UNIT VIII: BIOTECHNOLOGY

CHAPTER 4 PRINCIPLES AND PROCESSES OF BIOTECHNOLOGY

POINTS TO REMEMBER

- **Biotechnology** is science of development and utilization of biological processes.
- The term **biotechnology** was coined by Karl Ereky (Hungarian) in 1919.
- Two main heads of biotechnology - conventional or traditional biotechnology and modern biotechnology.
- Traditional Biotechnology is the kitchen technology developed by our ancestors.
- The study of fermentation and its practical uses is called zymology (1856)
- Louis Pasteur (French) demonstrated that fermentation was caused by **yeast**.
- **Primary metabolites** are Ethanol, citric, acid, lactic acid, acetic acid.
- **Secondary metabolites** are Amphotericin-B (*Streptomyces nodosus*), Penicillin (*Penicillium chryosogenum*) Streptomycin (*S. grises*), Tetracycline (*S. aureofacins*), alkaloids, toxic pigments, vitamins etc.
- Single cell proteins are dried cells of microorganism that are used as protein supplement.
- Recombination carried out artificially using modern technology is called recombinant DNA technology.
- Tools required for genetic engineering are the restriction enzymes, **DNA ligase** and **alkaline phosphatase**.
- A restriction enzyme or restriction endonuclease is an enzyme that cleaves DNA into fragments
- **Exonucleases** are Bal 31, Exonuclease III and **Endonucleases** are Hind II, EcoRI, Pvul, BamHI, TaqI.
- The restriction enzymes are called as **molecular scissors**.
- DNA ligase enzyme joins the sugar and phosphate molecules of double stranded DNA (dsDNA)
- Vector is also called **cloning vehicle** or **cloning DNA**.
- Plasmids are extra chromosomal , self replicating ds circular DNA molecules, found in the bacterial cells
- **pBR 322 - *p*** for plasmid, ***B***and ***R*** for scientist **B**oliver and **R**odriguez **322** no. of plasmid developed in laboratory.
- Ti plasmid is found in **Agrobacterium tumefaciens**, for inducing tumours in several dicot plants.
- Transposons are called **walking genes** or **jumping genes**.
- The use of transposons in *Arabidopsis thaliana* and *Escherichia coli*.
- Bt gene for insect resistance. The lacZ encodes the enzyme β-galactosidase
- β-galactosidase breaks a synthetic substrates called X-gal
- X-gal -- 5-bromo-4-chloro-indolyl-β-D-galacto-pyranoside.
- Agarose GEL Electrophoresis is used mainly for the purification of specific DNA fragments.

- In electrophoresis the bands of DNA in the gel are stained with the dye **Ethidium Bromide**
- Basta herbicide tolerant gene PPT (*L*-phosphinothricin) was isolated from *Medicago sativa* plant.
- Golden rice can control childhood blindness - **Xerophthalmia**.
- The Bt brinjal has been developed to give resistance against *Lepidopteron* insects, Flavr-Savr tomato, i.e., retaining the natural colour and flavor of tomato.
- Golden rice contains **beta-carotene** and **Vitamin-A** in the edible parts of rice
- Polylactic acid or polylactide is a biodegradable and bioactive thermoplastic.
- GFP refers to the protein first isolated from the **jellyfish** *Aequorea victoria*.
- Biopharming the production and use of transgenic plants to produce pharmaceutical substancesand also called **"molecular farming or pharming"**.
- The use of microorganisms or plants to clean up environmental pollution are called Bioremediation.
- *Botryococcus braunii* is normally used to produce **algal biofuel**.
- Biological hydrogen production by algae is *Chlamydomonas reinhardtii*
- Turmeric, neem and basmati rice are indigenous to the Indo-Pak subcontinent.
- Transgenic varieties of plants are Bt-cotton, rice, tomato, tobacco, cauliflower, potato and banana.
- Single cell protein from *Spirulina* is utilized in food industries.

Expanded forms

- '**lyc**' - lycopene cyclase
- '**psy**' - phytoene synthase
- **CRISPR**-Cas9- Clustered Regularly Interspaced Short Palindromic Repeats
- **DMH** - Dhara Mustard Hybrid
- **ELISA - Enzyme Linked Immumo Sorbent Assay**
- **EPSPS 5**- Eno Pyruvate Shikimate-3 Phosphate Synthase enzyme,
- **GFP** - Green Fluorescent Protein
- **GMH** - Genetically Modified Herbicide.
- **GMO** - Genetically Modified Organism
- **HT** - Herbicide Tolerant
- **HT** - Herbicide Tolerant
- MCS - Multiple Cloning Site or polylinker.
- **ori** - Origin of replication
- **PAT** - Phosphinothricin Acetyl Transferase
- **PHAs** - Poly Hydroxy Alkanoates
- **PHB** - Polyhydroxybutyrate
- **PLA** - Polylactic acid
- **PPT** - Phos PhinoThricin
- **RISC** - RNA induced silencing complex .
- **RNAi** - RNA Interference
- **siRNA** - short interfering RNA
- **SSC** - Sodium Saline Citrate

Book Evaluation

PART – A

(1 MARK)

1. Restriction enzymes are

a) Not always required in genetic engineering

b) Essential tools in genetic engineering

c) Nucleases that cleave DNA at specific sites

d) both b and c **Ans: d**

2. Plasmids are

a) circular protein molecules

b) required by bacteria

c) tiny bacteria

d) confer resistance to antibiotics **Ans: d**

3. EcoRI cleaves DNA at

a) AGGGTT b) GTATATC

c) GAATTC d) TATAGC **Ans: c**

4. Genetic engineering is

a) making artificial genes.

b) hybridization of DNA of one organism to that of the others.

c) production of alcohol by using micro organisms.

d) making artificial limbs, diagnostic instruments such as ECG, EEG etc) **Ans: b**

5. Consider the following statements:

I. Recombinant DNA technology is popularly known as genetic engineering is a stream of biotechnology which deals with the manipulation of genetic materials by man invitro

II. pBR322 is the first artificial cloning vector developed in 1977 by Boliver and Rodriguez from E.coli plasmid

III. Restriction enzymes belongs to a class of enzymes called nucleases.

Choose the correct option regarding above statements

a) I & II b) I & III

c) II & III d) I,II & III **Ans: d**

6. The process of recombinant DNA technology has the following steps

I. amplication of the gene

II. Insertion of recombinant DNA into the host cells

III. Cutting of DNA at specific location using restriction enzyme .

IV. Isolation of genetic material (DNA)Pick out the correct sequence of step for recombinant DNA technology.

a) II, III, IV, I b) IV, II, III, I

c) I, II, III, IV d) IV, III, I, II **Ans: d**

7. Which one of the following palindromic base sequence in DNA can be easily cut at about the middle by some particular restriction enzymes?

a) 5 CGTTCG 3 3 ATCGTA 5

b) 5 GATATG 3 3 CTACTA 5

c) 5 GAATTC 3 3 CTTAAG 5

d) 5 CACGTA 3 3 CTCAGT 5 **Ans: c**

8. pBR 322, BR stands for

a) Plasmid Bacterial Recombination

b) Plasmid Bacterial Replication

c) Plasmid Boliver and Rodriguez

d) Plasmid Baltimore and Rodriguez **Ans: c**

9. Which of the following one is used as a Biosensors?

a) Electrophoresis b) Bioreactors

c) Vectors d) Electroporation

Ans: b

10. Match the following :

	Column A	Column B
1	Exonuclease	a) add or remove phosphate
2	Endonuclease	b) binding the DNA fragments
3	Alkaline Phosphatase	c) cut the DNA at terminus
4	Ligase	d) cut the DNA at middle

	1	2	3	4
A)	a	b	c	d
B)	c	d	b	a
C)	a	c	b	d
D)	c	d	a	b

Ans: d

11. In which techniques Ethidium Bromide is used?

a) Southern Blotting techniques
b) Western Blotting techniques
c) Polymerase Chain Reaction
d) Agrose Gel Electroporosis **Ans: d**

12. Assertion : Agrobacterium tumifaciens is popular in genetic engineering because this bacterium is associated with the root nodules of all cereals and pulse crops

Reason: A gene incorporated in the bacterial chromosomal genome gets atomatically transferred to the cross with which bacterium is associated)

a) Both assertion and reason are true. But reason is correct explanation of assertion.
b) Both assertion and reason are true. But reason is not correct explanation of assertion.
c) Assertion is true, but reason is false.
d) Assertion is false, but reason is true.
e) Both assertion and reason are false. **Ans: d**

13. Which one of the following is not correct statement.

a) Ti plasmid causes the bunchy top disease
b) Multiple cloning site is known as Polylinker
c) Non viral method transfection of Nucleic acid in cell
d) Polylactic acid is a kind of biodegradable and bioactive thermoplastic) **Ans: a**

14. An analysis of chromosomal DNA using the southern hybridisation technique does not use

a) Electrophoresis
b) Blotting
c) Autoradiography
d) Polymerase Chain Reaction **Ans: d**

15. An antibiotic gene in a vector usually helps in the selection of

a) Competent cells b) Transformed cells
c) Recombinant cells d) None of the above
Ans: a

16. Some of the characteristics of Bt cotton are

a) Long fibre and resistant to aphids
b) Medium yield, long fibre and resistant to beetle pests
c) high yield and production of toxic protein crystals which kill dipteran pests.
d) High yield and resistant to ball worms **Ans: d**

PART – B, C AND D

(2,3 AND 5 MARKS)

17. How do you use the biotechnology in modern practice?

The use of the biotechnology in modern practice are

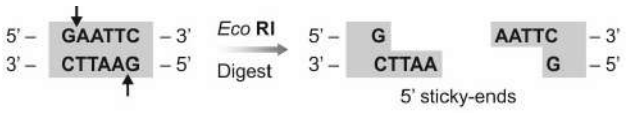
- Wide applications in various sectors like agriculture, medicine, environment and commercial industries.
- An invaluable outcome like **transgenic varieties** of plants e.g. transgenic cotton (Bt-cotton), rice, tomato, tobacco, cauliflower, potato and banana)
- The development of transgenics as pesticide, stress and disease resistant varieties of agricultural crops is the outcome of biotechnology.
- The synthesis of **human insulin** and blood protein in *E.coli* .
- Vaccines, enzymes, antibiotics, dairy products and beverages are the products of biotech industries.
- **Biochip** based biological computer is one of the successes of biotechnology.
- **Single cell protein** from *Spirulina* is utilized in food industries.
- Production of **secondary metabolites**, biofertilizers, biopesticides and enzymes.
- Biomass energy, biofuel, Bioremediation, phytoremediation for environmental biotechnology.

18. What are the materials used to grow microorganism like Spirulina?

Spirulina can be grown easily on materials like waste water from potato processing plants, straw, molasses, animal manure and even sewage.

19. You are working in a biotechnology lab with a becterium namely E.coli. How will you cut the nucleotide sequence? explain it.

- *Eco* R1 is restriction enzymes isolated from *E.coli* bacterium.
- The enzyme recognise and cuts specific DNA sequence.
- The given diagram is blunt or flush end of cutting using restriction enzymes *Eco*R1.
- They cut in a way producing protruding and recessed ends known as **sticky** or **cohesive end**)
- Such cut are called staggered or asymmetric cuts



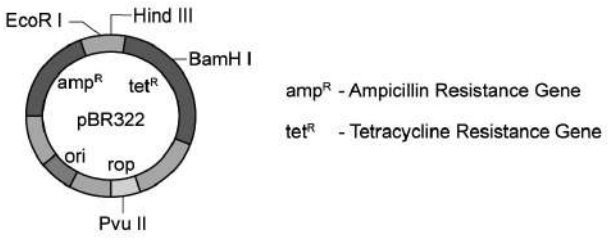
20. What are the enzymes you can used to cut terminal end and internal phospho di ester bond of nucleotide sequence?

Exonucleases:	
The enzymes which remove nucleotides one at a time from the end of a DNA molecule.	Example: Bal 31, Exonuclease III.
Endonucleases:	
They are enzymes which break the internal Phosphodiester bonds within a DNA molecule.	Example: Hind II, EcoRI, Pvul, BamHI, TaqI

21. Name the chemicals used in gene transfer.

Polyethylene glycol (PEG) and dextran sulphate are the chemicals used in gene transfer.

22. What do you know about the word pBR332?



- pBR 322 plasmid is a reconstructed plasmid
- It is most widely used as cloning vector.
- It contains 4361 base pairs.
- In pBR, ***p*** for plasmid, ***B***and ***R*** for scientist **B**oliver and **R**odriguez.**322** is the number of plasmid developed from their laboratory.
- It contains ampR and tetR two different antibiotic resistance genes .
- It is a recognition sites for several restriction enzymes(*Hind III, EcoRI, BamH I, Sal I, Pvu II, Pst I, Cla I*), ori and antibiotic resistance genes.
- Rop codes for the proteins involved in the replication of the plasmid

23. Mention the application of Biotechnology.

- Biotechnology is one of the most important applied interdisciplinary sciences of the **21st century**.
- It is the trusted area that enables us to find the beneficial way of life.
- It has wide applications in various sectors like agriculture, medicine, environment and commercial industries.
- This science has an invaluable outcome like **transgenic varieties** of plants e.g. transgenic cotton (Bt-cotton), rice, tomato, tobacco, cauliflower, potato and banana)
- The development of transgenics as pesticide, stress and disease resistant varieties of agricultural crops is the outcome of biotechnology.
- The synthesis of **human insulin** and blood protein in *E.coli* .
- Vaccines, enzymes, antibiotics, dairy products and beverages are the products of biotech industries.
- **Biochip** based biological computer is one of the successes of biotechnology.
- Genetic engineering involves genetic manipulation, tissue culture involves aseptic cultivation of totipotent plant cell into plant clones under controlled atmospheric conditions.
- **Single cell protein** from *Spirulina* is utilized in food industries.
- Production of **secondary metabolites**, biofertilizers, biopesticides and enzymes.
- Biomass energy, biofuel, Bioremediation, phytoremediation for environmental biotechnology.

24. What are restriction enzyme? Mention their type with role in Biotechnology.

A **restriction** enzyme is an enzyme that cleaves DNA into fragments at or near specific recognition sites. Restriction enzyme also called restriction endonuclease.

Based on their mode of action restriction enzymes are classified into.

a) Exonucleases:	
The enzymes which remove nucleotides one at a time from the end of a DNA molecule.	Example: Bal 31, Exonuclease III.
b) Endonucleases:	
They are enzymes which break the internal Phosphodiester bonds within a DNA molecule.	Example: Hind II, EcoRI, Pvul, BamHI, TaqI.

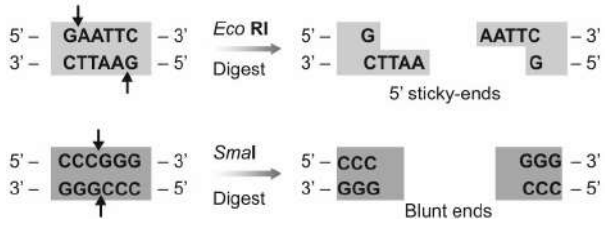
- The restriction enzymes are called as molecular scissors.
- These act as foundation of recombinant DNA technology.

Restriction endonuclease: Molecular scissors

- The restriction enzymes are called as molecular scissors.
- These act as foundation of recombinant DNA technology.
- There are three main classes of restriction endonuclease :
- Type I, Type II and Type III, which differ slightly by their mode of action.
- Only type II enzyme is preferred for use in recombinant DNA technology.
- They recognise and cut DNA within a specific sequence typically consisting of 4-8 bp.
- Examples of certain enzymes are given in table

Restriction enzyme	Microbial source	Recognition sequence	Fragments
Alu I	Arthrobacter luteus	5'AG/CT3' 3'TC/GA5'	A-G C-T / T-C G-A — Blunt ends
BamHI	Bacillus amyloliquefaciens	5'G/GATCC3' 3'CCTAG/G5'	G G-A-T-C-C / C-C-T-A-G G — Sticky ends
EcoRI	Escherichia coli	5'G/AATTC3' 3'CCTAG/G5'	G A-A-T-T-C / C-T-T-A-A G — Sticky ends
HaeIII	Haemophilus aegyptus	5'GG/CC3' 3'CC/GG5'	G-G C-C / C-C G-G — Blunt ends
HindIII	Haemophilus influenza	5'A/AGCTT3' 3'TTCGA/A5'	A A-G-C-T-T / T-T-C-G-A A — Sticky ends

- The restriction enzyme ***Hind II*** always cut DNA molecules at a point of recognising a specific sequence of six base pairs. This sequence is known as recognition sequence.
- The exact kind of cleavage produced by a restriction enzyme is important in the design of a gene cloning experiment.
- Some cleave both strands of DNA through the centre resulting in **blunt** or **flush end**
- These are known as symmetric cuts.
- Some enzymes cut in a way producing protruding and recessed ends known as **sticky** or **cohesive end**
- Such cut are called staggered or asymmetric cuts.



25. Is their any possibilities to transfer a suitable desirable gene to host plant without vector? Justify your answer.

- Yes. In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant without the help of a vector.
- The following are some of the common methods are

a) **Chemical mediated gene transfer:**

- Certain chemicals like polyethylene glycol (PEG) and dextran sulphate induce DNA uptake into plant protoplasts.

b) **Microinjection:**

- The DNA is directly injected into the nucleus using fine tipped glass needle or micro pipette to transform plant cells.

c) **Electroporation Methods of Gene Transfer:**

- A pulse of high voltage is applied to protoplasts, cells or tissues which makes transient pores in the plasma
- membrane through which uptake of foreign DNA occurs.

d) **Liposome mediated method of Gene Transfer:**

- Liposomes the artificial phospholipid vesicles are useful in gene transfer.
- The gene or DNA is transferred from liposome into vacuole of plant cells.
- It is carried out by encapsulated DNA into the vacuole.
- Liposome and tonoplast of vacuole fusion resulted in gene transfer.
- This process is called lipofection.

e. **Biolistics:**

- The foreign DNA is coated onto the surface of minute gold or tungsten particles (1-3 μm)
- and bombarded onto the target tissue or cells using a particle gun (also called as **gene gun/ micro projectile gun/shotgun**).
- Then the bombarded cells or tissues are cultured on selected medium to regenerate plants from the transformed cells.

26. How will you identify a vectors?

Identify a vectors based on the following properties

- Vectors are able to replicate autonomously to produce multiple copies of them along with their DNA insert in the host cell.
- It should be small in size and of low molecular weight, less than 10 Kb (kilo base pair) in size.
- so that entry/transfer into host cell is easy.
- Vector must contain an origin of replication so that it can independetly replicate within the host.
- It should contain a suitable marker such as antibiotic resistance, to permit its detection in transformed host cell.
- Vector should have unique target sites for integration with DNA insert
- It should have the ability to integrate with DNA insert it carries into the genome of the host cell.
- Most of the commonly used cloning vectors have more than one restriction site.
- These are Multiple Cloning Site (MCS) or polylinker.
- Presence of MCS facilitates the use of restriction enzyme of choice.

27. Compare the various types of Blotting techniques.

The compare of the various types of Blotting techniques are

	Southern blotting	Northern blotting	Western blotting
Separation of	DNA	RNA	Proteins
Denaturation	Needed	Not needed	Proteins
Membrane	Nitrocellulose/ nylon	Amino benzyloxymethyl	Nitrocellulose
Hybridisation	DNA-DNA	RNA-DNA	Protein-antibody
Visualising	Autoradiogram	Autoradiogram	Dark room

28. Write the advantages of herbicide tolerant crops.

The advantages of herbicide tolerant crops are

- Weed control improves higher crop yields
- Reduces spray of herbicide
- Reduces competition between crop plant and weed
- Use of low toxicity compounds which do not remain active in the soil
- The ability to conserve soil structure and microbes

29. Write the advantages and disadvantages of Bt cotton.

The advantages of Bt cotton are

- Yield of cotton is increased due to effective control of bollworms.
- Reduction in insecticide use in the cultivation of Bt cotton
- Potential reduction in the cost of cultivation.

