2020

BOTANY — HONOURS

Paper : DSE-B-1

(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 : 2×5

(a) Name two chemicals used for surface sterilization of explants for in vitro culture.

(a) Two chemicals commonly used for surface sterilization of explants for in vitro culture are **Sodium hypochlorite** and **Mercuric chloride**[123].

(b) What is cytodifferentiation?

(b) **Cytodifferentiation** is the process where a cell develops into a more specialized cell type. It involves the development of specialized cells (such as muscle, blood, or nerve cells) from undifferentiated precursors[4567].

(c) What do you mean by 'Super bug'?

© A **Superbug** is a strain of bacteria, viruses, parasites, or fungi that have developed the ability to resist most of the antibiotics and other medications commonly used to treat the infections they cause[891011].

OR

Cellular totipotency refers to the unique capability of a single, undifferentiated cell to give rise to all the cell types of an organism, including both embryonic and extraembryonic tissues. It is the highest level of developmental potential exhibited by a cell.

(d) What is edible vaccine?

(d) An **Edible vaccine** is a genetically manipulated food containing organisms or related antigens that may provide active immunity against infection. They are produced from one edible part of plants, such as fruits and vegetables, that can be ingested orally rather than injected like traditional vaccines[1213].

(e) Define cellular totipotency

(e) Cellular totipotency refers to the unique capability of a single, undifferentiated cell to give rise to all the cell types of an organism, including both embryonic and extraembryonic tissues. It is the highest level of developmental potential exhibited by a cell.. Spores and zygotes are examples of totipotent cells[1415].

(f) What is T-DNA?.

(f) **T-DNA**, or Transfer DNA, is the transferred DNA of the tumor-inducing (Ti) plasmid of some species of bacteria such as Agrobacterium tumefaciens and Agrobacterium rhizogenes. The T-DNA is transferred from bacterium into the host plant's nuclear DNA genome[1617].

(g) Define fusogen with an example

(g) A **Fusogen** is a substance that can cause cellular membranes to merge[181920]. An example of a fusogen is the protein hemagglutinin, which is found on the surface of the influenza virus and facilitates the fusion of the viral envelope with the host cell membrane.

(h) State two names of transgenic plants

(h) Two examples of transgenic plants are **Golden Rice**, which is genetically engineered to have a higher Vitamin A content, and **Bt Cotton**, which is genetically modified to be resistant to the pest bollworm[212223].

2. Answer any two questions :

(a) Briefly discuss the method of isolation of protoplast.

Protoplasts are plant cells that have had their cell wall removed, leaving only the plasma membrane. The isolation of protoplasts involves the removal of the cell wall in a controlled manner. This can be achieved through two main methods: mechanical and enzymatic[12].

Mechanical Method: In this method, large and highly vacuolated cells of storage tissues such as onion bulb scales, radish root, and beet root tissue could be used for isolation. The cells are plasmolysed in an iso-osmotic solution resulting in the evacuation of contents in the center of the cell. Eventually, the tissue is dissected and deplasmolyzed to release the preformed protoplasts[2]. However, this method is generally not followed because of certain disadvantages such as low yield of protoplasts, time-consuming process, and low viability of protoplasts due to the presence of substances released by damaged cells[2].

Enzymatic Method: This is the most commonly used method for protoplast isolation. The process involves the use of cell wall-degrading enzymes to remove the cell wall[12]. The source material, if it is from an in vivo grown plant, should be

properly surface sterilized using the proper method of sterilization[1]. Then, the enzymes are applied to isolate the protoplast and the tissue material should be placed in a proper osmoticum or plasmolyticum[1]. The most commonly used enzymes for this purpose are cellulase and macerozyme[3]. The use of these enzymes provides a stable osmotic environment which prevents bursting of protoplast[1].

The success of protoplast isolation depends greatly on the condition of the tissue and the combination of enzymes being used[4]. Protoplasts can be isolated directly from different parts of the whole plant which bears soft parenchymatous tissue (e.g., young fully expanded soft leaves) or indirectly from the in vitro grown plant tissue (e.g., callus tissue)[1].

