#### [3 Marks]

#### Q.1. What is GMO? List any five possible advantages of a GMO to a farmer.

Ans. Genetically modified organisms (GMOs) are plants, bacteria, fungi and animals whose genes have been altered by manipulation.

#### Genetic modification of crops have resulted in

- a. increased tolerance against abiotic stresses (cold, drought, salt, heat).
- b. reduced reliance on chemical pesticides (pest-resistant crops).
- c. reduced post-harvest losses.
- d. increased efficiency of minerals used by plants (this prevents early exhaustion of fertility of soil).
- e. enhanced nutritional value of food, e.g., vitamin 'A' enriched rice (golden rice).
- f. creation of tailor-made plants to supply alternative resources such as starches, fuels and pharmaceuticals to industries.

## Q.2. Name the process involved in the production of nematode-resistant tobacco plants, using genetic engineering. Explain the strategy adopted to develop such plants.

**Ans.** The process involved in the production of nematode-resistant plants is RNA interference or RNAi. Using *Agrobacterium* vectors, nematode-specific genes were introduced into the host plant. The introduction of DNA was such that it produced both sense and antisense RNA in the host cells. These two RNA's being complementary to each other formed a double stranded RNA (*ds*RNA) that initiated RNAi and thus, silenced the specific *m*RNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant, therefore, got itself protected from the parasite.

#### Q.3. Explain the synthesis of genetically engineered human insulin.

#### Ans. Genetically engineered insulin

- Insulin contains two short polypeptide chains—chain A and chain B linked by disulphide bridges.
- In mammals, insulin is synthesised as a pro-hormone (that needs to be processed to become mature and functional hormone). It contains an extra stretch called C peptide.
- C peptide is absent in mature insulin and is removed during maturation into insulin.
- Earlier, insulin was extracted from pancreas of slaughtered cattle and pigs but some patients began developing allergies.
- Production of insulin by *r*DNA techniques was achieved by an American company, Eli Lilly, in 1983. It prepared two DNA sequences corresponding to A and B chains of

human insulin and introduced them in plasmids of E. coli for production. The A and B chains produced, were separated, extracted and combined, by creating disulfide bonds to form human insulin.

### Q.4. Describe the various stages involved in gene transfer for the commercial production of human insulin by Eli Lilly.

Ans.

- a. Eli Lilly prepared two DNA sequences corresponding to the A and B chains of human insulin.
- b. Sticky ends were produced in the *Escherichia coli* plasmid and the insulin gene by treating them both with the same restriction endonucleases.
- c. These two are then joined together by the enzyme DNA ligase.
- d. The bacteria are then grown in sterilised bioreactors in the appropriate growth medium.
- e. The chains A and B are produced separately, extracted and purified.
- f. These two chains are then combined by creating disulfide bonds to form human insulin.

#### Q.5.

- a. What is gene therapy?
- b. Describe the procedure of such a therapy that could be a permanent cure for a disease. Name the disease.

#### Ans.

- Gene therapy is a collection of methods that allows correction of gene defects, diagnosed in a child or embryo.
- By insertion of normal genes, the defective mutant allele of the genes are replaced and non-functional gene is compensated.
- ADA is caused due to deletion of gene for adenosine deaminase.
- Lymphocytes from patient's blood were grown in a culture and functional ADA, cDNA was introduced in these lymphocytes using a retroviral vector.
- The lymphocytes were transferred into the patient's body. Periodic infusion of such genetically engineered lymphocytes is done because these cells are mortal.

### Q.6. Expand the name of the enzyme ADA. Why is this enzyme essential in the human body? Suggest a gene therapy for its deficiency.

#### Ans. ADA–Adenosine deaminase.

This enzyme is essential for immune system to function. ADA deficiency can be cured by gene therapy. Lymphocytes from the blood of the patients are extracted and cultured outside the body. A functional ADA *c*DNA (using a retroviral vector) is introduced into these lymphocytes and these lymphocytes are then returned to the patient's body.

However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.

Q.7.

- a. Name the deficiency for which first clinical gene therapy was given.
- b. Mention the causes of and one cure for this deficiency.

#### Ans.

- a. Adenosine deaminase deficiency (ADA).
- b. Cause: Deletion of ADA gene.
  Cure: Bone marrow transplantation/enzyme replacement therapy/giving functional ADA to patient by injection/infusion of genetically engineered lymphocytes/introducing gene isolated from marrow cells producing ADA into cells at early embryonic stages. (*Any one*)

#### Q.8. Describe the gene therapy procedure for an ADA-deficient patient.

**Ans.** Gene therapy is a method which corrects or replaces the defective genes. In 1900, first clinical gene therapy was given to a 4-year old girl with adenosine deaminase (ADA) deficiency. This enzyme plays an important role in functioning of immune system. This disorder is caused due to the deletion of the gene for adenosine deaminase. In gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body. A functional ADA *c*DNA (using a retroviral vector) is then introduced into these lymphocytes, which are returned to the patients. However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.