The disadvantages of Bt cotton are

- Cost of Bt cotton seed is high.
- Effectiveness up to 120 days after that efficiency is reduced
- Ineffective against sucking pests like jassids, aphids and whitefly.
- Affects pollinating insects and thus yield)

30. What is bioremediation? give some examples of bioremediation.

- The use of microorganisms or plants to clean up environmental pollution is called bioremediation.

- It is used in wastewater, industrial waste and solid waste.
- It is applied to the removal of oil, petrochemical residues, pesticides or heavy metals from soil or ground water.
- It is a cheaper, eco-friendly and remove contaminants more effectively.

Some examples of bioremediation technologies are:

- **Phytoremediation** - use of plants for remediation.
- **Mycoremediation** - use of fungi for remediation.
- **Bioventing** - increases the oxygen or air flow to accelerate the degradation of environmental pollutants.
- **Bioleaching** - use of microorganisms in solution to recover metal pollutants from contaminated sites.
- **Bioaugmentation** - the addition of selected microbes to speed up degradation process.
- **Composting** -the solid waste is composted by the use of microbes into manure .It acts as a nutrient for plant growth.
- **Rhizofiltration-** the uptake of metals by rhizosphere microorganisms.
- **Rhizostimulation** - the stimulation of plant growth by the rhizosphere by providing better growth condition or reduction in toxic materials.

31. Write the benefits and risk of Genetically Modified Foods.

The benefits of Genetically Modified Foods

- High yield without pest
- 70% reduction of pesticide usage
- Reduce soil pollution problem
- Conserve microbial population in soil

The risk of Genetically Modified Foods

- Affect liver, kidney function and cancer
- Hormonal imbalance and physical disorder
- Anaphylactic shock (sudden hypersensitive reaction) and allergies.
- Adverse effect in immune system because of bacterial protein.
- Loss of viability of seeds show in terminator seed technology of GM crops.

PART – A

ADDITIONAL QUESTIONS (1 MARK)

1. Polyethylene glycol method is used for

a) energy production from sewage
b) biodiesel production
c) gene transfer without a vector
d) seedless fruit production **Ans: c**

2. The linking of antibiotic resistance gene with the plasmid vector became possible with

a) DNA polymerase b) DNA ligase
c) Exonucleases d) Endonucleases **Ans: b**

3. Gel electrophoresis is used for

a) construction of recombinant DNA by joining with cloning vectors
b) cutting of DNA into fragments
c) isolation of DNA molecule
d) separation of DNA fragments according to their size **Ans: d**

4. Introduction of food plants developed by genetic engineering is not desirable because

a) this method is costly
b) economy of developing countries may suffer
c) there is danger of entry of viruses and toxins with introduced crop
d) these products are less tasty as compared to the already existing products **Ans: c**

5. Microbe used for biocontrol of pest butterfly caterpillars is

a) Bacillus thuringiensis
b) Trichoderma sp.
c) Streptococcus sp.
d) Saccharomyces cerevisiae **Ans: a**

6. Restriction endonucleases are enzymes which

a) restrict the action of the enzyme DNA polymerase
b) make cuts at specific positions within the DNA molecule
c) remove nucleotides from the ends of the DNA molecule
d) recognize a specific nucleotide sequence for binding of DNA ligase **Ans: b**

7. Which one of the following palindromic base sequences in DNA can be easily cut at about the middle by some particular restriction enzyme?

a) 5′.....GAATTC).....3′
3′.....CTTAAG.....5′

b) 5′.....CGTTCG.....3′
3′.....ATGGTA).....5′

c) 5′.....CACGTA).....3′
3′.....CTCAGT.....5′

d) 5′.....GATATG.....3′
3′.....CTACTA).....5′ **Ans: a**

8.Which one of the following is used as vector for cloning genes into higher organisms?

a) Rhizopus nigricans

b) Baculovirus

c) Retrovirus

d) Salmonella typhimurium **Ans: c**

9. Which one of the given options correctly identifies its certain component (s)?

a) Hind III, EcoRI - selectable markers

b) ori - original restriction enzyme

c) ampR, tetR - antibiotic resistance genes

d) rop-reduced osmotic pressure **Ans: c**

10. Agarose extracted from sea weeds finds use in:

a) PCR

b) Spectrophotometry

c) Gel electrophoresis

d) Tissue culture **Ans: c**

11. There is a restriction endonuclease called EcoRI. What does co. part in it stand for?

a) coenzyme b) colon

c) coli d) coelom **Ans: c**

12. Biolistics (gene-gun) is suitable for

a) Transformation of plant cells.

b) DNA finger printing.

c) Constructing recombinant DNA by joining with vectors.

d) Disarming pathogen vectors.

Ans: b

13. For transformation, micro-particles coated with DNA to be bombarded with gene gun are made up of:

a) Silicon or Platinum b) Silver or Platinum

c) Gold or Tungsten d) Platinum or Zinc

Ans: c

14. Which one is a true statement regarding DNA polymerase used in PCR

a) It is isolated from a virus

b) It is used to ligate introduced DNA in recipient cell

c) It remains active at high temperature

d) It serves as a selectable marker **Ans: c**

15. PCR and Restriction Fragment Length Polymorphism are the methods for:

a) DNA sequencing

b) Study of enzymes

c) Genetic Fingerprinting

d) Genetic transformation **Ans: c**

16. DNA fragments generated by the restriction endonucleases in a chemical reaction can be separated by:

a) Restriction mapping

b) Polymerase chain reaction

c) Centrifugation

d) Electrophoresis **Ans: c**

17. Which one of the following represents a palindromic sequence in DNA?

a) 5′ - CATTAG - 3′ 3′ - GATAAC - 5′

b) 5′ - GAATTC - 3′ 3′ - CTTAAG - 5′

c) 5′ - GATACC - 3′ 3′ - CCTAAG - 5′

d) 5′ - CCAATG - 3′ 3′ - GAATCC - 5′ **Ans: d**

18. The figure below shows three steps (A, B, C) of Polymerase Chain Reaction (PCR). Select the option giving correct identification together with what it represents?

a) C - Extension in the presence of heat stable DNA polymerase.

b) B - Denaturation at a temperature of about 98°C separating the two DNA strands.

c) A - Annealing with two sets of primers.

d) A - Denaturation at a temperature of about 50°C) **Ans: b**

19. In genetic engineering, the antibiotics are used

a) to keep the cultures free of infection.
b) as selectable markers.
c) as sequences from where replication starts.
d) to select healthy vectors. **Ans: a**

20. Genetically engineered bacteria have been successfully used in the commercial production of

a) thyroxine b) human insulin
c) melatonin d) testosterone **Ans: b**

21. Genes of interest can be selected from a genomic library by using

a) DNA probes b) Restriction enzymes
c) Gene targets d) Cloning vectors **Ans: c**

22. A trangenic food crop'which may help in solving the problem of night blindness in developing countries is

a) Bt Soybean b) Flavr Savr tomatoes
c) Golden rice d) Starlink maize
Ans: c

23. Bacillus thuringiensis (Bt) strains have been used for designing novel:

a) Bio-mineralization processes
b) Biofertilizers
c) Bioinsecticidal plants
d) Bio-metallurgical techniques **Ans: c**

24. Golden rice is a transgenic crop of the future with the following improved trait:

a) high protein content
b) insect resistance
c) high vitamin-A content
d) high lysine (essential amino acid) content
Ans: c

25. ELISA is used to detect viruses where the key reagent is

a) catalase b) RNase
c) DNA probe d) alkaline phosphatase
Ans: d

26. The genetically-modified (GM) brinjal in India has been developed for:

a) enhancing mineral content
b) insect-resistance
c) drought-resistance
d) enhancing shelf life **Ans: b**

27. Transgenic plants are the ones:

a) grown in artificial medium after hybridization in the field)
b) generated by introducing foreign DNA into a cell and regenerating a plant from that cell.
c) produced by a somatic embryo in artificial medium.
d) produced after protoplast fusion in artificial medium.
Ans: b

28. What is true about Bt toxin?

a) The concerned Bacillus has antitoxins.
b) Bt protein exists as active toxin in the Bacillus
c) The inactive protoxin gets converted into active form in the insect gut.
d) The activated toxin enters the ovaries of the pest to sterilise it and thus prevent its multiplication.
Ans: c

29. Which one of the following is commonly used in transfer of foreign DNA into crop plants?

a) Penicillium expansum
b) Meloidogyne incognita
c) Trichoderma harzianum
d) Agrobacterium tumefaciens **Ans: d**

30. Main objective of production/use of herbicide resistant GM crops is to

a) encourage eco-friendly herbicides
b) eliminate weeds from the field without the use of manual labour
c) reduce herbicide accumulation in food articles for health safety
d) eliminate weeds from the field without the use of herbicides
Ans: c

31. Continuous addition of sugars in 'fed batch' fermentation is done to:

a) purify enzymes b) produce methane
c) degrade sewage d) obtain antibiotics

Ans: a

32. The most common substrate used in distilleries for the production of ethanol is

a) ground gram b) corn meal
c) molasses d) soya meal **Ans: c**

33. Some of the characteristics of Bt cotton are:

a) high yield and production of toxic protein crystals which kill dipteran pests
b) long fibre and resistance to aphids
c) high yield and resistance to bollworms
d) medium yield, long fibre and resistance to beetle pests **Ans: c**

34. Genetic engineering has been successfully used for producing:

a) transgenic cow – rosie which produces high fat milk for making ghee
b) transgenic mice for testing safety of polio vaccine before use in humans
c) animals like bulls for farm work as they have super power
d) transgenic models for studying new treatments for certain cardiac diseases **Ans: b**

35. Which one of the following techniques made it possible to genetically engineer living organism?

a) Heavier isotope labelling
b) Recombinant DNA techniques
c) Hybridization
d) X-ray diffraction **Ans: b**

36. In history of biology, human genome project led to the development of:

a) bioinformatics b) biotechnology
c) biosystematics d) biomonitoring

Ans: a

37. Consider the following statements (A-D) about organic farming:

A) Utilizes genetically modified crops like Bt cotton
B) Uses only naturally produced inputs like compost
C) Does not use pesticides and urea
D) Produces vegetables rich in vitamins and minerals Which of the above statements are correct?

a) (B) and (C) only b) (B), (C) and (D)
c) (A) and (B) only d) (C) and (D) only

Ans: a

38. Which one of the following vectors is used to replace the defective gene in gene therapy?

a) Cosmid b) Ti plasmid
c) Ri plasmid d) Adenovirus

Ans: d

39. Which of the following Bt crops is being grown in India by the farmers?

a) Soyabean b) Cotton
c) Maize d) Brinjal **Ans: b**

40. Tobacco plants resistant to a nematode have been developed by the introduction of DNA that produced (in the host cells

a) an antifeedant
b) both sense and anti-sense RNA
c) a toxic protein
d) a particular hormone **Ans: b**

41. Consumption of which one of the following foods can prevent the kind of blindness associated with vitamin 'A' ndeficiency?

a) Golden rice b) 'Flaver Savr' tomato
c) Bt-Brinjal d) Canolla **Ans: a**

42. 250 g of *Methylophilus methylotrophus*, as its high rate of biomass production and growth, can be expected to produce.

a) 15 tonnes of protein b) 25 tonnes of protein
c) 35 tonnes of protein d) 45 tonnes of protein

Ans: b

43. How many restriction enzymes that have been isolated so far

a) more than 600 b) more than 700
c) more than 900 d) more than 500 **Ans: c**

Directions: In the following questions, a statement of assertion is followed by a statement of reason. Mark the correct choice as:

a) If both Assertion and Reason are true and Reason is the correct explanation of Assertion.
b) If both Assertion and Reason are true but Reason is not the correct explanation of Assertion.
c) If Assertion is true but Reason is false.
d) If both Assertion and Reason are false.

44. Assertion : Restriction enzymes cut the strand of DNA to produce sticky ends.

Reason : Stickiness of the ends facilitates the action of the enzyme DNA polymerase. Ans: c

45. Assertion : Plasmids are extrachromosomal DNA)

Reason : Plasmids are found in bacteria and are useful in genetic engineering. Ans. b

46. Assertion: Plasmids are single-stranded extra chromosomal DNA)

Reason: Plasmids are usually present in eukaryotic cells. Ans: c

47. Assertion : Insertion of recombinant DNA within the coding sequence of b-galactosidase results in colourless colonies.

Reason : Presence of insert results in inactivation of enzyme b-galactosidase known as insertional inactivation. Ans: a

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

Reason : A gene incorporated in the bacterial chromosomal genome gets automatically transferred to the crop with which the bacterium is associated) Ans: d

49. The crops engineered for glycophosate are resistant /tolerant to

a) insects b) herbicides
c) fungi d) bacteria **Ans.b**

50. Golden rice is genetically modified crop plant where the incorporated gene is meant for biosynthesis of

a) omega 3 b) vitamin B
c) vitamin A d) vitamin C **Ans.c**

51. The term bio-technology was coined by

a) Herbert boyer b) Smith
c) Karl Ereky d) Edward Tatum **Ans: c**

52. The method in which recombinant DNA is directly injected into the nucleus of cell is

a) Micro injection b.Ti plasmid
c.Ri Plasmid d.Both (b) and (c) **Ans: a**

53. E. Coli cloning vector pBR 322 contains restriction sites (Hind III, Eco R I, Bam H I, Sal I, PVU II, Past I, Cla I) ori, amp^R, tet^R and rop codes for the

a. antibiotic resistance genes
b. Foreign DNA
c. Slection of recombinants form non-recombinants
d. Proteins involved in the replication of the Plasmid **Ans: d**

54. By using which of the following vectors, specific genes were introduced into the host plant

a. *Agrocbacterium tumefaciens*
b. *Meloidegyne incognittia*
c. *Echerichia coli*
d. **Bacillus thuringiensis** **Ans: a**

55. The science deals with biomaterials and stem cells to repair or replace body tissue or organ is

a. Bio-informatics
b. Nano biotechnology
c. Tissue engineering
d. Bio-method engineering **Ans: d**

56. The identification of DNA as the genetic material by

a. karl Ereky
b. Avery Mac leod_ Mc Carky
c. Antoine lavoisien
d. Gilbert **Ans: b**

57. The use of Ti Plasmids to genetically transform plants in

a. 1982 b. 1984 c. 1983 d. 1985 **Ans: c**

58. The first plant genome sequenced in

a. *Arabidopsis* b. *Oryza*

c. Wheat d. None of the above

Ans: a

59. The development of artificial gene is functioning with in the living cells by

a. Karly Ereky b. H.G. Khorana

c. Mc Carty d. Gilbert **Ans**: **b**

60. The Micro-organisms used for the production of single cell protein are

a. *Cellulomonas* b. *Candida utilis*

c. *Spirulina* d. All of these **Ans: d**

61. The tool used in genetic engineering is (are)

a. Restriction enzymes

b. DNA ligase

c. Alkaline phosphatase

d. All of these **Ans: d**

62. In which type of enzyme is preferred for use in recombinant DNA technology as they recognize and cut DNA with in a specific sequence

a. Type I b. Type II

c. Type III d. Type IV **Ans: b**

63. Select the Microbial source of the restriction enzyme Hind III is

a. *Bacillus*

b. *E. coli*

c. *Haemophilus influenza*

d. *Arthobacter* **Ans: c**

64. Select the Microbial source of the restriction enzyme Hae III is

a. *Arthobacter luteus*

b. *Escherichia coli*

c. *Haemophilus aegyptus*

d. *Haemophilus influenza* **Ans: c**

65. The given Eco. RI is the restriction endonucleases are named by Standard procedure. The final romen numeral indicating the order of

a. Genus name b. Species name

c. Strain of organism d. Order of discovery

Ans: c

66. The restriction enzyme clave both strands of DNA through the center resulting in

a. blunt end b. flush end

c. sticky end d. both a & b **Ans: d**

67. Alkaline Phosphatase enzyme is purified from

a. Fungi b. bacteria

c. calf intestine d. both (b) and (c)

Ans: d

68. Vector is also called

a. Cloning vehicle b. Cloning RNA

c.Cloning DNA d. Both (a) and (c)

Ans: d

69. Read the statements

i. Restriction enzyme that claves DNA into fragments

ii. DNA ligase joins the sugar and phosphate of double stranded DNA

iii. Plasmid is not widely used as cloning vector

iv. Ti plasmid is stably integrated with plant RNA

a) i and ii only correct

b) i and iii only correct

a) ii and iii only correct

a) iii and iv only correct **Ans: .a**

70. Transponsons are called

a. Stick genes b. Jumping genes

c. Walking genes d. Both (a) and (c)

Ans: d

71. Match the following

	Column I		Column II
A.	Shuttle vector	i.	more user friendly
B.	Yeast artificial chromosome	ii.	pBlue scrift SK
C.	Phage mid vector	iii.	most of eukaryotic
D.	Bacterial artificial chromosome	iv.	Circular and linear forms

	A	B	C	D
a	iii.	iv.	ii.	i.
b	iii.	iv.	i.	ii.
c	ii.	iv.	iii.	i.
d	i.	ii.	iii.	iv.

Ans: a

72. Find out the mismatched pairs

a) PBR322 Plasmid - Bolívar and Rodríguez
b) Ti Plasmid - Inducing tumerours
c) Transposon as vector - Arabidopsis thaliana
d) Expression vector - Escherichia Coli

Ans: d

73.

A.	Chemical mediated gene transfer	i.	transient pores
B.	Microinjection	ii.	dextran sulphate
C.	Electroporation	iii.	Coated with gold or tungsten
D.	Biolistics	iv.	Glass needle / Micropipette

	A	B	C	D
a	ii.	iv.	i.	iii.
b	ii.	i.	iv.	iii.
c	i.	ii.	iii.	iv.
d	iv.	iii.	ii.	i.

Ans: a

74. The following one is the indirect / vector-mediated gene transfer

a) Agrobacterium tumefaciens
b) Liposome into vacuole
c) foreign DNA coated with tungsten
d) Polyethylene glycol **Ans: a**

75. In Gel electrophoresis +ve charged cations will move towards

a) anode b) cathode
c) both a) & b) d) none of these

Ans: b

76. The given one is preferred for the purification of smaller DNA fragments.

a) Ethidium Bromide b) Nitrocellulose
c) Agarose gel d) Polyacrylamide

Ans: c

77. The bands of DNA in the gel are stained with the dye

a) Ethidium Bromide b) Nitrocellulose
c) Agarose gel d) Polyacrylamide **Ans: a**

78. The process of transfer of RNA to nitrocellulose membrane

a) Southern Blotting b) Northern Blotting
c) Western Blotting d) None of these **Ans: b**

79. Find the mismatched pair

a)	Southern blotting Techniques	-	Southern
b)	Northern blotting Techniques	-	Alwin et al
c)	rDNA technology	-	Mc Carty
d)	PBR322	-	Bolívar and Rodríguez

Ans: c

80. Basta herbicide tolerant gene PPT was isolated from

a) *Bacillus thuringiensis*
b) *Narcissus pseudonarcissus*
c) *Agrobacterium tumefaciens*
d) *Medicago sativa* **Ans: d**

81. The Bt brinjal has been developed to give resistance against

a) Nematodes b) *Lepidopteron*
c) *Erwinia* d) None of these **Ans: b**

82. Dhara mustard hybrid-11 contains

a) Bar gene b) Barnase gene
c) Barstar gene d) all of these **Ans: d**

83. The following gene had made Dhara mustard hybrid plant resistant to herbicide named Basta

a) Bar gene b) Barnase gene
c) Barstar gene d) all of these **Ans: a**

84. The following one helps in delaying the ripening process of tomato during long storage and transportation

a) Polyethene glycol b) Polyethelene glycol
c) Polygalacturonase d) Sorbital **Ans: c**

85. In Golden rice, beta-carotene biosynthesis Gene namely

a) Psy b) Crt-I
c) lyc d) all of these **Ans: d**

86. Golden rice can control childhood disease called

a) Guiter b) Xerophthalmia
c) Rickets d) Rubella **Ans: b**

87. The following one is biodegradable and bioactive thermoplastic

a) Polylactic acid
b) Polyhydroxyalkanoates
c) Polyhydroxybutyrate
d) Phytoene Synthase **Ans: a**

88. Green Fluorescent Protein (GFP) first isolate from

a) *Alcaligenes eutrophus*
b) *Aequorea victoria*
c) *Erwinia auredorora*
d) *Bacillus megaterium* **Ans: b**

89. How many amino acid present in Green Fluorescent Protein is?

a) 234 b) 235 c) 237 d) 238 **Ans: d**

90. Bio-remediation process is applied to

i. to the removal of oil
ii. to the adding of petrochemical residues
iii. more expensive and Eco friendly approach
iv. use of microorganisms / plants to clean up

a) i. and ii. only correct b) ii. and iii. only correct
c) i. and iv. only correct d) i. and iii. only correct **Ans: c**

91. Match the following

A.	Bioventing	i.	addition of selected microbes
B.	Composting	ii.	solid waste
C.	Bio augmentation	iii.	use of microorganisms in solution
D.	Bioleaching	iv.	Increase the oxygen

	A	B	C	D
a	iv.	ii.	i.	iii.
b	iv.	i.	ii.	iii.
c	i.	ii.	iii.	iv.
d	iv.	iii.	ii.	i.

