Chapter 10: Biotechnology and Its Applications

Exercises — Chapter-end Exercises

Q1 Which part of the plant is best suited for making virus-free plants and why?

SOLUTION

The meristem (apical and axillary meristems) is best suited for making virus-free plants.

Even if a plant is infected with a virus, the meristematic tissue remains free of the virus. This is because viruses spread through the vascular system and plasmodesmata, but the meristem cells divide so rapidly and are so newly formed that the virus has not yet colonised them.

By removing the meristem and growing it in vitro under sterile conditions (tissue culture / micropropagation), one can obtain virus-free plants. Scientists have successfully cultured meristems of banana, sugarcane, potato and other important crop plants using this technique.

Answer: Meristem (apical/axillary) is virus-free even in infected plants; culturing it in vitro yields virus-free plants.

Q2 What is the major advantage of producing plants by micropropagation?

SOLUTION

Micropropagation is a method of producing thousands of plants through tissue culture from a single explant in a very short duration.

The major advantage of micropropagation is that it allows the rapid, large-scale propagation of genetically identical plants (somaclones) from a single parent plant. This means:

1. Thousands of plants can be produced in a short time from a small amount of parent material.
2. All produced plants are genetically identical to the original plant, preserving desirable traits.
3. Plants can be produced throughout the year, independent of season.
4. Virus-free plants can be raised by using meristematic tissue as the explant.
5. It enables commercial-scale production of important food plants like tomato, banana, apple, etc.

The ability to generate a whole plant from any cell or explant is based on the property called totipotency.

Answer: Micropropagation allows rapid production of thousands of genetically identical (somaclone) plants in a short time, enabling commercial-scale propagation while preserving desirable traits.

Q3 Find out what the various components of the medium used for propagation of an explant in vitro are?

SOLUTION

The nutrient medium used for in vitro propagation of an explant (tissue culture medium) contains the following components:

1. Carbon source: Sucrose is commonly used as the carbon and energy source for the growing cells.
2. Inorganic salts (macro- and micronutrients): These supply essential mineral elements such as nitrogen (as nitrate or ammonium), phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, zinc, boron, copper, molybdenum, etc.
3. Vitamins: Thiamine (B_1), nicotinic acid, pyridoxine (B_6) are commonly included to support cell growth and differentiation.
4. Amino acids: Certain amino acids may be added to serve as organic nitrogen sources.
5. Growth regulators (phytohormones): Auxins (such as IAA, NAA, 2,4-D) promote root differentiation; cytokinins (such as BAP, kinetin) promote shoot differentiation. The ratio of auxin to cytokinin determines the direction of differentiation.
6. Agar (gelling agent): Used to solidify the medium for callus and shoot culture (liquid medium is used in some protocols).
7. Water: Ultra-pure/distilled water forms the base of the medium.
8. The medium is sterilised (usually by autoclaving) and maintained at a controlled pH (around 5.7-5.8) to ensure sterile and optimal growth conditions.

Answer: The medium contains sucrose (carbon source), inorganic salts, vitamins, amino acids, growth regulators (auxins and cytokinins), agar, and sterile water at controlled pH.

Q4 Crystals of Bt toxin produced by some bacteria do not kill the bacteria themselves because –
(a) bacteria are resistant to the toxin
(b) toxin is immature;
(c) toxin is inactive;
(d) bacteria encloses toxin in a special sac.

SOLUTION

The correct answer is (c) toxin is inactive.

Bacillus thuringiensis (Bt) produces protein crystals during a particular phase of its growth. These crystals contain the Bt toxin protein, but within the bacterium the toxin exists as an inactive protoxin.

The protoxin is solubilised and converted into its active form only when ingested by an insect, because the gut of susceptible insects has an alkaline pH. The alkaline pH of the insect midgut solubilises the crystals, releasing the protoxin which is then processed (cleaved by gut proteases) into the active toxin.

Since the bacterium itself does not have this alkaline gut environment, the toxin remains in its

inactive (protoxin) form inside the bacterium and does not harm it.

