21 Feb 2020

Plant Cell and Tissue Culture

- Cultural techniques for **regeneration** of functional Plants from embryonic tissues, tissue fragments, calli, isolated cells, or protoplasts.
- The *in vitro* and aseptic **cultivation** of any plant part on a nutrient medium. This includes:
  - Cell culture
  - Tissue culture
  - Organ culture
- It is an essential part of **Plant Biotechnology** because
  - Genetic engineering of plants occurs at the level of a single cell.
  - The cell must develop into a whole plant through cell and tissue culture for applicable uses.

Plant Biotechnology

Hinges on this ability of plants - The **ability of a single plant cell to give rise to a whole plants**. This property is unique to plants.

Totipotent cells

Are cells that have retained the **ability to divide and differentiate into a mature plant** if placed in the appropriate environment.

Totipotency

The **ability of undifferentiated plant tissues to differentiate** into functional plants when cultured in vitro.

Competency

The **endogenous potential** of a given cell or tissue to develop in a particular way.

Organogenesis

The process of initiation and development of a structure that shows natural organ form and/or function.

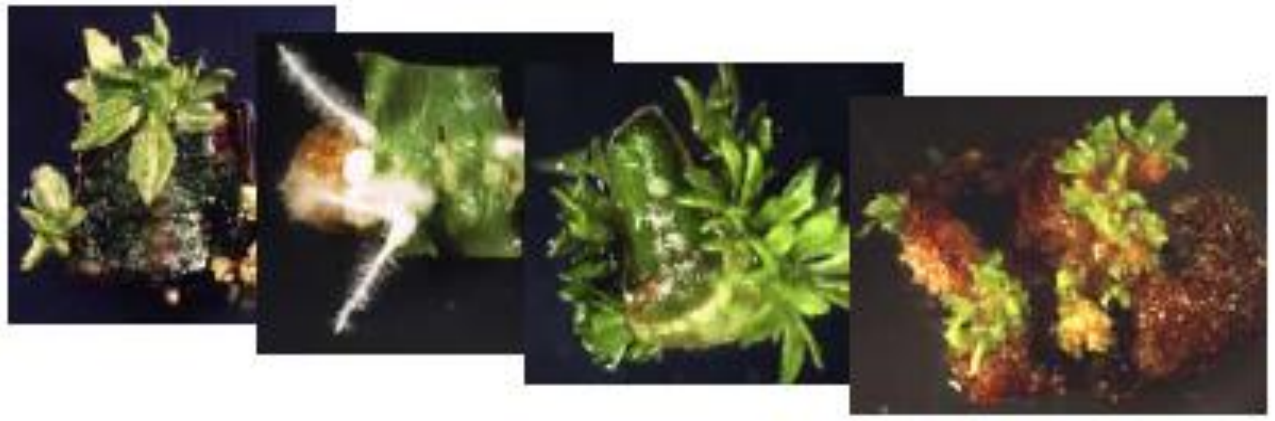
Embryogenesis

The process of initiation and development of embryos or embryolike structures from somatic cells (Somatic embryogenesis).

Differentiation

- The development of cells or tissues with a specific function and/or the regeneration of organs, organ-like structures (roots, shoots, etc.) or embryos.

- Development of organs (roots, buds, shoots, flowers, etc.) or embryos (embryo-like structures) from **points of origin**, including callus.



Basis for Plant Tissue Culture

- **Two Hormones** Affect Plant Differentiation:
  - Auxin: Stimulates Root Development
  - Cytokinin: Stimulates Shoot Development

- *Generally*, the ratio of these two hormones can determine plant development:
  - ↑ **Auxin** ↓**Cytokinin** = Root Development
  - ↑ **Cytokinin** ↓**Auxin** = Shoot Development
  - **Auxin = Cytokinin** = Callus Development

◙ Nutrient medium

Mixture of substances on or in which cells, tissues, or organs can grow.

