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

Semester	:	IV (New)	**Term**	:	II	**Academic year**	: 2018-19
Course No.	:	ELE BOT-242	**Title**	:	Micropropagation Technologies		
Credits	:	3 (1+2)					
Day & Date	:		**Time**	:	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 diagram wherever necessary.

MODEL ANSWER SHEET
SECTION "A"

Q.1 Define micropropagation. Describe its various stages.

Definition of micropropagation: It is the multiplication of genetically identical copies of a cultivar by *in vitro* techniques is called as micropropagation. **(1 mark)**

Invitro Clonal propagation is complicated process requires many steps or stages.

Murashinge (1978) proposed 4 different stages i.e. I-IV.

Stage I : Establishment of aseptic culture

Stage II : Multiplication

Stage III : Rooting an hardening

Stage IV : Transfer to the field / Green house conductions **(3 marks)**

Q.2 What is anther culture? Describe the importance of haploids in crop improvement.

Ans. The development of whol plant from anther or pollen under in vitro conditions is called as anther culture.

Importance of haploids in crop improvement :

1) For the Development of homozygous lines for the production of hybrids in self incompatible species.

2) Isolation of mutants.

3) Haploids are also of immense importance for the improvement of some crop plants like forest and fruit trees as they are highly heterozygous nature.

4) Haploid cell cultures are also useful material for studying the somatic cell genetics, especially for cell modification by the introduction of foreign genetic material.

Q.3 Define somatic embryogenesis. Describe developmental pattern of somatic embryogenesis. **(1 mark)**

Ans : A somatic embryo(SE) is an embryo derived from a somatic cell, other than zygote, usually on culture *in vitro*, and the process is known as somatic embryogenesis. In contrast, embryos developing from zygotes are called zygotic embryos or often simply embryos, while those derived from pollen are known as pollen embryos or androgenetic embryos. Somatic embryogenesis is reported form 80 species belonging to 33 families the list has expanded considerably.

Developmental pattern of SE: **(3 marks)**

SEs generally originate from single cells which divide to form a group of meristematic cells. Usually, this multicellular group becomes isolated by breaking cytoplasmic connections with the other cells around it and subsequently by cutinization of the outer walls of this differentiating cell mass. The cells of meristermatic mass continue to divide and give rise to globular (round ball shaped), heart shaped, torpedo and cotyeledonary stages. In general, the essential feratures of SE developments, especially after the globular stage, are comparable to those of zygotic embryos.

Somatic embryos are bipolar structures in that they have a radical and a plumule. The radicular end is always oriented toward the centre of callus or cell mass, while the plumular end always sticks out form the cell mass. In contrast, a shot bud is monopolar as it does not have a radicular end. SEs radical is suppressed so that they often do not produce roots; in such case, roots have to be regenerated from the shoots produced by germinating SEs.

SEs often show abnormal developmental features, e.g. 3 or more cotyledons, bell-shaped cotyledon, larger size etc. These problems are often overcome by the presence of ABA or a suitable concentration of mannitol. In some species, normal looking somatic embryos are formed but they fail to germinate; at least some SEs do not germinate in most of the species.

The SEs regenerating form explant or callus are termed as primary somatic embryos. In many cases, SEs regenerate form the tissues of other SEs or the parts of germinating SEs ;

Such SEs are called secondary somatic embryos. Ordinarily SEs originate form cells at surface of callus or explant.

Somatic embryogenesis is influenced by several factors such as.

1) GRS 2) Nitrogen source
3) Explant 4) Genotype

Q.4 Define somaclonal variation. Give the causes and applications of somaclonal variation.

Ans : Somaclonal variation: The heritable variation for quantitative traits present in the cell cultured in vitro is called as somaclonal variation. **(1 Mark)**

Application of somaclonal variation: . (3 Marks)

1. Novel variants can rise and these can be agronomic ally used. (New breeding lines)
2. It is useful in diseases resistance.
3. It is useful in abiotic stress resistance.
   a. Salt tolerance
   b. Aluminum tolerance.
   c. Drought tolerance.
   d. Herbicide tolerance.

Causes of somaclonal variation.

1. Changes in the chromosome structure
2. Changes in the chromosome number
3. Genetic mutation
4. Cytoplasmic genetic changes
5. Mitotic crossing over
6. Increased amount of DNA content
7. Transposable

Q.5 What is nutrient media. Describe an ideal composition of nutrient media.

Ans : Any media which supplements essential micro and macro nutrients to the plants each called as nutrient media. **(1 Mark)**

In general, the medium contains : *(i)* inorganic salts: *(ii)* vitamins ; *(iii)* growth regulators; *(iv)* Carbon source ; and (*v*) organic supplements.

Inorganic salts: These are divided into two groups : major and minor salts

Major salts : The salts of potassium, nitrogen, calcium, magnesium, phosphorus and sulphur constitute the the major salts.

Minor salts : The salts of iron, zinc, manganese, boron, copper, cobalt, molybdenum, iodine, etc. make up the minor salts.

Vitamins: The B- vitamins play an important role in growth of tissues

Growth regulators : Growth as well as differentiation of tissues *in vitro*

Auxins : Indole acetic acid, indole butyric acid naphthalene acetic acid, 2, 4-dichlorophenoxy acetic acid are the frequently use auxins.