### Q.9. Explain enzyme-replacement therapy to treat adenosine deaminase deficiency. Mention two disadvantages of this procedure.

Ans. Functional adenosine deaminase is given to the patient by injection.

- i. Lymphocytes from the blood of the patient are grown on culture outside the body.
- ii. A functional ADA, cDNA is then introduced into these lymphocytes using a retroviral vector.
- iii. The genetically engineered lymphocyte are returned to the blood of patient.

**Disadvantages:** Therapy is not completely curative as cells do not remain alive and periodic infusion of lymphocytes is required.

#### Q.10.

- a. How do organic farmers control pests? Give two examples.
- b. State the difference in their approach from that of conventional pest control methods.

Ans.

- a. By natural predation or biological control. **Examples:** Lady bird used to kill aphids, dragon flies used to kill mosquitoes, *Bacillus thuringiensis* used to kill cotton bollworm.
- b.

Conventional pest control	Organic farming based pest control
1. Use of chemical insecticides and	1. No chemical used.
pesticides.	
2. Harmful to non-target organisms.	2. Not harmful to non-target organisms.
3. Cause environmental pollution.	3. No adverse impact on environment.

#### Q.11.

- a. Why are transgenic animals so called?
- b. Explain the role of transgenic animals in (*i*) Vaccine safety and (*ii*) Biological products with the help of an example each.

#### Ans.

- a. Transgenic animals are so called because these animals have had their DNA manipulated.
- b.
- i. Vaccine safety: Transgenic mice are developed to test safety of polio vaccine before being used on humans.
- ii. Human protein ( $\alpha$ -1-antitrypsin) is used to treat emphysema.

### Q.12. Name the host plant and the part that *Meloidegyne incognitia* infects. Explain the role of *Agrobacterium* in production of *ds*RNA in host plant.

Ans. Meloidegyne incognitia infects the roots of tobacco plant.

- By using *Agrobacterium* vectors, nematode-specific genes were introduced into the host plants which produce both sense and anti-sense RNA in the host cells.
- These two RNAs are complementary to each other and form a double-stranded RNA (*ds*RNA) that initiates RNAi and hence silence the specific *m*RNA of the nematode.
- The parasite cannot survive in the transgenic host, so protects the plants from pests.

### Q.13. Why do lepidopterans die when they feed on Bt cotton plant? Explain how does it happen.

**Ans.** Bt cotton contains inactive toxin protein or protoxin. These are insecticidal protein in the form of crystal protein. Once the insect ingests its the inactive protoxins is converted into active form due to alkaline pH in the gut, which solubilise the crystals.

The activated toxins bind to the surface of midgut epithelial cells, thus creating pores which causes cell swelling and lysis, eventually leading to the death of the insect pest.

### Q.14. Name the pest that destroys the cotton bolls. Explain the role of *Bacillus thuringiensis* in protecting the cotton crop against the pest to increase the yield.

**Ans.** Cotton bollworms destroy the cotton bolls. *Bacillus thuringienesis* has Bt toxin genes. These genes produce toxic proteins that kill the pests. Bt toxins are initially inactive protoxins but after ingestion by the insect their inactive toxin becomes active due to the alkaline pH of the gut. The activated toxin binds to the surface of midgut epithelial cells thus killing the insects. Specific Bt toxins were isolated from *Bacillus thuringienesis* and incorporated into the cotton plants to make them pest resistant.

### Q.15. How did Eli Lilly synthesise the human insulin? Mention one difference between this insulin and the one produced by the human pancreas.

#### OR

How did Eli Lilly Company go about preparing the human insulin? How is the insulin thus produced different from that produced by the functional human insulin gene?

**Ans.** Eli Lilly prepared two DNA sequences corresponding to A and B chains of human insulin and introduced them in plasmids of *E. coli* to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin. Insulin in human pancreas is synthesised as a pro-hormone containing the C peptide, which is removed to form mature hormone. The synthesised insulin did not contain C peptide and was directly prepared in mature form.

## Q.16. List the three molecular diagnostic techniques that help detect pathogens from suspected patients. Mention one advantage of these techniques over conventional methods.

**Ans.** The three molecular diagnostic techniques that help to detect pathogens from suspected patients are:

- a. Recombinant DNA technology
- b. Polymerase chain reaction (PCR)
- c. Enzyme-linked immunosorbent assay (ELISA)

These techniques are better than the conventional methods because they help in early diagnosis of the disease even when the bacteria or virus concentration is very low.

## Q.17. Recombinant DNA-technology is of great importance in the field of medicine. With the help of a flow chart, show how this technology has been used in preparing genetically engineered human insulin.

