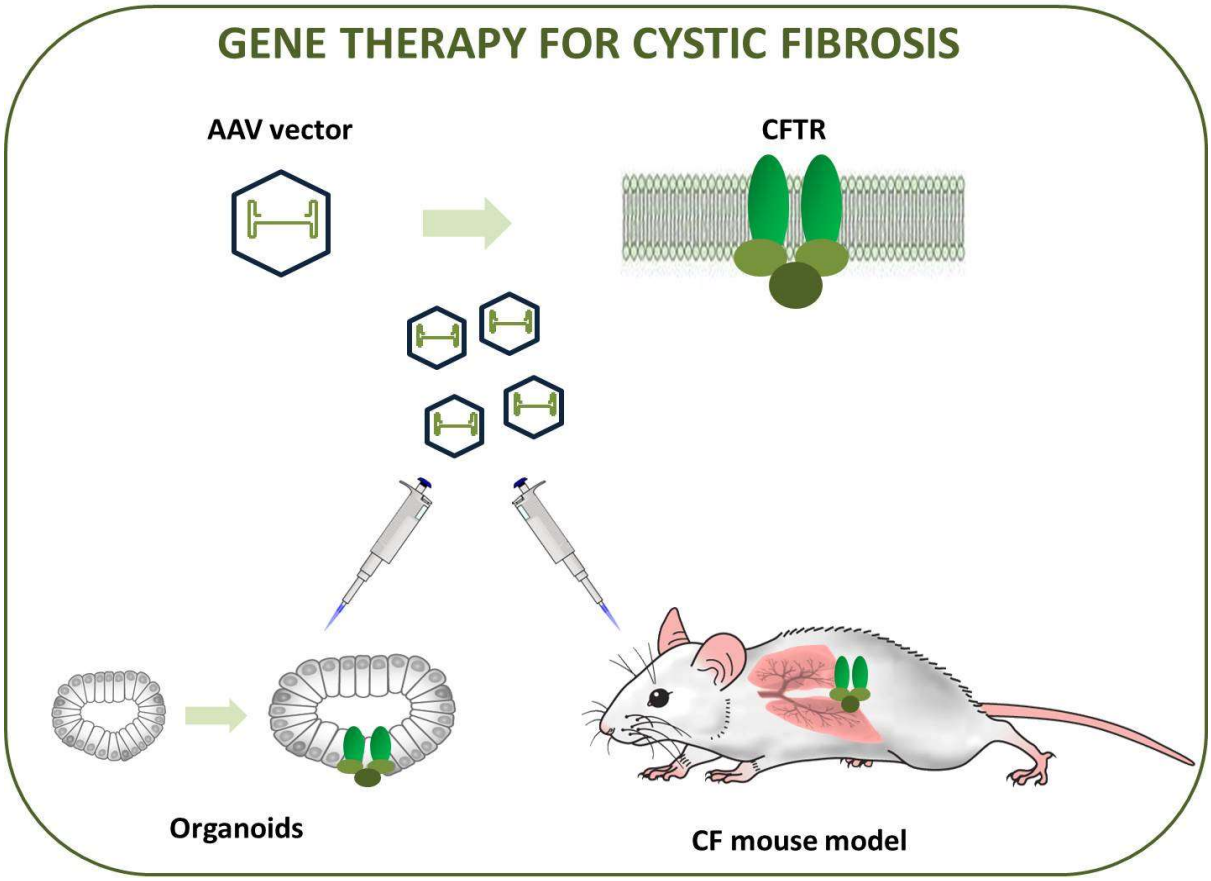
Genetically Engineered Insulin:

- Adult-onset diabetes can be controlled by taking insulin at regular intervals. The main source of this insulin was isolation of insulin from animals. Now a day's insulin can be obtained from bacterium using techniques of biotechnology.
- Insulin was earlier extracted from pancreas of slaughtered cattle and pigs but insulin from these sources develops allergy or other types of reactions to the foreign protein.
- Insulin consists of two short polypeptide chains- chain A and chain B, that are linked together by disulphide bridges.
- In humans, insulin is synthesized as a prohormone, which contains an extra stretch called C peptide, which is absent in mature insulin. The main challenge for production of insulin using rDNA technique was getting insulin assembled into a mature form.
- An American company, Eli Lilly in 1983 prepared two DNA sequence corresponding to A and B chain of human insulin and introduced them in plasmids of E.coli to produce insulin chain. Chain A and Chain B were produced separately, extracted and combined by creating disulphide bonds to form human insulin.



