2021 BOTANY — HONOURS Paper :

DSE-B-5

(Plant Biotechnology)

Full Marks : 50

The figures in the margin indicate full marks. Candidates are required to give their answers in their own words as far as practicable.

1. Answer any five questions :

(a) What is suspension culture

(a) **Suspension culture:** Suspension culture is a type of cell culture where cells are grown freely in a liquid medium, as opposed to being attached to a solid surface. In suspension culture, cells are suspended in the liquid medium and are typically agitated or stirred to ensure even distribution of nutrients and oxygen.

(b) State the role of osmoticum with an example

(b) **Role of osmoticum with an example:** Osmoticum refers to substances that help in maintaining the osmotic balance in cells during various biological processes. For example, in plant tissue culture, sucrose is often used as an osmoticum. It helps in maintaining the osmotic pressure and prevents plasmolysis of plant cells when they are transferred to a medium with a different osmotic potential.

(c) Give two examples of reporter genes

(c) **Examples of reporter genes:** Reporter genes are genes that are used to study the expression of other genes. Two examples of reporter genes are:

1. **Green Fluorescent Protein (GFP):** It produces a green fluorescent light, allowing researchers to track the location and expression of the gene to which it is linked.

2. **β-Galactosidase:** This enzyme can be detected using a chromogenic substrate, X-gal, producing a blue color. It is often used in combination with the lacZ gene as a reporter system.

(d) Differentiate between artificial seed and natural seed.

(d) **Difference between artificial seed and natural seed:**

- **Natural Seed:** Developed through sexual reproduction, contains a mature embryo, seed coat, and endosperm.

- **Artificial Seed:** Produced by encapsulating somatic embryos or other propagules with a protective coating, mimicking the appearance of a seed but not formed through sexual reproduction.

(e) What are vir-genes and where are they located?

(e) **Vir genes and their location:** Vir genes are found in Agrobacterium tumefaciens, a bacterium responsible for transferring genes into plants. These genes are located on the Ti (tumor-inducing) plasmid. Vir genes play a crucial role in the transfer of T-DNA (transfer DNA) into the plant host cells.

(f) Name two macroelements and two microelements present in plant tissue culture media.

(f) **Macroelements and microelements in plant tissue culture media:**

- **Macroelements:** Examples include nitrogen (N) and potassium (K).

- **Microelements:** Examples include iron (Fe) and zinc (Zn).

(g) Name the oncogenes present in the Ti Plasmid of Agrobacterium tumefaciens

(g) **Oncogenes in Ti Plasmid of Agrobacterium tumefaciens:** The oncogenes found in the Ti plasmid include genes like virulence genes (vir) and the T-DNA region, which can induce the formation of tumors in plants.

.. (h) What is somaclonal variation

(h) **Somaclonal variation:** Somaclonal variation refers to the genetic variability observed among plants derived from the same tissue culture. It can result from genetic mutations or epigenetic changes during the in vitro propagation of plant cells. Somaclonal variation can lead to phenotypic differences among the regenerated plants.

2. Answer any two questions :

(a) Discuss in brief the different sterilization techniques in plant cell culture.

Sterilization is a critical step in plant cell culture to eliminate contaminants and create aseptic conditions for the growth of plant cells and tissues. Various sterilization techniques are employed to ensure the removal of bacteria, fungi, viruses, and other microorganisms. Here are some commonly used sterilization techniques in plant cell culture:

1. ****Autoclaving**:**

- *Principle:* Exposure to high-pressure steam (usually at 121°C) kills microorganisms by denaturing their proteins and disrupting their cell structures.

- *Application:* Autoclaving is widely used for sterilizing solid and liquid culture media, glassware, and other heat-resistant materials.

2. ****Filtration**:**

- *Principle:* Filtration involves passing the culture medium through a filter with specific pore sizes that trap microorganisms.

