MAHARASHTRA AGRICULTURAL UNIVERSITIES EXAMINATION BOARD, PUNE
SEMESTER END THEORY EXAMINATION
B.Sc. (Hons.) Horticulture

Semester	: III (New)	Term	: I	Academic Year	: 2022-23
Course No.	: H/BIOT 231				
Credits	: 2(1+1)	Title	: Elementary Plant Biotechnology		
Day & Date	:	Time (hrs.)	: 2 hrs.	Total Marks	: 40

Note : 1. Solve ANY EIGHT questions from SECTION "A".
2. All questions from SECTION "B" are compulsory.
3. All questions carry equal marks.
4. Draw neat diagrams wherever necessary.

SECTION 'A'

Marking scheme

Q.1 Define plant biotechnology. Write down the scope and importance of plant biotechnology.

Ans: It is the controlled use of biological agents such as microorganisms of cellular components for beneficial use of human kind. 1

Scope of biotechnology. 3

1. Bioprocessing.
2. Engineering of organisms for specific indusial.
3. Genetically improvement/pharmaceuticals products.
4. Human gene theory.
5. Production of biopesticides/biofertilizer.
6. Production of monoclonal antibodies.
7. Transgenic plant.
8. Photosynthetic active plants.

Importance of biotechnology

1. Biotechnology is the third wave in biological science.
2. This discipline represents the fusion of basic and applied science.
3. The challenge to meet the incurved production of crops is now possible with biotechnology.

As the resources are limited, biotechnology is only answer to the increased population.

Q.2 What do you mean by somatic hybridization? Describe procedure for somatic hybridization.

Ans: The technique of hybrid production through the fusion of protoplast from different genetic backgrounds is known as somatic hybridization or parasexual hybridization and protoplast fusion.

The technique of somatic hybridization is of special significance for the improvement of vegetatively propagated plants such as banana, cassava, potato, sweet potato and sugarcane.

The production of somatic hybridization involve following steps:-

1. collection of explants
2. protoplast isolation
3. protoplast fusion
4. selection of Hybrid cells
5. culture of Hybrid cells

6. Regeneration of plants from hybrid tissue
7. Characterization of Hybrid and cybrid plants.

Q.3 What do you mean by QTL mapping? Enlist different methods of QTL mapping and explain any one in detail.

Ans: The gene mapping is a technique which is used to identify genes responsible for expression of specific traits. The QTL mapping refers to locating of genes that control expression of quantitative traits. 1

There are several methods of QTL mapping. 1

The important method include.

1. Single market analysis.
2. Interval mapping.
3. Composite interval mapping.
4. Multiple interval mapping and
5. Bayesian interval mapping.

(Explanation of any one method) 2

Q.4 What is micro propagation? Describe in brief stages of micro propagation and write its applications in Agriculture.

Ans: Micropropagation:

Multiplication of genetically identical copies of a cultivar by asexual reproduction is called as clonal propagation. Clonal propagation through tissue culture, popularly called as micropropagation. 1

Invitro clonal propagation is complicated process requires many steps or stages

Murashige(1978 a) proposed 4 different stages i.e. I – IV. 3

Stage : I –III - Invitro condition.
IV- Green house condition.

Debergh and Maene (1981) suggested additional stage 0 .

Stages of micropropagation:

0 – Selection and maintenance of stock plants for culture initiation.
I – Initiation and establishment of aseptic culture. (main step – explant isolation, surface sterilization and washing)
II – Multiplication of shoots or rapid somatic embryo formation using a defined culture media.
III – Germination of somatic embryo and / or rooting of regenerated shoot in vitro.
IV- Transfer of plantlet to sterilized soil for hardening under green house environment.

Applications of micropropagation:

1. It ensures pathogen free healthy status of the propagules.
2. It helps in rapid multiplication of material.
3. The material multiplied by this technique can be maintained in a small
4. place. The transportation of such propagules from one place to another is also easy.
5. The micropropagation can give results faster than conventional breeding. It is mostly used in horticulture, floriculture and forestry.

Q.5 Define molecular marker. Enlist different types of molecular marker. Write down its applications in crop improvement.

Ans: A DNA sequence used to mark or track a particular location (locus) on a

particular chromosome is called as molecular marker.