(b) Briefly discuss the Ti Plasmid

The Ti (Tumor-inducing) plasmid is an extrachromosomal genetic material found in pathogenic species of Agrobacterium, including A. tumefaciens, A. rhizogenes, A. rubi, and A. vitis[1]. This plasmid is essential for the bacteria to cause crown gall disease in plants[1].

The Ti plasmid has three important regions[21]:

- **T-DNA region**: This region carries genes responsible for inducing tumors in plants. Other foreign genes of interest can be inserted in this region[2].
- **Vir region**: This region consists of virulence genes. It is responsible for the excision, integration, and transfer of T-DNA into the plant chromosome[2].
- **Opine catabolism region**: This region catabolizes opines, which are specialized amino acids. It is responsible for catabolizing opines produced by the T-DNA region[2].

Based on the differences in the T-DNA region, there are two types of Ti plasmids[2]:

1. **Nopaline**: It has a continuous region of T-DNA, which is approximately 25 kb in length. It produces an opine known as Nopaline.
2. **Octopine**: It is subdivided into two regions which are 13 kb and 8 kb long. It produces an opine known as Octopine.

The Ti plasmid can be used as a vector for transformation involving Agrobacterium tumefaciens and is used for producing transgenic plants[2]. With the use of restriction enzymes, a particular gene of interest can be inserted into the plasmid and transformed[2]. It is used for the development of stress-tolerant plant varieties[2].

OR

The Ti plasmid, or tumor-inducing plasmid, is a circular DNA molecule found in the bacterium Agrobacterium tumefaciens. This plasmid is essential for the bacterium's ability to induce tumor or gall formation in the host

plants it infects. The Ti plasmid is extensively used as a vector in genetic engineering to introduce foreign genes into plants, a process known as Agrobacterium-mediated transformation.

Key features of the Ti plasmid include:

1. **Tumor-Inducing Genes (T-DNA):**

- The Ti plasmid contains a segment of DNA known as the T-DNA (Transfer DNA), which carries genes responsible for the induction of tumors or galls in infected plants.

- During infection, Agrobacterium transfers the T-DNA into the plant host's genome, leading to the expression of these genes and the formation of a tumor or gall.

2. **Virulence Genes:**

- The Ti plasmid also carries virulence (vir) genes that are involved in the transfer of T-DNA into the plant cells.

- Virulence genes help in the transformation process by facilitating the transport of the T-DNA across the bacterial and plant cell membranes.

3. **Origin of Replication:**

- Like other plasmids, the Ti plasmid contains an origin of replication that allows it to replicate independently within Agrobacterium cells.

4. **Selectable Marker Genes:**

- Modified Ti plasmids used in genetic engineering often carry selectable marker genes, such as antibiotic resistance genes.

- These markers help in identifying and selecting transformed plant cells during the regeneration process.

The process of Agrobacterium-mediated transformation involves the preparation of a Ti plasmid-based vector carrying the gene of interest flanked by T-DNA borders. This vector is then introduced into Agrobacterium, and the bacterium is used to infect plant tissues. The T-DNA is transferred to the plant cells, leading to the integration of the foreign gene into the plant genome.

The utilization of the Ti plasmid in genetic engineering has proven to be a powerful and widely adopted method for the creation of genetically modified plants. It has applications in crop improvement, the production of transgenic plants, and the study of gene function in plants.

(c) Write a short note on Bt cotton.

Bt cotton is a genetically modified variety of cotton that has been engineered to produce an insecticide to combat bollworms[12]. The modification involves the insertion of one or more genes from a common soil bacterium, Bacillus thuringiensis (Bt), into the cotton genome[12].

The Bt bacterium produces over 200 different Bt toxins, each harmful to different insects[1]. Most notably, Bt toxins are insecticidal to the larvae of moths and butterflies, beetles, cotton bollworms, and flies but are harmless to other forms of life[1]. When insects attack and eat the cotton plant, the Cry toxins or crystal protein

are dissolved due to the high pH level of the insect's stomach. The dissolved and activated Cry molecules bond to cadherin-like proteins on cells comprising the brush border molecules[1]. The death of such cells creates gaps in the brush border membrane[1].