- *Application:* Filtration is suitable for sterilizing heat-sensitive substances such as vitamins, amino acids, and hormones. It is commonly used for liquid media.

3. ****Chemical Sterilization (Antibiotics, Disinfectants)**:**

- *Principle:* Chemical agents like antibiotics and disinfectants can be added to the culture medium to inhibit or kill microorganisms.

- *Application:* Antibiotics like streptomycin or fungicides like benomyl are added to prevent bacterial or fungal contamination. Disinfectants like ethanol can be used for surface sterilization of equipment.

4. ****Flame Sterilization**:**

- *Principle:* Direct exposure to an open flame is used to sterilize tools, such as scalpels, forceps, and needles.

- *Application:* Flame sterilization is commonly employed for tools used in manipulating plant tissues or during the transfer of cultures.

5. **UV Sterilization**:**

- *Principle:* Ultraviolet (UV) light damages the DNA of microorganisms, preventing their replication.

- *Application:* UV sterilization is useful for sterilizing the air and surfaces in laminar flow hoods or other enclosed spaces.

6. **Germicidal Irradiation**:**

- *Principle:* Exposure to germicidal lamps emitting ultraviolet C (UVC) light kills microorganisms.

- *Application:* Used for sterilizing surfaces, air, and equipment in a controlled environment.

7. **Gamma Irradiation**:**

- *Principle:* Ionizing radiation disrupts the DNA of microorganisms, rendering them unable to replicate.

- *Application:* Gamma irradiation is used for the sterilization of disposable materials, such as plasticware and disposable culture vessels.

The choice of sterilization method depends on the specific requirements of the plant cell culture protocol and the materials being sterilized. A combination of techniques may be employed to achieve comprehensive sterility in plant cell culture laboratories.

(b) Write short notes on – (i) Golden rice (ii) Flavr Savr tomato.

Golden Rice is a genetically modified variety of rice (Oryza sativa) that has been engineered to biosynthesize **beta-carotene**, a precursor of vitamin A, in the edible parts of the rice[1]. This gives the rice its distinctive golden-yellow color[1].

The development of Golden Rice began as a Rockefeller Foundation initiative in 1982[1]. The scientific details of the rice were first published in 2000, the product of an eight-year project by Ingo Potrykus of the Swiss Federal Institute of Technology and Peter Beyer of the University of Freiburg[1].

Golden Rice is intended to be grown and consumed in areas with a shortage of dietary vitamin A[1]. Vitamin A deficiency causes a range of eye conditions from night blindness to more severe clinical outcomes such as keratomalacia and corneal scars, and permanent blindness[1]. Additionally, vitamin A deficiency also increases the risk of mortality from measles and diarrhea in children[1].

Despite meeting significant opposition from environmental and anti-globalization activists, more than 100 Nobel laureates in 2016 encouraged the use of genetically modified Golden Rice which can produce up to 23 times as much beta-carotene as the original Golden Rice[1].

Flavr Savr Tomato (also known as CGN-89564-2; pronounced "flavor saver") is a genetically modified variety of tomato that was the first commercially grown genetically engineered food to be granted a license for human consumption[1]. It was developed by the Californian company Calgene in the 1980s[1].