Gene Therapy:

- It is a collection of methods that allows correction of a gene defect that has been diagnosed in a child or embryo. This method is applied in a person with a hereditary disease. In this method, genes are inserted into a person's cells and tissues to treat a disease.
- The correction of gene defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for non-functional gene.
- The first clinical gene therapy was done in 1990 to a 4 year old girl with adenosine deaminase (ADA) deficiency. This disorder is caused due to the deletion of the gene for adenosine deaminase that is essential for immune system to function. This defect can be treated by enzyme replacement therapy in which functional ADA is given to the patient by injection or bone marrow transplant.
- In gene therapy method lymphocytes from the blood of the patient are grown in culture medium outside the body. A functional ADA cDNA is then introduced into these lymphocytes and returned to the patient. In this method periodic infusion of such genetically engineered lymphocytes is needed. If gene isolated from bone marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.



Molecular Diagnosis:

Conventional method of diagnosis such as serum or urine analysis is not able to early detection of disease-causing pathogens or virus.

Following methods can be used to diagnosed earlier:

- Recombinant DNA technology.
- Polymerase Chain Reaction (PCR).
- Enzyme Linked Immuno-sorbent Assay (ELISA).

Symptoms of disease appear only when the concentration of pathogen get increased significantly. Low concentration of bacteria and virus can be detected by amplification of nucleic acid by PCR. It detects the mutation in the gene in cancer patient. PCR is routinely used to detect the HIV in suspected AIDS patients. Genetic disorder can be also detected by using PCR technique.

A single stranded DNA or RNA having radioactive molecule is allowed to hybridize to its complementary DNA in a clone of cells followed by detection using autoradiography. The clone having the mutated gene will not appear on the photographic film.

ELISA is based on the principle of antigen-antibody interaction. Infection by pathogen can be detected by the presence of antigens like proteins, glycoproteins etc. or by detecting the antibodies synthesised against the pathogen.

Transgenic Animals:

Transgenic animals can be defined as those animals in which a new or altered gene has been experimentally inserted into the genome by genetic engineering technique.

Few examples of transgenic animals are rats, rabbits, pigs, sheep, cows, fish, etc. Among all other transgenic animals, the mouse is the existing transgenic animal.

The main aim behind the creation of transgenic animals are:

- For the production of biological products.
- To study the different types of diseases.
- To study the contribution of genes in the development of the disease.
- For testing the safety of vaccines and toxicity of drugs before they are used on humans.
- To study how genes are regulated and how do they affect the normal functioning of the body and its development.

Applications in Aquaculture:

Biotechnology applications help in the improvement of quality and quantity of fish. The gonadotropin-releasing hormone is introduced into the fish to enhance breeding. This helps in enhancing growth and improving their genetic characteristics. It also prevents a number of

diseases.

Production of Antibiotics:

Biotechnology helps in the production of vaccines, antibiotics and artificial hormones, using plants. Genes with desired characteristics are introduced into the plants to manufacture the encoded proteins. Edible vaccines are cost-effective, can be easily stored and administered in the body. These are used to cure diseases such as measles, hepatitis, cholera, etc.

Ethical Issues:

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

Biopiracy: The use of bio-resources found in any country by commercial and multinational companies and other organizations without taking appropriate authorization and permission from the countries and their people concerned and also without making the compensatory payment is biopiracy.

MIND MAP : LEARNING MADE SIMPLE

CHAPTER - 12

Biotechnology and its Applications

Transgenic animals

Advantages

- To study normal physiology and development.
- To study the contribution of genes in the development of a disease.
- Biological products.
- Vaccine safety testing.
- Chemical safety testing.