Cytokinins : Cytokinins have a profound effect on cell division and cell differentiation ex. Kinetin, Zeatin and 6 BAP.

Carbon source : Sucrose

Organic supplements : Coconut water **(3 Mark)**

Q.6 Define plant tissue culture. Describe the basic requirements in plant tissue culture laboratory.

Ans : Definition of plant tissue culture : It refers to the growth of living plant tissues in a suitable culture medium *(in vitro)* **(1 mark)**

Basic requirements of plant tissue culture are as below: (3 marks)

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. Subculturing

Q.7 Define sterilization. Describe different methods of sterilization. **(1 mark)**

Definition of sterilization: Sterilization is a procedure used for elimination of microorganisms and maintaining aseptic (or) sterile conditions for successful culture of plant tissues (or) organs.

The different techniques used for sterilization of plant tissue, culture, growth room chambers and instruments are :

Different types of sterilization: **(3 marks)**

1. Dry sterilization
2. Wet heat / autoclaving / stem sterilization
3. Ultra filtration (or) filter sterilization
4. Ultra violet sterilization
5. Flame sterilization
6. Surface sterilization (or) chemical sterilization.

Q.8 Define suspension culture. Describe the various phases of batch culture.

Ans : Definition of suspension culture: A culture consisting of cells or cell aggregates initiated by placing callus tissues or sometimes seedlings in an agitated liquid liquid medium is called as suspension culture.

There are two types of suspension culture. 1) Continuous culture 2) Batch culture **(1 Mark)**

The cells in culture exhibit the following five phases of a growth cycle. (3 Marks)

i) *Lag phase*, where cells prepare to divide.
ii) *Exponential Phase*, where the rate of cell division is highest.
iii) *Linear phase*, where cell division slows but the rate of cells expansion increases.
iv) *Deceleration phase*, where the rate of cell division and end elongation decreases.
v) *Stationary phase*, wher the number and size of cells remain constant.

Q.9 What is embryo culture ? Explain various applications of embryo culture.

Definition of embryo culture : in vitro culture of either of polarized egg, zygote, pre-embryo or matured embryo is call as embryo culture. **(1 Mark)**

Application of embryo culture : (3 Marks)

1) Production of rare hybrids from intergeneric and interspecific crosses.
2) Development of disease resistant plants.
3) Production of haploids Chromosome elimination technique for production of haploids in cereals / Bulbosum technique (barley and wheat).
4) Overcoming seed dormancy.
5) Shortening of Breeding cycle.

Q. 10 Write short notes on (Any two)

a) **1. Production of Secondary Metabolites (2 mark)**

Ans: Plants are a valuable source of a vast array of chemical compounds including fragrances, Flavors, natural sweeteners, industrial feedstock's, anti-microbial and pharmaceuticals. In most instances these compounds belong to a rather broad metabolic group, collectively referred to as secondary products of metabolites.

Despite limitations there are some measure advantages excpected fom cell coulture systems over the convention cultivation of whole plants for production of secondary metabolites. these are:

a. Independence from various environmental factors, including climate, paste and microbolites diseases, geographical and seasonal constraints
b. useful compounds called be produced under controlled conditions and geared more accurately to market demands.
c. any cell of a plant called be multiplied to yield specific metabolites
d. a consistent product quality called be assured with the use of characterized cell lines
e. there would be greater control over production levels sins they would not be so much at the mercy of political interference
f. cell growth could be automatically controlled and metabolic process could be regulated recently all contributing to the improvement of productivity and the reduction of labor and costs.
g. production of substance in chemical controlled environment faculties later processing and product recovery steps.
h. culture of cells may prove suitable in cases where plants are difficult or expensive to gow in the field due to their longlife cycles, eg. papaver bracteatum the source of thebaine takes two to three seasons to reach maturity.
i. New routs of synthesis can be recovered from mutant cell lines. These roots of synthesis can lead to novel products not previously found in whole plants
j. some cell cultures have the capacity for biotransformation of specific substrates to more valuable products by means of single or multiple step enzyme activity.

b) **2. Totipotency (2 mark)**

Ans: It is the basis of plant cell and tissue culture. Each living cell of amulticellular organism is capable of independent development.

Morgan (1901) coined the Totipotency to denote the capacity of a cell to develop into an organism. All the potential lies mainly in cellular differentiation; this indicates that all genes responsible for differentiation are present within individual's cells. Cell differentiation is the basic event of development in higher organisms.

c) **3. Problems in Micro Propagation: (2 mark)**

1 Microbail contamination :
2 Callusing :
3 Tissue culture induced variation:
4 Browning of medium :

SECTION "B"

Q.11 Define the following terms.

1. **Haploid** : Plant with half the number of chromosomes due to reduction division of the diploid I called as haploid.
2. **Callus** : An unorganized mass of cells is called as callus.
3. **Explant :** Any plant part which is use to start tissue culture is called as explant.
4. **Hardening :** The process of gradual acclimatization to *in vivo* condition of plants grown under *in vitro* is called as hardening.

Q.12 Give the contribution of following scientists. .

1 G. haberlandt : He is called as father of plant tissue culture

2 Kanta : First successful test tube fertilization in papaver rhoeas.

3 Murashige and Skoog : Development of MS nutrient media in 1962

4 Guha and maheshwari.: Production of haploid plants from pollen srains of *Datura*