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Biotechnology Applications

CBSE-XII

Biology

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hello@ +91-9939586130 ;
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www.aepstudycircle.com
2nd Floor, Satkoudi Complex, Thana Chowk, RAMGARH, JHARKHAND-829122

BIOLOGY
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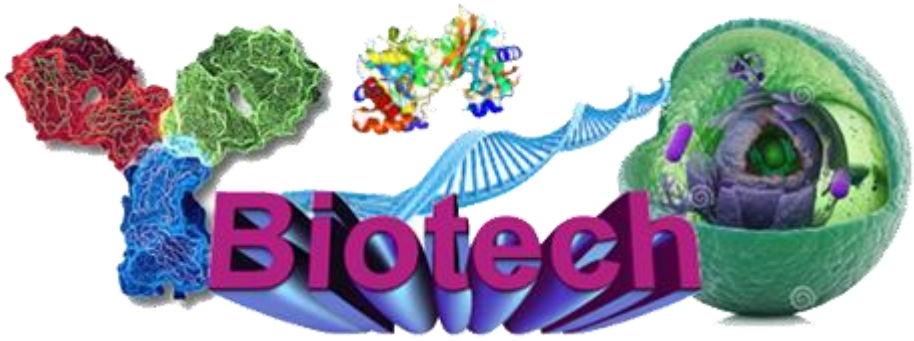


Applications

Recap Notes

- **Modern biotechnology** provides breakthrough solutions and technologies to combat debilitating and rare diseases, reduce our environmental footprint, feed the hungry, use less and cleaner energy, and have safer and more efficient industrial manufacturing processes.
- **Research areas of biotechnology** – Following are three research areas of biotechnology : -
  - Providing the best catalyst in the form of improved organism ; generally a microbe or pure enzyme.
  - Creating optimal conditions through engineering for a catalyst to act, and
  - **Downstream processing** technologies to purify the protein/organic compound.

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

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

Production of Transgenic Plants

- The plants in which foreign genes have been introduced through genetic engineering are called **transgenic plants.**
- The vector used to introduce new genes into plant cells is most often a plasmid from the soil bacterium *Agrobacterium tumefaciens*. This is the **Ti plasmid** (tumour inducing plasmid), so called because in nature, it induces tumours in broad leaf plants.
- The part of Ti plasmid transferred into plant cell DNA, is called the **T-DNA**. This T-DNA with desired DNA spliced into it, is inserted into the chromosomes of the host plant where it produces copies of itself, by migrating from one chromosomal position to another at random. Such plant cells are then **cultured**, induced to multiply and differentiate to form **plantlets**. Transferred into soil, the plantlets grow into mature plants, carrying the foreign gene, expressed throughout the new plant.

Bt cotton (Insect Resistance Plant)

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

Pest Resistant Plants

- A nematode *Meloidogyne* incognita infests the **roots of tobacco plants** and causes a great **reduction in yield**. A novel strategy was adopted to prevent this infection that was based on the process of **RNA interference (RNAi)**. RNA interference is the phenomenon of **inhibiting activity of a gene through production of sense and antisense RNA**.
- This method involves a **specific mRNA silencing**. The result was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plants thus got itself protected from the parasite.

Other Agricultural Applications

- Some other **agricultural applications** are :
  - The protein **hirudin** prevents blood clotting. Its gene was chemically synthesised and introduced in *Brassica napus*. The seeds of the latter came to have hirudin which could be extracted and purified and used as medicine.
  - **'Flavr Savr'** tomato was the first transgenic variety to reach the market. Here inactivation of gene which produces **polygalactouronase enzyme** has been done. The **non-availability of this enzyme prevents over-ripening** because this enzyme is essential for degradation of cell walls.
  - **Golden rice** is a transgenic variety of rice (*Oryza sativa*) which contains good quantities of β-carotene (provitamin A – inactive state of vitamin A).

BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

- The recombinant DNA technological processes have made great impact in the area of healthcare by mass production of safe and more effective therapeutic drugs. A large number of human genes encoding pharmaceutically valuable proteins have been cloned and expressed in microorganisms. Initially, *E. coli* was used as the host for obvious reasons of ease in cloning. But yeast is fast becoming the host of choice for production of **recombinant proteins.**

Production of Genetically Engineered Insulin

- Human insulin is made up of 51 amino acids arranged in two polypeptide chains, **A** having **21 amino acids** and **B** with **30 amino acids.** The two polypeptide chains are interconnected by two disulphide bridges or **S-S linkages.** An S-S linkage also occurs in A chain.
- In mammals, including humans, insulin is synthesised as a pro-hormone which contains an extra stretch called the **C peptide** (with 33 amino acids). This C peptide is not present in the mature insulin and is removed during maturation into insulin. The pro-hormone needs to be processed before it becomes a fully mature and functional hormone. Bacteria can not be made to synthesise insulin from its gene because of the presence of introns in eukaryotic genes. Bacteria do not possess enzymes for removing introns. It was challenging to produce insulin using rDNA technique and to assemble insulin into its mature form.

In 1983, **Eli Lilly,** an American company, prepared two DNA sequences corresponding to A and B chains of human insulin and introduced them in plasmids of *E.coli* to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulphide bonds to form **human insulin ('Humulin').**

Gene Therapy

- **Gene therapy** is the technique of genetic engineering to replace a **faulty gene** by a normal healthy functional gene. The first clinical gene therapy was given in 1990 to a 4-year old girl with **adenosine deaminase (ADA) deficiency**. This enzyme is very important for the immune system to function. **SCID** is caused due to defect in the gene for the enzyme adenosine deaminase. ADA deficiency can be cured by bone marrow transplantation and by enzyme replacement therapy, in which functional ADA is given to the patient by injection. But in both approaches the patients are not completely cured. However, if the isolated gene from bone marrow cells producing ADA is introduced into cells at early embryonic stages, it can be a permanent cure.

Vaccines

- **Vaccines** represent an invaluable contribution of biotechnology as they provide protection against even such diseases for which effective cures are not yet available. The effectiveness of vaccines may be appreciated from the fact that **small pox**, once a dreaded disease the world over, has been completely eradicated from the world.
- The various vaccines can be grouped under the following types :
  - ▶ Conventional vaccines (live vaccines, inactivated pathogens), purified antigen and
  - ▶ Recombinant vaccines (recombinant) proteins/polypeptides, DNA vaccines).
- Many new vaccines of great importance have been produced with the help of genetic engineering techniques. These are called 'second-generation vaccines'. Now-a-days even 'third generation vaccines' called synthetic vaccines, have been produced.

Boon for Solving Cases

- Biotechnology has proved to be a **boon in solving crimes**, legal disputes, etc. Establishing the identity of victims (*e.g.*, of murder, accidents, etc.), criminals (*e.g.*, in cases of rape, murder, etc.), father (in cases of paternity dispute), etc. is critical to solving the problems of crimes/cases.

TRANSGENIC ANIMALS

- A transgenic animal contains in its genome, a gene or genes introduced by one or the other technique of **transfection**. The gene introduced by transfection is known as **transgene**.

Advantages of Transgenic Animals

- Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease.
- Transgenic animals that produce useful **biological products** can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (α-1-antitrypsin) used to treat **emphysema**. Similar attempts are being made for treatment of **phenylketonuria** (PKU) and **cystic fibrosis**. In 1997, the first transgenic cow, **Rosie** produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow milk.
- **Transgenic mice** are being developed for use in testing the safety of vaccine before they are used on humans *e.g.* Polio vaccine.
- Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. **Toxicity testing** in such animals will allow us to obtain results in less time.
- Genetically modified salmon was the first transgenic animal obtained for food production.
- **Transgenic sheep** have been produced to achieve better growth and meat production. For example, human genes for blood clotting factor IX and for α1-antitrypsin have been

transferred in sheep and expressed in mammary tissue.