Definition

- Animals that have their DNA manipulated to possess or express an extra gene.
- E.g. Transgenic rats, rabbits, pigs, sheep, Rosie cow and fish.

GEAC

- Make decisions about the validity of GM research.
- Safety of GM-organisms for public services.

Biopiracy and biopatent

- Use of bio-resources by multinational companies and other organizations without proper authorization from the countries and people concerned is biopiracy.
- Patents granted for biological entities and for products derived from them are called biopatents.E.g. Basmati Rice.

In Agriculture

Advantages of GM Crops

- Makes crops more tolerant to abiotic stresses
- Reduces resilience on chemical pesticides.
- Helps to reduce post-harvest losses.
- Increases efficiency of mineral usage by plants.
- Increases nutritional value of food. E.g. Golden rice.

Pest resistance in Cotton plant

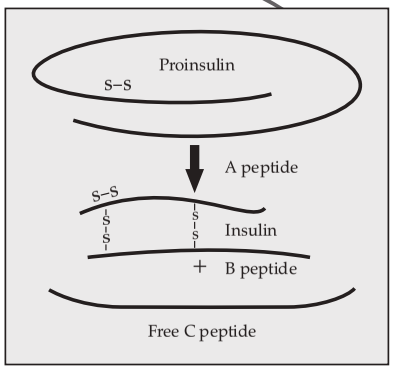
- *Bacillus thuringiensis* contain Bt toxin, which is in inactive form.
- It becomes active toxin in the alkaline pH of insect gut.
- The activated toxin binds to the surface of midgut epithelial cells of insect and creates pores.
- This causes cell swelling and lysis and eventually death of the insect.

Pest resistance in Tobacco plant

RNA interference (RNAi) strategy is used to prevent the infestation of tobacco plants by Nematode *(Meloidegyne incognitia)*.

In Medicine

Genetically Engineered insulin



Gene Therapy

- Technique for correcting a defective gene through gene manipulation.
- First clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency.

Molecular diagnosis

- Polymerase Chain Reaction.
- Enzyme Linked Immuno-sorbent Assay (ELISA).

Important Questions

➢ Multiple Choice Questions:

1. Bt cotton is not:

(a) A GM plant
(b) Insect resistant
(c) a bacterial gene expression system
(d) Resistant to all pesticides.

2. C-peptide of human insulin is:

(a) A part of mature insulin molecule
(b) Responsible for formation of disulphide bridges
(c) Removed during maturation of pro-insulin to insulin
(d) Responsible for its biological activity.

3. GEAC stands for:

(a) Genome Engineering Action Committee
(b) Ground Environment Action Committee
(c) Genetic Engineering Approval Committee
(d) Genetic and Environment Approval committee.

4. a-1 antitrypsin as:

(a) An antacid
(b) An enzyme
(c) Used to treat arthritis
(d) Used to treat emphysema.

5. A probe which is a molecule used to locate specific sequences in a mixture of DNA or RNA molecules could be:

(a) A single-stranded RNA
(b) A single-stranded DNA
(c) Either RNA or DNA
(d) Can be ss DNA but not ss RNA.

6. Choose the correct option regarding Retrovirus.

(a) An RNA virus that can synthesise DNA during infection
(b) A DNA virus that can synthesise RNA during infection
(c) An ssDNA virus
(d) AdsRNA virus.

7. The site of production of ADA in the body is:

(a) Bone marrow
(b) Lymphocytes
(c) Blood plasma
(d) Monocytes.

8. A protoxin is:

(a) A primitive toxin
(b) A denatured toxin
(c) Toxin produced by protozoa
(d) Inactive toxin.

9. Pathophysiology is the:

(a) Study of physiology of pathogen
(b) Study of normal physiology of host
(c) Study of altered physiology of host
(d) None of the above.