Ans: a

92. Normally used to produce algal biofuel by

a) *Erwinia auredorora*
b) *Narcissus pseudonarcissus*
c) *Botryococcus braunii*
d) *Chlamydomonas reinhardtii* **Ans: c**

93. Biological hydrogen production by algae is

a) *Erwinia auredorora*
b) *Narcissus pseudonarcissus*
c) *Botryococcus braunii*
d) *Chlamydomonas reinhardtii* **Ans: d**

94. Turmeric, neem and basmati, the products are indigenous to the

a) Europe
b) Unites States
c) Indo Pak Sub Continent
d) Asia **Ans: c**

95. Transgenic varieties of plants namely

a) Cotton b) Cauliflower
c) Rice d) all of these **Ans: d**

96. The environmental biotechnology is

a) Biomass energy b) bio fuel
c) phytoremediation d) all of these **Ans: d**

97. The production of acids, enzymes, alcohols, antibiotics, fine chemicals, vitamins and toxins are

a) Bio-fuels b) Fermentation
c) Enzymes d) Bio-mass **Ans: b**

98. First hormone produced artificially by culturing bacteria is

a) Insulin b) thyroxine
c) testosterone d) adrenaline **Ans: a**

99. Bt toxin obtained from

a) Prokaryotes b) Eukaryotes
c) both (a) & (b) d) none of these **Ans: a**

100. Bt Cotton is resistant to

a) Insects b) herbicides
c) Salt resistant d) drought resistant **Ans: a**

PART – B

ADDITIONAL QUESTIONS (2 MARKS)

1. Who coined the term biotechnology ?

The term biotechnology was coined by Karl Ereky, a Hungarian Engineer in 1919.

2. Mention the two main heads of biotechnology.

1. Conventional or traditional biotechnology
2. Modern biotechnology.

3. Write the extends preparation of conventional biotechnology

Alcoholic beverages like beer, wine, etc.

4. In which fields the biotechnological tools can apply for the product improvement.

Pharmaceutical companies, breweries, agro industries and other biotechnology based industries

5. Write the wide applications in various sectors of biotechnology

Agriculture, medicine, environment and commercial industries.

6. Define the meaning of fermentation in Latin.

Fermentation is derived from the Latin verb 'fervere' which means ' to boil'.

7. Define Fermentation

The metabolic process in which organic molecules (glucose) are converted into acids, gases or alcohol in the absence of oxygen.

8. What is meant by zymology ?

The study of fermentation and its practical uses is called zymology

9. Who demonstrated fermentation ? Where it occurs ?

French chemist Louis Pasteur demonstrated fermentation (1856) in yeast. It occurs in certain types of bacteria and fungi.

10. What is downstream process of fermentation ?

All the process after the fermentation process is known as the downstream process.

It includes distillation, centrifuging, filtration and solvent extraction

11. Name the two process involved in fermentation

Fermentation involves two process namely upstream and downstream process.

12. What are called microbial metabolites?

Microbes produce compounds that are very useful to man and animals are called microbial metabolites

13. What is meant by primary metabolites? Give examples.

Metabolites produced for the maintenance of life process of microbes are known as primary metabolites

Eg. Ethanol, citric, acid, lactic acid, acetic acid)

14. What the examples of secondary metabolites?

Amphotericin-B (*Streptomyces nodosus*), Penicillin (*Penicillium chryosogenum*) Streptomycin (*S. grises*) , Tetracycline (*S. aureofacins*), alkaloids, toxic pigments, vitamins etc)

15. Define single cell protein (SCP) .

The dried cells of microorganism that are used as protein supplement in human foods or animal feeds are called Single cell proteins .

16. What are the advancements in modern biotechnology ?

The genetic manipulations, protoplasmic fusion techniques are the advancements in modern biotechnology.

17. Define genetic engineering

The modification and transfer of DNA from one organism to another is called Genetic engineering.

It also called recombinant DNA technology or gene cloning

18. Define conventional recombination

It involves exchange or recombination of genes between homologous chromosomes during meiosis is called conventional recombination.

19. Define recombinant DNA technology

The recombination carried out artificially using modern technology is called recombinant DNA technology (r-DNA technology). It is also known as gene manipulation technique.

20. What is PCR ?

Polymerase Chain Reaction is a common laboratory technique used to make copies (millions) of a particular region of DNA.

21. What is palindromic?

Palindromic means that the sequence in both DNA strands at this site read same in 5′ – 3′ direction and in the 3′-5′ direction. Example: MALAYALAM: This phrase is read the same in either of the directions.

22. Name the tools for genetic engineering

The basic tools are enzymes, vectors and host organisms.

Enzymes are the restriction enzymes, DNA ligase and alkaline phosphatase.

23. What is DNA Ligase ?

DNA ligase enzyme joins the sugar and phosphate molecules of double stranded DNA (dsDNA) with 5′-PO_4 and a 3′-OH in an Adenosine Triphosphate (ATP) dependent reaction.

24. Name the enzymes that play an important role in recombinant DNA technology.

Restriction enzymes ,DNA ligase and alkaline phosphatase are the enzymes that play an important role in recombinant DNA technology

25. Name some of host cells are available for gene cloning.

E.coli, yeast, animal or plant cells are the host cells are available for gene cloning.

26. Write the different types of gene transfer methods in plants

- Direct or vector less gene transfer
- Indirect or vector – mediated gene transfer

27. What is meant by direct gene transfer method?

The foreign gene of interest is delivered into the host plant without the help of a vector.

This process is called direct gene transfer method) It also called vector less gene transfer.

28. What is Indirect or Vector-Mediated Gene Transfer ?

Gene transfer is mediated with the help of a plasmid vector is known as indirect or vector mediated gene transfer.

Example: The Ti-plasmid from *Agrobacterium tumefaciens*

29. What is an electrophoresis?

Electrophoresis is a separating technique used to separate different biomolecules with positive and negative charges.

30. Write the most efficient methods in detection of pathogens in plant tissues

Two of the most efficient methods are

1. ELISA (Enzyme Linked Immumo Sorbent Assay)
2. DNA Probes

31. ELISA- Expand

ELISA -Enzyme Linked Immumo Sorbent Assay

32. What is called Lambda genome

Lambda phage is a temperate bacteriophage that infects Escherichia coli.

The genome of lambda-Phage is 48502 bp long, i.e. 49Kb and has 50 genes.

33. Write types of blotting techniques

Blotting techniques are

1.Southern Blotting

2.Northern Blotting

3.Western Blotting

34. What is Southern Blotting techniques ?

The transfer of DNA from agarose gels to nitrocellulose membrane are called southern blotting techniques.

35. What is Northern Blotting techniques ?

The transfer of RNA to nitrocellulose membrane are called northern blotting techniques.

36. What is Western Blotting techniques ?

Electrophoretic transfer of Proteins to nitrocellulose membrane are called western blotting techniques.

37. Define autoradiography

A technique that captures the image formed in a photographic emulsion due to emission of light or radioactivity from a labelled component placed together with unexposed film.

38. What is transfection ?

Introduction of foreign nucleic acids into cells by non-viral methods are called **transfection**.

39. What is genome project ?

A project in which the whole genome of plant is analysed using sequence analysis and sequence homology with other plants are called Genome project.

40. How is termed the content in Genome of an organism ?

Genome content of an organism is expressed in terms of number of base pairs or in terms of the content of DNA is expressed in c-value.

41. What is genome sequencing ?

The location of genes on the entire diploid chromosome of an organism is called genome sequencing

42. What is transcription ?

The copying of genetic information from one strand of the DNA (called sense strand) by RNA is called transcription.

43. What is RNA interference ?

A biological process in which RNA molecules inhibit gene expression or translation is called RNA interference. This is done by neutralising targeted mRNA molecules.

44. Who developed Dhara Mustard Hybrid (DMH) ?

Dhara Mustard Hybrid (DMH) was developed by a team of scientists Centre for Genetic Manipulation of Crop Plants at Delhi University under Government sponsored project.

45. What is FlavrSavr ?

It is transgenic tomato variety which has blocked production of polygalactronase is called FlavrSavr.

46. What is Bioremediation ?

The use of microorganisms or plants to clean up environmental pollution are called Bioremediation.

47. What is Bioprospecting ?

The process of discovery and commercialization of new products obtained from biological resources is called Bioprospecting.

48. Who got patent right of neem?

The United States Department of Agriculture (USDA) and an American MNC (Multi Nation Corporation) W.R.Grace in the early 90's sought a patent from the European Patent Office (EPO).

49. What is the uses of the neem?

Neem and its oil in many ways to controlling fungal and bacterial skin infections

50. Who got patent right of Turmeric in 1995 ?

The United States Patent and Trademark Office, in the year 1995 granted patent to the method of use of turmeric as an antiseptic agent.

51. What are the uses of the turmeric ?

Turmeric is the Indians home remedy for the quick healing of the wounds and also for purpose of healing rashes.

52. Who got patent right of Basmati in 1997?

On September 2, 1997, the U.S. Patent and Trademarks Office granted Patent on "basmati rice lines and grains" to the Texas-based company RiceTec)

53. What is recognition sequence ?

The restriction enzyme ***Hind II*** always cut DNA molecules at a point of recognising a specific sequence of six base pairs. This sequence is knowns recognition sequence.

54. What is known as screening for recombinants ?

After the introduction of *r*-DNA into a suitable host cell, it is essential to identify those cells which have received the r-DNA molecule. This process is called screening.

55. In which plants genome projects have been undertaken

The genome projects have been undertaken in *Chlamydomonas*(algae), *Arabidopsis thaliana*, rice and maize plants.

56. Although SCP has high nutritive value why people hesitate to eat.

Due to their high nucleic acid content and slower in digestibility causes some hazardous health problems.

57. Why SCP used for Astronauts and Antarctica expedition scientists ?

SCP is used by Astronauts and Antarctica expedition scientists because of their protein content, carbohydrates, fats, vitamins and minerals.

PART – C

ADDITIONAL QUESTIONS (3 MARKS)

1. What is biotechnology ?

- Biotechnology is the science of applied biological process.
- It is science of development and utilization of biological processes, forms and systems for the benefit of mankind and other life forms.

2. What are the conventional or traditional biotechnology ?

- It is the kitchen technology developed by our ancestors

- This technology uses bacteria and other microbes in the daily usage for preparation of dairy products.
- Example: Curd, ghee, cheese and in preparation of foods like idli, dosa, nan, bread and pizza)

3. Mention the main features of Modern technology

- Ability to change the genetic material for getting new products with specific requirement through recombinant DNA technology
- Ownership of the newly developed technology and its social impact.

4. Write uses of the processes of fermentation

- are valuable to the food and beverage industries,
- with the conversion of sugar into ethanol to produce alcoholic beverages,
- the release of CO2 by yeast used in the leavening of bread,
- and with the production of organic acids to preserve and flavour vegetables and dairy products.

5. Describe bioreactor (Fermentor)

- Bioreactor (Fermentor) is a vessel or a container.
- It is designed that it can provide an optimum environment in which microorganisms or their enzymes interact with a substrate to produce the required product.
- In the bioreactor aeration, agitation, temperature and pH are controlled)

6.What is called upstream process of fermentation?

- All the process before starting of the fermenter are called upstream process.
- It includes sterilization of the fermenter, preparation and sterilization of culture medium and growth of the suitable inoculum .

7. What are the application of fermentation in industries?

Fermentation has industrial application such as

1. Microbial biomass production
2. Microbial metabolites
   a) Primary metabolites
   b) Secondary metabolites
3. Microbial enzymes
4. Bioconversion, biotransformation or modification of the substrate

8. What is meant by secondary metabolites? Give examples.

- Metabolites are not required for the vital life process of microbes.
- They have value added nature, this includes antibiotics are called **secondary metabolites** .
- E.g-Amphotericin-B (*Streptomyces nodosus*), Penicillin (*Penicillium chryosogenum*) Streptomycin (*S. grises*), Tetracycline (*S. aureofacins*), alkaloids, toxic pigments, vitamins etc)

9. What is microbial enzymes ?Give examples .

- **M**icrobes are cultured, they secrete some enzymes into the growth media are called microbial enzymes .
- These enzymes are industrially used in detergents, food processing, brewing and pharmaceuticals.
- Eg. protease, amylase, isomerase, and lipase.

10. What are the Bioconversion,biotransformation or modification of the substrate of microbial fermentation ?

The fermenting microbes has the capacity to produce valuable products,

Example:

- conversion of ethanol to acetic acid (vinegar),
- isopropanol to acetone,
- sorbitol to sorbose (this is used in the manufacture of vitamin C),
- sterols to steroids.

11. Write different examples of scp

- **Bacteria** - *Methylophilus methylotrophus*, *Cellulomonas, Alcaligenes*
- **Fungi** - *Agaricus campestris, Saccharomyces cerevisiae* (yeast), *Candida utilis*
- **Algae** - *Spirulina, Chlorella, Chlamydomonas*

12. How spirulina helps in reduces environmental pollution

- *Spirulina* can be grown easily on materials like waste water from potato processing plants, straw, molasses, animal manure and even sewage.

- This produce large quantities and can serve as food rich in protein, minerals, fats, carbohydrate and vitamins.
- Such utilization reduces environmental pollution.

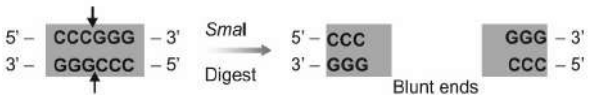
13. Write the technique involved in recombinant DNA technique.

- The recombinant DNA technique involves the transfer of DNA coding for a specific gene from one organism into another organism using specific agents like vectors or using instruments like electroporation, gene gun, liposome mediated, chemical mediated transfers and microinjection.

14. Describe the standard procedure naming of restriction endonucleases.

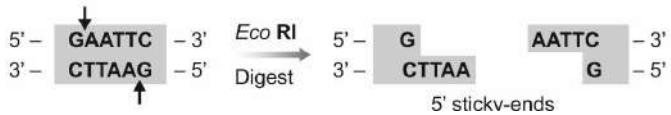
- The first letter of the enzymes indicates the genus name, followed by the first two letters of the species, then comes the strain of the organism and finally a roman numeral indicating the order of discovery.
- For example, ***EcoRI*** is from Escherichia (**E**) coli (**co**), strain RY 13 (**R**) and first endonuclease (**I**) to be discovered)

15. Observe, identify and explain the given diagram.



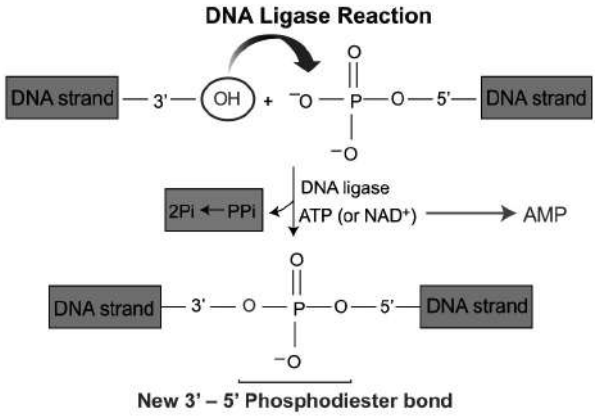
- The given diagram is blunt or flush end of cutting using restriction enzymes.
- They cleave both strands of DNA through the centre resulting in **blunt** or **flush end**)
- These are known as symmetric cuts.

16. Observe, identify and explain the given diagram.



- The given diagram is blunt or flush end of cutting using restriction enzymes.
- They cut in a way producing protruding and recessed ends known as **sticky** or **cohesive end**)
- Such cut are called staggered or asymmetric cuts.

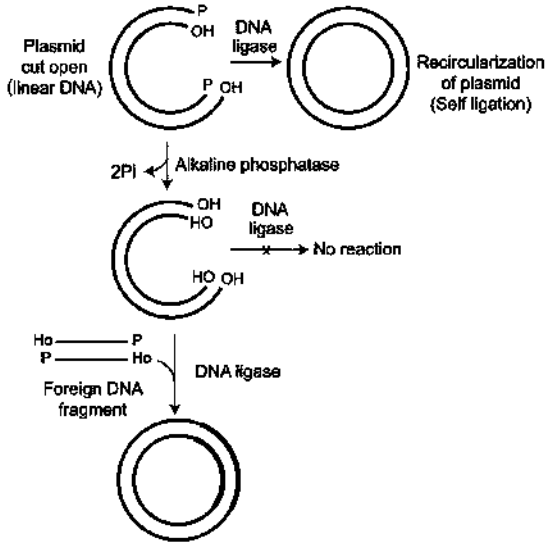
17. Draw the diagram of DNA ligase reaction which is olated from T4 phage.



18. Describe the alkaline phosphatase

- It is a DNA modifying enzymes.
- It adds or removes specific phosphate group at 5′ terminus of double strandedDNA (dsDNA) o r single stranded DNA (ssDNA) or RNA)
- Thus it prevents self ligation.
- This enzyme is purified from bacteria and calf intestine.

19. Draw the action of alkaline phosphatase diagram.



20. Describe the vectors or cloning vehicle or cloning DNA

- It is a major component of a gene cloning experiment.
- A Vector is a small DNA molecule capable of self-replication.
- It is used as a carrier and transporter of DNA fragment.

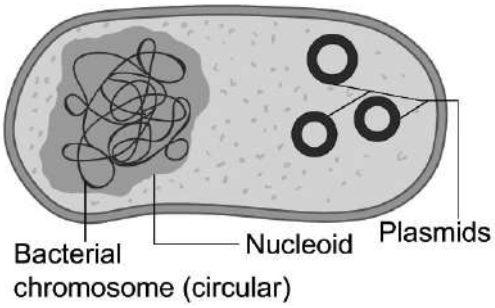
- It is insert for cloning experiments.
- Vector is also called **cloning vehicle** or **cloning DNA**)

21. Classify the vectors and describe them

- Vectors are of two types: i) Cloning Vector, and ii) Expression Vector.
- Cloning vector is used for the cloning of DNA insert inside the suitable host cell.
- Expression vector is used to express the DNA insert for producing specific protein inside the host

22. Define plasmid

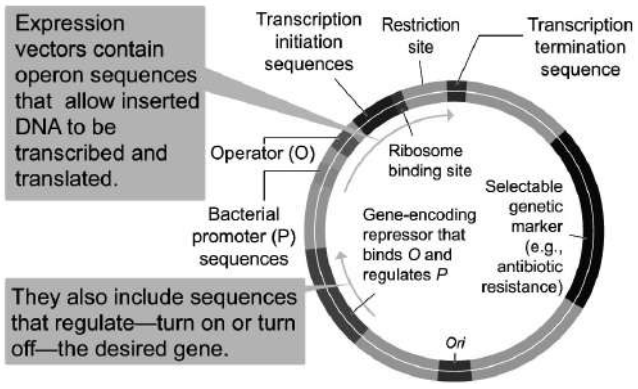
- Plasmids are extra chromosomal,self replicating ds circular DNA molecules, found in the bacterial cells.
- It contains Genetic information for their own replication.
- Plasmid is a major component of a gene cloning experiment is a vector.