Types of molecular marker : 1

1. Restriction Fragment Length Polymorphisms (RFLPs).
2. Randomly Amplified Polymorphic DNAs (RAPDs). 3
3. Inter Simple Sequence Repeats (ISSR).
4. Simple Sequence Repeats (SSRs).
5. Amplified Fragmented Length Polymorphisms (AFLPs).
6. or any other relevant systems.

Application of molecular markers in crop improvement are as below.

1. Resistance breeding.
2. Pyramiding of major/minor genes into cultivars for development of durable resistance/multiple resistance.
3. Improvement of qualitative characters.
4. Molecular markers for hybrid vigor.
5. The MAS is especially useful for the traits that are difficult and/or expensive to evaluate, such as male fertility restoring genes for cytoplasmic male sterility.
6. Molecular markers and abiotic resistance.

Q.6 Define somatic embryogenesis. Describe factors affecting somatic embryogenesis.

Ans: The process of embryo development is called embryogenesis. Under certain exceptional conditions, the cells of angiosperm sporophyte behave like a zygote and development into embryo like structure in culture *in vitro*. Since embryo like structures derived from the sporophytic or somatic cells of plant, is known as somatic embryogenesis. 1

Factors affecting somatic embryogenesis

1. Growth regulators
2. Nitrogen source
3. Genotype of explants
4. Explant.

Q.7 What do you mean by somaclonal variation? Write its causes and applications in crop improvement.

Ans: **Somaclonal variation:** The heritable variation for quantitative and qualitative traits present in the cell cultured in vitro. 1

Causes of somaclonal variation:

1) Karyotype changes. 3
2) Changes in chromosomes structure.
3) Single gene mutations.
4) Cytoplasmic genetic changes.
5) Mitotic crossing over.

Applications of somaclonal variation:

1) Novel variants can rise and these can be agronomically used. (New breeding lines)
2) It is useful in diseases resistance.
3) It is useful in abiotic stress resistance.

e.g. in rice somaclone T-42 has been released.

Q.8 What is plant tissue culture. Describe in detail the basic requirements in plant tissue culture.

Ans: Defn: "It refers to the growth of living plant tissues in a suitable culture medium. *(in vitro)*" 1

Definition of culture medium: It is the nutrient medium which contains all essential micro and macro nutrients, carbohydrates vitamins and hormones. 3

Basic requirements of plant tissue culture are as follows:

1. Explant
2. Surface sterilization
3. Sterilization
   a. Flame sterilization b. Dry heat d. Ethanol 70%
   e. Autoclaving f. Air filters
4. Nutrient medium
5. Environmental conditions
6. Subculture

Q.9 What is genetic transformation? Enlist the various methods of gene transfer and explain any one of them.

Ans: Genetic Transformation : The process of transfer, integration and expression of transgene (foreign gene) in an organism.

There are two methods of gene transfer.

A) Indirect gene transfer :

a. Vector mediated gene transfer

b. Virus mediated gene transfer

B) Direct gene transfer

a. Micro injection

b. Macro injection

c. Electroporation

d. Particle gun method

e. Liposome-mediated transformation

f. Silicon carbide fiber-mediated transformation

g. Ultrasound-mediated DNA transformation

h. DNA transfer via pollen

i. PEG-mediated gene transfer

j. Calcium phosphate coprecipitation

Vector mediated gene transfer :

Plasmid Method : Plasmids are extra chromosomal elements which are found in the bacterial cell. Plasmids are used as cloning vectors in their genetic engineering. The soil-borne bacterium *Agrobacterium tumifaciens* is used for development of transgenic plants. The *abgrobacterium* mediated genetic transformation consists of four main steps given below.

i) Gene Cloning : A technique of genetic engineering which is used to make several identical copies of a gene is called gene cloning. Plasmids of *Agrobacterium* contain tumour producing genes (Ti genes or oncogens). The foreign gene which has to be used for genetic transformation is incorporated in place of oncogenes for cloning. Thus several identical copies of the transgenic are produced by self replication of plasmids.

ii) Genetic Transformation : The process of transfer, integration and expression of transgene (foreign gene) in the host cells is known as genetic transformation. For genetic transformation, bacteria with cloned gene are mixed (in millions) in the cell culture or protoplast culture of host plant for a day or

two.

iii) Identification of transformed cells : The third important step is identification of transformed cells. A marker gene is included with gene under consideration for easy identification of transformed cells. Generally, either an antibiotic resistant gene, such as Kanamycin resistant gene, or a herbicide resistant gene is used for identification of transformed cells.

iv) Regeneration of Transformed cells : The final step is regeneration of transformed cells or protoplasts into whole plants. The transformed cells are transferred into culture medium for regeneration into whole plant. The regeneration of cell or protoplast into whole plant is a basic requirement of *Aggrobacterium* mediated gene transfer system.

