FINAL EXAMINATION – ANSWER KEY

ATTEMPT ALL THE QUESTIONS

OUT OF 100%

1. **Define the following terms. (20 Marks)**
   - i. **Plant tissue culture –** This is the aseptic culture of cells, tissues, organs and their components under defined physical and chemical conditions in vitro. It is also referred to as cell culture, invitro culture, axenic culture or sterile culture.
   - ii. **Totipotency –** This is the ability to regenerate the entire organism from a single somatic cell, that is, trigger the use of the genetic information present to direct the entire regenerative and developmental programs needed to create the whole organism from a single cell.
   - iii. **Organogenesis –** This is a major path of regeneration that involves the differential of culture cells or callus tissue into organs such as shoots and roots. Plant regeneration through the formation of shoots and roots is known as plant regeneration through organogenesis.
   - iv. **Embryogenesis –** This is also a major path of regeneration and development of plantlets for micropropagation or mass multiplication of specific plants. The cells, under a particular hormonal combination change into the physiological state similar to zygotes (somatic zygotes) and follow an embryonic path to development to form somatic embryos. These somatic embryos are similar to normal embryos such as the seed embryo developed from zygotes formed by sexual fertilization. These somatic embryos can develop into a complete plant and can be used for the production of artificial seeds.
   - v. **Invitro Micrografting –** this is a technique which involves the placement of a meristem or shoot tip explant onto a decapitated rootstock that has been grown aseptically from seed or from micro-propagated cultures.
   - vi. **Callus –** This is an unorganized, proliferative mass of differentiated plant cells, and usually occurs naturally as wound response
   - vii. **Protoplasts –** These are naked plants cells without cell wall but they possess plasma membrane and other cellular components. They represent the functional plant cells but for the lack of the barrier cell wall.
   - viii. **Cybrids –** these are cytoplasmic hybrids where the nucleus is derived from only one parent and the cytoplasm is derived from both parents. The phenomenon of formation of cybrids is regarded as cybridization. (Normally, cybrids are produced when protoplasts from two phylogenetically distinct species are fused. Genetically, cybrids are hybrids only for cytoplasmic traits)
   - ix. **Endomitosis –** This is the phenomenon of doubling the number of chromosomes without division of the nucleus.
   - x. **Soma clonal variation -** this is defined as the variation originating in cell and tissue culture

2. **Micro-propagation has become a suitable alternative to conventional methods of vegetative propagation of plants. Define the term micro-propagation and List and explain FIVE methods of Micro-propagation. (20 Marks)**

   Micro-propagation is the tissue culture technique used for rapid vegetative multiplication of ornamental plants and fruit trees. This method of tissue culture produces several plants. Each of these plants will be genetically identical to the original plant from where they were grown. This can also be defined as the practice of rapidly multiplying stock plant material to produce a large number of progeny plants using modern plant tissue culture methods.

   Methods of micropropagation

   1. **Meristem culture -** Here, meristematic tissues are used as explant for culturing purposes. Meristematic tissues are the type of tissues that can continuously divide and produce new cells. In this case, meristem tissue with a few leaf primordia is placed on a suitable media for the growth and development of plants. The size of the explant is essential to be considered depending on the goal of culturing before initiating the experiment. Meristem culture technique is best for virus elimination, gene conservation, genetic transformation, and plant breeding purposes.
   2. **Callus culture -** A callus is an undifferentiated mass of tissue. In vitro, the callus formation is induced by placing a piece of tissue in a growth culture media under favorable conditions. Then, the callus is transferred to another fresh media containing a high concentration of auxin or auxin and cytokinin (plant growth regulators) for organ development. Some limitations of callus culture include high biochemical variability and slow growth rate. These limitations hinder the utilization of the callus culture techniques at a higher level by the culturists.
   3. **Suspension culture -** The suspension culture method involves the formation of suspension by the multiplication of single cells or aggregates when agitated in an aerated and sterile liquid medium. The two types of suspension culture include batch culture and continuous culture. In batch culture, cells are grown in a fixed amount of culture medium under suitable environmental conditions. In continuous culture, fresh medium is added and the leftover nutrients are continuously removed from the culture

media in which cells are suspended. The most common problem in this method is the formation of clumps and failure of cells to separate after division.

4. **Embryo culture -** Embryo culture is the isolation of immature or mature embryos and culture them in a suitable growth media under favorable conditions. The zygotic or seed embryo is used as explant and the presence of nourishing tissue, endosperm, ensures the proper development of the plant. When the endosperms degenerate, as in the case of a cross between two distant species, the development of the embryo is hindered and the plants develop improperly. Therefore, endosperm has an essential role in embryo culture. To produce healthy plants in cases where endosperms degenerate, the process is followed by culturing the immature hybrid embryo. This process is called embryo rescue. It is done to save embryos that could be aborted.
5. **Protoplast culture -** Protoplast is a spherical naked living cell without a cell wall. It is obtained by stripping the cell wall of plants by using chemical, mechanical, or enzymatic processes. Protoplast culture is the isolation of plant cells followed by degradation of cell walls and culturing them in a liquid media under suitable physiological conditions. The cultured protoplasts form cell walls followed by calli that are transferred to solid media for the development of the whole plant. The explants used for protoplast culture are leaves, root tips, and embryos. Out of these three explants, leaves are the common source of protoplast.

