

1. Cell Culture Types-> (ye lo cutie, pdh lo)

Definition:

Cell culture is a fundamental aspect of plant tissue culture. It refers to the maintenance and growth of plant cells, tissues, or organs under sterile conditions on a suitable nutrient medium in a controlled environment. The aim is to keep the cells alive, dividing, and capable of differentiation.

1.1. Classification

The types of cell culture can be broadly classified based on the nature of the explant and the growth pattern:

A. Based on Explant

1. Organ Culture

- Involves the culture of whole organs or organ primordia such as shoot tips, roots, embryos, anthers, or ovules.
- The aim is to maintain the structural and functional integrity of the organ in vitro.
- For example, shoot tip culture retains the apical meristem's organization; embryo culture maintains the embryo's potential to grow into a plant.

2. Tissue Culture

- This method uses small tissue pieces from different parts of the plant (leaf, stem, node, root).
- The tissue undergoes dedifferentiation, forming an unorganized mass of cells known as callus.
- This callus can later be induced to undergo organogenesis (shoot/root formation) or embryogenesis.

3. Cell Culture

- Involves the isolation and culture of single cells or small cell aggregates.
- Single cells may be obtained mechanically or enzymatically by breaking down cell walls.
- These isolated cells are then cultured in a liquid medium (suspension culture) or solid medium to study cell behavior, totipotency, or produce secondary metabolites.

B. Based on Growth Pattern

1. Callus Culture

- A common technique where an explant forms a mass of undifferentiated parenchymatous cells when placed on an auxin-rich medium.
- Widely used for regeneration studies, induction of somaclonal variation, or secondary metabolite production.

2. Suspension Culture

- Callus pieces are agitated in a liquid medium, breaking into single cells or small clumps.
- Used for large-scale production of plant metabolites like alkaloids, flavors, or pigments.

3. Protoplast Culture

- A protoplast is a plant cell without a cell wall, isolated using enzymes like cellulase and pectinase.
- Cultured protoplasts can regenerate new cell walls and divide to form callus or somatic embryos.
- Also used for somatic hybridization (fusion of two different protoplasts).

Importance of Cell Culture

- Fundamental to understanding plant cell totipotency.
- Provides material for plant regeneration, genetic manipulation, and production of valuable compounds.
- Essential for conservation and propagation of rare or endangered plant species.

2. Meristem Culture (Detailed) -> (pdh rhi ho n cutie?)

Definition:

Meristem culture is the in vitro culture of the actively dividing shoot apical meristem or axillary meristem along with one or two leaf primordia. This method is primarily used to produce virus-free, genetically uniform plants.

2.1. Principle

The shoot apical meristem is generally free from systemic viruses because viruses are unable to invade the meristematic region due to the absence of vascular connections and the rapid cell division rate. This makes meristem culture an important technique for producing pathogen-free planting material.

2.2. Procedure

1. Selection of Mother Plant:

- A healthy, disease-free, elite plant is chosen to ensure high-quality clones.

2. Surface Sterilization:

- The explant is washed thoroughly and sterilized using disinfectants like 0.1% mercuric chloride solution for 2–5 minutes, followed by rinsing with sterile water.

3. Excision of Meristem:

- Under a stereo or dissecting microscope, the meristematic dome along with 1–2 leaf primordia (size 0.1–0.5 mm) is carefully excised to avoid damaging the growing point.

4. Inoculation on Culture Medium:

- The meristem is placed on a solid nutrient medium, typically Murashige and Skoog (MS) medium, supplemented with appropriate concentrations of cytokinins (BAP or Kinetin) for shoot proliferation and auxins (NAA or IAA) for rooting if required.

5. Multiplication:

- The meristem develops into shoots, which can be sub-cultured to produce multiple shoots from a single meristem.

6. Rooting:

- Shoots are transferred to an auxin-rich medium to induce root formation.

7. Acclimatization (Hardening):

- The rooted plantlets are gradually acclimatized to external conditions by initially keeping them in a high-humidity chamber or polyhouse before field transfer.
-

2.3. Applications

- Production of virus-free plants, especially for vegetatively propagated crops such as potato, sugarcane, banana, and ornamental plants.
 - Rapid clonal propagation of elite genotypes.
 - Conservation and exchange of germplasm.
 - Used in horticulture and floriculture industries for commercial production.
-

2.4. Limitations

- Requires skilled personnel and precise handling.
- Contamination can destroy cultures.
- High initial cost for setting up tissue culture labs.

3. Types of In-Vitro Techniques-> (dekh dekh dekh aalsi bacchi)

Definition:

In-vitro techniques include various methods to grow and manipulate plant cells, tissues, or organs under controlled laboratory conditions.

3.1. Major In-Vitro Techniques

1. Meristem Culture:

- Culturing shoot apical meristem to produce virus-free plants.

2. Callus Culture:

- Culture of explants that dedifferentiate into callus under the influence of plant growth regulators. Useful for plant regeneration and production of somaclonal variants.

3. Suspension Culture:

- Small callus pieces agitated in a liquid medium to produce dispersed single cells or small cell aggregates. Extensively used for secondary metabolite production on an industrial scale.

4. Protoplast Culture:

- Isolation and culture of protoplasts for studies on cell fusion, somatic hybridization, and genetic manipulation.

5. Embryo Culture:

- Culturing immature or mature embryos to rescue hybrid embryos that would normally abort due to incompatibility barriers in wide crosses.

6. Anther and Pollen Culture (Androgenesis):

- Culture of immature anthers or pollen grains to produce haploid plants, which can then be doubled to obtain homozygous lines for breeding programs.

7. Ovule and Ovary Culture:

- Culture of ovules or ovaries, especially for overcoming post-fertilization barriers in wide hybridization.

8. Somatic Embryogenesis:

- Induction of embryos directly from somatic (non-reproductive) cells. This method mimics zygotic embryogenesis and is used for large-scale propagation and production of synthetic seeds.

3.2. Importance

- Rapid and large-scale multiplication of superior genotypes.
- Development of disease-free planting material.
- Production of bioactive compounds such as alkaloids, flavors, and pigments.
- Source of genetic variation through somaclonal variation.
- Useful for genetic engineering, somatic hybridization, and conservation of endangered species.

4. Micropropagation — Steps & Stages-> (that's my girl, keep it up)

Definition:

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants using modern plant tissue culture techniques.

4.1. Stages of Micropropagation

Stage I — Establishment:

- Selection and sterilization of explant from a healthy mother plant.
- Introduction and establishment in sterile culture conditions.

Stage II — Multiplication:

- Induction of multiple shoot formation using cytokinin-rich medium (e.g., BAP, Kinetin).
- Repeated sub-culturing to maintain and multiply shoots.

Stage III — Rooting:

- Individual shoots are transferred to a medium containing higher concentrations of auxins (e.g., IAA, NAA, IBA) to induce root formation.

Stage IV — Acclimatization (Hardening):

- Gradual adaptation of plantlets to external environmental conditions by transferring them from in-vitro to ex-vitro conditions.
 - Initially grown in greenhouses or mist chambers before field planting.
-

4.2. Advantages

- Provides disease-free, true-to-type plants in large numbers.
 - Rapid propagation of elite varieties that are difficult to propagate conventionally.
 - Independent of seasonal constraints.
 - Important for conservation and exchange of germplasm.
-

4.3. Limitations

- High initial investment and maintenance cost.
- Requires skilled manpower and strict aseptic conditions.
- Some species may develop somaclonal variation which affects genetic fidelity.