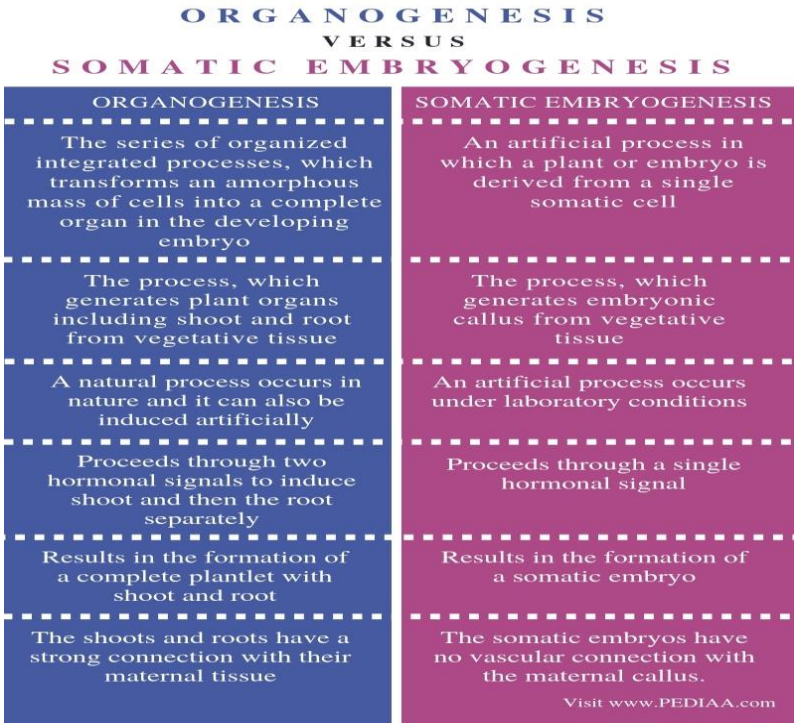
The Flavr Savr Tomato was engineered to have an improved shelf-life, increased fungal resistance, and a slightly increased viscosity compared to its non-modified counterpart[1]. It was meant to be harvested ripe for increased flavor for long-distance shipping[1].

The Flavr Savr Tomato contains two genes added by Calgene; a reversed antisense polygalacturonase gene which inhibits the production of a rotting enzyme and a gene responsible for the creation of APH (3')II[1]. This gene confers resistance to certain aminoglycoside antibiotics including kanamycin and neomycin[1].

The Flavr Savr Tomato was first sold in 1994, and was only available for a few years before production ceased in 1997[1]. Despite its initial success, mounting costs prevented the company from becoming profitable, and it was eventually acquired by Monsanto Company[1].

Through genetic engineering, Calgene hoped to slow down the ripening process of the tomato and thus prevent it from softening too early, while still allowing the tomato to retain its natural color and flavor[1]. This would allow it to fully ripen on the vine and still be shipped long distances without it going soft[1].

(c) Differentiate between organogenesis and somatic embryogenesis stating advantages of each.



(d) What is androgenesis? Briefly discuss pollen culture technique and state its advantages.

Androgenesis is a biological process that results in an individual genetically coming exclusively from a male nucleus[1]. It occurs when a zygote is produced with only paternal nuclear genes[1]. During standard sexual reproduction, one female and one male parent each produce haploid gametes (such as a sperm or egg cell, each containing only a single set of chromosomes), which recombine to create offspring with genetic material from both parents[1]. However, in androgenesis, there is no recombination of maternal and paternal chromosomes, and only the paternal chromosomes are passed down to the offspring[1].

Pollen Culture Technique: Pollen culture is an in vitro technique by which pollen grains, preferably at the uni-nucleated stage, are squeezed out aseptically from the intact anther and then cultured on nutrient medium[2]. Here's a general procedure[2]:

1. Collect anthers (at least 50) from flower buds in a small beaker containing 20ml of the liquid basal medium (MS or Nitsch medium).
2. Press anthers against the side of the beaker to squeeze out pollens.
3. The homogenized anthers are then filtered through a nylon sieve to remove the anther tissue debris.
4. Centrifuge the pollen suspension at 500-800 rpm/min for five minutes. Discard the supernatant and keep the pellet containing pollens. Then suspend it in a liquid media and wash it twice by centrifugation. Repeat the process twice.
5. Pipette off a 2.5ml pollen suspension and spread it on a Petri dish with a soft agar medium. You can also use a liquid medium to grow pollens.
6. Incubate cultures at 27-30 degrees Celsius under the low intensity of white cool light. After 30 days, you will observe young embryoids that you can use to regenerate plantlets[2].