23. Describe expression vectors

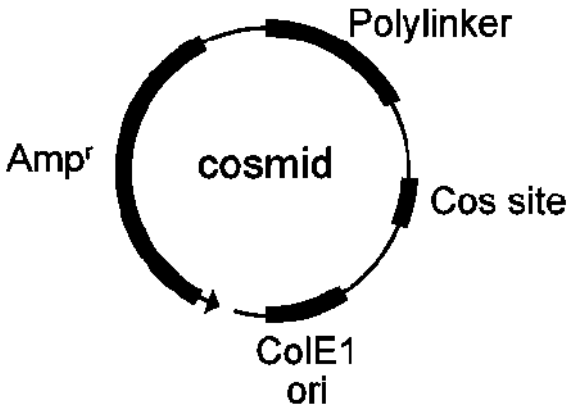
- Vectors which are suitable for expressing foreign proteins are called expression vectors.
- This vector consists of signals necessary for transcription and translation of proteins in the host.
- This helps the host to produce foreign protein in large amounts. Example: pUC 19.

24. Draw the *E.coli* expression vectors



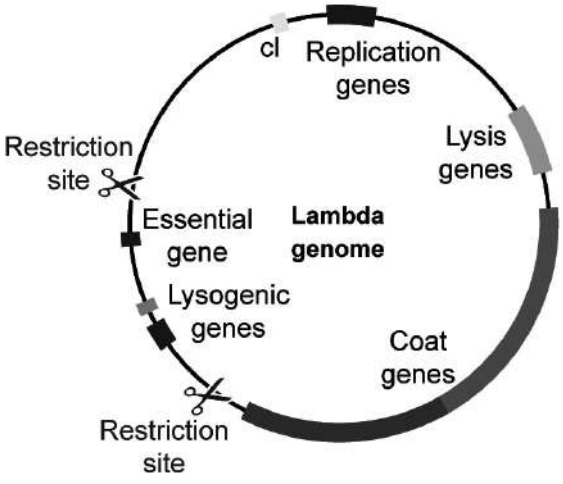
25. Describe cosmid vectors

- Cosmids are plasmids containing the 'cos' - Cohesive Terminus, the sequence having cohesive ends.
- They are hybrid vectors derived from plasmids having a fragment of lambda phage DNA with its Cos site and a bacterial plasmid)



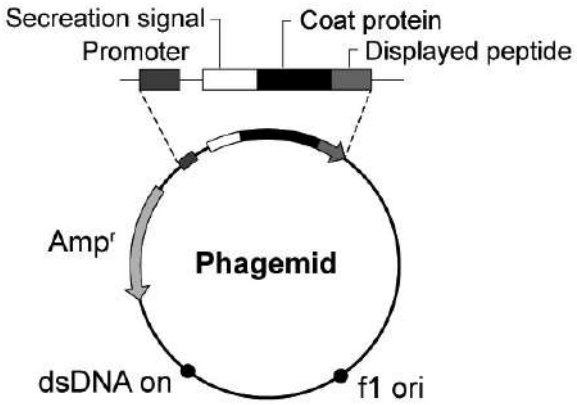
26. Describe Bacteriophage Vectors

- Bacteriophages are viruses that infect bacteria)
- The most commonly used *E. coli* phages are l phage (Lambda phage) and M13 phage.
- Phage vectors are more efficient than plasmids - DNA upto 25 Kb can be inserted into phage vector.
- **Lambda genome**
- Lambda phage is a temperate bacteriophage that infects Escherichia coli.
- The genome of lambda-Phage is 48502 bp long, i.e. 49Kb and has 50 genes.



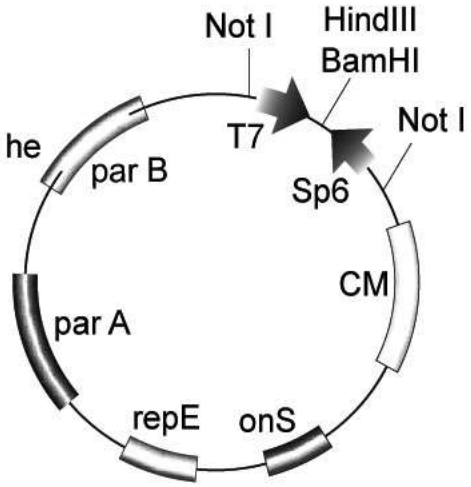
27. Describe Phagemid Vectors

- Phagemids are reconstructed plasmid vectors, which contain their own origin - 'ori' gene and also contain origin of replication from a phage.
- pBluescript SK (+/–) is an example of phagemid vector.



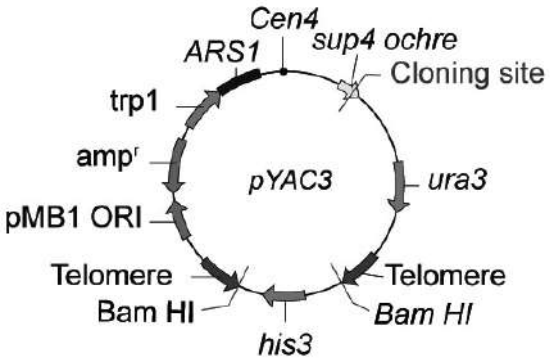
28. Describe Bacterial Artificial Chromosome (BAC) Vector

- BAC is a shuttle plasmid vector, created for cloning large-sized foreign DNA)
- BAC vector is one of the most useful cloning vector in r-DNA technology they can clone DNA inserts of upto 300 Kb and they are stable and more user-friendly.



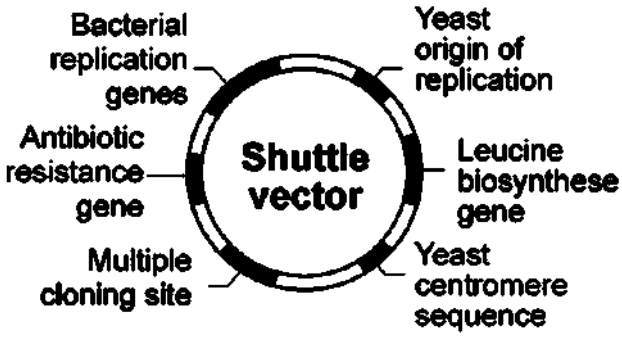
29. Describe Yeast Artificial chromosome (YAC vector

- YAC plasmid vector behaves like a yeast chromosome.
- It occurs in two forms, i.e. circular and linear.
- The circular YAC multiplies in Bacteria and linear YAC multiplies in Yeast Cells.



30. Describe Shuttle Vectors

- The shuttle vectors are plasmids designed to replicate in cells of two different species.These vectors are created by recombinant techniques.
- The shuttle vectors can propagate in one host and then move into another host without any extra manipulation.
- Most of the Eukaryotic vectors are Shuttle Vectors.



31. *E.coli* is the most widely used organism for vectors.give reasons.

- Its genetic make-up has been extensively studied)
- It is easy to handle and grow.
- It can accept a range of vectors and has also been studied for safety.
- *E.coli* to be preferred as a host cell because under optimal growing conditions the cells divide every 20 minutes.

32. Describe Antibiotic resistant markers

- An antibiotic resistance marker is a gene that produces a protein that provides cells with resistance to an antibiotic)

- Bacteria with transformed DNA can be identified by growing on a medium containing an antibiotic)
- Recombinants will grow on these medium as they contain genes encoding resistance to antibiotics such as ampicillin, chloro amphenicol, tetracycline or kanamycin, etc),
- while others may not be able to grow in these media, hence it is considered useful selectable marker.

33. What is the principle of electrophoresis?

- By applying electricity (DC) on electrophoresis the molecules migrate according to the type of charges they have.
- The electrical charges on different molecules are variable.

+ve	charged	**Cations**	will move towards	–ve	**Cathode**
–ve	charged	**Anions**	will move towards	+ve	**Anode**

34. Write the advantages of agarose gel electrophoresis

- The advantages of agarose gel electrophoresis are
- The DNA bands can be readily detected at high sensitivity.
- The bands of DNA in the gel are stained with the dye **Ethidium Bromide**
- DNA can be detected as visible fluorescence illuminated in UV light will give orange fluorescence, which can be photographed)

35. Describe the two of the most efficient methods in detection of pathogens in plant tissues

➢ **ELISA -Enzyme Linked Immumo Sorbent Assay**

- Elisa is a diagnostic tool for identification of pathogen species by using antibodies and diagnostic agents.

Use

- In plant pathology especially for weeding out virus infected plants from large scale planting is well known.

➢ **DNA Probes**

- Isotopic and non-isotopic (Northern and Southern blotting) are popular tools for identification of viruses and other pathogens

36. Describe the southern blotting techniques - DNA

- The transfer of denatured DNA from Agarose gel to Nitrocellulose Blotting or Filter Paper technique.
- It was introduced by Southern in 1975.
- This technique is called Southern Blotting Technique.

37. Describe Nucleic Acid Hybridization - Blotting Techniques

- The analytical tools for the specific identification of desired DNA or RNA fragments from larger number of molecules.
- Blotting refers to the process of immobilization of sample nucleic acids or solid support (nitrocellulose or nylon membranes.)
- The blotted nucleic acids are then used as target in the hybridization experiments for their specific detection.

38. Describe Northern blotting techniques

- It was found that RNA is not binding to cellulose nitrate. It was introduced by Alwin e*t al*. (1979).
- The RNA bands are transferred from the agarose gel into nitrocellulose filter paper.
- This transfer of RNA from gel to special filter paper is called Northern Blot hybridization.
- The filter paper used for Northern blot is Amino Benzyloxymethyl Paper.
- Amino Benzyloxymethyl Paper can be prepared from Whatman 540 paper.

39. Describe Western blotting techniques

- It refers to the electrophoretic transfer of proteins to blotting papers.
- Nitrocellulose filter paper can be used for western blot technique.
- A particular protein is then identified by probing the blot with a radio-labelled antibody.
- It binds on the specific protein to which the antibody was prepared)

40. Describe genome

- The whole complement of gene that determine all characteristic of an organism is called genome.
- The genome may be nuclear genome, mitochondrial genome or plastid genome.
- Genome of many plants contain both functional and non-expressive DNA proteins.

41. What is barcode in genetic term ?

- Barcode in genetic term refer to the identify of the taxon based on its genetic makeup.
- In practice, it is an optical, machine-readable representation of data which describes about the characters of any plants or any objects

42. How is evolutionary pattern assessed using DNA)

- The evolutionary relationship between different plant taxa is assessed using DNA content as well as the similarities and differences in the DNA sequence (sequence homology).
- Based on such analysis the taxa and their relationship are indicated in cladogram.
- Such cladogram will show the genetic distance between two taxa)
- It is also showed antiquity or modernity of any taxon with respect to one another

43. Describe genome editing.

- The ability to change an organism's DNA are called Genome editing or gene editing.
- These allow genetic material to be added, removed, or altered at particular locations in the genome.
- Several approaches to genome editing have been developed)

44. Describe CRISPR - Cas9.

- A recent genome editing is known as CRISPR-Cas9,
- It is short form of **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
- This CRISPR-associated protein 9.
- The CRISPR-Cas9 generated a lot of excitements.
- They are faster, cheaper, more accurate, and more efficient than other existing genome editing methods.

45. Explain re-engineer rice plants using the technic of CRISPR-Cas9

- Rice, was the first plants of CRISPR-mediated targeted mutagenesis and gene replacement.
- The gene editing tool CRISPR can be used to make hybrid rice plants that can clone their seed)
- A new study which clearly shows one can re-engineer rice to switch it from a sexual to an asexual mode.
- This was reported by Imtiyaz Khand and Venkatesan Sundaresan and colleagues.

46. Explain the simplified model for the RNAi pathway

- A simplified model for the RNAi pathway is based on two steps, each involving ribonuclease enzyme.
- In the first step, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into a short interfering RNA (siRNA)by the RNase II enzymes called Dicer and Drosha)
- In the second step, siRNAs are loaded into the effector complex RNA-induced silencing complex (RISC).
- The siRNA is unwound during RISC assembly and the single-stranded RNA hybridizes with mRNA target.
- This RNAi is seen in plant feeding nematodes.

47. How is genetically modified variety of Herbicide Tolerant (HT) mustard is created?

- **Dhara Mustard Hybrid** (DMH) -11 is transgenic mustard)
- It is genetically modified variety of Herbicide Tolerant (HT) mustard)
- It was created by using "barnase/ barstar" technology for genetic modification by adding genes from soil bacterium that makes mustard, a self-pollinating plant.
- DMH -11 contains three genes viz. Bar gene, Barnase and Barstar sourced from soil bacterium.
- The bar gene had made plant resistant to herbicide named Basta)

48. How is genetically modified variety of virus resistance ?

- Many plants are affected by virus attack resulting in series loss in yield and even death.
- Biotechnological intervention is used to introduce viral resistant genes into the host plant.
- So that they can resist the attack by virus.
- This is by introducing genes that produce resistant enzymes which can deactivate viral DNA)

49. Write the benefits of genetically modified (GM) food

- High yield without pest

- 70% reduction of pesticide usage
- Reduce soil pollution problem
- Conserve microbial population in soil

50. Write the risks believed to genetically modified (GM) food

- Affect liver, kidney function and cancer
- Hormonal imbalance and physical disorder
- Anaphylactic shock (sudden hypersensitive reaction) and allergies.
- Adverse effect in immune system because of bacterial protein.
- Loss of viability of seeds show in terminator seed technology of GM crops.

51.Explain the term polylactic acid (PLA)

- Polylactic acid or polylactide (PLA) is a biodegradable and bioactive thermoplastic)
- It is an aliphatic polyester derived from corn starch, cassava root, chips or starch or sugarcane.
- The production of PLA, two main monomers are used
- lactic acid, and 2.The cyclic diester lactide.
- The most common route is the ring-opening polymerization of lactide with metal catalysts like tin octoate in solution. The metal-catalyzed reaction results in equal amount of *d* and polylactic acid)

52. What are the limitations of bioremediation

- Only biodegradable contaminants only can be transformed)
- It must be specifically made in accordance to the conditions at the contaminated site.
- Small-scale tests on a pilot scale must be performed before carrying out the procedure at the contaminated site.
- The use of genetic engineering technology to create genetically modified microorganism .

53. Describe algal fuel

- Algae is a source of energy-rich oils.
- Algal fuel, also known as algal biofuel, or algal oil,.
- It is an alternative to liquid fossil fuels.
- *Botryococcus braunii* is normally used to produce algal biofuel.
- Algal fuels are an alternative to commonly known biofuel sources obtained from corn and sugarcane.
- The energy crisis and the world food crisis have initiated interest in algal culture (farming algae) for making biodiesel and other biofuels using land unsuitable for agriculture.

54. Describe biological hydrogen production by algae

- The biological hydrogen production with algae is a method of photo biological water splitting.
- In normal photosynthesis the alga, *Chlamydomonas reinhardtii* releases oxygen.
- When it is deprived of sulfur, it switches to the production of hydrogen during photosynthesis and the electrons are transported to ferredoxins.
- [Fe]-hydrogenase enzymes combine them into the production of hydrogen gas.

55. What is Biopiracy?Give examples .

- Biopiracy refers to the use of bio resources by multinational and other organisations without proper authorisation from the countries and concerned people without compensatory payments .
- Examples: Recent patents granted by the U.S. Patent and Trademarks Office to American companies on turmeric, 'neem' and, , 'basmati' rice.
- All three products are indigenous to the Indo-Pak subcontinent.

56. How the turmeric US patent was cancelled ?

- The journal article published by the Indian Medical Association, in the year 1953, proved that the use of turmeric as an antiseptic is not new to the world and is not a new invention.
- The objection in this case US patent and trademark office was upheld and traditional knowledge of the Indians was protected)
- It is another example of Biopiracy.

57. How the turmeric US patent was cancelled ?

- India had periled the United States to take the matter to the WTO as an infringement of the TRIPS agreement, In the year 2002, the final decision was taken.
- Rice Tec dropped down 15 claims, resulting in clearing the path of Indian Basmati rice exports to the foreign countries.

PART – D

ADDITIONAL QUESTIONS (5 MARKS)

1. What are the major focus of biotechnology? Expain.

The major focus of biotechnology are

- **Fermentation** for production of acids, enzymes, alcohols, antibiotics, fine chemicals, vitamins and toxins
- **Biomass for** bulk production of single cell protein , alcohol, and biofuel
- **Enzymes** as biosensors, in processing industry
- **Biofuels** for production of hydrogen, alcohol, methane
- **Microbial inoculants** as bio fertiliser, and nitrogen fixers
- **Plant and animal cell culture** for production of secondary metabolites, monoclonal antibodies
- **Recombinant DNA technology** for production of fine chemicals, enzymes, vaccines, growth hormones,
- antibiotics, and interferon
- **Process engineering** – tools of biotechnology is used for effluent treatment, water recycling.

2. Explain the procedure of fermentation

- Depending upon the type of product, bioreactor is selected)
- A suitable substrate in liquid media is added at a specific temperature, pH and then diluted)
- The organism, such as microbe, animal/ plant cell, sub-cellular organelle or enzyme is added to it.
- Then it is incubated at a specific temperature for the specified time.
- The incubation may either be aerobic or anaerobic)
- Withdrawal of product using downstream processing methods

3. Explain the industrial application fermentation

The industrial application fermentation are

➢ **Microbial biomass production**

- Microbial cells like algae, bacteria, yeast, fungi are grown, dried)
- It is used as source of a complete protein called 'single cell protein (SCP)'.
- It serves as human food or animal feed)

➢ **Microbial metabolites**

- Microbes produce compounds that are very useful to man and animals are called metabolites,
- It can be grouped into two categories

✓ **Primary metabolites:**

- Metabolites produced for the maintenance of life process of microbes are known as primary metabolites
- Eg. Ethanol, citric, acid, lactic acid, acetic acid)

✓ **Secondary metabolites:**

- Metabolites are not required for the vital life process of microbes.
- They have value added nature, this includes antibiotics are called **secondary metabolites**.
- E.g -Amphotericin-B (*Streptomyces nodosus*), Penicillin (*Penicillium chryosogenum*) Streptomycin (*S. grises*), Tetracycline (*S. aureofacins*), alkaloids, toxic pigments, vitamins etc

➢ **Microbial enzymes**

- When microbes are cultured, they secrete some enzymes into the growth media)
- These enzymes are industrially used in detergents, food processing, brewing and pharmaceuticals.
- Eg. protease, amylase, isomerase, and lipase.

➢ **Bioconversion, biotransformation or modification of the substrate**

- The fermenting microbes has the capacity to produce valuable products,
- Example: conversion of ethanol to acetic acid (vinegar),

o isopropanol to acetone,

o sorbitol to sorbose (this is used in the manufacture of vitamin C),

o sterols to steroids.

4. List out the applications of Single-Cell Protein

- It is used as protein supplement

- It is used in cosmetics products for healthy hair and skin
- It is used in poultry as the excellent source of proteins and other nutrients,
- It is widely used for feeding cattle, birds, fishes etc)
- It is used in food industry as aroma carriers, vitamin carrier, emulsifying agents to improve the nutritive value of baked products, in soups, in ready-to-serve-meals, in diet recipes
- It is used in industries like paper processing, leather processing as foam stabilizers.

5. Write the steps involved in recombinant DNA technology

The steps involved in recombinant DNA technology are:

- Isolation of a DNA fragment containing a gene of interest that needs to be cloned)
- This is called an **insert**.
- Generation of recombinant DNA (rDNA) molecule by insertion of the DNA fragment into a carrier molecule called a **vector.** It can self-replicate within the host cell.
- Selection of the transformed host cells that is carrying the rDNA and allowing them to multiply thereby multiplying the rDNA molecule.
- The entire process thus generates either a large amount of rDNA or a large amount of protein expressed by the insert.
- Wherever vectors are not involved the desired gene is multiplied by PCR technique.
- The multiple copies are injected into the host cell protoplast or it is shot into the host cell protoplast by shot gun method)

6. Enumerate the properties of vectors

- Vectors are able to replicate autonomously to produce multiple copies of them along with their DNA insert in the host cell.
- It should be small in size and of low molecular weight, less than 10 Kb (kilo base pair) in size.
- so that entry/transfer into host cell is easy.
- Vector must contain an origin of replication so that it can independetly replicate within the host.
- It should contain a suitable marker such as antibiotic resistance, to permit its detection in transformed host cell.
- Vector should have unique target sites for integration with DNA insert
- It should have the ability to integrate with DNA insert it carries into the genome of the host cell.
- Most of the commonly used cloning vectors have more than one restriction site.
- These are Multiple Cloning Site (MCS) or polylinker.
- Presence of MCS facilitates the use of restriction enzyme of choice.