Bt cotton was first approved for field trials in the United States in 1993, and first approved for commercial use in the United States in 1995[1]. In 2002, a joint venture between Monsanto and Mahyco introduced Bt cotton to India[2]. In 2011, India grew the largest GM cotton crop at 10.6 million hectares[1].

The adoption of Bt cotton has led to a significant increase in cotton production, a drastic reduction in the application of chemical insecticides for bollworm control, higher profits for farmers, and conservation of biological control agents and other beneficial organisms[2]. However, it has also led to some challenges, such as the high cost of seeds, the need for new stock for every growing season, and the development of resistance to Bt cotton by pests like the pink bollworm[2].

(d) What is callus culture? State its application

Callus culture is a laboratory technique used in plant tissue culture where an unorganized, proliferating mass of cells, known as a callus, is produced from isolated plant cells, tissues, or organs[1][2]. The callus is grown aseptically on an artificial nutrient medium under controlled experimental conditions[1][2]. The nutrient medium and methods of callus culture depend on the type of explant and the hormone requirements[1].

The process of callus culture involves the following steps:

1. **Initiation:**

- Plant tissues (explants) are taken from a donor plant and cultured on a nutrient medium supplemented with auxins, usually 2,4-dichlorophenoxyacetic acid (2,4-D) or naphthaleneacetic acid (NAA).

- These auxins stimulate the dedifferentiation of cells, leading to the formation of a mass of undifferentiated cells known as callus.

2. **Proliferation:**

- The callus continues to grow and proliferate in culture. The cells in the callus remain in an undifferentiated state and exhibit rapid cell division.

3. **Subculture:**

- Periodically, the callus can be subcultured onto fresh nutrient medium to maintain its growth and prevent differentiation.

Applications of Callus Culture:

1. **Plant Regeneration:**

- Callus culture is often used as an intermediate step in the regeneration of whole plants from isolated cells or tissues. Under certain conditions, callus can be induced to differentiate into shoots, roots, and eventually, complete plants.

2. **Genetic Transformation:**

- Callus culture plays a crucial role in genetic engineering and biotechnology. Genetic modifications can be introduced into callus cells using various techniques, such as Agrobacterium-mediated transformation or direct gene transfer methods.

3. **Secondary Metabolite Production:**

- Some plant species accumulate valuable secondary metabolites, such as alkaloids, flavonoids, or terpenoids. Callus culture can be employed to produce these compounds in larger quantities than what the intact plant might naturally produce.

4. **Somatic Embryogenesis:**

- Callus culture can be induced to undergo somatic embryogenesis, a process in which somatic cells differentiate into structures resembling embryos. These somatic embryos can be further developed into complete plants.

5. **Micropropagation:**

- Callus culture is a valuable tool for the mass production of plants through a process known as micropropagation. In this method, callus is induced from explants, and the resulting shoots or plantlets are then regenerated, allowing for the rapid multiplication of plants with desirable traits.

Callus culture is a versatile technique with various applications in plant biology, tissue culture, and biotechnology. It has contributed significantly to advancements in plant propagation, genetic engineering, and the production of valuable plant-derived compounds.

3. **Answer any three questions :**

Anther culture is a technique in plant tissue culture that involves the isolation and in vitro culture of anthers, the male reproductive structures in flowers. This method is particularly used for the production of haploid plants, which have only one set of chromosomes. Anther culture is often employed in plant breeding and genetic studies. Below is a flowchart outlining the steps of anther culture and its application:

Start

|

Anther isolation

|

Surface sterilization

|

Culture on nutrient medium with plant growth regulators (PGRs)

|

Induction of callus

|

Subculture and regeneration

|

Rooting and acclimatization

|

Haploid plantlet

|

Application in plant breeding and genetic studies

|

End

1. **Anther Isolation:**

- Anthers are isolated from the flowers at a specific developmental stage.