Answer: (c) toxin is inactive — Bt toxin exists as an inactive protoxin inside the bacterium and becomes active only in the alkaline pH of an insect's gut.

Q5 What are transgenic bacteria? Illustrate using any one example.

SOLUTION

Transgenic bacteria are bacteria whose DNA has been altered by the introduction of a foreign gene (from another organism) using recombinant DNA (rDNA) technology. These bacteria are capable of expressing the foreign gene and producing the corresponding protein.

Example — Production of Human Insulin:

In 1983, the American company Eli Lilly used E. coli as a transgenic bacterium to produce human insulin. Human insulin consists of two polypeptide chains: Chain A and Chain B, linked by disulphide bonds.

The procedure involved:
1. Two separate DNA sequences corresponding to chain A and chain B of human insulin were chemically synthesised.
2. Each sequence was separately introduced into plasmids of E. coli.
3. The bacteria expressed the genes and produced insulin chain A and chain B separately.
4. Both chains were extracted and combined in vitro by creating disulphide bonds between them to form the mature, functional human insulin.

This insulin (marketed as Humulin) is structurally identical to natural human insulin and does not trigger immune reactions, unlike insulin extracted from cattle or pigs.

Answer: Transgenic bacteria have foreign genes introduced via rDNA technology. Example: E. coli was engineered to carry human insulin genes (chains A and B separately), producing human insulin identical to the natural molecule.

Q6 Compare and contrast the advantages and disadvantages of production of genetically modified crops.

SOLUTION

Advantages of Genetically Modified (GM) Crops:

1. Abiotic stress tolerance: GM crops can be made more tolerant to cold, drought, salt, and heat stress, enabling cultivation in harsh conditions.
2. Pest resistance: Crops like Bt cotton carry the Bt toxin gene, reducing reliance on chemical pesticides and lowering production costs.
3. Reduced post-harvest losses: Genetic modification can slow ripening or improve shelf life, reducing losses after harvest.
4. Improved mineral utilisation: GM crops can be engineered for efficient use of soil minerals,

preventing early exhaustion of soil fertility.
5. Enhanced nutritional value: e.g., Golden Rice is enriched with Vitamin A (β-carotene) to combat nutritional deficiencies.
6. Industrial applications: GM crops can be designed to produce alternative resources such as starches, fuels and pharmaceuticals.
7. Higher yields: Reduce crop loss due to pests and diseases, increasing overall yield.

Disadvantages of Genetically Modified (GM) Crops:

1. Ecological risks: Introducing GM organisms into ecosystems can have unpredictable consequences — potential gene flow to wild relatives, creation of superweeds or resistance in pests.
2. Reduction in biodiversity: Widespread adoption of a few GM varieties may reduce agricultural biodiversity.
3. Unknown long-term effects: Long-term effects on human health and the environment have not been fully established.
4. Ethical concerns: Genetic manipulation of living organisms raises moral and ethical questions for many communities.
5. Biopiracy and patent issues: Companies can patent GM varieties derived from traditional crop varieties, depriving farming communities of their rights.
6. Dependence on corporations: Farmers may become dependent on seed companies for patented GM seeds, increasing costs.
7. Regulatory complexity: Requires careful governmental oversight (e.g., GEAC in India) to ensure safety, adding to development costs and time.

Answer: GM crops offer higher yields, pest resistance, stress tolerance, and enhanced nutrition, but carry risks of ecological disruption, loss of biodiversity, ethical concerns, and potential corporate dependency.

Q7 What are Cry proteins? Name an organism that produce it. How has man exploited this protein to his benefit?

SOLUTION

Cry proteins (Crystalline insecticidal proteins) are toxic insecticidal proteins produced by the bacterium Bacillus thuringiensis (Bt). These proteins are encoded by genes called cry genes (e.g., crylAc, crylIAb, crylAb).

The organism that produces Cry proteins: Bacillus thuringiensis (a gram-positive, soil-dwelling bacterium).