7. What are the features that are required to facilitate cloning into a vector

The following are the features that are required to facilitate cloning into a vector.

➢ **Origin of replication (ori):**

- This is a sequence from where replication starts.
- The piece of DNA when linked to this sequence can be made to replicate within the host cells.

➢ **Selectable marker**:

- In addition to **ori** the vector requires a selectable marker.
- It helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants.

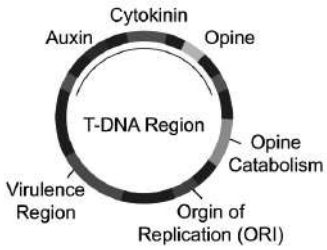
➢ **Cloning sites**:

- In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes.

8. Why is *Agrobacterium tumefaciens* a good cloning vector?

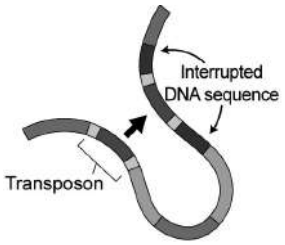
- Ti plasmid is found in *Agrobacterium tumefaciens*,
- This bacteria responsible for inducing tumours in several dicot plants.
- The plasmid carries transfer (tra) gene which help to transfer T- DNA from one bacterium to other bacterial or plant cell.
- It has Onc gene for oncogenecity,

- ori gene for origin for replication and inc gene for incompatibility.
- T-DNA of Ti-Plasmid is stably integrated with plant DNA)
- Agrobacterium plasmids have been used for introduction of genes of desirable traits into plants.



9. How Transposons (Transposable elements or mobile elements) used as a genetic tool – explain with diagram

- Transposons are DNA sequence able to insert itself at a new location in the genome without having any sequence relationship with the target locus. These transposons are called **walking genes** or **jumping genes**.



Uses

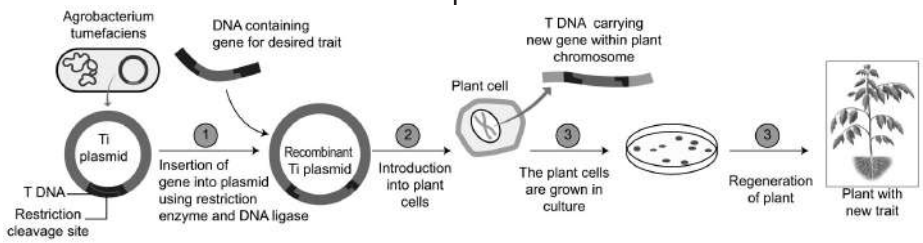
- They are used as genetic tools for analysis of gene and protein functions.
- They produce new phenotype on host cell.
- The use of transposons is well studied in *Arabidopsis thaliana* and bacteria such as *Escherichia coli*.

10. How does Competent refer to in competent cells used in transformation ?

- The DNA is a hydrophilic molecule,
- It cannot pass through cell membranes.
- In order to force bacteria to take up the plasmid)
- The bacterial cells must first be made competent to take up DNA)
- This is done by treating them with a specific concentration of a divalent cation such as calcium.
- Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42^{0}C (heatshock). Then putting them back on ice.
- This enables bacteria to take up the Recombinant DNA)

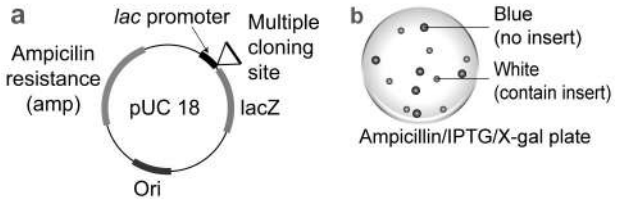
11. *Agrobacterium tumefaciens is* known as the natural genetic engineer of plants. What could be the reason ?

- The Ti-plasmid from *Agrobacterium tumefaciens* has been used extensively for plant transformation.
- This bacterium has a large size plasmid, known as Ti plasmid (Tumor inducing).
- A portion of it referred as T-DNA (transfer DNA) is transferred to plant genome in the infected cells and cause plant tumors (crown gall).
- This bacterium has the natural ability to transfer T-DNA region of its plasmid into plant genome, upon infection of cells at the wound site.
- So it is known as the natural genetic engineer of plants.



12. Explain Insertional inactivation - Blue- White colony selection method

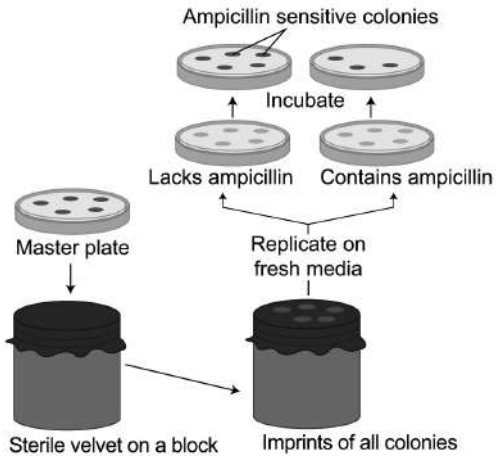
- It is a powerful method used for screening of recombinant plasmid)
- In this method, a reporter gene **lacZ** is inserted in the vector.
- The lacZ encodes the enzyme β-galactosidase.
- It contains several recognition sites for restriction enzyme.
- β-galactosidase breaks a synthetic substrates called X-gal (5-bromo-4-chloro-indolyl-β-D-galacto-pyranoside) into an insoluble blue coloured product.
- If a foreign gene is inserted into lacZ, this gene will be inactivated)
- Therefore, no-blue colour will develop (white) because β-galactosidase is not synthesized due to inactivation of lacZ.
- Therefore, the host cell containing r-DNA form white coloured colonies on the medium contain X-gal, whereas the other cells containing non-recombinant DNA will develop the blue coloured colonies.
- On the basis of colony colour, the recombinants can be selected)



a. Plasmid vector designed for blue-white screening.
b. Blue-white colony selection method.

13. How transformed cells can be selected by Replica plating technique.Explain

- A technique in which the pattern of colonies growing on a culture plate is copied)
- A sterile filter plate is pressed against the culture plate and then lifted)
- Then the filter is pressed against a second sterile culture plate.
- This results in the new plate being infected with cell in the same relative positions as the colonies in the original plate.
- Usually, the medium used in the second plate will differ from that used in the first. It may include an antibiotic or without a growth factor.
- In this way, transformed cells can be selected)

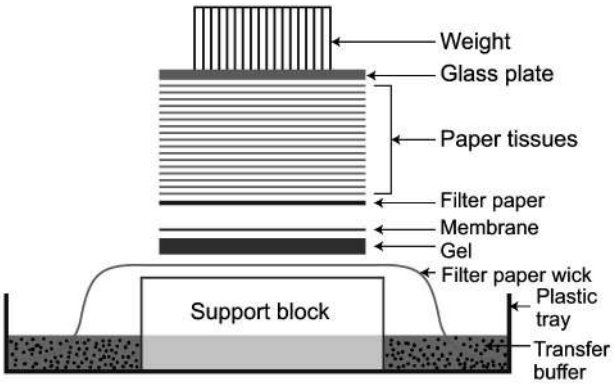


14. Expain the mechanism of Agarose GEL Electrophoresis

- It is used mainly for the purification of specific DNA fragments.
- Agarose is convenient for separating DNA fragments ranging in size from a few hundred to about 20000 base pairs.
- Polyacrylamide is preferred for the purification of smaller DNA fragments.
- The gel is complex network of polymeric molecules.
- DNA molecule is negatively charged molecule - under an electric field DNA molecule migrates through the gel. The electrophoresis is frequently performed with marker DNA fragments of known size which allow accurate size determination of an unknown DNA molecule by interpolation.

15. Explain the Steps in southern blotting techniques.

- The transfer of DNA from agarose gel to nitrocellulose filter paper is achieved by Capillary Action.
- A buffer Sodium Saline Citrate (SSC) is used, in which DNA is highly soluble
- It can be drawn up through the gel into the Nitrocellulose membrane.
- By this process ss-DNA becomes '**Trapped**' in the membrane matrix.
- This DNA is hybridized with a nucleic acid and can be detected by autoradiography.



Diagramatic representation of southern blotting techniques

16. Explain bioassay for target gene effect

- Target gene is target DNA, foreign DNA, passenger DNA, exogenous DNA, gene of interest or insert DNA that is to be either cloned or specifically mutated)
- Gene targeting experiments have been targeting the nuclei.
- This leads to 'gene knock-out'. For this purpose, two types of targeting vectors are used)
- They are

➢ **Insertion vectors**

- These are entirely inserted into targeted locus as the vectors are linearized within the homology region.
- Initially, these vectors are circular but during insertion, become linear.
- It leads to duplication of sequences adjacent to selectable markers.

➢ **The replacement vector**

- It has the homology region and it is co-linear with target. This vector is linearized prior to transfection outside the homology region and then consequently a crossing over occurs to replace the endogenous DNA with the incoming DNA)

17. Herbicide Tolerant – Glyphosate- explain

- Weeds are a constant problem in crop fields.
- Weeds not only compete with crops for sunlight, water, nutrients and space but also a carrier for insects and diseases.
- If left uncontrolled, weeds can reduce crop yields significantly.
- **Transgenic plants** contain a novel DNA introduced into its genome.
- **Glyphosate herbicide** produced by Monsanto, USA company.
- The trade name 'Round up' kills plants by blocking the 5-enopyruvate shikimate-3 phosphate synthase (EPSPS) enzyme.
- An enzyme involved in the biosynthesis of aromatic amino acids, vitamins and many secondary plant metabolites.
- There are several ways by which crops can be modified to be glyphosate-tolerant.
- One strategy is to incorporate a soil bacterium gene that produces a glyphosate tolerant form of EPSPS.
- Another way is to incorporate a different soil bacterium gene that produces a glyphosate degrading enzyme.

18. Describe herbicide tolerant - basta

- 'Basta' refers herbicide containing the chemical compound phosphinothricin.
- Basta herbicide tolerant gene PPT (*L*-phosphinothricin) was isolated from *Medicago sativa* plant.
- It inhibits the enzyme glutamine synthase which is involved in ammonia assimilation.
- The PPT gene was introduced into tobacco and transgenic tobacco produced was resistant to PPT.
- Similar enzyme was also isolated from *Streptomyces hygroscopicus* with bar gene encodes for PAT (Phosphinothricin acetyl transferase
- It was introduced into crop plants like potato and sugar-beet and transgenic crops have been developed)

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

- Bt cotton is a genetically modified pest resistant plant cotton variety
- It produces an insecticide activity to bollworm.
- Strains of *Bacillus thuringiensis* produce over 200 different Bt toxins, each harmful to different insects.

- Most Bt toxins are insecticidal to the larvae of moths and butterflies, beetles, cotton bollworms and gatflies but are harmless to other forms of life.
- The genes are encoded for toxic crystals in the Cry group of endotoxin.
- When insects attack and eat the cotton plant the Cry toxins are dissolved in the insect's stomach.
- The epithelial membranes of the gut block certain vital nutrients thereby sufficient regulation of potassium ions are lost in the insects.
- It results in the death of epithelial cells in the intestine membrane which leads to the death of the larvae.

20. The Bt brinjal has been developed to give resistance against *Lepidopteron* insects- explain briefly

- The Bt brinjal is another transgenic brinjal
- It created by inserting a crystal protein gene (Cry1Ac) from the soil bacterium *Bacillus thuringiensis* into the genome of various brinjal cultivars.
- The insertion of the gene, along with other genetic elements such as promoters, terminators and an antibiotic resistance marker gene into the brinjal plant is accomplished using *Agrobacterium*-mediated genetic transformation.
- The Bt brinjal has been developed to give resistance against *Lepidopteron* insects, in particular the Brinjal Fruit and Shoot Borer (*Leucinodes orbonalis*).

21. Describe FlavrSavr Tomato

- *Agrobacterium* mediated genetic engineering technique produce Flavr-Savr tomato.
- This retaining the natural colour and flavor of tomato.
- Through genetic engineering, the ripening process of the tomato is slowed down.
- It prevent from softening and to increase the shelf life.
- The gene transfer mechanism by *Agrobacterium,* introducing an antisense gene into tomato .
- It produce the enzyme polygalacturonase
- It helps in delaying the ripening process of tomato during long storage and transportation.

22. Describe Golden rice - biofortification

- Golden rice is a variety of *Oryza sativa* (rice) produced through genetic engineering.
- It is developed by Ingo Potrykus and his group.
- The aim is to produce Vitamin-A in the edible parts of rice.
- Golden rice differs from its parental by the addition of three beta-carotene biosynthesis genes.
- 1.'psy' (phytoene synthase) from daffodil plant
- 2. *Narcissus pseudonarcissus* and 'crt-1' gene from the soil bacterium *Erwinia auredorora* and
- 3.'lyc' (lycopene cyclase) gene from wild-type rice endosperm.
- The endosperm of normal rice, does not contain beta-carotene.
- Golden-rice has accumulates Beta-carotene.
- This has been done using Recombinant DNA technology.
- Golden rice can control childhood blindness - Xerophthalmia)

23. How is Polyhydroxybutyrate (PHB) protect our environment ?

- Synthetic polymers are non-degradable and pollute the soil .
- when burnt add dioxin in the environment which cause cancer.
- So, efforts were taken to provide an alternative eco-friendly biopolymers.
- Polyhydroxyalkanoates (PHAs) and polyhydroxybutyrate (PHB) are group of degradable biopolymers.
- They have several medical applications such as drug delivery, scaffold and heart valves.
- PHAs are biological macromolecules and thermoplastics which are biodegradable and biocompatible. Some of the microorganisms utilized to produce different types of PHAs
- Gram-positive like *Bacillus megaterium*, *Bacillussubtilis* and *Corynebacterium glutamicum*,

- Gram-negative bacteria like group of *Pseudomonas* sp. and *Alcaligenes eutrophus*.
- **Name the microorganisms utilized to produce different types of PHAs.**
- Some of the microorganisms utilized to produce different types of PHAs
- Gram-positive like *Bacillus megaterium*, *Bacillussubtilis* and *Corynebacterium glutamicum*,
- Gram-negative bacteria like group of *Pseudomonas* sp. and *Alcaligenes eutrophus*.

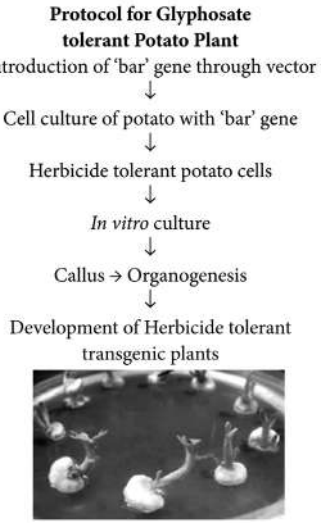
24. Describe green fluorescent protein (GFP)

- The green fluorescent protein (GFP) is a protein containing 238 amino acid residues of 26.9 k Da)
- It exhibits bright green fluorescence when exposed to blue to ultraviolet range (395 nm).
- GFP refers to the protein first isolated from the jellyfish *Aequorea victoria*)
- GFP is an excellent tool in biology due to its ability to form internal chromophore without requiring any accessory cofactors, gene products, enzymes or substrates other than molecular oxygen.
- In cell and molecular biology, the GFP gene is frequently used as a reporter of expression.
- It has been used in modified forms to make biosensors.

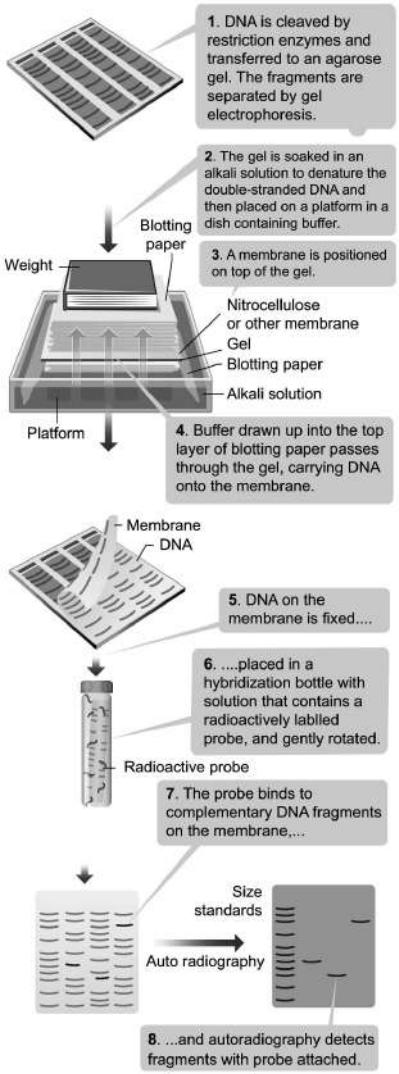
25. Explain briefly the biopharming or molecular farming or pharming

- Biopharming also known as molecular pharming is the production.
- Use of transgenic plants genetically engineered to produce pharmaceutical substances for use of human beings.
- This is also called "**molecular farming or pharming**".
- These plants are different from medicinal plants which are naturally available.
- The use of plant systems as bioreactors is gaining more significance in modern biotechnology.
- Many pharmaceutical substances can be produced using transgenic plants. Example: Golden rice

26. Write protocol for Glyphosate tolerant potato plant.



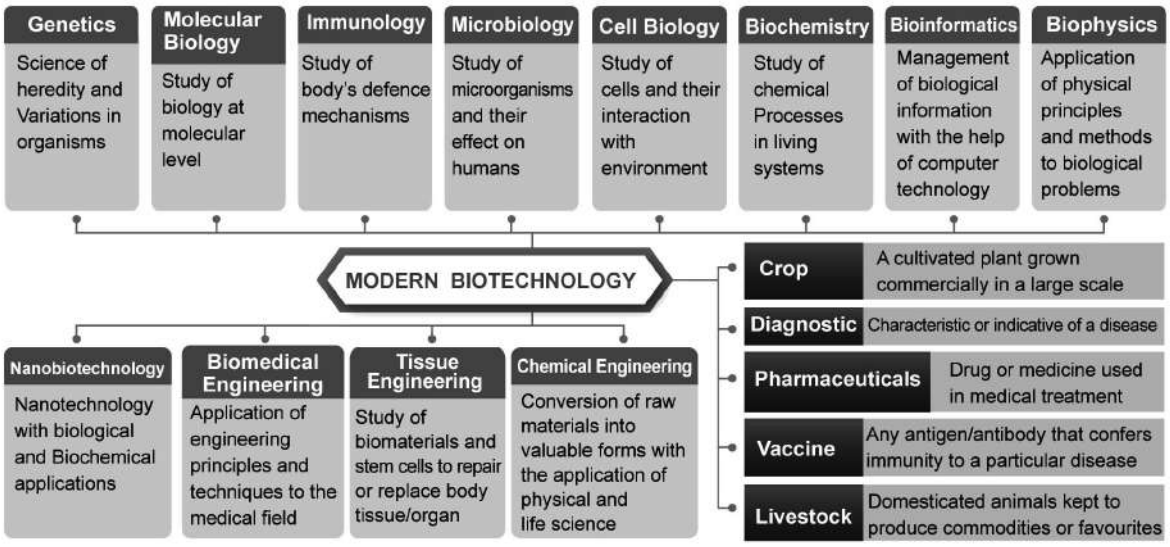
27. Explain steps involved in southern blotting technique



28. Explain flow chart of Inter disciplinarity Fields of Biotechnology

Interdisciplinarity Fields of Biotechnology

Biotechnology is one of the most important applied interdisciplinary sciences of the **21st century**. It is the trusted area that enables us to find the beneficial way of life. Biotechnology has wide applications in various sectors like agriculture, medicine, environment and commercial industries.