Q.10 Write short notes on (Any two)

a) **1. DNA fingerprinting :-** 2

Ans: It was invented in 1984 by Professor Sir Alec Jeffreys. DNA fingerprinting is the process of determining an individual's DNA characteristics. DNA analysis intended to identify a species, rather than an individual, is called DNA barcoding.

DNA profiling is a forensic technique in criminal investigations, comparing criminal suspects' profiles to DNA evidence so as to assess the likelihood of their involvement in the crime. It is also used in paternity testing, to establish immigration eligibility, and in genealogical and medical research. DNA profiling has also been used in the study of animal and plant populations in the fields of zoology, botany, and agriculture. To identify and protect the commercial crop and livestock types. To figure out an organism's evolutionary history and the relationships between different groupings of species.

Following are the steps involved in DNA fingerprinting:

1. Isolating the DNA.
2. Digesting the DNA with the help of restriction endonuclease enzymes.
3. Separating the digested fragments as per the fragment size by the process of electrophoresis.

b) **2. Nanotechnology** 2

Ans:

Nanotechnology refers to the branch of science and engineering devoted to designing, producing, and using structures, devices, and systems by manipulating atoms and molecules at nanoscale, i.e. having one or more dimensions of the order of 100 nanometres (100 millionth of a millimetre) or less. The term 'nanotechnology' was first coined by scientist **Norio Taniguchi** in 1974.

The National Nanotechnology Initiative, which defined nanotechnology as the manipulation of matter with at least one dimension sized from 1 to 100 nanometers.

It employs nanoscale principles and techniques to understand and transform biosystems (living or non-living) and uses biological principles and materials to create new devices and systems integrated from the nanoscale. The integration of nanotechnology with biotechnology as well as with cognitive science and infotechnology is expected to expand exponentially in the next few

years.

Application Nanotechnology in Agriculture.

Nanotechnology for the management of crops is used as an essential technology for enhancing crop productivity. Nanomaterials and nanostructures, such as carbon nanotubes, nanofibers, and quantum dots are now exploited in agriculture research as biosensors for evaluating the quality of soil and fertilizer distribution. Nanoparticles can be used to turn off or change particular genes responsible for adverse characteristics, thereby reducing the risk of diseases. Nanotechnology has emerged as a groundbreaking and modern technique. Nanoparticle-mediated gene delivery is superior to conventional biomolecular approaches because it enhances the transformation efficiency for both temporal (transient) and permanent (stable) genetic modifications in various plant species

3. Blotting techniques

Ans: Blotting techniques involve the separation (via electrophoresis) and transfer of DNA, RNA, or proteins onto a blotting membrane. The target DNA is then attached to a molecule in order to aid detection. **2**

Types :-

Southern blotting.

Western blotting.

Northern blotting.

Steps in Southern blotting

Southern Blotting

Digest the DNA with an appropriate restriction enzyme.

Run the digest on an agarose gel.

Denature the DNA (usually while it is still on the gel).

Transfer the denatured DNA to the membrane.

Probe the membrane with labeled ssDNA.

Visualize your radioactively labeled target sequence.

SECTION 'B'

Q.11 Define the following terms.

Ans:

1. **Plasmid** :-A plasmid is a small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently.
2. **Totipotency** : -Totipotency is the genetic potential of a plant cell to produce the entire plant.
3. **Clone** :-Cell or organism that is genetically identical to the original cell or organism from which it is derived.
4. **Cybrid:-** Cybrid is a eukaryotic cell line produced by the fusion of a whole cell with a cytoplast.

Q.12 Give the contribution of following scientists.

Ans:

1. Karl Erkey :- Coined term 'biotechnology'

2. Haberlandt :- Father of plant tissue culture
3. Paul Berg :- Father of Genetic Engineering
4. E.M. Southern :- Southern blotting technique

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