- **Dogie** is a transgenic dog with excellent smelling power.

ETHICAL ISSUES

- Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms. Therefore, the Indian Government has set up organisations such as **GEAC (Genetic Engineering Approval Committee),** which will make decisions regarding the validity of GM research and the safety of introducing GM research, the safety of introducing GM-organisms for public services.
- Ethics include a set of standards by which a community regulates its behaviour and decides as to which activity is legitimate and which is not. Therefore, **bioethics** may be viewed as a set of standards that may be used to regulate our activities in relation to the biological world.
- The main bioethical concerns pertaining to biotechnology are briefly mentioned as follows:
  - Introduction of a transgene from one species into another species **violates the 'integrity of species'.**
  - Biotechnology may pose unforeseen risks to the environment, including risk to biodiversity. Thus it could **disturb the existing ecological balance.**
  - Transfer of human genes into animals (and vice-versa) **dilutes the concept of 'humanness'.**
  - **Use of animals** in biotechnology causes great suffering to them.
  - When animals are used for production of pharmaceutical proteins, they are virtually reduced to the status of a 'factory'.
  - Biotechnology is disrespectful to living beings, and only exploits them for the **benefit of human beings.**
- A **patent** is the right granted by a government to an inventor to prevent others from commercial use of his invention. When patents are granted for biological entities and for products derived from them, these patents are called **biopatents**. Patents have been taken out on plants such as black pepper (*Piper nigrum*), basmati rice (*Oryza sativa*), Indian mustard (*Brassica campestris*), pomegranate (*Punica granatum*), turmeric and neem.
- The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiative.
- Some organisations and multinational companies exploit and/or patent biological resources or bioresources of other nations without proper authorisation from the countries concerned, this is called **biopiracy**. For example, a patent granted in U.S.A. covers the entire 'basmati' rice germplasm indigenous to our country.
- **Biowar** is the use of highly infectious pathogens, their spores and toxins as agents of biological weapons against humans, crops and animals of enemy country. The agent **(virus, bacteria, fungus)** is kept in a special container that keeps it in active/virulent state during delivery by missile or aircraft. Spray and powder are two common forms in which the bioweapon agent is delivered. Potential pathogens for bioweapons are *Bacillus anthracis*, *Vibrio cholerae*, small pox virus *Pasteurella / Yersinia pestis*, Botulinum toxin, etc.
- Bioweapons are almost invisible, low cost weapons which can cause more casualities than conventional or chemical weapons. Possible defence against them is gas mask or respirator, protective shelter, vaccination, antibiotics, decontamination and sensitive detection systems.

OBJECTIVE TYPE QUESTIONS

Multiple Choice Questions (MCQs)

1. What triggers the activation of protoxin to active toxin of *Bacillus thuringiensis* in boll worm?
(a) Acidic pH of stomach
(b) Body temperature
(c) Moist surface of midgut
(d) Alkaline pH of gut

2. Which of the following is true for Golden rice?
(a) It has yellow grains, because of a gene introduced from a primitive variety of rice.
(b) It is vitamin A enriched, with a gene from daffodil.
(c) It is pest resistant, with a gene from *Bacillus thuringiensis*.
(d) It is drought tolerant, developed using *Agrobacterium* vector.

3. In RNAi, the genes are silenced using
(a) dsRNA (b) ssDNA
(c) ssRNA (d) dsDNA.

4. Identify the incorrect statement regarding Bt cotton.
(a) Dried spores of *Bacillus thuringiensis* are sprayed on its vulnerable leaves.
(b) It releases toxin that causes swelling in the gut of insect that ingest its leaves.
(c) It is a transgenic plant.
(d) It can produce toxin due to gene introduced in it by transgenesis.

5. The plasmid of which one of the following has '*nif*' gene in it?
(a) *Rhizobium*
(b) *Agrobacterium tumefaciens*
(c) *Bacillus thuringiensis*
(d) *Salmonella typhimurium*

6. The introduction of T-DNA into plants involves
(a) exposing the plants to cold for a brief period
(b) allowing the plant roots to stand in water
(c) infection of the plant by *Agrobacterium tumefaciens*
(d) altering the pH of the soil, then heat-shocking the plants.

7. Protein encoded by gene *cry*IAb controls the infestation of which of the following insects?
(a) Cotton bollworm (b) *Anopheles* mosquito
(c) Corn borer (d) *Aedes* mosquito

8. RNA interference which is employed in making tobacco plant resistant to *Meloidogyne incognita* is essentially involved in
(a) preventing the process of replication of DNA
(b) preventing the process of translation of *m*RNA
(c) preventing the process of splicing of hnRNA
(d) preventing the process of transcription.

9. Which of the following Bt crops is being grown in India by the farmers?
(a) Brinjal (b) Soybean
(c) Maize (d) Cotton

10. Consumption of which one of the following foods can prevent the kind of blindness associated with vitamin 'A' deficiency?
(a) 'Flavr Savr' tomato (b) Canolla
(c) Golden rice (d) Bt-brinjal

11. *Bt* brinjal is an example of transgenic crops. In this, *Bt* refers to
(a) *Bacillus tuberculosis*
(b) biotechnology
(c) β-carotene
(d) *Bacillus thuringiensis*.

12. The genetically-modified (GM) brinjal in India has been developed for
(a) insect-resistance
(b) enhancing shelf life
(c) enhancing mineral content
(d) drought-resistance.

13. Some of the characteristics of Bt cotton are
(a) long fibre and resistance to aphids
(b) medium yield, long fibre and resistance to beetle pests
(c) high yield and production of toxic protein crystals which kill dipteran pests
(d) high yield and resistance to bollworms.

14. An improved variety of transgenic basmati rice
(a) does not require chemical fertilisers and growth hormones
(b) gives high yield and is rich in vitamin A
(c) is completely resistant to all insect pests and diseases of paddy
(d) gives high yield but has no characteristic aroma.

15. *cry*II*Ab* and *cry*I*Ab* produce toxins that control
(a) cotton bollworms and corn borer respectively
(b) corn borer and cotton bollworms respectively
(c) tobacco budworms and nematodes respectively
(d) nematodes and tobacco budworms respectively

16. Which of the following is commonly used as a vector for introducing a DNA fragment in human lymphocytes?
(a) Retrovirus (b) Ti plasmid
(c) λ phage (d) pBR322

17. Which kind of therapy was given in 1990 to a four-year-old girl with adenosine deaminase (ADA) deficiency?
(a) Gene therapy
(b) Chemotherapy
(c) Immunotherapy
(d) Radiation therapy

18. Select the wrong statement.
(a) Human insulin is being commercially produced from a transgenic species of *Escherichia coli.*
(b) Bt toxin genes *cry*IAc control the corn borer.
(c) Human protein, α-1-antitrypsin is used to treat emphysema.
(d) The first transgenic cow, Rosie, produced alpha lactalbumin, enriched milk.

19. The first human hormone produced by recombinant DNA technology is
(a) insulin (b) estrogen
(c) thyroxin (d) progesterone.

20. The 51 amino acids of insulin are arranged in
(a) single polypeptide
(b) two polypeptides, having 21 and 30 amino acids in chain A and B respectively
(c) two polypeptides having 25 and 26 amino acids
(d) two polypeptides having 18 and 33 amino acids.