3. **List FIVE some of the advantages and disadvantages of micropropagation?**

Advantages (10 Marks)

i. Rapid multiplication of plants within a short period and on small space.
ii. Plants are obtained under controlled conditions, independent of seasons.
iii. Sterile plants or plants which cannot maintain their characters by sexual reproduction are multiplied by this method.
iv. The rare plant and endangered species are multiplied by this method and such plants are saved.
v. Production of virus free plants like potato, sugarcane, banana and apple for horticulture and agriculture.

Disadvantages (10 Marks)

i. Labour may make up 50%-69% of operating costs.
ii. A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
iii. An infected plant sample can produce infected progeny. This is uncommon as the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.
iv. Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant.
v. Sometimes plants or cultivars do not come true to type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line.
vi. Some plants are very difficult to disinfect of fungal organisms.

4. **State and explain FOUR factors affecting vitro clonal propagation also known as micropropagation. (10 Marks)**
   i. **Genotype of the plant –** selection of the right genotype of the plant species (by screening) is necessary for improved micro-propagation. In general, plants with vigorous germination and branching capacity are more suitable for micro-propagation.
   ii. **Physiological status of the explants –** Explants (plant materials) from more recently produced parts of plants are more effective than those from older regions. Good knowledge of donor plants' natural propagation process with special reference to growth stage and seasonal influence will be useful in selecting explants.
   iii. **Culture media –** The standard plant tissue culture media are suitable for micro-propagation during stage I and stage II. However, for stage III, certain

modifications are required. Addition of growth regulators (auxins and cytokinin) and alterations in minerals compositions are required. This is largely dependent on the type of culture (meristem, bud etc.,).

iv. **Culture environment**

- **Light –** Photosynthetic pigment in cultured tissue does absorb light and thus influence micro-propagation. The quality of light is also known to influence invitro growth of shoots e.g., blue light induced bud formation in tobacco shoots. Variations in diurnal illumination also influence micro-propagation. In general, an illumination of a few hours a day and 8 hours a night is satisfactory for shoot proliferation.
- **Temperature –** Majority of the culture for micro-propagation requires an optimal temperature around 25^0 C. There are however some exceptions where some would go as low as 18^0C
- **Composition of gas phase –** The constitution of the gas phase in the cultured vessels also influences micro-propagation. Unorganized growth of cells is generally promoted by ethylene, Oxygen, Carbon dioxide, ethanol and acetaldehyde.

5. State FIVE applications of micro-grafting. (10 Marks)

i. Virus and viroid elimination
ii. Production of plants resistant to pests and diseases
iii. Assessment of graft incompatibility
iv. Improvement of plant regeneration
v. Mass multiplication
vi. Indexing viral disease
vii. Safe germplasm exchange

6. Protoplasts have a wide range of applications. State FIVE of them. (10 Marks)

i. The protoplast in culture can be regenerated into a whole plant
ii. Hybrids can be developed from protoplast fusion
iii. It is easy to perform single cell cloning with protoplasts
iv. Genetic transformations can be achieved through genetic engineering of protoplasts DNA
v. Protoplasts are excellent materials for ultra-structural studies
vi. Isolation of cell organelles and chromosomes is easy from protoplasts
vii. Protoplasts are useful for membrane studies (transport and uptake processes)
viii. Isolation of mutants from protoplast cultures is easy

7. State and explain FIVE applications of Haploid plants (10 Marks)

i. Development of homozygous lines – it is now possible to develop homozygous lines within a span of a few months or a year by employing anther/pollen culture. This is in contrast to the conventional plant breeding programme that might take several years. In this way, production of haploids is highly useful for research related to plant genetics and breeding.
ii. Generation of exclusive male plants - By the process of androgenesis to produce haploids, followed by chromosome doubling, it is possible to develop exclusive male plants. The male plants are particularly useful when their productivity and applications are much more than female plants.
iii. Induction of mutations - In general, majority of induced mutations are recessive and therefore are not expressed in diploid cells (due to the presence of dominant allele). Haploids provide a convenient system for the induction of mutations and selection of mutants with desired traits. In fact, the haploid cells can be cultured and handled in a fashion similar to microorganisms. Mutants from several plant species that are resistant to antibiotics, toxins, herbicides etc. have been developed.
iv. Production of disease resistant plants - Disease resistance genes can be introduced while producing haploids. The so developed haploids are screened for the desired resistance, and then diploidized.

v. Production of insect resistant plants - Some varieties of rice resistant to insects have been developed e.g., the Hwacheongbyeo rice is resistant to brown plant hopper. Other varieties of rice that are resistant to pests have also been produced

vi. Production of salt tolerance plants - The plant species with salt tolerance are needed for their cultivation in some areas. Anther cultures have resulted in some varieties of rice and wheat with good salt tolerance.

vii. Cytogenetic research - Haploids are useful in several areas of cytogenetic research. These include;

   a. Production of aneuploids.
   b. Determination of the nature of ploidy
   c. Determination of basic chromosome number
   d. Evaluation of origin of chromosomes.

viii. Induction of genetic variability - Besides the development of haploid mutants, it is also possible to produce plants with various ploidy levels through androgenesis.

ix. Doubled haploids in genome mapping - Genome mapping, a recent development in molecular biology, can be more conveniently achieved by using doubled haploid plant species.

x. Evolutionary studies - A comparison of di-haploids (doubled haploids) with diploid wild plant species will be useful to trace the evolutionary origin of various plants. The close evolutionary relationship between tomato and potato has been evaluated by this approach.