10. The trigger for activation of toxin of Bacillus thuringiensis is:

(a) Acidic pH of stomach
(b) High temperature
(c) Alkaline pH of gut
(d) Mechanical action in the insect gut.

11. Golden rice is:

(a) A variey of rice grown along the yellow river in China
(b) Long stored rice having yellow colour tint
(c) A transgenic rice having gene for p-carotene
(d) Wild variety of rice with yellow coloured grains.

12. In RNAi, genes are silenced using:

(a) ssDNA
(b) dsDNA
(c) dsRNA
(d) ssRNA.

13. The first clinical gene therapy was done for the treatment of:

(a) AIDS
(b) Cancer
(c) Cystic fibrosis
(d) SCID.

14. ADA is an enzyme which is deficient in a genetic disorder SCID. What is the full form of

ADA?

(a) Adenosine deoxy aminase
(b) Adenosine deaminase
(c) Aspartate deaminase
(d) Arginine deaminase.

15. Silencing of a gene could be achieved through the use of:

(a) Short interfering RNA (RNAi)
(b) Antisense RNA
(c) By Both
(d) None of the above.

➢ Very Short Question:

1. Name the technique based on the principle of antigen-antibody interaction used in detection of a virus (HIV).

2. Development of a transgenic food crop may help in solving the problem of night blindness in the developing countries, name this crop plant.

3. Which nematode infects the roots of tobacco plant and causes a great reduction in yield?

4. The first transgenic cow, produced human protein – enriched milk. Name the cow and the protein found in milk.

5. The insulin produced using recombinant DNA technology is more advantageous than the insulin extracted from pancreas of slaughtered cattle and pigs. How?

6. Name two pest resistant plants produced by using recombinant DNA technology.

7. Name the genetically engineered human Insulin?

8. Write the Scientific name of nematode that attacks the root of tobacco plant?

9. Define a patent?

10. Expand GEAC.

➢ Short Questions:

1. What are the two methods for correcting ADA deficiency in a child?

2. Some crop plants are modified genetically by manipulating their genes. How are they made beneficial?

3. GEAC is one of the organisation set up by Indian Government. Write its full form. Give its two objectives.

4. "Industrialised nations are exploiting the bioresources of under industrialised nations . Justify the statement with a suitable example.

5. What is Golden rice? What is its advantage?
6. What are the three critical research areas in the field of Biotechnology?
7. What are the advantages of molecular diagnostics over conventional methods?
8. What do you mean by "Biopiracy" Give an example?

➢ Long Questions:

1. Mention any six fields of application of biotechnology for human welfare.
2. "Specific Bt Toxin gene is incorporated into the cotton plant so as to control the infestation of Bollworm". Mention the organism from which the gene was isolated and explain its mode of action.
3. How is the ELISA test carried out?

➢ Assertion and Reason Questions:

1. Two statements are given-one labelled Assertion and the other labelled Reason. Select the correct answer to these questions from the codes (a), (b), (c) and (d) as given below.

a) Both assertion and reason are true and reason is the correct explanation of assertion.

b) Both assertion and reason are true but reason is not the correct explanation of assertion.

c) Assertion is true but reason is false.

d) Both assertion and reason are false.

Assertion: Flavr-Savr tomato was the first transgenic commercial crop that entered the market.

Reason: Roundup variety of soybean was prepared through breeding.

2. Two statements are given-one labelled Assertion and the other labelled Reason. Select the correct answer to these questions from the codes (a), (b), (c) and (d) as given below.

a) Both assertion and reason are true and reason is the correct explanation of assertion.

b) Both assertion and reason are true but reason is not the correct explanation of assertion.

c) Assertion is true but reason is false.

d) Both assertion and reason are false.

Assertion: GM foods are facing widespread resistance by the people.