21. How many recombinant therapeutics have been used for human diseases throughout the world?
(a) 12 (b) 24 (c) 30 (d) 56

22. Which one of the following vectors is used to replace the defective gene in gene therapy?
(a) Adenovirus (b) Cosmid
(c) Ri plasmid (d) Ti plasmid

23. Human proteins can be produced in the milk or semen of farm animals. True or false?
(a) True
(b) False, proteins cannot be produced in milk
(c) False, proteins cannot be produced in semen
(d) False, animals are not used for protein production

24. The genetic defect, adenosine deaminase (ADA) deficiency may be cured permanently by
(a) administering adenosine deaminase through injection
(b) bone marrow transplantation
(c) enzyme replacement therapy
(d) introducing isolated gene from marrow cells producing ADA into the cells at early embryonic stages

25. During the processing of the prohormone "proinsulin" into the mature "insulin"
(a) C-peptide is added to proinsulin
(b) C-peptide is removed from proinsulin
(c) B-peptide is added to proinsulin
(d) B-peptide is removed from proinsulin.

26. The illegal and unlawful development of biomaterials without payment to the inhabitants of their origin is called
(a) biopatent (b) biotechnology
(c) biowar (d) biopiracy.

27. Some of the steps involved in the production of humulin are given below. Choose the correct sequence.
(i) Synthesis of gene (DNA) for human insulin artificially.
(ii) Culturing recombinant *E.coli* in bioreactors.
(iii) Purification of humulin.
(iv) Insertion of human insulin gene into plasmid.
(v) Introduction of recombinant plasmid into *E.coli*.
(vi) Extraction of recombinant gene product from *E.coli*.
(a) ii, i, iv, iii, v, vi (b) i, iii, v, vi, ii, iv
(c) i, iv, v, ii, vi, iii (d) iii, v, ii, i, vi, iv

28. Transgenic animals are extensively used for all of the following procedures except
(a) bioremediation (b) chemical safety
(c) vaccine safety (d) toxicity test.

29. Which of the following statements about transgenic animals is/are false?
(i) Transgenic animals are designed to study how genes are regulated.
(ii) They are specially made to serve as models for human diseases.
(iii) Transgenic cow Rosie was created to produce the human protein α-1-antitrypsin.
(iv) Transgenic mice are used to test the safety of vaccines.
(a) (iii) only (b) (i) and (iii) only
(c) (ii) only (d) (ii) and (iii) only

30. Which one of the following is not a GMO?
(a) Bt brinjal (b) Golden rice
(c) Tracy (d) Dolly

31. Read the following four statements (A-D) about certain mistakes in two of them.
(A) The first transgenic buffalo, Rosie produced milk which was human alpha-lactalbumin enriched.
(B) Restriction enzymes are used in isolation of DNA from other macromolecules.
(C) Downstream processing is one of the steps of rDNA technology.
(D) Disarmed pathogen vectors are also used in transfer of rDNA into the host.
Which of the two statements have mistakes?
(a) B and C (b) C and D
(c) A and C (d) A and B

32. Maximum number of existing transgenic animals is of
(a) fish (b) mice
(c) cow (d) pig.

33. Genetic engineering has been successfully used for producing
(a) transgenic mice for testing safety of polio vaccine before use in humans
(b) transgenic models for studying new treatments for certain cardiac diseases
(c) transgenic cow-Rosie which produces high fat milk for making ghee
(d) animals like bulls for farm work as they have super power.

34. In India, the organisation responsible for assessing the safety of introducing genetically modified organisms for public use is
(a) Indian Council of Medical Research (ICMR)
(b) Council for Scientific and Industrial Research (CSIR)
(c) Research Committee on Genetic Manipulation (RCGM)
(d) Genetic Engineering Approval Committee (GEAC).

35. A 'new' variety of rice was patented by a foreign company, though such varieties have been present in India for a long time. This is related to
(a) Co-667 (b) Sharbati Sonora
(c) Lerma Rojo (d) Basmati.

36. Use of bioresources by multinational companies and organisations without authorisation from the concerned country and its people is called
(a) bio-infringement (b) biopiracy
(c) biodegradation (d) bioexploitation.

37. Which one of the following is now being commercially produced by biotechnological procedures?
(a) Nicotine (b) Morphine
(c) Quinine (d) Insulin

38. Which of the following genes do not occur naturally in living organisms?
(a) Bt genes
(b) RNA*i* genes
(c) Cry genes
(d) Endogenous cytoplasmic defense genes

39. Which of the following is known as 'Flavr Savr'?
(a) Specific variety of pesticide
(b) Breed of chicken
(c) Transgenic tomato
(d) Toxic insecticidal protein

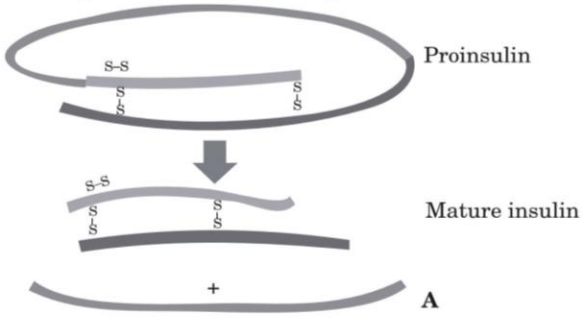
40. Basic principle of developing transgenic plants and animals is to introduce the gene of interest into the nucleus of
(a) somatic cell
(b) vegetative cell
(c) germ cell
(d) body cell.

Case Based MCQs

Case I : Read the following passage and answer the questions from 41 to 45 given below.

Insulin used to cure diabetes was earlier extracted from pancreas of slaughtered cattle and pigs. Insulin extracted from an animal source, though caused some patients to develop allergy or other types of reactions to the foreign protein. Human insulin consists of two short polypeptide chains : chain A and chain B, that are linked together by disulphide bridges. In mammals including humans, insulin is synthesised as a pro-hormone which contains an extra stretch called the C-peptide. This C peptide is not present in mature insulin and is removed during maturation into insulin.

41. Identify A in the given figure.



(a) Polypeptide chain A
(b) Polypeptide chain B
(c) Polypeptide chain C
(d) None of these

42. The following is a list of some stages involved in producing human insulin from genetically engineered bacteria.
1. The bacteria are cultured in a fermenter for large scale production.
2. Recombinant insulin is extracted from the bacterial cells that expresses insulin gene.
3. The same restriction enzyme is used again to cut the bacterial plasmid for insertion of the human insulin gene.
4. Bacteria take up the plasmid carrying the insulin gene.
5. A restriction enzyme is used to cut human DNA to extract the insulin gene.

Select the correct order of these stages.
(a) 1, 5, 3, 4, 2 (b) 2, 4, 3, 5, 1
(c) 4, 5, 3, 2, 1 (d) 5, 3, 4, 1, 2

43. To insert the insulin gene into bacterial DNA, both the bacterial plasmid and the human chromosome containing the insulin gene are treated with the same restriction enzyme. Using the same restriction enzyme ensures that
(a) DNA ligase is able to join the segments of human and bacterial DNA
(b) the exact length of nucleotides matching the insulin gene is removed from the plasmid
(c) both the bacterial and human DNA will contain sticky ends
(d) sticky ends in the cut plasmid and insulin gene are complementary.

44. Why is the fermentor important for the production of human insulin by transgenic bacteria?
(a) It provides optimal conditions for the transgenic to multiply rapidly.
(b) It facilitates the extraction and purification of insulin from the transgenic bacteria.
(c) It maximise the rate of fermentation of the transgenic bacteria.
(d) It provides the low-oxygen conditions that are important for insulin production.