Nature of Cry proteins:
B. thuringiensis forms protein crystals during a particular phase of growth. These crystals contain Cry proteins as inactive protoxins. When ingested by susceptible insects, the alkaline pH of the insect midgut solubilises the crystals and activates the protoxin into its active form. The activated toxin binds to the surface of the midgut epithelial cells, creates pores, causes cell

swelling and lysis, and ultimately kills the insect.

How man has exploited Cry proteins:
1. Bt crops (bio-pesticides): Specific cry genes have been isolated from B. thuringiensis and introduced into crop plants such as cotton, corn, rice, tomato, potato, and soyabean to make them pest-resistant — e.g., Bt cotton (containing cryIAc and cryIIAb to control bollworms) and Bt corn (cryIAb to control corn borer).
2. These transgenic Bt crops produce Cry proteins in their tissues, making them toxic to specific insect pests without the need to spray chemical insecticides.
3. The use of Bt crops reduces environmental pollution from pesticides and lowers the cost of crop protection.
4. Since different Cry proteins are specific to different insect groups (lepidopterans, coleopterans, dipterans), appropriate genes can be chosen for the target pest.

Answer: Cry proteins are insecticidal crystalline proteins from Bacillus thuringiensis; their cry genes have been introduced into crop plants (e.g., Bt cotton) to create pest-resistant transgenic crops that reduce pesticide use.

Q8 What is gene therapy? Illustrate using the example of adenosine deaminase (ADA) deficiency.

SOLUTION

Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child or embryo. It involves the insertion of a functional (normal) gene into a person's cells and tissues to treat a disease, especially hereditary diseases, by compensating for the non-functional or absent gene.

Illustration — ADA (Adenosine Deaminase) Deficiency:

ADA deficiency is caused by the deletion of the gene encoding adenosine deaminase, an enzyme crucial for the proper functioning of the immune system. Without ADA, the immune system is severely compromised (Severe Combined Immunodeficiency — SCID).

The first clinical gene therapy was given in 1990 to a 4-year-old girl with ADA deficiency. The procedure was as follows:

1. Lymphocytes (white blood cells) were isolated from the patient's blood and cultured outside the body (ex vivo).
2. A functional ADA cDNA was introduced into these lymphocytes using a retroviral vector as the delivery vehicle.
3. The genetically modified lymphocytes (now carrying the functional ADA gene) were then returned (infused) into the patient's body.
4. These cells could now produce functional ADA enzyme and help restore immune function.

Limitations:
- Since the lymphocytes are not immortal, periodic infusion of genetically engineered lymphocytes is required.

- For a permanent cure, the ADA gene would need to be introduced into bone marrow stem cells at an early embryonic stage so that all daughter cells carry the functional gene throughout the patient's life.

Alternative treatments include bone marrow transplantation and enzyme replacement therapy (injection of functional ADA), but neither is completely curative.

Answer: Gene therapy corrects hereditary defects by inserting functional genes into patient cells. In ADA deficiency, lymphocytes were taken from the patient, a functional ADA cDNA was inserted via retroviral vector, and the cells were returned to restore immune function.

Q9 Digrammatically represent the experimental steps in cloning and expressing an human gene (say the gene for growth hormone) into a bacterium like E. coli?

SOLUTION

A diagram cannot be drawn here, but the experimental steps in cloning and expressing a human gene (e.g., growth hormone gene) in E. coli are described below with the key labels/components that would appear in a diagram:

Step 1 — Isolation of the desired gene:
- The mRNA for human growth hormone (hGH) is isolated from pituitary cells.
- Reverse transcriptase is used to make a complementary DNA (cDNA) from the mRNA.
- The cDNA represents the coding sequence of the growth hormone gene.

Step 2 — Insertion into a vector:
- A suitable restriction endonuclease is used to cut both the cDNA and a plasmid vector (e.g., pBR322) at specific restriction sites, generating compatible sticky ends.
- The cDNA is ligated into the plasmid using DNA ligase, creating a recombinant DNA (rDNA) molecule.