2. **Surface Sterilization:**
   - Anthers are subjected to surface sterilization to eliminate contaminants.
3. **Culture on Nutrient Medium with PGRs:**
   - Anthers are cultured on a nutrient medium supplemented with plant growth regulators (PGRs), usually auxins and cytokinins. This induces the formation of callus.
4. **Induction of Callus:**
   - Callus is induced from the anther cells. This callus may contain both haploid and diploid cells.
5. **Subculture and Regeneration:**
   - The callus is subcultured onto fresh medium to promote further growth and differentiation. Regeneration of haploid plantlets may occur during this stage.
6. **Rooting and Acclimatization:**
   - Haploid plantlets are induced to develop roots, and they are subsequently acclimatized to the external environment.
7. **Haploid Plantlet:**
   - The end result is the production of haploid plantlets with a single set of chromosomes.
8. **Application in Plant Breeding and Genetic Studies:**
   - Haploid plants obtained through anther culture can be used in plant breeding programs to develop new crop varieties with desirable traits. They are also valuable for genetic studies and research.

Anther culture is particularly useful in obtaining homozygous lines faster than traditional breeding methods. The resulting haploid plants can be doubled to obtain fertile, homozygous diploid plants. This technique facilitates the rapid development of new plant varieties with improved characteristics and is widely used in crop improvement programs.

(b) Write short notes : (i) Reporter gene (ii) Artificial seed (iii) De-differentiation (iv) Suspension culture. 2½×4

****Reporter Gene:****

A reporter gene is a gene used in molecular biology and genetic research to easily monitor and identify the expression of a target gene or the activity of a promoter. Reporter genes produce a readily detectable product, such as a fluorescent or luminescent protein, an enzyme, or a color-changing pigment, allowing researchers to visualize and quantify gene expression or promoter activity in living cells or organisms.

Common types of reporter genes include:

1. **Green Fluorescent Protein (GFP):**
- GFP is widely used as a reporter gene. When fused to the gene of interest, it produces a green fluorescence that can be observed under fluorescence microscopy.

2. **β-Galactosidase (lacZ):**
- The lacZ gene codes for β-galactosidase, an enzyme that cleaves a substrate, resulting in a color change. X-gal, a substrate for β-galactosidase, can be used to detect β-galactosidase activity by turning blue.

3. **Luciferase:**
- Luciferase produces bioluminescence in the presence of its substrate luciferin. This allows for sensitive and quantitative measurements of gene expression or promoter activity.

4. **Discosoma sp. Red Fluorescent Protein (DsRed):**
- DsRed is a red fluorescent protein that can be used as a reporter gene for visualizing cellular structures or monitoring gene expression.

Applications of Reporter Genes:

1. **Gene Expression Studies:**
- Reporter genes help researchers study the spatial and temporal patterns of gene expression in different tissues or developmental stages.

2. **Promoter Analysis:**
- Reporter genes are employed to investigate the activity of promoters and regulatory elements. This is crucial for understanding how genes are regulated.

3. **Screening and Selection:**
- In genetic engineering and biotechnology, reporter genes are often used to screen for successfully transformed cells or organisms. The visible or measurable reporter signal simplifies the identification of successful transformations.

4. **Drug Discovery and Development:**
- Reporter genes are used in drug screening assays to study the effects of potential drugs on specific cellular processes or gene expression.

5. **Biotechnology and Transgenic Organisms:**
- Reporter genes are frequently utilized in the development of transgenic organisms, allowing researchers to track the expression of foreign genes introduced into the organisms.

Overall, reporter genes are invaluable tools in molecular biology and biotechnology, providing a means to visualize and quantify the activity of specific genes or regulatory elements in various experimental systems.

Artificial seeds- also known as synthetic seeds, are seed-like structures that are produced experimentally[123]. They are created by encapsulating somatic embryos or other vegetative parts such as shoot buds, cell aggregates, auxiliary buds, or any other micropropagules in a hydrogel[1234]. These encapsulated structures can be sown directly into soil as a substitute for natural seeds[1234].