29. The major historical events for the development of Biotechnology

The major historical events for the development of Biotechnology, as an interdisciplinary field with multidisciplinary applications are listed below:

i) Before common era and pre-20th century.

Before Common Era

6000 BC – 3000 BC – Bread making, fermentation of fruit juices and plant exudates to produce alcoholic beverages using yeast.

Pre – 20th Century

1770 – Antoine Lavoisier gave chemical **basis of alcoholic fermentation.**

1798 – Edward Jenner uses **first viral vaccine** to inoculate a child from smallpox.

1838 – **Protein** discovered, named and recorded by Gerardus Johannes Mulder and Jons Jacob Berzelius.

1871 – Ernst Hoppe, Seyler discovered **enzyme invertase**, which is still used for making artificial sweeteners.

1876 – Louis Pasteur identified **role of microorganisms in fermentation.**

ii) 20th Century

20th Century

1919 – The **term biotechnology** was coined by Karl Ereky

1928 – **Discovery of Penicillin** by Alexander Fleming

1941 – Experiment with *Neurospora crassa* resulting in **one gene one enzyme hypothesis** by George Beadle and Edward Tatum.

1944 – Identification of **DNA as the genetic material** Avery–MacLeod–McCarty

1953 – Discovery of **double helix structure of DNA** by James Watson and Francis Crick.

1972 – Discovery of **Restriction enzymes** by Arber, Smith and Nathans.

1973 – Fragmentation of DNA-combined with Plasmid DNA, **r-DNA technology** - Genetic engineering -Modified gene by Stanley Cohen, Annie Chang, Robert Helling and Herbert Boyer.

1975 – Production of **Monoclonal antibodies** by Kohler and Milstein

1976 – Sanger and Gilbert developed **techniques to sequence DNA**

1978 – Production of **human insulin** in E.Coli

1979 – **Development of Artificial gene** – functioning within the living cells by H.G. Khorana

1982 – U.S approved **humulin** (human insulin) the first pharmaceutical product of rDNA technology, for human use.

1983 – Use of **Ti plasmids** to genetically transform plants

1986 – Development of **Polymerase Chain Reaction (PCR)** technology by Kary Mullis.

1987 – Gene transfer by **biolistic transformation**

1992 – First chromosomes of yeast is sequenced

1994 – U.S approved the first **Genetically Modified food: Flavr Savr tomato.**

1997 – The **first transgenic animal,** mammalian sheep, Dolly developed by **nuclear cloning** by Ian Wilmet.

2000 – **First plant Genome** of *Arabidopsis thaliana* sequenced

ii) 21st Century

21st Century

2001 – Human genome Project creates a **draft of the human genome sequence.**

2002 – **First crop plant genome sequenced** in *Oryza sativa*

2003 – **Human genome project is completed,** providing information on the locations and sequence of human genes on all 46 chromosomes.

2010 – Sir Robert G. Edwards developed ***in vitro* fertilization in animal.**

2016 – Stem cells injected into stroke patients re-enable patient to walk – **Stem cell therapy**

2017 – **Blood stem cells** grown in lab.

2018 – James Allison and TasukuHonjo **discovered protein found in immune cells.** This found a new role in cancer therapy.

UNIT VIII: BIOTECHNOLOGY

CHAPTER 5

PLANT TISSUE CULTURE

POINTS TO REMEMBER

- *in vitro* growth of plant protoplasts, cells, tissues and organs called tissue culture .
- Tissue culture techniques are used for commercial production of plants as well as for plant research
- Gottlieb Haberlandt (1902) the German Botanist is called father of tissue culture.
- The development of whole plant from isolated cells or tissue in in vitro condition is called Totipotency.
- Basic concepts of plant tissue culture are totipotency, differentiation, dedifferentiation and redifferentiation.
- Sterilization is the technique employed to get rid of microbes from the culture medium, vessels and explants.
- Surface sterilization agents are 0.1% mercuric chloride, 70% ethanol .
- MS nutrient medium (Murashige and Skoog 1962) is commonly used.
- The marine algae used as solidifying agent in media preparation are called **Agar**
- The pH of medium is normally adjusted between **5.6 to 6.0** for the best result.
- Temperature of the cultures in incubated normally at of **25°C ± 2°C** for optimal growth.
- **Callus** is a mass of unorganized growth of plant cells or tissues in *in vitro* culture medium.
- The callus cells undergoes differentiation and produces somatic embryos, known as **Embryoids**.
- The plantlets developed *in vitro* are transferred to normal environmental conditions called **Hardening** .
- Fusion of protoplast done through a suitable **fusogenic agent** called PEG (Polyethylene Glycol).
- The cultures are incubated in continuous light **1000-2000 lux at 25°C**.
- Small aggregates of cells *in vitro* in liquid medium is known as cell **suspension culture**.
- Secondary metabolites are not required by the plant for normal growth and development
- Plants can be regenerated by somatic **embryogenesis** or **organogenesis**.
- Somatic embryoids can be used for the production of synthetic seeds
- Synthetic seeds are produced by encapsulation of embryoids in agarose gel or calcium alginate
- The morphological changes occur in the callus produce of shoot and roots is called **organogenesis**.
- Auxin and cytokinins induce shoot and root formation.
- Micropropagation of plants at industrial level maintains high standards of homogeneity
- Shoot meristem tip culture is the method to produce **virus-free** plants
- Cryopreservation (Cryo-conservation) is very low temperature of –196°C using liquid nitrogen.
- The IPR is protected by different ways like patents, copyrights, trade secrets and trademarks, designs and geographical indications.
- A patent is a personal property which can be licensed or sold by the person or organisation just like any other property.

- **ELSI** which represents **ethical**, **legal** and **social implications** of biotechnology
- Biosafety is the prevention of large-scale loss of biological integrity, focusing both on ecology and human health.
- Bioethics refers to the study of ethical issues emerging from advances in biology and medicine.
- The GEAC is also responsible for approval of proposals relating to release of genetically engineered organisms

Expanded forms

- **GEAC** - Genetic Engineering Appraisal Committee
- **BRL-I** - Biosafety Research Level trial-I
- **BRL-II** - Biosafety Research Level trial-II .
- **ELSI** - Ethical, Legal, and Social Implications
- **IBSCs** - Institutional Bio-safety Committees
- **RCGM** - Review Committee on Genetic Manipulation (RCGM
- **DBT** - Department of Biotechnology (DBT
- **IPR** - Intellectual Property Right

Book Evaluation

PART – A

(1 MARK)

Choose the correct Answer from the given option:

1. Totipotency refers to

a) capacity to generate genetically identical plants.

b) capacity to generate a whole plant from any plant cell / explant.

c) capacity to generate hybrid protoplasts.

d) recovery of healthy plants from diseased plants.

Ans: a

2. Micro propagation involves

a) vegetative multiplication of plants by using micro-organisms.

b) vegetative multiplication of plants by using small explants.

c) vegetative multiplication of plants by using microspores.

d) Non-vegetative multiplication of plants by using microspores and megaspores.

Ans: b

3. Match the following :

Column A		Column B	
1)	Totipotency	A)	Reversion of mature cells into meristerm
2)	Dedifferentiation	B)	Biochemical and structural changes of cells
3)	Explant	C)	Properties of living cells develops into entire plant
4)	Differentiation	D)	Selected plant tissue transferred to culture medium

	1	2	3	4
a)	C	A	D	B
b)	A	C	B	D
c)	B	A	D	C
d)	D	B	C	A

Ans: a

4. The time duration for sterilization process by using autoclave is ___minutes and the temperature is ___

a) 10 to 30 minutes and 125° C

b) 15 to 30 minutes and 121° C

c) 15 to 20 minutes and 125° C

d) 10 to 20 minutes and 121° C

Ans: b

5. Which of the following statement is correct

a) Agar is not extracted from marine algae such as seaweeds.

b) Callus undergoes differentiation and produces somatic embryoids.

c) Surface sterilization of explants is done by using mercuric bromide

d) P^H of the culture medium is 5.0 to 6.0 **Ans: b**

6. Select the incorrect statement from given statement

a) A tonic used for cardiac arrest is obtained from Digitalis purpuria

b) Medicine used to treat Rheumatic pain is extracted from Capsicum annum

c) An anti malarial drug is isolated from Cinchona officinalis.

d) Anti-cancinogenic property is not seen in Catharanthus roseus. **Ans: d**

7. Virus free plants are developed from

a) Organ culture

b) Meristem culture

c) Protoplast culture

d) Cell suspension culture **Ans: b**

8. The prevention of large scale loss of biological interity

a) Biopatent b) Bioethics

c) Biosafety d) Biofuel **Ans: c**

9. Cryopreservation means it is a process to preserve plant cells, tissues or organs

a) at very low temperature by using ether.

b) at very high temperature by using liquid nitrogen

c) at very low temperature of -196 by using liquid nitrogen

d) at very low temperature by using liquid nitrogen **Ans: c**

10. Solidifying agent used in plant tissue culture is

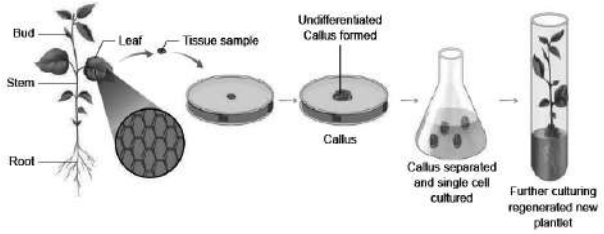
a) Nicotinic acid b) Cobaltous chloride

c) EDTA d) Agar **Ans: d**

PART – B,C AND D

(2,3 AND 5 MARKS)

11. What is the name of the process given below? Write its 4 types.



The name of the process given below is basic steps in plant tissue culture technology

1.Isolation of cells 2.Culture condition 3.Induction of callus 4.Embryogenesis.

12. How will you avoid the growing of microbes in nutrient medium during culture process? What are the techniques used to remove the microbes?

Sterilization is a process to avoid the growing of microbes in nutrient medium during culture process.

The techniques used to remove the microbes in nutrient medium

- Culture media are dispensed in glass containers, plugged with non-absorbent cotton or sealed with plastic closures.
- Then sterilized using autoclave at 15 psi (121°C) for 15 to 30 minutes.
- The plant extracts, vitamins, amino acids and hormones are sterilized by passing through Millipore filter with 0.2 mm pore diameter.
- Then added to sterilized culture medium inside Laminar Airflow Chamber under sterile condition

13. Write the various steps involved in cell suspension culture.

- The culture of single cells or aggregates of cells *in vitro* in liquid medium is known as cell suspension culture.
- The cell suspension is prepared by transferring a portion of callus to the liquid medium and agitated using rotary shaker instrument.

- The cells are separated from the callus tissue and used for cell suspension culture.

14. What do you mean Embryoids? Write its application.

- The formation of embryos from the callus tissue directly are called somatic embryogenesis.
- These embryos are called Embryoids

Applications of somatic embryogenesis

- It provides potential plantlets which after hardening period can establish into plants.
- Somatic embryoids can be used for the production of synthetic seeds.
- Somatic embryogenesis is now reported in many plants.
- Example: *Allium sativum*, *Hordeum vulgare, Oryza sativa, Zea mays*.
- Synthetic seeds are produced by encapsulation of embryoids in agarose gel or calcium alginate.

15. Give the examples for micro propagation performed plants.

Pineapple, banana, strawberry and potato are the examples for micro propagation performed plants.

16. Explain the basic concepts involved in plant tissue culture.

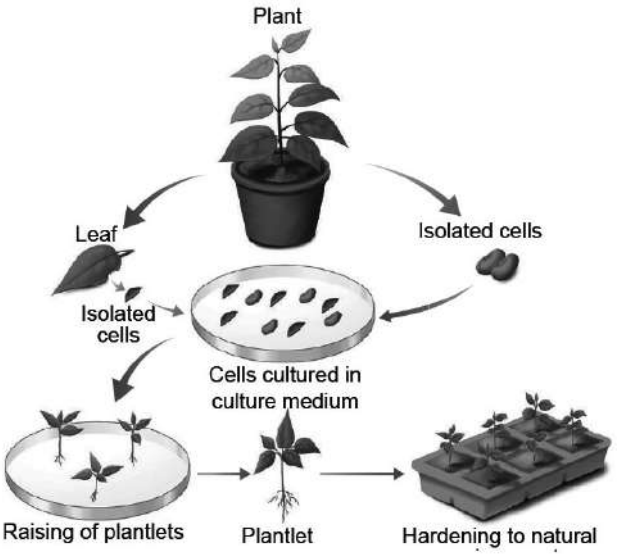
Basic concepts of plant tissue culture are totipotency, differentiation, dedifferentiation and redifferentiation.

- **Totipotency**
- The property of live plant cells that they have the genetic potential when cultured in nutrient medium to give rise to a complete individual plant.
- **Differentiation**
- The process of biochemical and structural changes by which cells become specialized in form and function
- (Meristematic tissue into mature cells i.e simple or complex tissue).
- **Dedifferentiation**
- The reversion of mature cells to the meristematic state leading to the formation of callus is called dedifferentiation.
- (Mature cells into the meristematic i.e formation of callus)
- **Redifferentiation**
- The component cells of callus have the ability to form a whole plant in a nutrient medium is called
- redifferentiation.(Callus into whole plant)

17. Based on the material used, how will you classify the culture technology? Explain it.

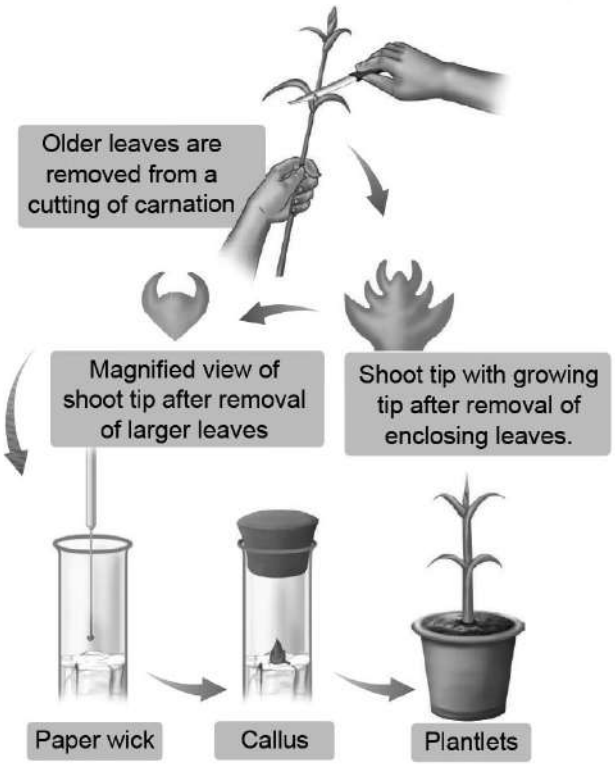
Based on the explants the culture technology types are

- 1.Organ culture
- 2.Meristem culture
- 3.Protoplast culture i).Isolation ii).Fusion iii). Culture iv).Selection of somatic hybrid cells
- 4.Cell culture.



- **1.Organ culture:**
- The culture of embryos, anthers, ovaries, roots, shoots or other organs of plants on culture media is called organ culture.
- **2.Meristem Culture:**
- The culture of any plant meristematic tissue on culture media is called meristem culture.
- **3.Protoplast Culture:**
- Protoplasts are cells without a cell wall but bounded by a plasma membrane.
- Using protoplasts, regenerate whole plants from single cells and also develop somatic hybrids.

The steps involved in protoplast culture.



✓ **Isolation of protoplast:**

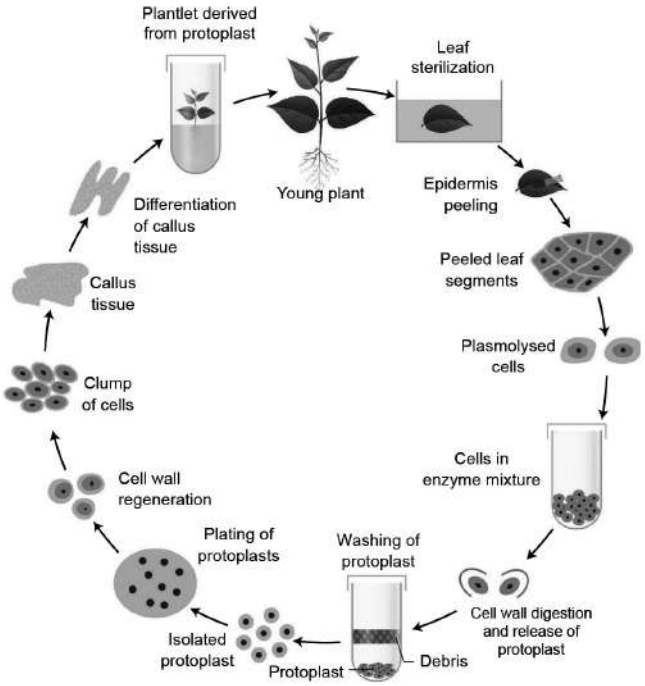
- The leaf tissue are used for isolation of protoplast.
- The leaf tissue is immersed in 0.5% Macrozyme and 2% Onozuka cellulase enzymes dissolved in 13% sorbitol or mannitol at pH 5.4.
- It is then incubated over-night at 25°C.
- After, protoplasts are obtained.
- These are then transferred to 20% sucrose solution to retain their viability.
- They are then centrifuged to get pure protoplasts.

✓ **ii. Fusion of protoplast:**

- It is done through a suitable fusogen of PEG (Polyethylene Glycol).
- The isolated protoplast are incubated in 25 to 30% concentration of PEG with Ca++ ions.
- The protoplast shows agglutination (the formation of clumps of cells) and fusion.

✓ **iii. Culture of protoplast:**

- MS liquid medium is used with some modification in droplet, plating or micro-drop array techniques.
- Protoplast viability is tested with fluorescein diacetate before the culture.
- The cultures are incubated in continuous light 1000-2000 lux at 25°C.
- The cell wall formation occurs within 24-48 hours.
- The first division of new cells occurs between 2-7 days of culture.



Protoplast Culture

✓ **iv. Selection of somatic hybrid cells:**

- The fusion product of protoplasts without nucleus of different cells is called a cybrid.
- Following this nuclear fusion happen. This process is called somatic hybridization.

- **4. Cell Suspension Culture**

- The culture of single cells or aggregates of cells *in vitro* in liquid medium is known as cell suspension culture.
- The cell suspension is prepared by transferring a portion of callus to the liquid medium and agitated using rotary shaker instrument.
- The cells are separated from the callus tissue and used for cell suspension culture.

18. Give an account on Cryopreservation.

- The protoplasts, cells, tissues, organelles, organs, extracellular matrix, enzymes or any other biological materials are subjected to

preservation by cooling to very low temperature of –196°C using liquid nitrogen are called cryopreservation.

- It also known as Cryo-conservation.
- At this extreme low temperature any enzymatic or chemical activity of the biological material will be totally stopped. This leads to preservation of material in dormant status.
- Later these materials can be activated by bringing to room temperature slowly for any experimental work.
- Protective agents like dimethyl sulphoxide, glycerol or sucrose are added before cryopreservation process.
- Protective agents are protect the cells, or tissues from the stress of freezing temperature.

19. What do you know about Germplasm conservation. Describe it.

- The conservation of living genetic resources like pollen, seeds or tissue of plant material are called germplasm conservation.
- It maintained for the purpose of selective plant breeding, preservation in live condition.
- It is used for many research works.
- It is a part of collection of seeds and pollen that are stored in seed or pollen banks.
- So as to maintain their viability and fertility for any later use. Example: Hybridization and crop improvement.
- It may also involve a gene bank, DNA bank of elite breeding lines of plant resources for the maintenance of biological diversity and also for food security.

20. Write the protocol for artificial seed preparation.

- Artificial seeds or synthetic seeds (synseeds) are produced by using embryoids (somatic embryos).
- This is obtained through in vitro culture.
- They may even be derived from single cells from any part of the plant.
- Later divide to form cell mass containing dense cytoplasm, large nucleus, starch grains, proteins, and oils etc.,
- To prepare the artificial seeds different inert materials are used for coating the somatic embryoids like agrose and sodium alginate.