Advantages of Pollen Culture:

1. The explants i.e., microspores or pollens are all haploid cells[3].
2. The sequence of androgenesis can be observed starting from a single cell[3].
3. The microspores are ideal for uptake, transformation, and mutagenic studies, and the microspores are evenly exposed to chemicals and physical mutagens[3].
4. Higher yields of plants/anther could be obtained[3].
5. Requires minimal time and enables the production of numerous haploids within a short period[2].
6. Highly receptive, the majority of anthers subjected to cultivation exhibit a response[2].
7. By relying on pollen culture, the possibility of chimerism and callus formation from the anther walls is eliminated[2].

3. Answer any three questions :

(a) What is somatic embryogenesis? Briefly discuss the induction and development of somatic embryos in culture.

Somatic Embryogenesis is an artificial process where a plant or embryo is derived from a single somatic cell[12]. Somatic embryos are formed from plant cells that are not normally involved in the development of embryos, i.e., ordinary plant tissue[12]. No endosperm or seed coat is formed around a somatic embryo[12]. The cells derived from potential source tissues are cultured to form an undifferentiated mass of cells called a callus[12]. Plant growth regulators in the tissue culture medium can be manipulated to induce callus formation and subsequently changed to induce embryos to form the callus[12].

Induction and Development of Somatic Embryos in Culture: The process of somatic embryogenesis is a three-step procedure, which causes the induction of embryogenesis, development of the embryo, and its maturation[1]. The principle of somatic embryogenesis finds its basis on the topic of totipotency of the plant cells[1].

1. **Induction**: The induction of somatic embryogenesis in the cells is one of the most spectacular achievements in plant tissue culture[3]. The cells which are derived from potential source tissues are subject to a culture medium for the formation of an undifferentiated cluster of cells referred to as the callus[1]. In the tissue culture medium, the plant growth regulators can be formed for the induction of the formation of calluses and hence modified to induce the embryos for the formation of calluses[1].
2. **Development**: Somatic embryos were induced by zygotic embryos germinating on callus induction medium (MS media with 1.54 mg L −1 2,4-D and 0.43 mg L −1 kinetin) in dark[4]. Thereafter, the compact embryogenic callus differentiated up on MS media with 0.1 mg L −1 NAA and 0.5 mg L −1 kinetin supplemented with various concentrations of ancymidol[4].
3. **Maturation**: Somatic embryos usually do not mature properly. Instead, due to environmental factors such as constant contact with inducing medium, somatic embryos often deviate from the normal developmental pattern by bypassing embryo maturation producing callus, undergoing direct secondary embryogenesis and/or germinating precociously[5].

Somatic embryogenesis is a significant biotechnological tool illustrating vital merits in the streams of clonal propagation, genetic transformation, etc., which when applied are most propitious of their applications[1].

(b) Describe the particle gun technique for transgenic plant development. Explain briefly one more technique for direct gene transfer in plants.

Particle Gun Technique for Transgenic Plant Development: The particle gun technique, also known as biolistics or microprojectile bombardment, is a method

used for direct gene transfer in plants[1]. This method works by shooting DNA into the plant cells[1]. Microscopic gold or tungsten particles are coated with hundreds of copies of the gene(s) to be introduced[1]. In earlier versions of the gene gun, DNA coated metal was loaded into a 22-caliber cartridge and then shot into plant tissue culture cells[1]. Current versions place the tissue culture cells in a vacuum chamber and then propel the metal particles with a high pressure gas that is released in a sudden burst much like a popped balloon[1]. Once inside the nucleus of a cell, the genes dissolve off of the gold particle and can potentially insert into a chromosome[1].