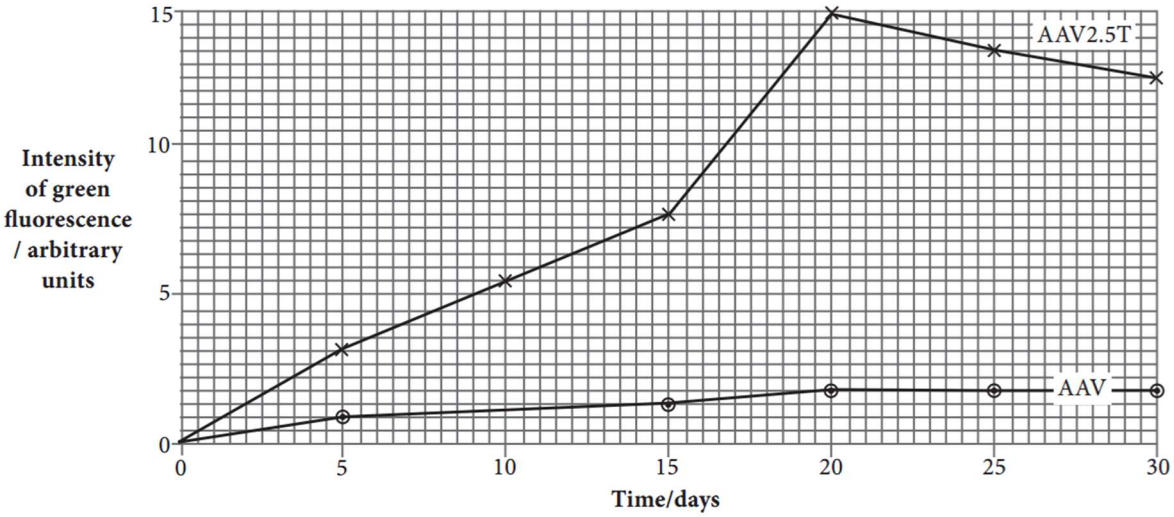
Reason: GM foods have mutated genes which cause infections and allergies.

➢ Case Study Questions:

1. Read the following and answer any four questions from (i) to (v) given below:

One approach of gene therapy to treat cystic fibrosis uses viruses to deliver normal alleles of the CFTR gene into epithelial cells of the airways. A team of researchers in the USA developed

a new strain of non-pathogenic adeno-associated virus (AAV), AAV2.ST. Genes for the CFTR protein and the enzyme luciferase were inserted into the DNA of the viruses. Luciferase catalyses the production of a green fluorescent protein when luciferin is added. The normal AAV strain and the AAV2.5T strain were added to cultures of epithelial cells from the airways. After adding luciferin, the number of cells that had taken up the viral genes was estimated using the intensity of the green fluorescence which developed. The result are shown in the given graph.



(i) What could be the probable reason for inserting a gene for luciferase by researchers into the viral DNA?

a) Infected cells are able to produce luciferase.
b) It is easy to identify the infected cells that have taken up viral DNA under fluorescent lamp.
c) THe non-infected cells were easily identified under fluorescent lamp as they will glow.
d) Both (a) and (b).

(ii) Select the incorrect statement with respect to the graph given.

a) Both AAV and AAV2.ST can infect epithelial cells.
b) Intensity of green fluorescence increases more in AAV 2.5 T as compared to normal AAV.
c) AA V infect cells more readily than AAV 2.5 T.
d) None of these.

(iii) The name of first transgenic cow is:

a) cDNA does not contain non-coding regions such as intrans.
b) cDNA can be transcribed and translated directly.
c) There is no need of post-trancriptional modification such as splicing, etc.
d) All of these.

(iv) There is a decrease in intensity of green fluorescence in cells infected with AAV 2.ST during the last 10 day. This is because:

a) Green fluorescent protein was broken down.
b) Luciferin was used up.
c) Infected cells die.
d) All of these.

(v) Which of the following best describe gene therapy?

a) Mutating the sequence of a particular gene.
b) Replacing a healthy gene with a defective gene.
c) Replacing a defective gene with a functional gene.
d) Transferring a healthy gene from one species with heathy gene of other species.