PART – A

ADDITIONAL QUESTIONS (1 MARK)

1. Who is the father of tissue culture?

a) hildbrandt b) haberlandt
c) melchers d) gautheret **Ans: b**

2. The production of secondary metabolites require the use of

a) artificial seed b) cell suspension
c) meristem culture d) auxillary buds **Ans: b**

3. Artificial seed is produced by encapsulating somatic embryoids with

a) sodium chloride b) sodium alginate
c) sodium acetate d) sodium nitrate **Ans: b**

4. Which of the hormone pair required for a callus to differentiate are

a) auxin and cytokinin
b) gibberllin and ethylene
c) auxin and absiccic acid
d) cytokinins and gibberllin **Ans: a**

5. Dimethyl sulfoxide is used as

a) fusogenic agent b) alkaylating agent
c) cellulycing agent d) cryoprotectant **Ans: d**

6. The most widely used chemical for protoplast fusion, as fusogens, is

a) manitol b) sorbitol
c) poly ethane glycine- d) Poly ethylene glycol (PEG) **Ans: d**

7. Cybrids are produced by

a) fusion of two different nuclei from two different species
b) fusion of two same nuclei from same species
c) fusion of cytoplasm from both the parent species
d) none of the above **Ans: c**

8. Callus is defined as

a) tissue that forms embryo
b) an insoluble carbohydrate
c) tissue that grows to form embryoid
d) un organised actively dividing mass of cells maintained in cultured **Ans: d**

9. The tissue of plant used for culturing is called
a) scion b) explant
c) stock d) callus **Ans: b**

11. A medium which is composed of chemically defined compound is called
a) natural media b) synthetic media
c) artificial media d) none of these **Ans: b**

12. To obtain haploid plant, we culture
a) entire anther b) nucleus
c) embryo d) apical bud **Ans: a**

13. Somaclonal variations are the ones
a) caused by mutagens
b) produce during tissue culture
c) caused by gamma rays
d) induced during sexual embryogeny **Ans: b**

14. Which of the following plant cell will show totipotency?
a) xylem vessels b) sieve tube
c) meristem d) cork cells **Ans: c**

15. Plant tissue culture technique is a redefined method of _____
a) hydridization
b) vegetative propagation
c) asexual reproduction
d) selection **Ans: b**

16. Polyethylene glycol is
a) fusogenic chemical
b) electrofusion stimulant
c) callus stimulant
d) differentiation stimulant **Ans: a**

17. Somatic hybridization is achieved through
a) grafting
b) protoplast fusion
c) conjugation
d) recombinant DNA technology **Ans: b**

19. The enzymes required to obtain wall-free / naked protoplasts are
a) cellulase and proteinase
b) cellulase and pectinase
c) cellulase and amylase
d) amylase and pectinase **Ans: b**

20. The concept of invitro cell culture was developed by
a) kotte and Robins b) haberlandt
c) hanning d) knop **Ans: b**

21. The first report of developed in vitro production of haploid embryos from anthers of Datura by
a) nitch
b) guha and maheswary
c) maheswary
d) bourgin and nitch **Ans: b**

22. Totipotency means
a) flowering in culture medium
b) development of fruit from a flower in a culture
c) development of an organ from a cell in culture medium
d) all of these **Ans: c**

23. Haploid plants can be obtained from
a) bud culture b) leaf culture
c) root culture d) anther culture **Ans: d**

24. In plant tissue culture, which of the following shows totipotency?
a) meristem b) sieve tube
c) xylem vessel d) collenchyma **Ans: a**

25. In plant tissue culture, the callus tissues can be regenerated into complete plantlets primarily by altering the concentration of
a) sugars b) vitamins
c) amino acids d) hormones **Ans: d**

26. The final stage in the tissue culture programme before the new plants are taken out for cultivation in the fields is known as
a) hardening b) Sterilisation
c) embryo culture d) induction of callus **Ans: a**

27. A single explant can be multiplied into several thousand plants in short period by
a) Genetic engineering b) Tissue culture
c) Somatic hybridization d) all of these **Ans: b**

28. Haberlandt is a first person to culture plant cells in artificial condition using the mesophyll cells of
a) *Lantana camara* b) *Catharanthus roseus*
c) *Lamium Purpureum* d) *Digitalis purpurea* **Ans: c**

29. Coconut water in plant tissue culture work and obtained cell proliferation from carrot explants by

a) F. C. Steward b) Mavel and Martin
c) Kanta el al d) Yamada el at **Ans: a**

30. The scientist who produced test tube fertilization in flowering plants is

a) F. C. Steward b) Mavel and Martin
c) Kanta el al d) Yamada el at **Ans: d**

31. Who proposed the concept of totipotency,

a) P. R. White b) Haberlandt
c) Takebe et al d) Carlson **Ans: b**

32. The Knop's Nutrient solution used in growth experiments of plants which contains

a) Calcium nitrate b) Potassium nitrate
c) Magnesium Sulphate d) all of these **Ans: d**

33. The component cells of callus have the ability to form a whole plant by

a) Differentiation b) Redifferentiation
c) Dedifferentiation d) Totipotency **Ans: b**

34. Growing the explant inoculated into culture tubes at

a) 22 - 28ºC b) 22 - 26ºC
c) 23 - 28ºC d) 24 - 28ºC **Ans: a**

35. The chemicals used for the sterilization process is

a) Mercuric chloride b) Calcium chloride
c) Ethanol d) both a) and b)**Ans: d**

36. Which of the culturing medium is commonly used?

a) MS medium b) B5 medium
c) White medium d) Nitsch's medium
Ans: a

37. Agar, the solidification gelling agent is

a) Red algae b) Brown algae
c) Green algae d) Blue-Green algae
Ans: a

38. The ph of culture condition is normally

a) 5.6 to 6.6 b) 5 to 6.0
c) 5.6 to 6.0 d) 5 to 6.6 **Ans: c**

39. The cultures require 50-60% relative humidity and the light intensity is

a) 1000 lux b) 1100 lux
c) 1200 lux d) 1300 lux **Ans: a**

40. The growth hormone present in the murashige and skoog culturing medium is

a) auxin b) gibbrallin
c) kinetin d) ethylene **Ans: c**

41. During Protoplast Culture, the isolated Protoplast are incubated in 25 to 30% concentration of

a) PEG with Na++ ions b) PEG with Ca++ ions
c) PEG with Mg++ ions d) None of these **Ans: b**

42. The fusion product of protoplasts without nucleus of different cells is called

a) hybrid b) cybrid
c) both a) and b) d) none of these **Ans: b**

43. i. The cultures are incubated in continuous light 1000 – 2000 lux at 25ºC

ii. Secondary metabolites are required for normal growth

iii. The culture of single cell in vitro in liquid medium is called suspension culture

iv. Bio synthesis and isolation of indole alkaloids from *Catharanthus roseus*.

a) i.,ii and iii. are correct
b) i. and ii. only correct
c) i., iii and iv. are correct
d) ii.,iii and iv. are correct **Ans: b**

44. i. Cell suspension culture can be useful for the production of secondary metabolites

ii. *Digitalis purpurea* produce Digoxin

iii. Plants can be regenerated by Somatic embryogenesis

iv. Formation of the shoot is called rhizogenesis

a) i.,ii. and iii. only correct
b) ii.,iii. and iv. only correct
c) iii., iv. and i. only correct
d) all are correct **Ans: a**

45.

Column A		Column B	
A.	Digoxin	i.	Cardiac tonic
B.	Codeina	ii.	Anti-Carcinogenic
C.	Capsaicin	iii.	Analgesic
D.	Vincristine	iv.	Rheumatic pain treatment

	A	B	C	D
a	i.	iii.	iv.	ii.
b	i.	iii.	ii.	iv.
c	i.	iv.	iii.	ii.
d	i.	ii.	iii.	iv.

Ans: a

46. Somatic embryogenesis is now reported in

a) *Allium sativum* b) *Oryza sativa*
c) *Zea mays* d) all of these **Ans: d**

47. Synthetic seeds are produced by encapsulation of embryoids in

a) agarose gel b) calcium alginate
c) both a) and b) d) none of these **Ans: c**

48. The term organogenesis means

a) formation of shoot and root
b) formation of root
c) formation of shoot
d) none of these **Ans: a**

49. How many days will take induction of shoots in micropropagation protocol for banana

a) 165 days b) 166 days
c) 167 days d) 168 days **Ans: d**

50. Virus free plants are produced from

a) Chemical methods
b) Artificial seeds
c) Shoot meristem cultures
d) all of these **Ans: c**

51. The Cryoprotectant agent is (are)

a) dimethyl sulphoxide b) glycerol
c) sucrose d) all of these **Ans: d**

52. Which has the power to permit the use of Genetically Modified Organisms.

a) GEAC b) RCGM
c) ELSI d) IBSC **Ans: a**

PART – B

ADDITIONAL QUESTIONS (2 MARK)

1. Write the the main applications of Plant tissue culture

The main applications of Plant tissue culture are clonal propagation of elite varieties, conservation of endangered plants, production of virus-free plants, germplasm preservation, industrial production of secondary metabolites. etc.,

2. What is Totipotency ?

The development of whole plant from isolated cells or tissue in in vitro condition is called Totipotency. This is unique to plant cells.

3. Define the term *'in vitro'*

The plant cells cultured in artificial condition in culture medium called in vitro (inside glass)

4. What is callus ?

During tissue culture , the unorganized growth of cells and tissue are called callus

5. Describe the Knop's solution

Nutrient solution used in growth experiments of plants which contains: Calcium nitrate 3.0 g Magnesium sulfate 1.0 g Potassium nitrate 1.0 g Dibasic Potassium phosphate 1.0 g Sucrose 50.0 g (optimal) Deionized water 1000.0 ml

6. What are the basic concepts of tissue culture

Basic concepts of plant tissue culture are totipotency, differentiation, dedifferentiation and redifferentiation.

7. Describe totipotency

The property of live plant cells that they have the genetic potential when cultured in nutrient medium to give rise to a complete individual plant.

8. Describe differentiation

The process of biochemical and structural changes by which cells become specialized in form and function (Meristematic tissue into mature cells i.e simple or complex tissue).

9. Describe dedifferentiation

The reversion of mature cells to the meristematic state leading to the formation of callus is called dedifferentiation.

(Mature cells into the meristematic i.e formation of callus)

10. Describe redifferentiation

The component cells of callus have the ability to form a whole plant in a nutrient medium is called redifferentiation. (Callus into whole plant)

11. What is meant by plant tissue culture (PTC)

The *in vitro* and aseptic growth of any plant part on a tissue culture medium is called plant tissue culture (PTC)

12. What is meant by explant?

The tissue taken from a selected plant transferred to a culture medium often to establish a new plant.

13. What is Sterilization?

The technique employed to get rid of microbes such as bacteria and fungi in the culture medium, vessels and explants are called Sterilization.

14. What is Agar?

A complex mucilaginous polysaccharide obtained from marine algae (sea weeds) used as solidifying agent in media preparation are called agar.

15. Write composition of MS (Murashige and Skoog) Medium

The composition of MS (Murashige and Skoog) medium are macronutrients, micronutrients, minor nutrient, iron stock, vitamins, growth hormones and solidifying agent

16. Write the iron stock found in MS (Murashige and Skoog) medium

- Na EDTA 37.25 mg/l
- Ferrous Sulphate ($FeSO_4$ $7H_2O$) 27.85 mg/l

17. Write the solidifying agent found in MS (Murashige and Skoog) medium

- Agar 8.0 g/l

18. What is embryogenesis ?

- The callus cells undergoes differentiation and produces somatic embryos, known as Embryoids.
- The embryoids are sub-cultured to produce plantlets.

19. Describe hardening ?

- The plantlets developed *in vitro* are transferred to greenhouse or hardening chamber.
- Then the plantlets expose to normal environmental conditions.

20. What is meant by hardening ?

The gradual exposure of *in vitro* plantlets in humid chambers then grow under normal field conditions are called hardening

21. What is inoculation?

Transferring the explants to sterile glass tube containing nutrient medium is called inoculation.

22. What is callus ?

Callus is a mass of unorganized growth of plant cells or tissues in *in vitro* culture medium

23. What is known s as embryoids|?

The callus cells undergoes differentiation and produces somatic embryos, known as Embryoids.

Write the types of plant tissue cultures based on the explants

Based on the explants plant tissue culture types are

- Organ culture
- Meristem culture
- Protoplast culture
- Cell culture.

24. What is organ culture?

- The culture of embryos, anthers, ovaries, roots, shoots or other organs of plants on culture media is called organ culture.

25. What is meristem culture?

- The culture of any plant meristematic tissue on culture media is called meristem culture.

26. What is Protoplast culture?

- Protoplasts are cells without a cell wall but bounded by a plasma membrane.
- Using protoplasts, regenerate whole plants from single cells and also develop somatic hybrids.

27. What is cybrid ?

The fusion product of protoplasts without nucleus of different cells is called a cybrid.

28. State the term somatic hybridization

The fusion of protoplasts without nucleus are followed by nuclear fusion.

This process is called somatic hybridization.

29. What is meant by cell suspension culture ?

The culture of single cells or aggregates of cells *in vitro* in liquid medium is known as cell suspension culture.

30. What is meant by plant regeneration pathway

From the explants, plants can be regenerated by somatic embryogenesis or organogenesis are called Plant regeneration pathway.

31. Define caulogenesis

The differentiation of shoots from callus is called caulogenesis.

32. Define rhizogenesis

The differentiation of roots from callus is called rhizogenesis.

33. Define synseeds

synthetic seeds are called synseeds.

34. Where is somaclonal variations are found ?

Somatic variations found in plants regenerated in vitro i.e. variations found in leaf, stem, root, tuber or propagule.

35. Where is gametoclonal variations are found ?

Gametophytic variations found in plants regenerated in vitro gametic origin i.e. variations found in gametes and gametophytes.

36. What is meant by germplasm conservation ?

The conservation of living genetic resources like pollen, seeds or tissue of plant material are called germplasm conservation.

37. What is called cryoprotectants ?

Before cryopreservation process, the protective agents like dimethyl sulphoxide, glycerol or sucrose are added.

These protective agents are called cryoprotectants

38. Shoot meristem tip culture is the method to produce virus-free plants. Why ?

Shoot meristem tip culture is the method to produce virus-free plants, because the shoot meristem tip is always free from viruses.

39. State the term biosafety

The prevention of large-scale loss of biological integrity, focusing both on ecology and human health are called Biosafety

40. What is bioethics

The study of ethical issues emerging from advances in biology and medicine is called bioethics.

41. How is IPR protected?

The IPR is protected by different ways like patents, copyrights, trade secrets and trademarks, designs and geographical indications.

PART – C

ADDITIONAL QUESTIONS (3 MARK)

1. Write the definitions of Tissue Culture

- Growing plant protoplasts, cells, tissues or organs away from their natural or normal environment, under artificial condition, is known as tissue Culture.
- It is also known as *in vitro* growth of plant protoplasts, cells, tissues and organs (*In vitro* is a Latin word, it means that - in glass or in test-tube).

2. Describe the botanist Gottlieb Haberlandt (1902)

- Gottlieb Haberlandt (1902) is the the German Botanist.
- He proposed the concept Totipotency .
- He was the first person to culture plant cells in artificial condition.
- He using the mesophyll cells of *Lamium purpureum* in culture medium and obtained cell proliferation.
- He is regarded as the father of tissue culture.

3. State the three fundamental principles of plant tissue culture

- The plant part (explant) must be selected and isolated from the rest of plant body.
- The explant must be maintained in controlled physically and chemically defined conditions.
- Aseptic condition must be maintained, in the laboratory.

4. List the technique involved in PTC

1.Sterilization

2.Media Preparation

3.Culture condition

4.Induction of Callus

5.Embryogenesis

6.Hardening

5. What do you know about the media preparation for the success of tissue culture

The success of tissue culture contains

- In the composition of the growth medium,
- Plant growth regulators and
- Culture conditions such as temperature, pH, light and humidity.

6. Name the different culture media used for plant tissue culture

- MS nutrient medium(Murashige and Skoog medium1962)
- B5 medium (Gamborg medium1968)
- White medium (white medium1943)
- Nitsch's medium (Nitsch medium1969)

7. What do you know about culture media for plant tissue culture

- No single medium is capable. Suitable nutrient medium is prepared and used.
- It has nutrients, with suitable vitamins and hormones.
- A medium may be solid or semisolid or liquid.
- For solidification, a gelling agent such as agar is added.

8. Write the macronutrients found in MS (Murashige and Skoog) medium

- Ammonium nitrate (NH4NO3) 1650.0 mg/l
- Potassium nitrate (KNO3) 1900.0 mg/l
- Calcium chloride (CaCl2 2H2O) 440.0 mg/l
- Magnesium sulphate (MgSO4 6H2O) 370.0 mg/l
- Potassium dihydrogen phosphate (KH2PO4) 170.0 mg/l

9. Write the micronutrients found in MS (Murashige and Skoog) medium

- Manganese sulphate (MnSO4 4H2O) 22.3 mg/l
- Zinc sulphate (ZnSO4 4H2O) 8.6 mg/l
- Boric acid (H3BO3) 6.2 mg/l
- Potassium iodide (KI) 0.83 mg/l

10. Write the minor nutrient found in MS (Murashige and Skoog) medium

- Sodium molybdate (Na2 MO4 2H2O) 0.250 mg/l
- Cupric sulphate (CuSO4 5H2O) 0.025 mg/l
- Cobaltous chloride (CoCl2 6H2O) 0.025 mg/l

11. Write the vitamins found in MS (Murashige and Skoog) medium

- Glycine 2.0 mg/l
- Nicotinic acid 0.5 mg/l
- Pyridoxin HCl 0.5 mg/l
- Thaiamine HCl 0.1 mg/l

12. Write the growth hormones found in MS (Murashige and Skoog) medium

- IAA 1.30 mg/l Kinetin 0.4–10.0 mg/l
- Myo-inositol 100.0 mg/l
- Sucrose 30.0 g/l

13. Describe the induction of callus

- Explant of 1-2 cm sterile segment selected from leaf, stem, tuber or root.
- The explant is inoculated in the MS nutrient medium supplemented with auxins.
- It should be incubated at 25°C ± 2°C in an alternate light and dark period of 12 hours to induce cell division.
- soon after, the upper surface of explant develops into callus.

14. Write the steps involved in fusion of protoplast in protoplast culture.

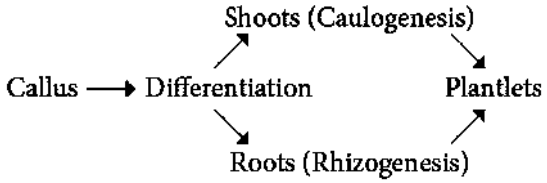
- It is done through a suitable fusogen of PEG (Polyethylene Glycol).
- The isolated protoplast are incubated in 25 to 30% concentration of PEG with Ca++ ions.
- The protoplast shows agglutination (the formation of clumps of cells) and fusion.

15. Describe cell suspension culture

- The culture of single cells or aggregates of cells *in vitro* in liquid medium is known as cell suspension culture.
- The cell suspension is prepared by transferring a portion of callus to the liquid medium and agitated using rotary shaker instrument.
- The cells are separated from the callus tissue and used for cell suspension culture.

16. Describe organogenesis

The morphological changes occur in the callus leading to the formation of shoot and roots is called organogenesis.



- Organogenesis can be induced *in vitro* by introducing plant growth regulators in the MS medium.
- Auxin and cytokinins induce shoot and root formation.

17. What is meant by cryopreservation ?

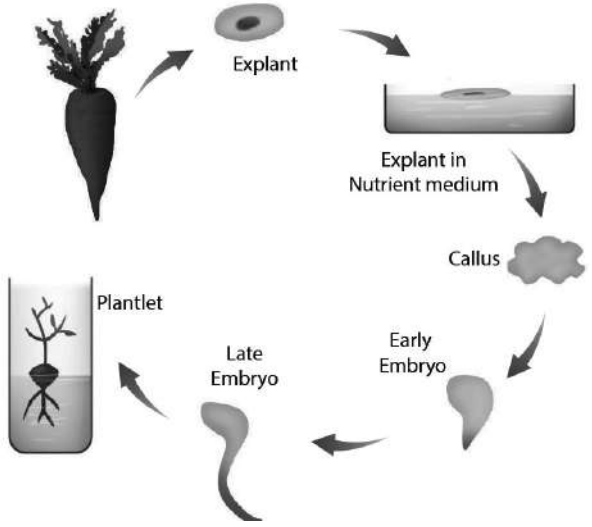
The protoplasts, cells, tissues, organelles, organs, extracellular matrix, enzymes or any other biological materials are subjected to preservation by cooling to very low temperature of –196°C using liquid nitrogen are called cryopreservation.