- ***Inorganic nutrients***
  - Macronutrients, N, P, K, Ca, Mg, Cl, Na
  - Micronutrients, Cu, Zn, Mn, Fe, Bo, Mo, Co, I
- ***Organic nutrients***
  - Vitamins
  - Amino acids
  - Complex organic supplements, Coconut milk, yeast extract
- ***Carbon source*** – sucrose
- ***Growth regulators*** (hormones)
- ***Agar*** (to make the medium semi-solid)

Factors Affecting Plant tissue culture

- **Growth Media** – Minerals, Growth factors, Carbon source, Hormones
- **Environmental Factors** – Light, Temperature, Photoperiod, Sterility, Media.
- **Explant Source** – Usually, the younger, less differentiated the explant, the better for tissue culture.
- **Genetics**

– Different species show differences in amenability to tissue culture.

– In many cases, different genotypes within a species will have variable responses to tissue culture.

Plant tissue culture techniques

1. Micropropagation
2. Somaclonal breeding
3. Embryo culture
4. Anther/Microspore culture
5. Organogenesis
6. Embryogenesis
7. Protoplast isolation, culture and fusion

1. Micropropagation

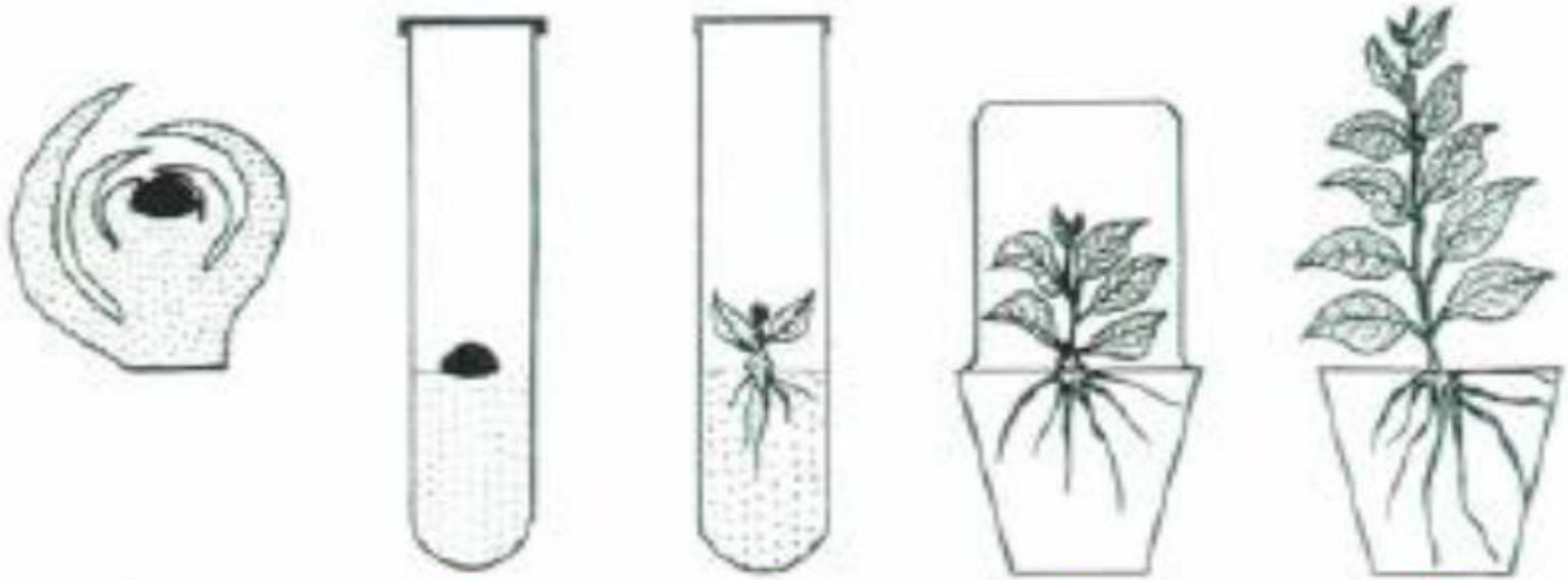
- The **art and science of plant multiplication *in vitro***