The production of artificial seeds involves the following steps[1]:

1. Selection of suitable plant material
2. Surface sterilization of plant material
3. Excision of anthers from the flower bud
4. Culture of anthers on nutrient medium
5. Induction of callus or embryoids from microspores
6. Regeneration of haploid plantlets from callus or embryoids
7. Transfer of plantlets to rooting medium
8. Hardening and transfer of plantlets to soil

Artificial seeds have a variety of applications in plant biotechnology[2567]:

- **Large scale clonal propagation**: Artificial seeds can be used for mass propagation of elite plant genotypes[25].
- **Germplasm conservation**: They can be used for the conservation of germplasm of commercially important crops[25].
- **Breeding of plants**: Artificial seeds can be used in the breeding of plants in which propagation through normal seeds is not possible[25].
- **Genetic uniformity**: They can be used to maintain genetic uniformity and varieties of crops[25].
- **Easy storage and transportation**: Artificial seeds do not require special storage conditions and can be easily transported[25].
- **Production of transgenic plants**: Artificial seeds can be used for the multiplication of transgenic plants[5].
- **Preservation of endangered species**: They can be used to propagate endangered plant species[7].

****De-differentiation in Plant Biotechnology**:**

De-differentiation plays a crucial role in plant biotechnology, particularly in the context of plant tissue culture and regeneration. This process involves the reversal of cellular differentiation, leading to the formation of undifferentiated cells that have the potential to develop into various cell types, including organs and whole plants. Here are key points regarding de-differentiation in plant biotechnology:

1. **Callus Formation:**

- De-differentiation in plants often results in the formation of callus. Callus is a mass of undifferentiated cells that can be induced from plant explants, such as leaves, stems, or roots, under specific in vitro culture conditions.

2. **Induction of Embryogenic Cultures:**

- De-differentiated cells, especially those within callus tissue, can be further induced to form embryogenic cultures. Embryogenic cultures mimic the developmental process of somatic embryogenesis, where somatic cells differentiate into structures resembling embryos.

3. **Somatic Embryogenesis:**

- De-differentiated cells within callus or embryogenic cultures have the potential to undergo somatic embryogenesis, a process wherein embryos develop directly from somatic cells. This is a valuable tool in plant biotechnology for mass production of plants from a single, de-differentiated cell source.

4. **Genetic Transformation:**

- De-differentiated cells are often used as the target for genetic transformation in plant biotechnology. Foreign genes can be introduced into the de-differentiated cells, leading to the generation of transgenic plants with desired traits.

5. **Micropropagation:**

- De-differentiation is exploited in micropropagation, a technique for rapidly multiplying plants. Explants from de-differentiated cultures can be induced to form shoots and roots, allowing for the efficient production of genetically identical plants.

6. **Regeneration of Whole Plants:**

- De-differentiated cells, such as those in callus or embryogenic cultures, possess the potential to regenerate into whole plants. This regeneration capability is a fundamental aspect of plant tissue culture and has applications in crop improvement and genetic studies.

7. **Stress Response:**

- De-differentiation can be induced by various stress conditions, including physical damage or exposure to certain hormones. This natural response is leveraged in tissue culture techniques to initiate the formation of de-differentiated cells.

8. **Applications in Crop Improvement:**

- De-differentiation is a key component in the development of improved crop varieties. Through tissue culture and regeneration techniques, plant biotechnologists can introduce and propagate desirable traits in crops.

Understanding and manipulating de-differentiation processes in plant cells are essential for advancements in plant biotechnology, enabling applications ranging from genetic engineering to the production of disease-resistant and high-yielding crops.

SUSPENSION CULTURE

Suspension culture is a type of cell culture in which single cells or small aggregates of cells multiply while suspended in an agitated liquid medium[12]. It is also referred to as cell culture or cell suspension culture[12]. The cells in suspension culture are free-floating and do not attach to a surface, making it different from adherent culture[2].