45. A bacteriologist carries out his first attempt at engineering *E.coli* with the gene for human insulin. During the process, he realises that his stock of DNA ligase has depleted but decides to continue anyway. What is a likely consequence of his decision?
(a) Bacteria with the rDNA will not be able to form colonies in a fermenter.
(b) The resulting plasmids are not able to enter the *E.coli* bacteria even after applying heat shock.
(c) The resulting *E.coli* bacteria do not contain the human insulin gene.
(d) The bacterial plasmids do not have sticky ends and are unable to accommodate the human gene.

Case II : Read the following passage and answer the questions from 46 to 50 given below.

Transgenic cows have extra gene or genes inserted into their DNA. Firstly the genes for the desired product is identified and sequenced. Then a gene construct containing this desired gene is introduced into female cow cells. Transgenic bovine cells are selected and fused with bovine oocytes that have had all of their chromosomes removed. Once fused with the oocyte, the transgenic cells chromosomes are reprogrammed to direct development which can be implanted into a recipient cow. The resulting transgenic cow only express the transgene in her milk. This is because expression of the transgene is controlled by a promoter specific to lactating mammary cells.

46. The gene construct with desired gene is introduced into female cow cells by
(a) transformation (b) transduction
(c) transfection (d) transplantation.

47. Production of transgenic cow fulfill the objective of
(a) increased milk production
(b) increased meat production
(c) molecular farming
(d) all of these.

48. The name of first transgenic cow is
(a) Tracy (b) Dolly
(c) Rosie (d) ANDI.

49. Transgenic cow is produced through the implantation of _____ containing transgene into recipient cow.
(a) ova (b) embryo
(c) mammary cell (d) both (a) and (b)

50. Read the given statements and select the correct option.
Statement I : Transgenes only express in the mammary glands of transgenic cow.
Statement II : Transgenes are present in chromosomes of every cell in transgenic cow.
(a) Both statements I and II are true.
(b) Both statements I and II are false.
(c) Statement I is true but statement II is false.
(d) Statement I is false but statement II is true.

Assertion & Reasoning Based MCQs

For question numbers 51-60, 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) Assertion is false but reason is true.

51. **Assertion :** Transgenic food may cause toxicity or produce allergy.
Reason : Transgenic plants have high nutrient content.

52. **Assertion :** Genetically modified microbes help in crop protection.
Reason : Transgenic bacteria control insects by producing endotoxins.

53. **Assertion :** Biopatents are awarded for biological entities and all products derived from them.
Reason : Patent on use of turmeric in wound healing was cancelled in 2008.

54. **Assertion :** *Agrobacterium tumefaciens* is popular in genetic engineering because this bacterium is associated with the roots of all cereal and pulse crops.

Reason : *Agrobacterium tumefaciens*, is pathogen of several dicot plants is able to cause crown gall tumors. These tumors are incited by the conjugative transfer of DNA segment (T DNA).

55. Assertion : Mouse is the most preferred mammal for studies on gene transfers.

Reason : Mouse possesses features like short oestrous cycle and gestation period, relatively short generation time, production of several offspring per pregnancy, etc.

56. Assertion : *Agrobacterium tumefaciens* is called natural genetic engineer.

Reason : *Agrobacterium tumefaciens* infects all broad-leaved agricultural crops but does not infect cereal crops.

57. Assertion : ADA deficiency cannot be cured permanently by gene therapy.

Reason : The lymphocytes from the blood of patient are grown in culture outside the body.

58. Assertion : PCR is routinely used for early diagnosis of HIV in suspected AIDS patients.

Reason : PCR can detect low amounts of DNA.

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

Reason : Roundup variety of soybean was prepared through breeding.

60. Assertion : Molecular probes are available for diagnosing genetic disorders, *e.g.,* Duchenne muscular dystrophy, cystic fibrosis, Tay-sach's disease.

Reason : The molecular probes are usually double stranded pieces of DNAs, labelled with radioisotopes such as ^{32}P.

SUBJECTIVE TYPE QUESTIONS

Very Short Answer Type Questions (VSA)

1. What was the speciality of the milk produced by the transgenic cow Rosie ?
2. State the role of C peptide in human insulin.
3. Biotechnologists refer to *Agrobacterium tumefaciens* as a natural genetic engineer of plants. Give reasons to support the statement.
4. What is a patent ?
5. Name two transgenic microorganisms that have been used for large scale production of amino acids.
6. Give the full form of SCID.
7. Give an example of bioweapon.
8. Name the *cry* genes that control cotton bollworm and corn borer respectively.
9. What is gene therapy?
10. Name the transgenic plant from which hirudin is extracted.

Short Answer Type Questions (SA-I)

11. What is a transgenic crop ? Which plant is used to produce blood anticoagulant protein ? What is this protein called?
12. Biopiracy should be prevented. State why and how?
13. Write the functions of
    (a) *cry*IAc gene
    (b) RNA interference (RNAi).
14. What is the full form of SCID? Mention the cure of this disorder. Mention any one point how SCID is different from AIDS.
15. What are transgenic animals? Give an example.
16. What are *cry* proteins? Name an organism that produces it. How has man exploited this protein to his benefit?

17. Write the full form of ELISA. Give an example of the clinical application of ELISA test.

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

19. List any two molecular diagnostic techniques and write one application of each of them.

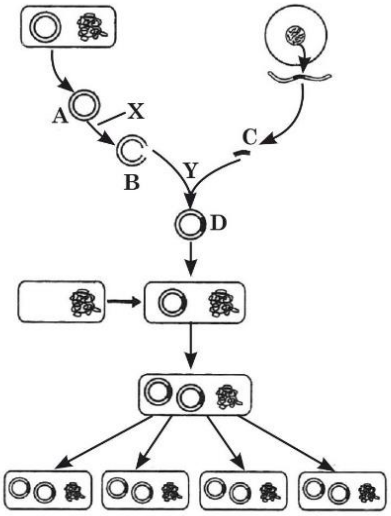
20. What is special of "Flavr Savr" variety of tomato ? Why is it preferred to its normal native variety ?

Short Answer Type Questions (SA-II)

21. What is the full form of GEAC? Describe the main objectives of GEAC set up by the Indian government.

22. Explain the structure of human insulin with the help of a diagram.

23. Refer to the given figure and answer the following questions.



(a) Name the process shown in the given figure.

(b) Identify A to D in the given figure.

(c) Name the processes X and Y. Also mention the enzymes involved in both steps.

24. Explain the various steps involved in the production of artificial insulin.

25. (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.

26. Name the soil bacterium that produces a protein/chemical that is toxic to insect pests. Show with example that these are encoded by different forms of the genes.

27. What are transgenic plants? Explain any two disadvantages of transgenic plants.

28. Two children, A and B aged 4 and 5 years respectively visited a hospital with a similar genetic disorder. The girl A was provided enzyme replacement therapy and was advised to revisit periodically for further treatment. The girl, B was, however, given a therapy that did not require revisit for further treatment.

(a) Name the ailments the two girls were suffering from.

(b) Why did the treatment provided to girl A required repeated visits?

(c) How was the girl B cured permanently?

29. Name the nematode that damages the roots of tobacco plants. How is transgenic tobacco plant made resistant to nematode using biotechnology?

30. Diagrammatically show steps involved in RNA interference.

31. List advantages of genetically modified plants.

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

33. Explain how β-carotene rich rice varieties are produced.

34. How is the Bt cotton plant created as a GM plant? How is it protected against bollworm infestation?

35. (a) Name the deficiency for which first clinical gene therapy was given.