Step 3 — Introduction into E. coli (Transformation):
- The recombinant plasmid is introduced into competent E. coli cells by transformation (e.g., heat shock method or electroporation).
- Transformed colonies are selected using antibiotic resistance markers present on the plasmid.

Step 4 — Screening and selection:
- Colonies carrying the recombinant plasmid with the growth hormone gene are identified (e.g., by colony hybridisation with a labelled probe, or by blue-white screening).

Step 5 — Expression:
- The selected E. coli clones are cultured in a fermenter/bioreactor.
- The promoter in the plasmid drives transcription of the growth hormone gene; the mRNA is translated by E. coli ribosomes to produce human growth hormone protein.

Step 6 — Extraction and purification:
- The human growth hormone protein is extracted from the bacterial cells and purified by downstream processing (chromatography, etc.).

Key labels for a diagram: human cell → mRNA → cDNA (via reverse transcriptase) → restriction digestion → ligation with plasmid → recombinant plasmid → transformation into E. coli → selection of transformed colonies → culture in bioreactor → extraction of hGH protein.

Answer: The steps are: isolate hGH mRNA → make cDNA via reverse transcriptase → cut with restriction enzyme → ligate into plasmid vector → transform into E. coli → select recombinant colonies → culture and express → purify hGH protein.

Q10 Can you suggest a method to remove oil (hydrocarbon) from seeds based on your understanding of rDNA technology and chemistry of oil?

SOLUTION

Oils (lipids/fats) stored in seeds are synthesised by specific enzymes encoded by genes involved in fatty acid biosynthesis pathways. Using rDNA technology, it is possible to silence or knock out the genes responsible for oil (triglyceride) synthesis in seeds.

Suggested Method — using RNA interference (RNAi) or antisense technology:

1. Identify the key genes encoding enzymes in the oil biosynthesis pathway in the seed (e.g., genes for fatty acid synthase, acyl-ACP thioesterase, or diacylglycerol acyltransferase — the enzyme that catalyses the final step of triglyceride synthesis).
2. Using rDNA technology, construct an antisense RNA or siRNA (small interfering RNA) complementary to the mRNA of the target enzyme gene.
3. Introduce this antisense/siRNA gene into the plant via a suitable vector (e.g., Agrobacterium tumefaciens) so that the plant cells produce the antisense or double-stranded RNA.
4. The dsRNA triggers RNA interference (RNAi), leading to degradation or translational silencing of the target enzyme mRNA in the seed.
5. Without the functional enzyme, oil biosynthesis is blocked or greatly reduced, resulting in seeds with little or no oil content.

Alternatively, the gene encoding a lipase enzyme could be introduced into the seed, so that the stored oil is hydrolysed within the seed itself before harvest.

This approach has practical applications in producing low-fat or oil-free crop varieties and in metabolic engineering of seeds for altered lipid profiles.

Answer: Using RNAi/antisense technology via rDNA methods, genes encoding key enzymes of oil biosynthesis (e.g., diacylglycerol acyltransferase) can be silenced in the seed, blocking triglyceride accumulation and producing oil-free seeds.

Q11 Find out from internet what is golden rice.

SOLUTION

Golden Rice is a genetically modified variety of rice (Oryza sativa) that has been engineered to produce β-carotene (pro-vitamin A) in its edible endosperm, giving the grain a characteristic golden-yellow colour.

Key facts about Golden Rice:

1. Normal white rice does not contain β-carotene in the endosperm (the part that is eaten after milling). Golden Rice was developed to address Vitamin A deficiency, which affects millions of people — especially children — in developing countries and can lead to blindness and increased susceptibility to infections.
2. The first Golden Rice ('Golden Rice 1') was developed in the late 1990s by Ingo Potrykus and Peter Beyer. Genes encoding phytoene synthase (from daffodil) and carotene desaturase (from the bacterium Erwinia) were introduced into rice using Agrobacterium-mediated transformation, enabling the rice endosperm to produce β-carotene.
3. An improved version, 'Golden Rice 2', was developed using the maize phytoene synthase gene, which produces around 23 times more β-carotene than the original.
4. Upon consumption, β-carotene is converted to Vitamin A in the human body.
5. The chapter text refers to Golden Rice as an example of GM crops with enhanced nutritional value: 'Vitamin A enriched rice'.
6. Golden Rice is considered a humanitarian project aimed at reducing malnutrition in developing countries, not a commercial product.