Ans.



Maturation of proinsulin into insulin after removal of C-peptide

#### Q.18. Why is proinsulin so called? How is insulin different from it?

Ans. Proinsulin is called so because it is an inactive form of insulin.

S.No.	Insulin	Proinsulin
( <i>i</i> ) ( <i>ii</i> )	It is made up of two short polypeptide chains A and B linked by disulphide bridges	Along with the two polypeptide chains in insulin. It contains an extra stretch called C peptide
(")	It is functional.	It is non-functional.

### Q.19. Plasmid is a boon to biotechnology. Justify this statement quoting the production of human insulin as an example.

**Ans.** Plasmids are extra-chromosomal, self-replicating, usually circular, doublestranded DNA molecules found naturally in many bacteria.

In 1983, Eli Lilly an American company, first 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 disulfide bonds to form functional human insulin (humulin).

### Q.20. How did the process of RNA interference help to control the nematode from infecting roots of tobacco plants? Explain.

**Ans.** Using *Agrobacterium* vectors, nematode specific genes are introduced into host plant. The introduction of DNA produced both sense and anti sense RNA in host cells. These two RNA's being complementary formed a double stranded RNA (*ds*RNA) that initiated RNAi and silenced the specific *m*RNA of the nematode. As a result, the parasite could not survive in the transgenic host expressing specific interfering RNA.

#### Q.21. Answer the following questions:

#### Q. List any four beneficial effects of GM plants.

#### Ans.

- i. Increased tolerance against abiotic stresses (cold, drought, salt, heat).
- ii. Reduced reliance on chemical pesticides (pest-resistant crops).
- iii. Reduced post-harvest losses.
- iv. Increased efficiency of minerals used by plants (this prevents early exhaustion of fertility of soil).
- v. Enhanced nutritional value of food, *e.g.*, vitamin 'A' enriched rice (golden rice).

### Q. Explain how has Bacillus thuringiensis contributed in developing resistance to cotton bollworms in cotton plants.

#### Ans. Bt cotton

- Some strains of Bacillus thuringiensis produce proteins that kill some insects like lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes).
- B. thuringiensis forms protein crystals which contain a toxic insecticidal protein.
- The toxin is coded by a gene called *cry* which is of various types. For example, proteins encoded by the genes *crylAc* and *crylIAb* control the cotton bollworms and that of *crylAb* control corn borer.

#### Short Answer Questions-II (OIQ)

#### [3 Marks]

### Q.1. What are genetically modified organisms? Name two factors on which their behaviour depends.

**Ans.** Plants , fungi, bacteria and animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as genetically modified organisms. The two factors on which the behaviour of such organisms depend are:

- i. nature of gene transferred.
- ii. nature of the host cell.

#### Q.2. Mention some transgenic plants and their potential applications.

Ans. Some transgenic plants and their potential applications are given below:

S.	Transgenic plants	Useful applications
No.		
(1)	Flavr Savr tomato	Better nutrient quality.
( <i>ii</i> )	Brassica napus	Contains hirudin (a protein) that prevents blood clotting. Hirudin is synthesised chemically and it is transferred
		into Brassica napus.
(iii)	Bt cotton	It has resistance to bollworm infestation, tolerance to
		herbicide, high yielding.
( <i>iv</i> )	Wheat	Resistant against herbicide PPT (Commercial name "Basta" — 26 per cent PPT).
( <i>V</i> )	Potato	Content of starch increased by about 20–40 per cent.
(vi)	Corn, brinjal	Insect resistance.
(vii)	Maize, soyabean	Herbicide resistance.
(viii)	Golden rice	Rich in vitamin-A.

### Q.3. Biotechnology has helped farmers to get pest resistant cotton crops. Explain the technique adopted along with its mode of action. (Mention six points)

**Ans.** The technique involves the use of a popularly known biopesticide Bt toxin produced by bacteria *Bacillus thuriengiensis*. Bt toxin protein when ingested by the insect gets converted to its active form due to alkaline pH of the gut. The activated toxin binds to the surface of midgut epithelial cells. It creates pores in these cells that cause swelling and lysis and eventually kills the insect. The genes (cry genes) encoding this protein are isolated from the bacterium and incorporated into crop plants like cotton. The proteins encoded by these cry genes control the pest.

Specifically, *cryIAc* and *cryIIAb* control cotton bollworm (*Helicoverpa armigera*), an insect belonging to Lepidoptera which earlier used to destroy the whole crop.

#### Q.4.

- i. Give the scientific name of the soil bacterium which produces crystal (Cry) proteins.
- ii. How are these proteins useful in agriculture?
- iii. What do the differently written terms 'Cry' and 'cry' represent respectively?