(b) Mention the cause and one cure for this deficiency.

Long Answer Type Questions (LA)

36. Neeraj was having a debate with Mohit regarding the advantages and disadvantages of transgenic animals. Neeraj was of the view that production of transgenic animals violates the integrity of species and animals suffer from cruelty so, it is unethical. On the other hand, Mohit emphasised the benefits that transgenic animals provide to the human race in various fields especially medicine.

(a) How do transgenic animals benefit humans?

(b) List the ethical issues related with the production of transgenic animals.

37. How did the process of RNA interference help to control the nematode from infecting the roots of tobacco plants?

38. Briefly explain the principle, procedure and the role of ELISA.

39. (a) Write short note on the following:

(i) Transgenic tomato

(ii) ANDI

(iii) Transgenic sheep

(b) To which virus is transgenic chicken resistant?

ANSWERS

OBJECTIVE TYPE QUESTIONS

1. (d) : Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as lepidopterans and dipterans. *B. thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein which exists as inactive protoxins but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilises the crystals.

2. (b) : Rice is a staple food in many countries, particularly in Asia, but does not contain vitamin A or its immediate precursors. By inserting two genes from daffodil and one gene from a bacterial species into rice plants, Swiss researchers have produced rice capable of synthesising β-carotene, the precursor of vitamin A. Vitamin A is required by all individuals as it is present in retina of eyes. Deficiency of vitamin A causes night blindness and skin disorders. This rice is called 'Golden rice' because of yellow colour of rice grains due to the presence of β-carotene.

3. (a) : RNAi or RNA interference is a novel strategy which prevents the infection by nematode *Meloidogyne incognita* in the roots of tobacco plants. RNAi involves silencing of specific *m*RNA. Using *Agrobacterium* vectors, nematode specific genes are introduced into the host plant (tobacco plant). The introduction of DNA is such that it produces both sense and anti-sense RNA in the host cells. These two RNAs, being complementary to each other form a dsRNA (double stranded RNA), that initiates RNAi by first forming siRNA (small interfering RNAs). The siRNA first unwinds and then binds to specific complementary *m*RNA molecules of the nematode. These sites are then cut by RISC (RNA-induced silencing complex), thereby destroying *m*RNA of host. Hence, no toxin proteins are synthesised.

4. (a)

5. (a) : The *nif* gene is a nitrogen fixing gene found in bacterium *Rhizobium*.

6. (c) : Ti plasmid (tumor inducing) from the soil bacterium *Agrobacterium tumefaciens* is effectively used as vector for gene transfer to plant cells. The part of Ti plasmid transferred into plant cell DNA, is called the T-DNA. This T-DNA with desired DNA spliced into it, is inserted into the chromosomes of the host plant where it produces copies of itself, by migrating from one chromosomal position to another at random. Such plant cells are then cultured, induced to multiply and differentiate to form plantlets. Transferred into soil, the plantlets grow into mature plants, carrying the foreign gene, expressed throughout the new plant.

7. (c) : Protein encoded by gene *cry*IAb controls the infestation of corn borer insects in Bt corn.

8. (b) **9. (d)**

10. (c) **11. (d)**

12. (a) : The genetically modified (GM) Bt brinjal in India has been developed mainly for insect resistance. Through genetic engineering Bt toxin genes were isolated from *Bacillus thuringiensis* and incorporated into the several crop plants such as cotton, brinjal etc.

13. (d) : Bt toxin genes were isolated from *Bacillus thuringiensis* and incorporated into cotton plant. The genetically modified crop is called Bt cotton. Bt cotton has

the following useful characteristics: pest resistance, herbicide tolerance, high yield and resistance to boll worm infestation.

14. (b) **15. (a)**

16. (a) : Retroviruses cause cancer in animals including humans. So modified retroviruses are used to transfer desirable genes into animal cells. It is used in gene therapy, in which lymphocytes from blood of patient are grown in culture medium outside the body, a functional gene is introduced by using a retroviral vector into these lymphocytes.

17. (a) : Gene therapy is a technique of genetic engineering which involves replacement of a faulty/disease causing gene by a normal healthy functional gene. The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency. This enzyme is very important for the immune system to function. The deficiency of this enzyme can lead to severe combined immune deficiency (SCID).

18. (b)

19. (a) : The recombinant DNA technological processes have made great impact in the area of health care by mass production of safe and more effective therapeutic drugs. 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 *Escherichia coli* to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin (humulin).

20. (b) : 51 amino acids of insulin are arranged in two polypeptide chains, chain A having 21 amino acids and chain B having 30 amino acids. The two polypeptide chains are interconnected by two disulphide bridges.

21. (c)

22. (a) : Gene therapy is a corrective therapy that is given to patients of diseases caused by some gene defects. Here, genes are inserted into a person's cells and tissues to treat disease by replacing the defective gene. The normal gene delivered into the individual or embryo takes over the function of and compensate for the normal gene. Viral vectors like adenovirus are generally used to deliver the normal gene.

23. (a) : Several human genes have been successfully transferred in animals and are expressed in the mammary tissue; the protein is secreted in milk from where it is easily harvested. These genes can also be made to express in reproductive tissues and thus proteins can also be harvested from semen ejaculate of male organisms. *E.g.*, hGH is being produced from a transgenic mouse variety's semen.

24. (d) : Adenosine deaminase enzyme is crucial for the immune system to function. The disorder ADA deficiency is caused due to deletion of the gene for adenosine deaminase. ADA deficiency can be cured by bone marrow transplantation or by enzyme replacement therapy, in which functional ADA is given to the patient by injection. But both of these approaches are not completely curative as the patient requires periodic infusion of such genetically engineered lymphocytes or hormonal injections. However, if the gene isolated from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

25. (b) : Human insulin is made up of 51 amino acids arranged in two polypeptide chains, A having 21 amino acids and B with 30 amino acids. The hormone develops from a storage product called pro-insulin. Pro-insulin has three chains, A, B and C. C – chain with 33 amino acids is removed prior to insulin formation. This C peptide is not present in the mature insulin and is removed during maturation into insulin.

26. (d) **27. (c)**

28. (a)

29. (a) : Several human genes have been successfully transferred in cows and expressed the mammary tissue, the protein is secreted in milk from where it is easily harvested.

30. (d) : Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO). They contain and express one or more useful foreign genes (transgenes). Bt brinjal, golden rice, tracy, rosie, etc., are genetically modified organisms. Dolly was a cloned sheep (first mammal to be cloned) born on 5th July 1996 at Roslin Institute in Edinburgh, Scotland.

31. (d) : In 1997, the first transgenic cow, Rosie, produced human protein enriched milk. The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow-milk. Isolation of DNA from other macromolecule is achieved by treating the bacterial cells/plant or animal tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus).

32. (b) : Animals that have their DNA manipulated to possess and express an extra (foreign) gene are known as transgenic animals. Transgenic mice, rabbits, pigs, sheep, cows and fish have been produced, although over 95 per cent of all existing transgenic animals are mice.

33. (a) : Many transgenic animals are designed to increase our understanding of how genes contribute to the development of diseases. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible. Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's. Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans. Transgenic mice are being used to test the safety of the polio vaccine.

34. (d) : Indian Government has set up organisation such as GEAC (Genetic Engineering Approval Committee) which makes decisions regarding the validity of GM research and safety of introducing GM organisms for public services.