2. Read the following and answer any four questions from (i) to (v) given below:

European patent office, Munich granted patent for fungicidal use of neem oil, to firm of W.R. Grace & Co. It was challenged by Vandana Shiva and Ajay Phadke who had researched neem in India and it was shown that Grace & Co. had not unveiled any novelty factor in neem's properties. Ethics include rules of conduct by which a community regulates the behaviour and decides as to which activity is lawful and which is not.

(i) _____ allow private, monopoly right over animals and plants.
a) Bioethics.
b) Biopatents.
c) Bioweapons.
d) Either (a) or (b).

(ii) Which of the following is not a criteria of granting patent?

a) Novelty.
b) Utility.
c) Non-obviousness.
d) Prior art.

(iii) On which plants patents have been granted?

a) Piper nigrum.
b) Brassica campestris.
c) Punica granataum.
d) All of these.

(iv) Rules of conduct that may be used to regulate our activities in relation to biological world are included in _____.

a) Bioethics.
b) Biopiracy.
c) Biopatents.
d) Biowar.

(v) Assertion: Genes and cells should not be patented.

Reason: Genes and cells are not an inventions.

a) Both assertion and reason are true and reason is the correct explanation of assertion.
b) Both assertion and reason are true but reason is not the correct explanation of assertion.
c) Assertion is true but reason is false.
d) Both assertion and reason are false.

✓ Answer Key-

➢ Multiple Choice Answers:

1. (c) a bacterial gene expression system
2. (c) Removed during maturation of pro-insulin to insulin
3. (c) Genetic Engineering Approval Committee
4. (d) Used to treat emphysema.
5. (c) Either RNA or DNA
6. (a) An RNA virus that can synthesise DNA during infection
7. (a) Bone marrow
8. (d) Inactive toxin.
9. (c) Study of altered physiology of host
10. (c) Alkaline pH of gut
11. (c) A transgenic rice having gene for p-carotene
12. (c) dsRNA
13. (d) SCID.
14. (b) Adenosine deaminase
15. (c) By Both

➢ Very Short Answers:

1. ELISA (Enzyme linked immuno – sorbent Assay)
2. Golden Rice
3. Meloidegyneincognitia.
4. Rosie, alpha-lactalbumin
5. Insulin obtained from animal source causes allergy.
6. Bt Cotton, Bt Corn, BtBrinjal.
7. Humulin
8. Meloidogyneincognitia.

9. Patent is the government protection to the inventor of biological material, Securing to him for a specific time the exclusive right of manufacturing, exploiting, using & selling an invention.

10. Genetic Engineering Approval Committee.

➢ Short Answer:

1. Bone marrow transplantation having functional ADA enzyme and Enzyme replacement therapy.
2. More tolerant to abiotic stresses; pest resistant; reduction in post harvest losses; increased nutritional value of food.
3. GEAC – Genetic Engineering approval committee. Objectives of GEAC as below:

   (i) To make decisions regarding validity of GM research.

   (ii) Safety of introducing GMO for public use.
4. Industrialised nations are collecting and patenting the genetic resources of under industrialised country like India. An American Company got patent rights on Basmati rice.

   Valuable biomolecules obtained from bioresources are patented and used for commercial purposes.
5. Golden rice is a transgenic variety of rice which contains a gene which codes for Vitamin A precursor. This variety have green yellow coloured grains and is rich in Vitamin A & thus nutritionally very advantageous.
6.

   i. providing best catalyst in the form of improved organism usually in the form of microbe or pure enzyme.

   ii. Creating optimal conditions through engineering for a catalyst to function.

   iii. downstream processing to purify the protein / organic compound.
7. In conventional methods, presence of pathogen is normally suspected only when pathogen has produced a disease symptom. By this time the concentration of pathogen is already very high in Body which could be harmful but with molecular diagnostics, Small amount of pathogen could be detected by amplification by PCR.
8. Biopiracy refers to the use of bio-resources lay multinational companies & other organizations without proper authorizations from the countries & people concerned eg. Basmati rice grown in India is distinct for its unique flavor & aroma but an American company got patent rights on Basmati through US patent.

