PLANT TISSUE CULTURE

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Definition

Tissue culture is in vitro cultivation of plant cell or tissue under aseptic and controlled environmental conditions, in liquid or on semisolid well defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant.

Tissue culture relies on three fundamental abilities of plant there are:

Totipotency

Dedifferentiation

competency

- **1902: Haberlandt attempted to the culture mesophyll tissue and root hair cells.**

  This was the first attempt of *in vitro* culture.
- **1904: Haning attempted to culture excised embryos from mature seeds.**
- **1922: Kotte was successful in obtaining growth from isolated root tips on inorganic media.**
- **Robbins reported similar success from root tip and stem tip.**
- **1934: Used yeast extract (vit B) with inorganic salts to repeatedly culture root tips of tomato by White.**

Contd..

- **1939: Tobacco crown gall culture, callus obtained: called as Plant Cancer.**
- **1940: Coconut milk used in plant cultures to obtain heart-shaped embyos.**
- **1950:Skoog used adenine sulfate to obtain buds on tobacco segments:**
- **identified: kinetin**
- **1958: Stewart and Reinert obtained somatic embryos from carrot cells.**
- **1950-60s: Botanists turned to plant tissue culture to study plant development.**

Contd..

- **1960: Jone, Hildebrandt, Ricker and Wu examined growth in hanging drop cultured of seperated cells from a callus of hybrid tobaccos.**
- **1962: Murashige and Skoog developed MS media for tobacco.**
- **1966: Guha and Maheshwari obtained first haploid plants (Delhi Univ., India)**
- **1970: Discovery of restriction endonuclease (Daniell Nathan). Plasmids were already known.**

Contd..

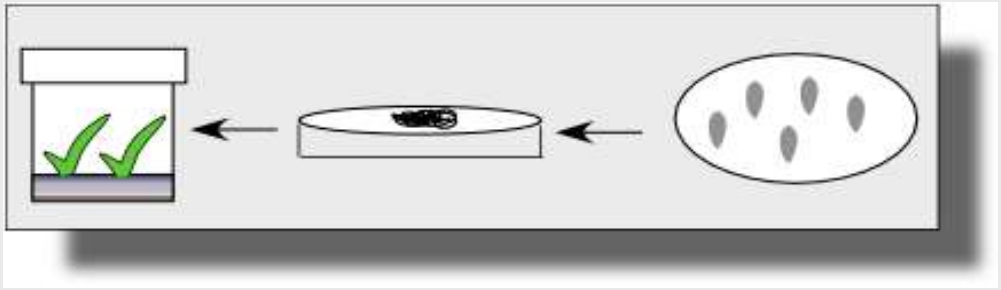
- **1972-73: First recombinant molecule created by Stanley Cohen, Stanford Univ.**
- **1974: Discovery of Ti plasmid in *Agrobacterium tumefaciens* (by Zaenen in Ghent Univ., Belgium)**
- **1970-80s:Ti plasmid analysis (Nester, Seattle; Van Montagu, Ghent)**
- **1983: First transgenic plant. (Monsanto, Ghent, Washington Univ).**
- **1985: Leaf disk transformation method (Monsanto)**