Four Techniques for Direct Gene Transfer in Plants:

1. **Plant Cell Transformation by Ultra-Sonication**: This technique uses ultra-sonication for gene transfer in plants such as wheat, tobacco, and sugarbeet[2]. When the cultured explants are sonicated with plasmid DNA carrying marker genes and sonicated cells transferred to selective medium, shoots have grown successfully[2].
2. **Liposome Mediated Gene Transfer**: Liposomes are microscopically small-sized lipid bags. They are bound by phospholipid bilayers and contain an aqueous chamber which may contain water soluble molecules such as plasmids or foreign DNA[2]. By using polyethylene glycol (PEG), plasmid containing liposomes may be stimulated to fuse with protoplast[2].
3. **Electroporation**: Electroporation is used to transfer the foreign DNA into the fragile cells[2]. Brief pulses of high voltage electricity (about 350 V) is applied to protoplast suspension containing naked or recombinant plasmids. The electric pulses induce the formation of large pores in the plasma membrane. These pores give a passage through which the foreign DNA can enter into the protoplasts and thus, increase in transformation frequency[2].
4. **Particle Bombardment Gun**: It was developed by Prof. Stanford and co-workers at Cornell University (USA) in 1987[2]. As the term denotes, it shoots foreign DNA into plant cells or tissues at a very high speed[2].

(c) What is somatic hybridization? State the genetic consequences of somatic hybridization with the help of a flowchart and suitable diagram. Define fusogen.

Somatic Hybridization: Somatic hybridization is a technique that allows the fusion of two different plants to form a new hybrid plant with characteristics from both plants[1]. This process involves the in vitro fusion of protoplasts (cells without any cell wall) to form a hybrid cell and culturing the hybrid cell to form a hybrid plant[1]. The fusion of protoplasts can be achieved by three methods: mechanical fusion, spontaneous fusion, and induced fusion[1].

Genetic Consequences of Somatic Hybridization:

1. **Isolation of Protoplasts:**

   - Protoplasts are isolated from different plant species with desirable traits.

2. **Cell Fusion:**

   - Protoplasts from different species are induced to fuse using chemical or electrical methods, creating a heterokaryotic cell.

3. **Nuclear Fusion:**

   - Nuclei from the fused protoplasts may undergo fusion, resulting in a cell with a combined set of chromosomes.

4. **Heteroplasmy:**

   - The fused cell is initially heteroplasmic, containing organelles (such as mitochondria and chloroplasts) from both parent species.

5. **Cell Culture and Selection:**

   - The fused cells are cultured and subjected to a selection process to identify and isolate cells with stable chromosome numbers.

6. **Chromosome Elimination:**

   - In some cases, one set of chromosomes may be eliminated through a process known as chromosome elimination, leading to a stabilized hybrid cell.

7. **Regeneration of Somatic Hybrid Plant:**

   - The stabilized hybrid cells are induced to regenerate into whole plants through suitable tissue culture methods.

8. **Genetic Variation:**

- Somatic hybrid plants exhibit genetic variation resulting from the combination of traits from the parent species.

9. **Stable Hybrid Genome:**

- Over subsequent generations, the somatic hybrid may stabilize its genome, resulting in a stable hybrid plant with a combination of desirable traits from the parent species.

10. **Potential for Novel Traits:**

- Somatic hybridization can lead to the expression of novel traits that were not present in either of the parent species.

It's important to note that somatic hybridization can result in a wide range of genetic outcomes, and the success of obtaining stable and fertile hybrids depends on factors such as the compatibility of parent species, the efficiency of cell fusion and regeneration processes, and the stability of the hybrid genome over generations.

Fusogen: A fusogen is a substance that can cause cellular membranes to merge[4]. In the context of somatic hybridization, fusogens such as polyethylene glycol (PEG) and sodium nitrate (NaNO3) are used to induce the fusion of protoplasts[1].

(d) What is a synthetic seed? Discuss briefly how the different types of synthetic seeds are produced in culture.

Synthetic Seed: A synthetic seed is an artificial seed that includes encapsulated somatic embryos, shoot buds, cell aggregates, or any other meristematic tissue having the potential to regrow after storage conditions[1]. Synthetic seeds are used to grow plants from scratch, improve their characteristics, or preserve their genetics[1].