35. (d) : In 1997, a Texas company got patent rights on Basmati rice through the US Patent and Trademark Office. This allowed the company to sell a 'new' variety of Basmati, in the US and abroad. This new variety of Basmati had actually been derived from Indian farmers' varieties. Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty. It caused a brief diplomatic crisis between India and United States with India threatening to take the matter to WTO (World Trade Organisation) as a violation of TRIPS (Trade Related Aspects of Intellectual Property Rights). Both voluntarily and due to review decisions by United States patent office, Rice Tec lost most of the claims of the patent.

36. (b) : Some organisations and multinational companies exploit or patent biological resources or bioresources of other nations without proper authorisation from the countries concerned. This is called biopiracy.

37. (d) : Insulin is now being commercially produced by genetic engineering.

38. (d) : Bt genes, RNA*i* genes and *cry* genes occur naturally in living organisms. Bt toxic gene of bacterium *Bacillus thuringiensis* has been cloned from bacteria and expressed in plants to provide resistance to insects without the need of insecticides. The choice of genes depends upon the crop and the targeted pest as most Bt toxins are insect group specific. The toxin is coded by a gene named *cry*. RNAi takes place in all eukaryotic organisms as a method of cellular defense this method involves silencing of a specific *m*RNA due to a complementary dsRNA molecule that binds to and prevents translation of the *m*RNA (silencing).

39. (c) **40. (c)**

41. (c) : A represents polypeptide chain C which is removed prior to insulin formation.

42. (d)

43. (d) : Each particular restriction enzyme produces unique sticky ends. Using the same enzyme for both the bacterial and human DNA will produce complementary sticky ends that can bind together by complementary base pairing. This would allow the human insulin gene to be inserted into the plasmid.

44. (a) : The optimal temperature, pH, oxygen and nutrient conditions in the fermenter allow the bacteria containing the insulin gene to reproduce quickly and produce large quantities of it.

45. (c) : DNA ligase forms strong hydrogen bonds between the DNA bases on the human insulin gene and the bacterial plasmid, producing a continuous double stranded DNA loop. Without DNA ligase, the human insulin gene, despite being able to undergo complementary base pairing with the bacterial DNA at the sticky ends would not be securely inserted into the plasmid. Thus, the resulting *E.coli* bacteria would receive plasmids that lack the human insulin gene.

46. (c)

47. (d) : The two chief objectives of transgenic cow production are as follows : (i) increased milk and meat production and (ii) molecular farming.

48. (c)

49. (b) : Transgenic bovine cells are selected and fused with bovine oocytes that have had all its chromosomes removed. Once fused with oocyte, the transgenic cells chromosomes are reprogrammed to direct development into an embryo which is implanted into recipient cow.

50. (a)

51. (b) : Plant in which foreign genes have been introduced through genetic engineering are called transgenic plants. Transgenic food from such plants may cause toxicity and or produce allergies. The enzyme produced by the antibiotic resistance genes can cause allergies because it is a foreign protein. GM plants enhance nutritional value of food, *e.g.*, Golden rice.

52. (b)

53. (c) : Biopatents are awarded for biological entities and all products derived from them. 1995 patent on 'use of turmeric in wound healing' was cancelled in 1998.

54. (d) : *A. tumefaciens* causes crown gall disease on a wide range of dicot (broad leaved) plants especially members of rose family by transferring its own DNA into plant cells. But in laboratory, the ability to move all sorts of genes into plants has made the microbe the standard tool for investigating plant genetics and modifying crops.

55. (a) : Mouse is the most preferred mammal for studies on gene transfers due to its many favourable features like (i) short oestrous cycle and gestation period, (ii) relatively short generation time, (iii) production of several offspring per pregnancy, (iv) convenient *in vitro* fertilisation, successful culture of embryos *in vitro*.

56. (b) : Ti plasmid (tumor inducing) from *Agrobacterium tumefaciens* is effectively used as a vector for gene transfer to plant cells. In nature, it induces tumors in broad leaf crops, *e.g.*, tomato, soybean, cotton, etc.

57. (d) : As a first step towards gene therapy, lymphocytes, a kind of white blood cells, are extracted from the bone marrow of the patient and are grown in a culture outside the body. A functional ADA cDNA (using a retroviral vector) is then

introduced into these lymphocytes, which are reinjected to the patient's bone marrow. But as these cells do not always remain alive, the patient requires periodic infusion of such genetically engineered lymphocytes. However, if the isolated gene from bone marrow cells producing ADA is introduced into cells at early embryonic stages, it can be permanent cure.

58. (b)

59. (c) : The Flavr-Savr tomato, was the first genetically modified fruit/vegetable to reach the market in USA. Roundup ready soybean is a genetically modified herbicide tolerant cultivar.

60. (c) : DNA probes are stretches of single-stranded DNA used to detect presence of complementary nucleic acid sequence (target sequences) by hybridisation. They are usually labelled with radioisotopes, to enable their detection.

SUBJECTIVE TYPE QUESTIONS

1. The first transgenic cow, Rosie produced human protein enriched milk which contained the human alpha lactalbumin and was nutritionally more balanced product for human babies than natural cow milk.

2. The C-peptide joins the A-peptide with B-peptide in the proinsulin. It is not present in mature insulin and is removed during processing of proinsulin to insulin.

3. *Agrobacterium tumefaciens* is called natural genetic engineer of plants because genes carried by its plasmid produce effect in several parts of the plant.

4. A patent is the right granted by a government to an inventor to prevent others from commercial use of his/her invention.

5. Two transgenic microorganisms used for large scale amino acid production are:

(i) *Bacillus amyloliquefaciens*

(ii) *Lactobacillus casei*

6. Severe combined immunodeficiency disease.

7. *Bacillus anthracis*

8. The genes *cry*IAc and *cry*IIAb control the cotton bollworms, whereas *cry*IAb controls corn borer.

9. Gene therapy is the technique of genetic engineering which involves replacement of a faulty gene by a normal healthy functional gene.

10. *Brassica napus*

11. Transgenic crops or genetically modified crop contain and express one or more useful foreign genes or transgenes. *Brassica napus* is used to produce blood anticoagulant protein. The protein is called hirudin.

The protein hirudin is an anticoagulant which is present in leech. Its gene was chemically synthesized and introduced in *Brassica napus*. The seeds of the latter came to have hirudin which could be extracted and purified.

12. Some multinational companies of industrialised nations have a good economic status but are poor in biodiversity and are exploiting biodiversity of developing and underdeveloped countries without authorisation and proper compensation.

There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore, some nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge.

13. (a) *cryIA*c gene controls cotton bollworms in Bt cotton.

(b) RNA interference (RNAi) takes place in all eukaryotic organisms as a method of cellular defense. This method involves silencing of a specific *m*RNA.

14. SCID stands for severe combined immunodeficiency disease. The SCID patient has a defective gene for the enzyme adenosine deaminase. He/she lacks functional T-lymphocytes and therefore fails to fight with infecting pathogens. It can be cured by gene therapy.

In SCID, the patient lacks functional T-lymphocytes and it is congenital while in AIDS only T-helper cells are destroyed by virus, but is not congenital.

15. Transgenic animals are those animals which contain in their genome, a foreign gene introduced by recombinant DNA technology. Such gene is called transgene. Examples of transgenic animals are transgenic mice and transgenic rabbit etc.

16. The bacterium *Bacillus thuringiensis* is a common soil bacterium which produces a protein toxin that kills certain insects. The toxin is a crystal (Cry) protein. There are several kinds of Cry proteins which are toxic to different groups of insects. The gene encoding Cry protein is called *cry* gene.