The process of suspension culture involves the following steps[1]:

1. Selection of suitable plant material
2. Surface sterilization of plant material
3. Transfer of pre-established callus tissue to a conical flask containing autoclaved liquid medium
4. Placement of the flasks within the clamps of a rotary shaker moving at 80-120 rpm
5. After 7 days, the contents of each flask are poured through a sterilized sieve to collect the filtrate, which contains only free cells and cell aggregates
6. The filtrate is allowed to settle for 10-15 minutes or centrifuged at 500 to 1,000 rpm, and the supernatant is finally poured off[1].

Applications of suspension culture include[134]:

- **Production of Secondary Metabolites**: Suspension culture is an excellent tool for examining the secondary metabolites in a variety of plants[4].
- **Study of Cellular Processes**: Suspension culture provides a system to study cellular processes like cell division, differentiation, and development[1].
- **Plant Breeding**: Suspension culture is used to make mutant cell clones, which can then be used in plant breeding[4].

- **Pharmaceuticals and Cosmetics**: Plant cell suspension cultures are used to obtain secondary metabolites in pharmaceuticals and cosmetics[3].
- **Food Industry**: Suspension cultures are used in the food industry[3].

© Briefly discuss the steps of T-DNA transfer process from Agrobacterium tumifaciens cell to plant cell

The transfer of T-DNA (Transfer DNA) from Agrobacterium tumefaciens to a plant cell is a complex process that results in the genetic modification of the plant. Here are the key steps involved in the T-DNA transfer process:

1. **Attachment and Recognition:**

- Agrobacterium tumefaciens recognizes wounded plant tissues. The bacteria attach to the plant cell surface, facilitated by the interaction between bacterial surface proteins and specific plant receptors.

2. **Induction of Virulence (vir) Genes:**

- Upon attachment, the bacterium perceives signals from the plant, leading to the activation of its virulence (vir) genes. These genes are crucial for the transfer of T-DNA and the establishment of infection.

3. **Formation of T-DNA Transfer Complex:**

- Virulence proteins produced by Agrobacterium facilitate the processing and transfer of T-DNA. The T-DNA, along with associated proteins, forms a complex known as the T-complex.

4. **T-DNA Transfer to Plant Cell:**

- The T-complex is transferred from Agrobacterium into the plant cell through a specialized structure called the type IV secretion system. This system spans both bacterial and plant cell membranes, creating a direct channel for the transfer of the T-DNA.

5. **Integration into Plant Genome:**

- Once inside the plant cell, the T-DNA is transported to the nucleus. The T-DNA integrates into the plant genome with the help of host factors and the activity of bacterial and plant proteins.

6. **Expression of Genes on T-DNA:**

- The integrated T-DNA contains specific genes, such as those for the production of plant hormones (auxins and cytokinins) and genes responsible for the synthesis of opines (novel compounds used by Agrobacterium as a nutrient source). The expression of these genes leads to the formation of a tumor or gall.

7. **Formation of Crown Gall or Tumor:**

- The integrated T-DNA induces uncontrolled cell division in the infected plant cells, resulting in the formation of a tumor or gall. The transformed cells continue to grow and differentiate, producing a mass of undifferentiated tissue.

8. **Opine Synthesis and Bacterial Nutrient Source:**

- The transformed plant cells start producing opines, which serve as a nutrient source for Agrobacterium. The bacteria can then utilize these opines as a specific carbon and nitrogen source.

The overall outcome of the T-DNA transfer process is the genetic modification of the plant cells, leading to the formation of a tumor or gall. Researchers have harnessed this natural process for genetic engineering applications, introducing desired genes into the T-DNA to achieve specific traits or modifications in the transformed plants.