Production of Different Types of Synthetic Seeds: There are two types of synthetic seeds currently being developed: desiccated synthetic seeds and hydrated synthetic seeds[21].

1. **Desiccated Synthetic Seeds**: This involves the encapsulation of multiple somatic embryos followed by desiccation[21]. The encapsulating material used in this case is polyoxyethylene (Polyox). This material doesn't allow for the growth of the microorganisms and is non-toxic to embryos[21]. In this case, a mixture is prepared by using equal volumes of embryo suspension and a 5% (w/v) solution of polyox to give a final concentration of 2.5% polyox. Then by using a pipette the suspension is dispensed as 0.2 ml drops onto Teflon sheets. Then the drops are dried till they themselves leave the Teflon sheet[2].
2. **Hydrated Synthetic Seeds**: This involves the encapsulation of a single somatic embryo in hydrogel capsules[21]. This technique is used in those plants which are recalcitrant for the somatic embryogenesis and sensitive to desiccation[21]. The most popular method of forming hydrated synthetic seeds is using Ca-alginate encapsulation[2]. The procedure followed to produce hydrated synthetic seed involves the mixing of somatic embryos with a 2% (w/v) solution of Na-alginate. Then, the embryo is dropped into a 100 mM solution of Ca (NO3)2 by using a plastic pipette[2].

(e) What do you mean by callus culture? How callus culture is developed from an explant? State briefly the applications of callus culture.

Callus Culture:

Callus culture is a type of in vitro plant tissue culture in which undifferentiated and proliferating mass of cells, known as callus, is induced from an explant (a piece of plant tissue) under sterile conditions. Callus is often formed in response to injury or stress and consists of rapidly dividing, dedifferentiated cells. It serves as a source of cells for subsequent regeneration into whole plants.

Development of Callus Culture from an Explant:

1. **Initiation:**

- An explant, typically derived from plant tissues like leaves, stems, or roots, is isolated and placed on a nutrient-rich medium containing plant growth regulators (auxins, cytokinins).

2. **Induction of Callus:**

- The presence of specific plant growth regulators in the culture medium stimulates the explant cells to undergo dedifferentiation and initiate cell division, leading to the formation of a mass of undifferentiated cells, known as callus.

3. **Subculture:**

- The callus is subcultured onto fresh medium periodically to maintain its growth and prevent differentiation.

4. **Regeneration:**

- Under appropriate conditions, the callus can be induced to differentiate and regenerate into various plant organs, such as shoots and roots.

Applications of Callus Culture:

1. **Micropropagation:**

- Callus culture serves as a source of cells for micropropagation, allowing for the rapid multiplication of plants with desirable traits.

2. **Somatic Embryogenesis:**

- Callus can undergo somatic embryogenesis, leading to the development of somatic embryos that can be converted into whole plants.

3. **Genetic Transformation:**

- Callus culture is often used as a target tissue for introducing foreign genes (genetic transformation) to create genetically modified plants.

4. **Secondary Metabolite Production:**

- Callus cultures can be induced to produce secondary metabolites, such as alkaloids or phenolic compounds, which may have pharmaceutical or industrial applications.

5. **Stress Tolerance Studies:**

- Callus culture provides a controlled environment for studying the effects of various stress factors, such as drought or salinity, on plant cells and tissues.

6. **Germplasm Conservation:**

- Callus culture is employed in the conservation of plant germplasm, allowing for the storage of genetic material under in vitro conditions.

7. **Disease Studies:**

- Callus cultures can be used to study plant-pathogen interactions and test the resistance or susceptibility of different plant varieties to diseases.

Callus culture is a versatile technique with applications in plant biotechnology, crop improvement, and research on plant physiology and development. It provides a valuable tool for manipulating plant cells and tissues in a controlled laboratory environment.