➢ Long Answer:

1. Applications of Biotechnology:

   i. Therapeutics

ii. Genetically modified crops

iii. Molecular diagnostics

iv. Processed food items

v. Bioremediation

vi. Biological waste treatment

vii. Energy production.

2.

- Specific Bt toxin genes isolated from Bacillus Thuringiensis are incorporated into cotton. Cry I AC and Cry II AC control the bollworm.
- Bt gene forms protein crystals that contain a toxin insecticidal protein.
- It is in an inactive state.
- The inactive toxin is converted into active form due to the alkaline pH of the gut which solubilizes the crystal.
- Activated Bt-toxin binds to the surface of midgut epithelial cells and creates pores that cause cell swelling and lysis. It finally leads to the death of the insect.

3. ELISA (Enzyme-Linked Immunosorbent Assay Test):

i. It is a technique of detecting a very small amount of protein (antibody or antigen) with the help of enzyme peroxidase or alkaline phosphatase and stain-producing substrates like 5-aminosalicylic acid or orthophenylene diamine.

ii. The serum is sorbed to the surface of the ELISA plate.

iii. An antibody is specific to the antigen for diagnosis placed over an immobilized antigen.

iv. The spot is washed to remove the free antibody.

v. Antibody bound to the enzyme is poured over the spot so as to react with com¬plex antibody.

vi. The area is washed again to remove the free antibody-enzyme complex.

vii. Chromagen is added. It will produce a stain showing the antigen was present.

viii. ELISA is a quick method of diagnosis of pregnancy (by detection hCG in urine), AIDS, hepatitis, STDs, thyroid disorder, and Rubella virus.

➢ Assertion and Reason Answers:

1. (c) Assertion is true but reason is false.

Explanation:

The Flavr-Savrtomato, was the first genetically modified fruit/vegetable to reach the market in USA. Roundup ready soybean is a genetically modified herbicide tolerant cultivar.

2. (a) Both assertion and reason are true and reason is the correct explanation of assertion.

Explanation:

The GM crops are fast becoming a part of agriculture throughout the world because of their contribution to the increased crop productivity and to global food, feed and fiber security, besides their use in health-care and industry.

However, GM foods are facing widespread resistance by the people from all over the world. It is because transgenes in commercial crops can endanger native species. For example, the gene for Bt toxin expressed in pollen might endanger pollinators like honeybees. These crops cause problems in human health by supplying allergens and transfer of antibiotic resistance markers. The GM crops may change the fundamental vegetable nature of plants as the genes from animals (e.g., fish or mouse) are being introduced into crop plants. GM foods also have a bad effect on environment and biodiversity.

➢ Case Study Answers:

1.

(i) (d) Both (a) and (b).

Explanation:

Luciferase catalyses the production of a green fluorescent protein when luciferin is added.

(ii) (c) AA V infect cells more readily than AAV 2.5 T.

Explanation:

AAV 2.5T infects cells more readily than AAV.

(iii) (d) All of these.

Explanation:

cDNA is a DNA copy produced from the m RNA by reverse transcriptase.

(iv) (d) All of these.

(v) (c) Replacing a defective gene with a functional gene.

Explanation:

Gene therapy refers to the insertion of genes into an individual cells and tissues to treat a disease. Essentially defective/mutant genes are replaced with healthy, functional ones.

2.

(i) (b) Biopatents.

Explanation:

A patent is the right granted by a government to an inventor to prevent others from commercial use of his invention. Biopatent system allows private, monopoly rights over cells, genes, animals and plants.

(ii) (d) Utility.

Explanation:

Patents are supposed to satisfy three criteria of novelty, non-obviousness and utility.

(iii) (d) All of these.

Explanation:

Patents have been taken out on plants such as black pepper, basmati rice, Indian mustard, Pomegranate, Turmeric and neem.

(iv) (a) Bioethics.

(v) (a) Both assertion and reason are true and reason is the correct explanation of assertion.

Explanation:

Genes, cells, micro-organisms, plants and animals are not inventions and hence must not be patented.