Biotechnologists have been able to isolate the gene responsible for production of toxin and introduce it into a number of plants to produce genetically modified plants resistant to insects, *e.g.*, Bt cotton (resistant to bollworm) and GM tobacco (resistant to hornworms).

17. Enzyme-linked immunosorbent assay (ELISA) is a non isotopic immunoassay. ELISA is based on the immunochemical principles of antigen-antibody reaction. ELISA is used for determining small quantity of proteins (hormones, antigens and antibodies). ELISA is also used for diagnosis of HIV.

18. Bt cotton plant contains *Bacillus thuringiensis*, which produces Bt toxin, an insecticidal protein. This Bt toxin protein exist as inactive form but once an insect ingest the inactive toxin, it is converted into active form of toxin due to alkaline pH of the insects gut which solublise the crystals. The activated toxin binds to surface of midgut epithelial cells and creates

pores that cause cell swelling and lysis and causes the death of the insect.

19. The two molecular diagnostic technique are as follows :

(i) PCR is polymerase chain reaction. It is used to detect HIV infection and mutations in genes in suspected cancer patient.

(ii) ELISA is Enzyme- Linked Immunosorbent Assay. It is used to detect infections by pathogens.

20. Flavr Savr, a variety of tomato is a genetically modified plant. In this variety one gene, which produces polygalactouronase enzyme is inactivated. The non availability of this enzyme prevents over ripening. Thus, fruit remains fresh for long time and it also retains flavour, superior taste and higher quantity of total soluble solids. So it prevents post harvest and over ripening losses. Thus, it is preferred over normal native variety.

21. GEAC is Genetic Engineering Approval Committee. It makes decisions regarding the validity of GM research and the safety of introducing GM organisms for public services. The objectives of setting up GEAC by our government is as follows:

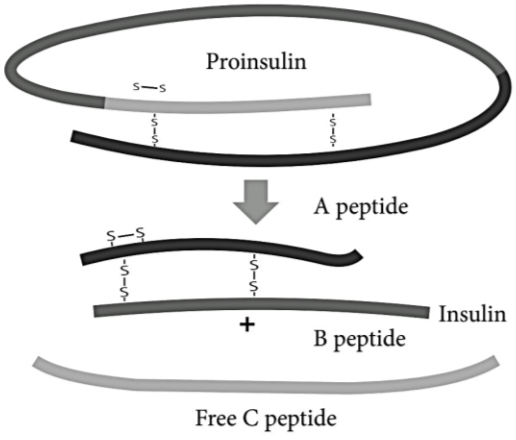
(i) To permit the use of GM organisms and their products for commercial applications.

(ii) To adopt procedures for restriction, production, import, export and application of GM organisms.

(iii) To approve for conduct of large scale field trials and release of transgenic crops in the environment.

(iv) To curb and take punitive action against agencies if they disturb ecological balance.

22. Insulin consists of two short polypeptide chains: chain A and chain B, that are linked together by disulphide bridges. In mammals, including humans, insulin is synthesised as a prohormone which contains an extra stretch called the C peptide. This C peptide is not present in the mature insulin and is removed during maturation into insulin. The given diagram explains the maturation of proinsulin into insulin:



23. (a) The given figure shows the steps involved in recombinant DNA technology.

(b) In the given figure, A is plasmid vector, B is cut plasmid, C is fragment of DNA containing gene of interest and D is recombinant DNA.

(c) Process X is restriction digestion and Y is annealing. Enzymes involved in X and Y are restriction endonuclease and DNA ligase respectively.

24. The steps involved in the production of artificial insulin or humulin are as follows:

(i) Isolation of donor or DNA segment – A useful DNA segment is isolated from the donor organism.

(ii) Formation of recombinant DNA (rDNA) – Both the vector and donor DNA segments are cut in the presence of restriction endonuclease. In the presence of ligase DNA segments of both are joined to form rDNA.

(iii) Production of multiple copies of rDNA – In this process multiple copies of this recombinant DNA are produced.

(iv) Introduction of rDNA in the recipient organism – The rDNA is inserted into a recipient organism.

(v) Screening of the transformed cells – The recipient (host) cells are screened in the presence of rDNA and the product of donor gene. The transformed cells are separated and multiplied.

25. (a) *Refer to answer 15.*

(b) (i) Genetically modified organisms such as mice are being formed for use in testing the safety of vaccines before they are used on human beings. Transgenic mice are being used to test the safety of the polio vaccine.

(ii) Transgenic animals that produce useful biological products can be created by the introduction of the DNA segment (or gene) which code for a particular product such as human protein (α-1-antitrypsin) used to treat emphysema. Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis.

26. Soil bacterium *Bacillus thuringiensis* produces proteins that kill certain insects like lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes), etc., *B. thuringiensis* forms some protein crystals. These crystals contain a toxic insecticidal protein. This toxin does not kill the *Bacillus* (bacterium) because it exists as inactive protoxins in them. But, once an insect ingests the crystals, it is converted into an active form of toxin due to the alkaline pH of the alimentary canal that solubilises the crystals.

The activated toxin binds to the surface of mid gut epithelial cells and creates pores which cause cell swelling and lysis and finally cause death of the insect.

cry genes code for certain crystal (cry) proteins that are toxic to insect larvae. The genes *cry*IAc and *cry*IAb control cotton bollworm. When these genes are introduced into cotton

plants through genetic engineering, these plants become resistant to the attack of cotton bollworm.

27. The plants in which foreign genes have been introduced through genetic engineering are called transgenic plants.

Following are the disadvantages of transgenic plants :

(i) Gene transfer to non-target species : Transgenic crop plants can crossbreed with weeds, resulting in the transfer of transgene. These "super weeds" can then be difficult to eradicate. Other non-transgenic crops can also get the transgenes by cross breed.

(ii) Allergies : The transgenic food may cause toxicity or produce allergies to human beings. The enzyme produced by the antibiotic resistance gene can cause allergies, because it is a foreign protein.

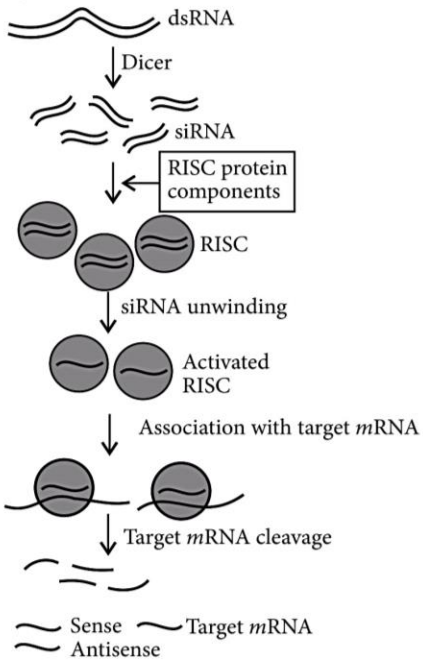
28. (a) Both the girls A and B were suffering from SCID (Severe Combined Immuno Deficiency) syndrome produced by the deficiency of enzyme Adenosine deaminase (ADA).

(b) The treatment provided to girl A required repeated visits because enzyme replacement therapy is not permanent cure. This is because these patients do not have functional T-lymphocytes, therefore they cannot provide immune responses against invading pathogens.

(c) The girl B was treated by the transplanted stem cells that are injected into the bloodstream. They will then become healthy white blood cells that replenish immune functions - essentially building a whole new, functional immune system for the girl B. The immune system regains complete function and hence girl B was permanently cured.