Answer: Golden Rice is a GM rice variety engineered to produce β-carotene (pro-vitamin A) in its endosperm, developed to combat Vitamin A deficiency in populations dependent on rice as a staple food.

Q12 Does our blood have proteases and nucleases?

SOLUTION

No, blood plasma does not normally contain active proteases and nucleases in significant amounts under healthy conditions.

This is relevant to the production and oral administration of recombinant protein drugs:

1. Blood plasma does contain some enzymes, but free active proteases and nucleases are generally not present in the circulating blood under normal physiological conditions. Coagulation factors (which are serine proteases) circulate as inactive zymogens and are only activated during clotting.

2. The relevance to biotechnology: This question is posed in the context of why insulin and other protein pharmaceuticals cannot be administered orally. The digestive tract (not the blood) is rich in proteases (pepsin, trypsin, chymotrypsin) that would degrade ingested proteins before they reach the bloodstream.

3. If blood did contain active proteases and nucleases, recombinant protein therapeutics administered intravenously would be rapidly degraded in the blood, making their use impossible.

Therefore, blood does not have significant active proteases and nucleases, which is why recombinant proteins like human insulin can be safely administered by injection — they remain

intact in the bloodstream long enough to exert their effect.

In contrast, oral administration of proteins fails because proteases in the stomach and intestine digest the protein before absorption.

Answer: No, blood does not have active proteases and nucleases under normal conditions. This is why protein drugs like insulin are given by injection (not orally) — they survive in the blood but would be digested in the gut.

Q13 Consult internet and find out how to make orally active protein pharmaceutical. What is the major problem to be encountered?

SOLUTION

Making proteins orally active is a major challenge in pharmaceutical science. Current approaches to develop orally active protein pharmaceuticals include:

1. Encapsulation / microencapsulation: Enclosing the protein in pH-sensitive polymeric capsules (e.g., enteric coatings) that protect the protein from the acidic stomach environment and release it in the small intestine for absorption.
2. Nanoparticle delivery: Using biodegradable nanoparticles (e.g., PLGA, chitosan) to encapsulate and protect the protein from enzymatic degradation, and to enhance mucosal absorption.
3. Liposome encapsulation: Enclosing the protein in lipid bilayer vesicles (liposomes) to protect it and facilitate cellular uptake in the gut.
4. Chemical modification: PEGylation (attaching polyethylene glycol chains) or other chemical modifications can protect proteins from degradation and improve their stability.
5. Protease inhibitors: Co-administering protease inhibitors to reduce enzymatic breakdown in the GI tract.
6. Fusion with receptor-targeting peptides: Fusing the protein to peptides that bind gut epithelial receptors (e.g., Fc receptor-binding domains, vitamin B_{12}-binding domains) to exploit active transport across the intestinal wall.
7. Plant-based oral vaccines: Expressing antigenic proteins in edible plant parts so that they can be consumed directly.

The major problem to be encountered:
The principal challenge is the degradation of the protein by proteolytic enzymes (proteases) in the gastrointestinal tract — pepsin in the stomach and trypsin, chymotrypsin, and other proteases in the small intestine — before the protein can be absorbed. Additionally, the acidic pH of the stomach denatures most proteins, and the large molecular size of proteins makes it difficult for them to cross the intestinal epithelium into the bloodstream. Achieving sufficient and reproducible bioavailability remains the central unsolved problem.

Answer: Orally active protein pharmaceuticals can potentially be made using encapsulation, nanoparticles, or liposomes; the major problem is degradation by GI tract proteases (pepsin, trypsin) and the acidic stomach environment before absorption.