- Usually derived from **meristems** (or vegetative buds) **without a callus stage**
  - Tends to reduce or eliminate somaclonal variation, resulting in true clones

- Can be derived from **other explants or callus** (but these are often problematic)

Steps of Micropropagation

- **Stage 0** – **Selection** and **preparation** of the mother plant
  – sterilization of the plant tissue takes place
- **Stage I - Initiation of culture**
  – explant placed into growth media
- **Stage II - Multiplication**
  – explant transferred to shoot media; shoots can be constantly divided
- **Stage III - Rooting**
  – explant transferred to root media
- **Stage IV - Transfer to soil**
  – explant returned to soil; hardened off



A: Apical meristem showing section to be excised
B: Excised meristem on agar medium
C: Plantlet regenerated from excised meristem tip
D: Plantlet transferred to sterile soil
E: Virus-free plant growing in soil

2. Somaclonal Variation and Mutant Breeding

- **Somaclonal variation** is a general phenomenon of all plant regeneration systems that **involve a callus** phase

- **Two general types**:
1. **Heritable**, genetic changes (alter the DNA)
2. **Stable**, but non-heritable changes (alter gene expression, AKA epigenetic)

- **Callus Growth** in Tissue Culture
  - Can screen large number of individual cells
  - Chromosomal aberrations, point mutations
  - Uncover genetic variation in source plant

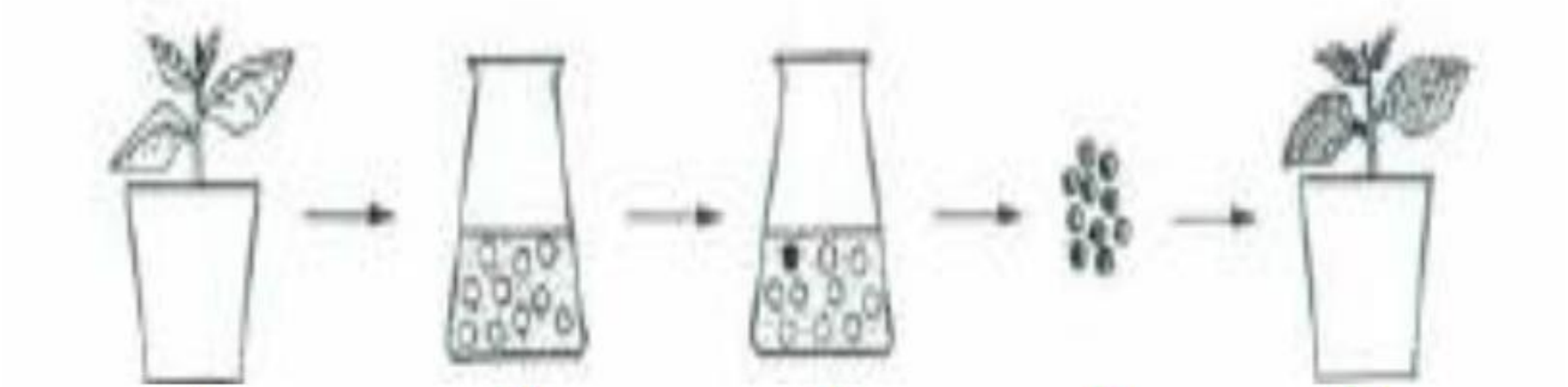
Somaclonal Breeding Procedures

- **Use plant cultures as starting material**
  - Idea is to target single cells in multi-cellular culture
  - Usually suspension culture, but callus culture can work (want as much contact with selective agent as possible)

- **Optional: apply physical or chemical mutagen**

- **Apply selection pressure to culture**
  - Target: very high kill rate, you want very few cells to survive, so long as selection is effective

- **Regenerate whole plants from surviving cells**



A: Haploid plant from cultured tissue

B: Cell suspension from haploid plant

C: Mutant cell in suspension culture

D: Aggregate of mutant cells

E: Haploid plantlet that exhibits the mutant character