29. A nematode *Meloidogyne incognita* infects the roots of tobacco plants and causes a great reduction in yield. A novel strategy that was adopted to prevent this infection was based on the process of RNA interference (RNAi). RNA interference (RNAi) is the phenomenon of inhibiting activity of a gene by synthesis of RNA molecules complementary to the *m*RNA. The normal (*in vivo* synthesised) *m*RNA of a gene is said to be "sense" because it carries the codons that are "read" during translation. Normally, the complement to the *m*RNA "sense" strand will not contain a sequence of codons that can be translated to produce a functional protein; thus, this complementary strand is called "anti-sense RNA". The anti-sense RNA and *m*RNA molecules will anneal to form duplex RNA molecules that can not be translated. Thus, the presence of anti-sense RNA will block translation of the *m*RNA of the affected gene. The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate *via* an RNA intermediate. Using *Agrobacterium* vectors, nematode-specific anti-sense genes are introduced into the host plant. The introduction of DNA produces anti-sense RNA in the host cells. The transgenic host plants expresses anti-sense RNA. As in consequence, nematode infestation fails in the transgenic plants because the complementary anti-sense RNA forms a double stranded RNA (ds RNA) which interferes or blocks the translation and thus, silences the *m*RNA of the nematode. The result was that the parasite could not survive in transgenic plant. In such way, the transgenic plant gets protected from the parasite.

30. The steps in RNA interference (RNAi)



31. Advantages of genetically modified plants are as follows:

(i) Genetically modified plants are resistant to (a) diseases resulting from viral, bacterial and fungal infections (b) pests, such as nematodes and insects and (c) pesticides.

(ii) GM plants can tolerate adverse abiotic stresses such as cold, drought, salt, heat.

(iii) GM plants show increased efficiency of mineral usage (this prevents early exhaustion of fertility of soil).

(iv) GM plants have high nutritional value, *e.g.*, vitamin A enriched rice.

(v) Plants such as poplar (*Populus*) trees have been genetically engineered to clean up heavy pollution from contaminated soil.

(vi) These plants helped to reduce post harvest losses, *e.g.*, Flavr savr transgenic tomato.

32. Adenosine deaminase (ADA) enzyme is crucial for the immune system to function. Its deficiency is caused due to the deletion of the gene for adenosine deaminase. In some patients, ADA deficiency can be cured by the bone marrow transplantation. It can be treated by enzyme replacement therapy, in which functional ADA is given to the patient by injection.

Two disadvantages of enzyme replacement therapy are :

(i) It is not permanent cure because the replacement patient of ADA deficiency do not have functional T-lymphocytes, they cannot provide immune responses against invading pathogens.

(ii) It is a costly method.

33. Golden rice is a transgenic variety of rice (*Oryza sativa*) which contains good quantities of β-carotene (provitamin A-inactive state of vitamin A). β-carotene is a principal source of vitamin A. Since the grains (seeds) of the rice are yellow in colour due to β-carotene, the rice is commonly called golden rice. β-carotene (provitamin A) is converted into vitamin A. Thus, golden rice is rich in vitamin A. It is required by all individuals as it is present in retina of eyes. Deficiency of vitamin A causes night blindness and skin disorder. Since the contents of vitamin A are very low in rice, vitamin A is synthesised from β-carotene which is precursor of vitamin A. Prof. Ingo Potrykus and Peter Beyer produced genetically engineered rice by introducing three genes associated with synthesis of carotene. The grains (seeds) of transgenic rice are rich in provitamin.

34. Two genes *cry*IAc and *cry*IIAb control cotton bollworms. These two genes were isolated from *Bacillus thuringiensis* and incorporated into cotton plant. The genetically modified plant is called Bt cotton as it contains Bt toxin genes. The bacterium *Bacillus thuringiensis* produces Bt toxin proteins in mature form. When the insect larvae ingest any plant part, toxin becomes active in the alkaline pH of the gut and kills the insect pests. That is how Bt cotton attains resistance against bollworm.

35. (a) Adenosine deaminase (ADA) deficiency

(b) Adenosine deaminase enzyme is necessary for the proper functioning of our immune system. ADA deficiency is caused by the defect in the gene coding for enzyme adenosine deaminase. The possible treatments that can be given to a patient exhibiting adenosine deaminase (ADA) deficiency are:

(i) bone marrow transplantation

(ii) enzyme replacement therapy.

36. (a) Benefits derived from transgenic animals are as follows:

(i) They produce useful biological products, that can be created by introduction of portion of gene, which codes for a particular product such as human protein (α -1- antitrypsin) from transgenic sheep is used to treat emphysema.

(ii) Transgenic mice are being developed for use in testing the safety of vaccine before they are used in humans.

(iii) They carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to toxic substances and the effects are studied.

(b) The ethical issues concerned with the production of transgenic animals include:

(i) Introduction of a transgene from one species into another species violates the 'integrity of species'.

(ii) Transfer of human genes into animals (and *vice-versa*) dilutes the concept of 'humanness'.

(iii) When animals are used for production of pharmaceutical proteins, they are virtually reduced to the status of a 'factory'.

(iv) Use of animals in biotechnology causes great suffering to them.

(v) It is disrespectful to living beings, and only exploits them for the benefit of human beings.

37. *Refer to answer 29.*

38. Enzyme-linked immunosorbent assay (ELISA) is a non-isotopic immunoassay. ELISA is based on the immunochemical principles of antigen antibody reaction. The stages of ELISA are summarized as follows:

(i) The antibody against the protein to be determined is fixed on an inert solid such as polystyrene. The biological sample containing the protein to be estimated is applied on the antibody coated surface. The protein antibody complex is then reacted with a second protein specific antibody to which an enzyme is covalently linked. Peroxidase, amylase and alkaline phosphatase are commonly used.

(ii) After washing the unbound antibody linked enzyme, the enzyme bound to the second antibody complex is assayed. The enzyme activity is determined by its action on a substrate to form a product (usually coloured). This is related to the concentration of the protein being estimated.

ELISA is widely used for the determination of small quantities of proteins (hormones, antigens, antibodies) and other biological substances. The most commonly used pregnancy test for the detection of human chorionic gonadotropin (hCG) in urine is based on ELISA. ELISA is also been used for diagnosis of HIV viruses in AIDS patient.

39. (a) (i) Flavr Savr is a transgenic variety of tomato. In this variety native tomato gene, which produces enzyme polygalacturonase is inactivated. The non-availability of this enzyme prevents over-ripening because the enzyme is essential for degradation of cell walls. Thus, fruit remains fresh for long time and it also retains flavour, superior taste and higher quantity of total soluble solids. So, it prevents post harvest and over ripening losses, and is preferred over normal native variety.

(ii) ANDI - DNA of a fluorescent jelly fish was introduced into an unfertilised egg of a Rhesus monkey in the

test tube. The diploid egg underwent cleavage and the early embryo was implanted in a surrogate mother. It has been named ANDI, the acronym of "inserted DNA". This work would be helpful for curing diseases such as breast cancer, Alzheimer's disease, diabetes and AIDS.

(iii) Tracy, the transgenic ewe was born in Scotland. Transgenic sheep have been produced to achieve better growth and meat production. For example, human genes for blood clotting factor IX and for α_1-antitryspin have been transferred in sheep and expressed in mammary tissue. This was achieved by fusing the genes with the mammary tissue-specific promoter of the bovine β-lactoglobulin gene. Human growth hormone gene has also been introduced in sheep in order to promote growth and meat production. However, they also showed several undesirable effects like joint pathology, skeletal defects, gastric ulcers, infertility, etc.

(b) Transgenic chicken is resistant to avian leukosis virus (ALV).

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