(d) Starting from an explant give an outline of different steps involved to raise a plant in vitro. State the essential components of any plant tissue culture medium

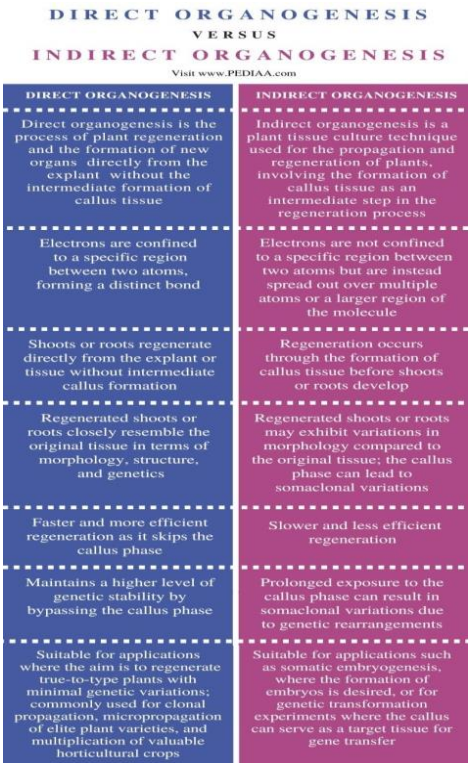
The process of raising a plant in vitro from an explant involves several steps[12]:

1. **Selection of Explant**: The first step is the selection of suitable plant material, known as an explant, which can be a piece of leaf, stem, root, or even a single cell[1].
2. **Surface Sterilization**: The explant is then sterilized to remove any surface contaminants, including bacteria and fungi[1].
3. **Inoculation**: The sterilized explant is transferred to a nutrient medium under aseptic conditions[1].
4. **Incubation**: The inoculated culture is incubated under controlled conditions of light and temperature[1].
5. **Callus Formation**: Under the influence of plant hormones in the medium, the explant cells divide and form a callus, an unorganized mass of cells[1].
6. **Organogenesis or Embryogenesis**: The callus is then induced to form shoots and roots, either directly (organogenesis) or through the formation of somatic embryos (embryogenesis)[1].
7. **Plantlet Formation**: The shoots and roots develop into a complete plantlet[1].
8. **Hardening**: The plantlet is gradually acclimatized to environmental conditions outside the tissue culture lab[1].
9. **Transfer to Soil**: Finally, the hardened plantlet is transferred to soil where it grows into a mature plant[1].

A plant tissue culture medium provides the nutrients necessary for plant growth and development[34]. It generally contains the following components[34]:

- **Macronutrients**: These are elements required in large amounts, such as nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur[34].
- **Micronutrients**: These are elements required in trace amounts, such as iron, manganese, zinc, boron, copper, molybdenum, and others[34].
- **Vitamins**: These include thiamine, riboflavin, niacin, pyridoxine, and others[34].
- **Amino Acids or Nitrogen Supplements**: These provide an easily assimilated source of nitrogen[34].
- **Carbohydrates or Sugars**: These provide a source of energy for the plant cells[34].
- **Solidifying Agents or Supporting Systems**: These help to provide a solid or semi-solid support for the growing plant cells[34].
- **Growth Regulators**: These are plant hormones that control the growth and development of plant cells[34].

(e) Distinguish between direct and indirect organogenesis. Define rhizogenesis. What is somatic embryo?



Rhizogenesis:

- **Definition:** Rhizogenesis refers to the induction and development of roots in plant tissue culture. It is a specific aspect of organogenesis that focuses on the generation of roots from the explant or callus.
- **Characteristics:** Rhizogenesis involves the manipulation of hormonal conditions in the culture medium to encourage the initiation and elongation of roots. This process is crucial for the development of a complete, well-established plantlet with a functional root system.

Somatic Embryo:

- **Definition:** A somatic embryo is an embryo that develops from somatic (non-reproductive) cells in plant tissue culture, rather than through the fertilization of gametes.
- **Characteristics:** Somatic embryos exhibit structures similar to zygotic embryos, including shoot and root meristems, cotyledons, and embryonic axes. They can develop into complete plants when provided with appropriate conditions for germination and growth.
- **Applications:** Somatic embryogenesis is utilized in plant micropropagation, genetic transformation, and the production of synthetic seeds. It allows for the clonal propagation of plants and the regeneration of whole plants from a small piece of tissue.