#### Ans.

- i. Bacillus thuringiensis.
- ii. These Cry proteins are toxic to certain larvae of insects and thus provide resistance against them. The gene encoding Cry proteins are used in several crop plants (Bt toxin). Such a crop plant is resistant to the particular insect pest.
- iii. Cry represents crystal protein while *cry* refers to the gene encoding the Cry protein.

### Q.5. How does a transgenic organism differ from the rest of its population? Give any two examples of such organism for human advantage.

**Ans.** A transgenic organism contains foreign gene, hence it differs from the rest of the population in having one or more extra genes apart from the gene pool of that population showing an additional phenotype.

#### Example,

- i. Transgenic *E.coli*, with gene for human insulin.
- ii. Transgenic mouse with gene for human growth hormone.

## Q.6. Explain the steps involved in the production of genetically engineered insulin. Name the source from which insulin was extracted earlier. Why is this insulin no more in use by diabetic people?

#### Ans. Genetically engineered insulin

- Insulin contains two short polypeptide chains—chain A and chain B linked by disulphide bridges.
- In mammals, insulin is synthesised as a pro-hormone (that needs to be processed to become mature and functional hormone). It contains an extra stretch called C peptide.
- C peptide is absent in mature insulin and is removed during maturation into insulin.
- Earlier, insulin was extracted from pancreas of slaughtered cattle and pigs but some patients began developing allergies.
- Production of insulin by rDNA techniques was achieved by an American company, Eli Lilly, in 1983. It prepared two DNA sequences corresponding to A and B chains of human insulin and introduced them in plasmids of E. coli for production. The A and B chains produced, were separated, extracted and combined, by creating disulfide bonds to form human insulin.

Earlier, insulin was extracted from pancreas of slaughtered cattle and pig. This insulin is not in use as some patients developed allergic reaction to this foreign protein.

# Q.7. What do you understand by the term biopesticide? Name and explain the mode of action of a popular biopesticide. Biopesticides are methods of controlling pests that rely on natural predation rather than introduced chemicals/or living organisms used to kill pests.

#### Ans. Biopesticide is a pesticide which is:

- a. not chemical in nature.
- b. more specific in action against the pest.
- c. safer for environment than chemical pesticides.

A popularly known biopesticide is Bt toxin, which is produced by a bacterium called *Bacillus thuringiensis*. Bt toxin gene has been cloned from this bacterium and

expressed in plants. Bt toxin protein when ingested by the insect, gets converted to its active form due to the alkaline pH of the gut. The activated toxin binds to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis and eventually kills the insect.

### Q.8. Gene expression can be controlled with the help of RNA molecule. Explain the method with an example.

**Ans.** Gene expression can be controlled by using RNA molecule and this technology is called RNA interference or RNAi. It is used to block the expression of certain genes and also referred to as gene silencing. During this process a complementary RNA to the *m*RNA being produced by the gene is introduced into the cell. This RNA binds to the *m*RNA making it double stranded and therefore stops translation. Resistance to nematode *Meloidegyne incognita* in tomato has been achieved by this method.

### Q.9. List the disadvantages of insulin obtained from the pancreas of slaughtered cow and pigs.

#### Ans.

- i. Insulin being a hormone is produced in very little amounts in the body. Hence, a large number of animals need to be sacrificed for obtaining small quantities of insulin. This makes the cost of insulin very high, demand being manyfold higher than supply.
- ii. Slaughtering of animal is also not ethical.
- iii. There is potential of immune response in humans against the administered insulin which is derived from animals.
- iv. There is possibility of slaughtered animals being infested with some infectious micro organism which may contaminate insulin.

Q.10. You have identified a useful gene in a bacteria. Make a flow chart of the steps that you would follow to transfer this gene to a plant.

Ans. After identifying a useful genein bacteria, following steps should be undertaken:

Isolation of useful gene using restriction endonuclease.

Transferring the gene to a suitable vector to create a recombinant DNA molecule.

Transfer of these recombinant DNA molecules to the target cells.

Screening of cell for transformation.

Selection of transformed cells.

Regeneration of plants from the transformed cells to get transgenic plants.

#### Q.11. Answer the following questions:

#### Q. What is plasmid?

**Ans.** Plasmid is a circular extra-chromosomal DNA molecule present in a bacterial cell, which replicates autonomously independent of bacterial chromosomal DNA.

### Q. What is meant by ADA deficiency? How is gene therapy a solution to this problem? Why is it not a permanent cure?

#### Ans. Gene therapy

- ADA is caused due to deletion of gene for adenosine deaminase.
- Lymphocytes from patient's blood were grown in a culture and functional ADA, *c*DNA was introduced in these lymphocytes using a retroviral vector.
- The lymphocytes were transferred into the patient's body. Periodic infusion of such genetically engineered lymphocytes is done because these cells are mortal.
- For permanent cure, gene isolated from the bone marrow cells producing ADA, at early embryonic stage can be a possible cure.