18. The scope of bioethics

The scope of bioethics is directly related to biotechnology, including cloning, gene therapy, life extension, human genetic engineering, astroethics life in space, and manipulation of basic biology through altered DNA, RNA and proteins.

19. Draw the diagrammatic representation of IPR is protected by different ways.

20. Observe the given diagram.Write the name of the process and define



- The name of this process is **Totipotency**.
- The development of whole plant from isolated cells or tissue in in vitro condition is called Totipotency.
- This is unique to plant cells.

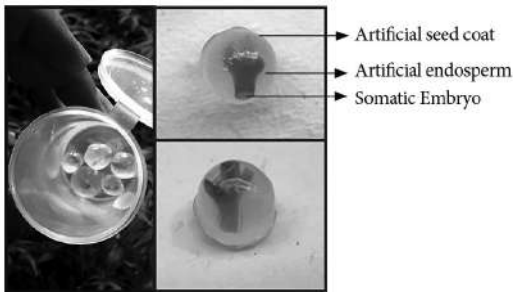
21. Observe the given diagram.Write the name of the process and describe

- The name of this process is **Embryogenesis**
- The callus cells undergoes differentiation and produces somatic embryos, known as **Embryoids**.
- The embryoids are sub-cultured to produce plantlets.

22. Observe the given diagram.Write the name of the process and their importance

- The name of this process is **Micropropagation of Banana**
- Micropropagation of plants at industrial level maintains high standards of homogeneity

23. Observe the given diagram is artificial seeds. Label the parts a, b and c

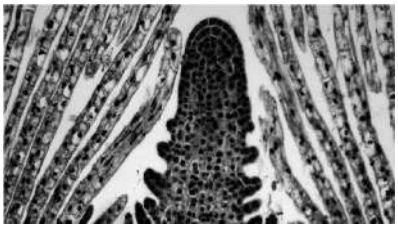


a. Artificial seed coat

b. Artificial endosperm

c. Somatic Embryo

24. Observe the given diagram.Write the name of the process and their importance



- The name of this process is **Shoot meristem tip culture**
- It is the method to produce virus-free plants, because the shoot meristem tip is always free from viruses.

PART – D

ADDITIONAL QUESTIONS (5 MARK)

1. List out the milestones in plant tissue culture

- **H aberlandt** (1902) - proposed *-in vitro* (inside glass) in Knop's salt solution - developed callus - the concept Totipotency
- **P.R.White** (1934) - developed root cultures, used Knop's solution with three vitamins like pyridoxine, thiamine and nicotinic acid
- **F.C.Steward** (1948) used coconut water in plant tissue culture and obtained cell proliferation from carrot explants (Cellular totipotency).
- **Morel** and **Martin** (1952, 1955) developed virus-free *Dahlia* and potato plants using shoot meristem culture. **Murashige** and **Skoog** (1962) formulated tissue culture medium, a land mark in plant tissue culture
- **Kanta** *et al.* (1962) produced test-tube fertilization in flowering plants.
- **Yamada** *et al*. (1963) produced *calli* and free cells in tissue culture of *Tradescantia reflexa.*
- **Guha** and **Maheshwari** (1964) developed *in vitro* production of haploid embryos from anthers of *Datura*.
- **Vasil** and **Hildbrandt** (1965) achieved differentiation of tobacco plants from single, isolated cells in micro propagation.
- **Takebe** *et al*. (1971) regenerated tobacco plants from isolated mesophyll protoplasts.
- **Carlson** and co-workers obtained protoplast fusion between *Nicotiana glauca* and *Nicotiana longsdorffii* and developed first interspecific somatic hybrid in 1971.
- **Melchers** and co-workers in 1978 developed intergenic hybrid between potato and tomato called pomato.
- **Chilton** (1983) produced transformed tobacco plants from single cell transformation and gene insertion.
- **Horsh** *et al*. (1984) developed transgenic tobacco by Agrobacterium mediated gene transfer.

2. What are the laboratory facilities for plant tissue culture PTC ?

For PTC, the laboratory must have the following facilities:

- Washing facility for glassware and ovens for drying glassware.
- Medium preparation room with autoclave, electronic balance and pH meter.
- Transfer area sterile room with laminar air-flow bench.
- A positive pressure ventilation unit called High Efficiency Particulate Air (HEPA) filter to maintain aseptic condition.

Culture facility:

- Growing the explant inoculated into culture tubes at 22-28° C.
- Light illumination is 2400 lux,

- The photoperiod of 8-16 hours and a relative humidity of about 60%.

3. Explain different types of sterilization methods used during the plant tissue culture.

i. Maintenance of Aseptic Environment:

- Sterilization of glassware, forceps, scalpels,
- All accessories in wet steam sterilization by autoclaving at 15 psi (121°C) for 15 to 30 minutes.
- Otherwise dipping in 70% ethanol followed by flaming and cooling.

ii. Sterilization of culture room:

- Floor and walls are washed first with detergent.
- Then washed with 2% sodium hypochlorite or 95% ethanol.
- The cabinet of laminar airflow is sterilized by 95% ethanol
- Then exposure of UV radiation for 15 minutes.

iii. Sterilization of Nutrient Media:

- Culture media are dispensed in glass containers, plugged with non-absorbent cotton or sealed with plastic closures.
- Then sterilized using autoclave at 15 psi (121°C) for 15 to 30 minutes.
- The plant extracts, vitamins, amino acids and hormones are sterilized by passing through Millipore filter with 0.2 mm pore diameter.
- Then added to sterilized culture medium inside Laminar Airflow Chamber under sterile condition.

iv. Sterilization of Explants:

- The plant materials surface sterilized in running tap water.
- Then treating with agents like 0.1% mercuric chloride, 70% ethanol.
- It should be under aseptic condition inside the Laminar Air Flow Chamber.

4. What are the culture condition need for the plant tissue culture? Explain them.

The culture condition need for the plant tissue culture pH, temperature, humidity , light intensity and aeration

- **pH**
- The pH of medium is adjusted between 5.6 to 6.0 for the best result.
- **Temperature**
- The normal constant temperature is 25°C ± 2°C for optimal growth.
- **Humidity and Light Intensity**
- Require 50-60% relative humidity.
- 16 hours of photoperiod by the illumination of cool white fluorescent tubes with 1000 lux.
- **Aeration**
- Aeration to the culture can be provided by
- The flasks or tubes of liquid culture on automatic shaker provide aeration.
- Otherwise aeration of the medium by passing with filter-sterilized air.

5. Write the steps involved in isolate of protoplast in protoplast culture.

- The leaf tissue are used for isolation of protoplast.
- The leaf tissue is immersed in 0.5% Macrozyme and 2% Onozuka cellulase enzymes dissolved in 13% sorbitol or mannitol at pH 5.4.
- It is then incubated over-night at 25°C.
- After, protoplasts are obtained.
- These are then transferred to 20% sucrose solution to retain their viability.
- They are then centrifuged to get pure protoplasts.

6. Write the steps involved in culture of protoplast in protoplast culture

- MS liquid medium is used with some modification in droplet, plating or micro-drop array techniques.
- Protoplast viability is tested with fluorescein diacetate before the culture.
- The cultures are incubated in continuous light 1000-2000 lux at 25°C.
- The cell wall formation occurs within 24-48 hours.
- The first division of new cells occurs between 2-7 days of culture.

7. Secondary metabolites are not required by the plant for growth and development. Justify your Answer.

- Cell suspension culture can be useful for the production of secondary metabolites.
- Example: Alkaloids, flavonoids, terpenoids, phenolic compounds and recombinant proteins.
- Secondary metabolites are not required by the plant for growth and development.
- but they are produced in the plant as 'byproducts' of cell metabolism.
- Example: Biosynthesis and isolation of indole alkaloids from *Catharanthus roseus* plant cell culture.
- It can be scaled up and automated using bio-reactors for commercial production.
- Many strategies such as biotransformation, elicitation and immobilization have been used to make cell suspension cultures more efficient in the production of secondary metabolites.

8. Tabulate the examples,plant sources and uses of secondary metabolites

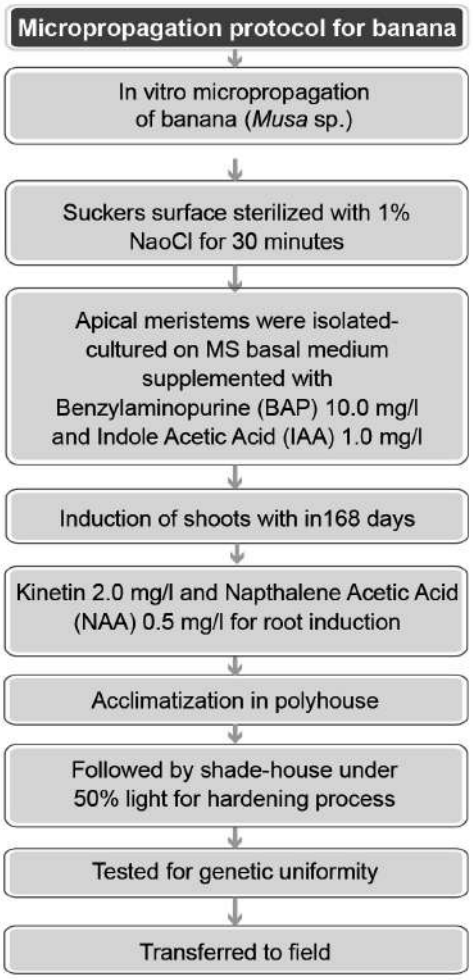
Secondary metabolites	Plant source	Uses
Digoxin	*Digitalis purpuria*	Cardiac tonic
Codeine	*Papaver sominiferum*	Analgesic
Capsaicin	*Capsicum annum*	Rheumatic pain treatment
Vincristine	*Catharanthus roseus*	Anti-carcinogenic
Quinine	*Cinchona officinalis*	Antimalarial

9. Write the applications of Plant tissue culture

Plant tissue culture techniques have several applications such as:

- Improved hybrids production through somatic hybridization.
- Somatic embryoids produce synthetic seeds (synseeds). It helps in conservation of plant biodiversity.
- Production of disease resistant plants through meristem and shoot tip culture.
- Production of stress resistant plants like herbicide tolerant, heat tolerant plants.
- Micropropagation technique to obtain both crop and tree species.
- It is useful in forestry within a short span of time and all through the year.
- Production of secondary metabolites from cell culture utilized in pharmaceutical, cosmetic and food industries.

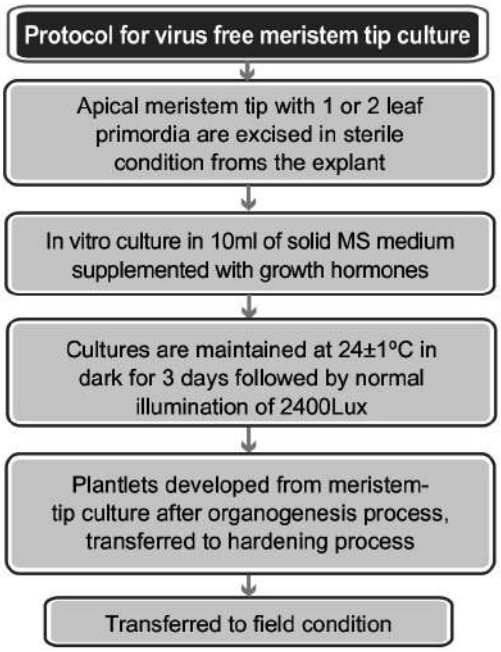
10. Write the schematic diagrammatic representation of micro propagation protocol for Banana.



11. Enumerate the advantages of artificial seeds

- Artificial seeds have many advantages over the true seeds
- Millions of artificial seeds can be produced at any time at low cost.
- They provide an easy method to produce genetically engineered plants with desirable traits.
- It is easy to test the genotype of plants.
- They can potentially store for long time under cryopreservation method.
- Artificial seeds produce identical plants.
- The period of dormancy is greatly reduced and growth is faster with a shortened life cycle.

12. Write the schematic diagrammatic representation of micro propagation protocol for virus-free meristem tip culture.



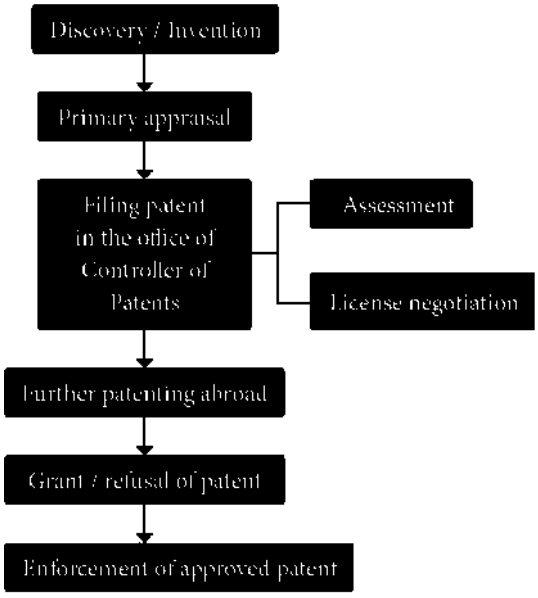
13. Explain the Intellectual Property Right (IPR)

- IPR is a category of property that includes intangible creation of the human intellect, and primarily consists of copyrights, patents, and trademarks.
- It also includes other types of rights, such as trade secrets, publicity rights, moral rights, and rights against unfair competition.
- In biotechnology, the transformed microorganisms and plants and technologies for the production of commercial products are exclusively the property of the discoverer.
- The discoverer has the full rights on his property.
- It should not be neglected by the others without legal permission.
- The right of discoverer must be protected and it does by certain laws framed by a country.
- The IPR is protected by different ways like patents, copyrights, trade secrets and trademarks, designs and geographical indications.

14. Explain the term patents

- It is a special right to the discoverer/inventor that has been granted by the government through legislation for trading new articles.
- A patent is a personal property which can be licensed or sold by the person or organisation just like any other property.
- Patent terms give the inventor the rights to exclude others from making, using or selling his invention.
- It is difficult to keep secret certain inventions and therefore, guidance should be obtained from a qualified patent attorney.
- A patent consists of three parts: the grant, specifications and claims.
- **The grant**
- It is filled at the patent office which is not published.
- It is a signed document, actually the agreement that grants patent right to the inventor.
- **The specification**
- The specification and claims are published as a single document which is made public from the patent office.
- The specification part is narrative in which the subject matter of invention is described as how the invention was carried out.
- **The claim**
- It is specifically defines the scope of the invention to be protected by the patent which the others may not practice.

15. Give the flow chart of general steps in patenting



16. Explain biosafety

- The prevention of large-scale loss of biological integrity, focusing both on ecology and human health are called Biosafety.
- These prevention mechanisms include conduction of regular reviews of the biosafety in laboratory settings, as well as strict guidelines to follow.
- Biosafety is used to protect from harmful incidents.
- Many laboratories handling biohazards employ an ongoing risk management assessment and enforcement process for biosafety.
- Failures to follow such protocols can lead to increased risk of exposure to biohazards or pathogens.
- Human error and poor techniques contribute to unnecessary exposure to hazards and compromise the best safeguards set into place for protection.

17. Enumerate the potential risks and consideration for safety aspects

- Pathogenicity of living organisms and viruses - natural and genetically modified - to infect humans, animals and plants to cause diseases.
- Toxicity of allergy associated with microbial production.
- Increasing number of antibiotic resistant pathogenic microorganisms.
- Problems associated with the disposal of spent microbial biomass and purification of effluent from biotechnological process.
- Safety aspects associated with contamination, infection or mutation of process strains.
- Safety aspects associated with the industrial use of microorganisms containing *in vitro* recombinants.

18. Name the organisations set for biosafety. Write their guidelines.

Biosafety guidelines are being implemented by

- Institutional Bio-safety Committees **(IBSCs**) - monitor the research activity at institutional level.
- Review Committee on Genetic Manipulation (**RCGM**) - functioning in the Department of Biotechnology (**DBT**) monitors the risky research activities in the laboratories.
- Genetic Engineering Approval Committee (**GEAC**)of Ministry of Environment and Forest - has the power to permit the use of Genetically Modified Organism (**GMO**) at commercial level and open field trials of transgenic materials including agricultural crops, industrial products and health care products.

19. Explain Bioethics

- The study of ethical issues emerging from advances in biology and medicine is called bioethics.
- It is also a moral discernment as it relates to medical policy and practice.
- Bioethicists are concerned with the ethical questions that arise in the relationships among life sciences, biotechnology and medicine.
- It includes the study of values relating to primary care and other branches of medicine.
- The scope of bioethics is directly related to biotechnology, including cloning, gene therapy, life extension, human genetic engineering, astroethics life in space, and manipulation of basic biology through altered DNA, RNA and proteins.
- These developments in biotechnology will affect future evolution, and may require new principles, such as biotic ethics, that values life and its basic biological characters and structures.

20. Describe ELSI

- The Ethical, Legal, and Social Implications (ELSI) program was founded in 1990 as an integral part of the Human Genome Project.
- The mission of the ELSI program was to identify and address issues raised by genomic research that would affect individuals, families, and society.
- A percentage of the Human Genome Project budget at the National Institutes of Health and the U.S. Department of Energy was devoted to ELSI research.

21. Describe ethical issues in genomic research

- Privacy and fairness in the use of genetic information, including the potential for genetic discrimination in employment and insurance.
- The integration of new genetic technologies, such as genetic testing, into the practice of clinical medicine.
- Ethical issues surrounding the design and conduct of genetic research with people, including the process of informed consent.

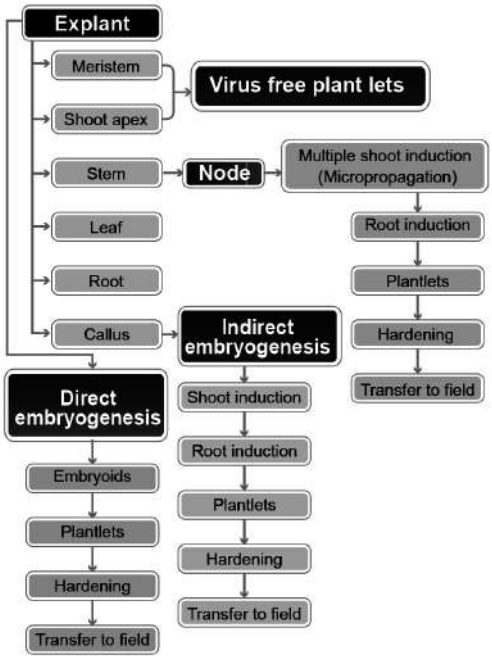
22. Write the functional role of genetic engineering appraisal committee (GEAC)

- GEAC is an apex body under Ministry of Environment, Forests and Climate change for regulating manufacturing, use, import, export and storage of hazardous microbes or genetically modified organisms (GMOs) and cells in the country.
- It was established as an apex body to accord approval of activities involving large scale use of hazardous microorganisms and recombinants in research and industrial production.
- The GEAC is also responsible for approval of proposals relating to release of genetically engineered organisms and products into the environment including experimental field trials (Biosafety Research Level trial-I and II known as BRL-I and BRL-II).

23. Biotechnological revolution-discuss.

- Biotechnology has become a comprehensive scientific venture from the point of academic and commercial angles, within a short time with the sequencing of human genome and genome of some important organisms.
- The future developments in biotechnology will be exciting.
- The development in biotechnology will lead to a new scientific revolution that would change the lives and future of people.
- Like industrial and computer revolution, biotechnological revolution will also promise major changes in many aspects of modern life.

24. Draw the flow chart of plant regeneration pathway



25. Draw the diagram representation of plant regeneration pathway