Tissue Culture: De-differentiation to Regeneration

Wheat inflorescence

seeds

embryos



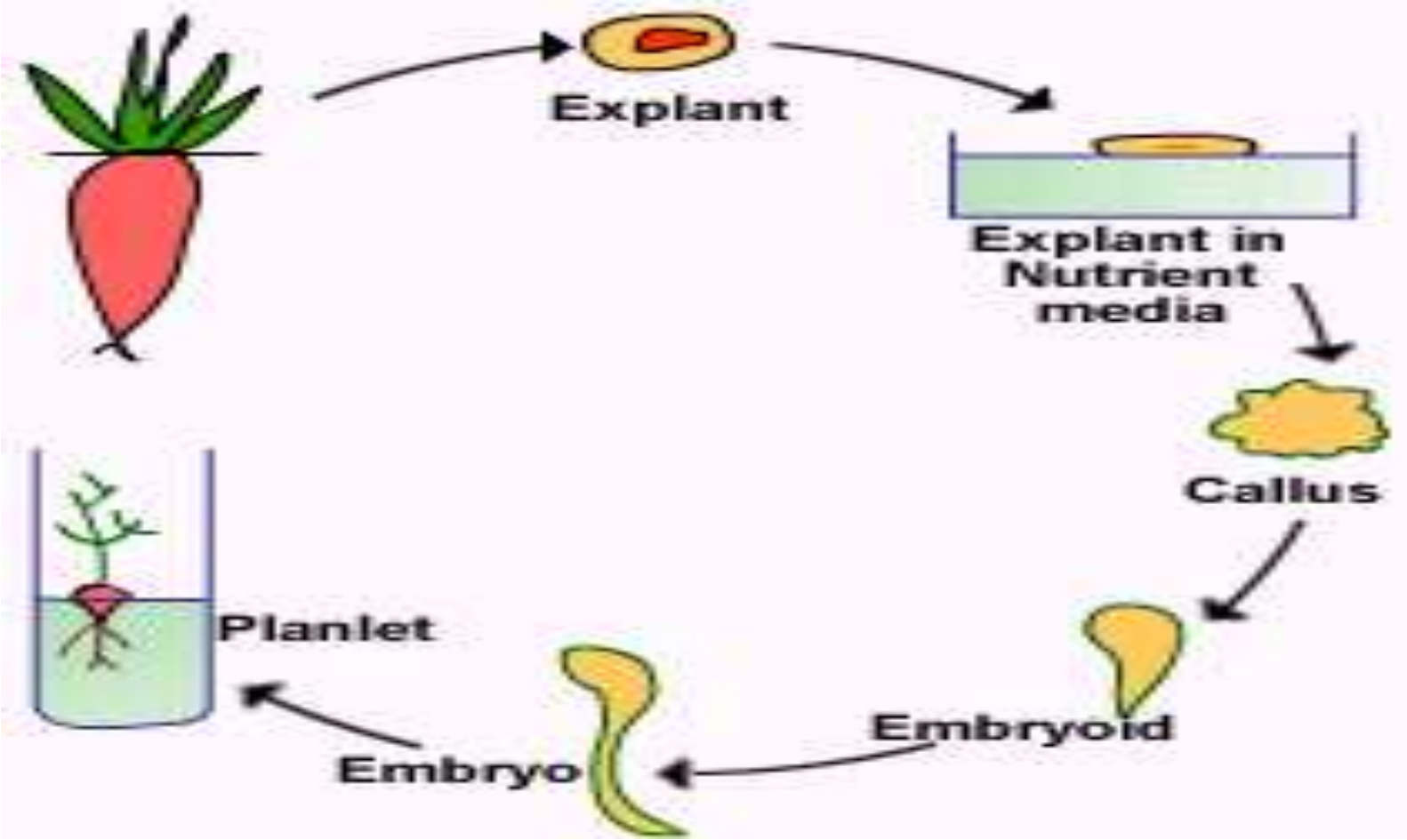
+ auxin

Callus

- auxin/ + cytokinin

Plant cells are totipotent

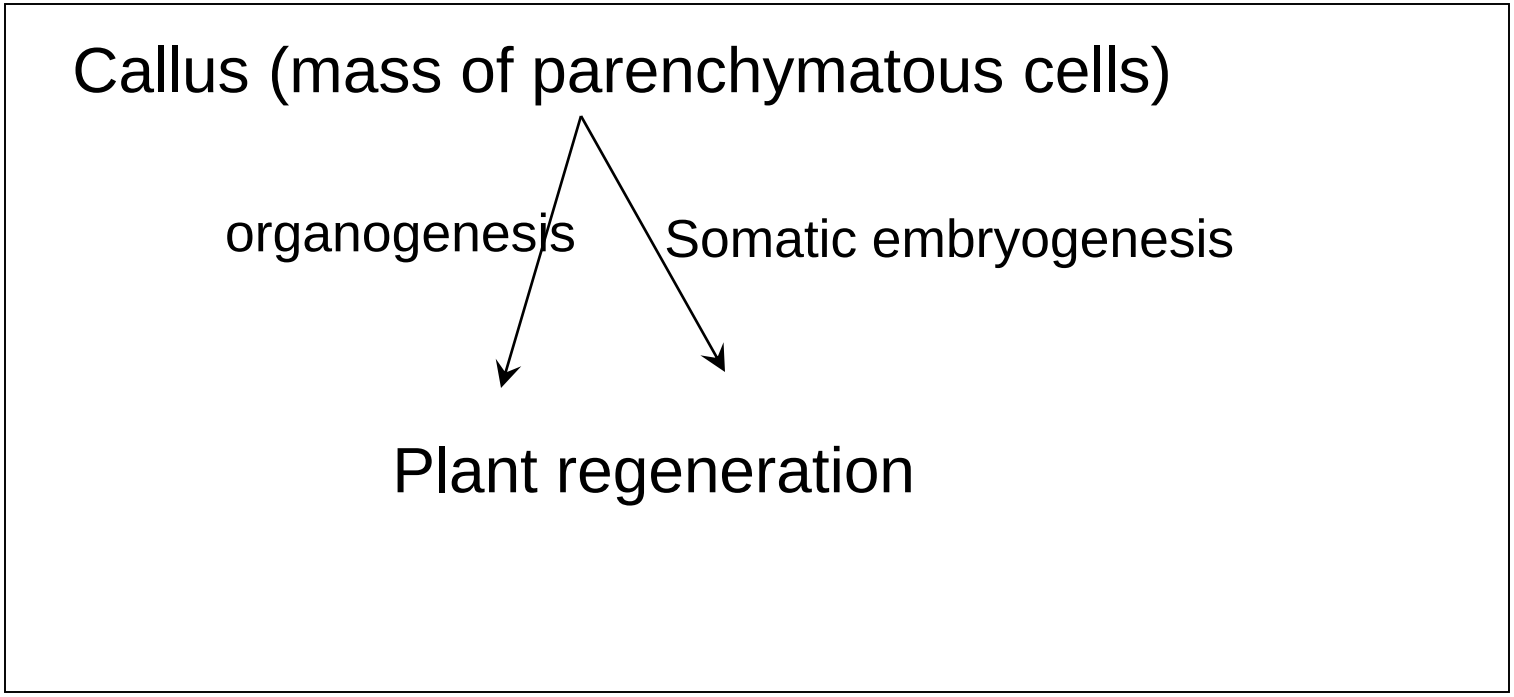
Totipotency: Ability of a cell or tissue or organ to grow and develop into a fully differentiated organism.



Explant: any living tissue: leaf, root, zygotic embryos

Culture Media or Nutrients:

1. Inorganic salts
2. vitamins
3. Carbon source
4. Plant Growth Regulators
5. Organic supplements



Organogenesis

Unique to plants. Plant tissue *in vitro* may produce many types of primordia such as shoot and root

Explant → Callus → meristemoid → organ primordia

Explant → meristemoid → organ primordia

Explant → de-differentiation → induction → differentiation → organ

Applications of Plant Tissue Culture

1. **Haploid production (rice, wheat and barley)**
2. **Triploid production (fruits)**
3. **Embryo Rescue/ Wide hybridization (numerous examples)**
4. **Somatic hybridization (scientific examples, few commercial products)**
5. **Somaclonal Variations (Tomato with altered color, taste and texture by Fresh World Farms; Imidazolinone resistant maize.**

Contd..

6. Production of disease resistant plants.
7. Clonal propagation
8. Secondary metabolite production (eg. Taxol production from cell cultures derived from the bark cuttings of pacific yew tree)
9. Germplasm conservation (cryopreservation)

Applications in detail

- To conserve rare or endangered plant species.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.

Contd..

- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants, and in vitro flowering studies.
- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).

Contd..

- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids.
- This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.

Contd..

- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.