DEPARTMENT OF BIOTECHNOLOGY

SOMATIC EMBROYOGENESIS

Ms. Payal Talekar

Zygotic Embryogenesis

Male Gametes (n)

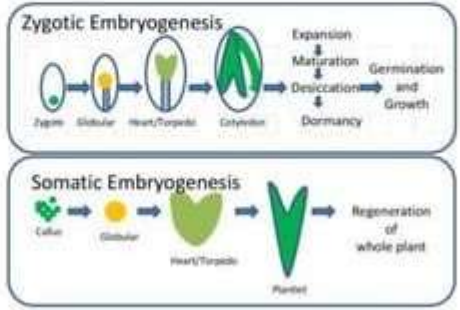
Female Gametes (n)

Zygote (2n)

Mitosis

Embryo

New plant.



Somatic Embryogenesis

Somatic or body cell (2n)

In-vitro.

Callus

Embryoids

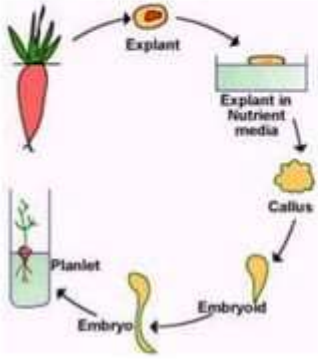
New plant.

Somatic Embryogenesis:

A process where an embryo is derived from a **single somatic cell or group of somatic cells**. Somatic embryos (SEs) are formed from plant cells that are not normally involved in embryo formation.

- ✓ Embryos formed by somatic embryogenesis are called **Embryoids**.
- ✓ The process was discovered for the first time in ***Daucas carota*** L. (carrot) by **Steward** (1958), **Reinert** (1959).

Cell (single) 2n → Callus / Nutrient medium → Induces (PGR) → Embryoids (Non- Zygotic) *In-vitro* → Plant



NOTE:

- Differ without membranous

Eg. Seed coat, endosperm

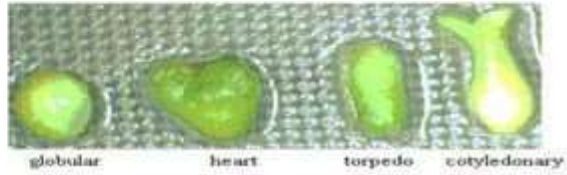
- But functional.

Process of formation of somatic embryos:

Two main phases of somatic embryogenesis include:

1) **Inductive phase**: In this phase, the somatic cells acquire embryonic competence and proliferate as embryonic cells

2) **Expressive phase**:
   a) Development
   b) Maturation



1) Inductive phase

- In this phase, the somatic cells acquire embryonic competence and proliferate as embryonic cells
- An auxin, particularly **2,4-D**, is generally necessary to induce embryogenesis.
- Pro embryogenic masses are formed.
- Once transferred to a medium with low or no auxin, these cells can start to develop into mature embryos.
- For callus induction **MS medium** supplemented with different concentration of 2,4-D(0, 1.0, 1.5, 2.5, 3.5 and 5 mg/l) was used in which **3.5 , 5 mg/l 2,4-D** showed high callus induction percentage.

2)Expressive phase

a) Development

- Globular structure
- Heart- shaped structure
- Torpedo-shaped structure

Auxin must be removed, continuous use of auxin inhibits embryogenesis

b) Maturation

- Cotyledonary
- Germination

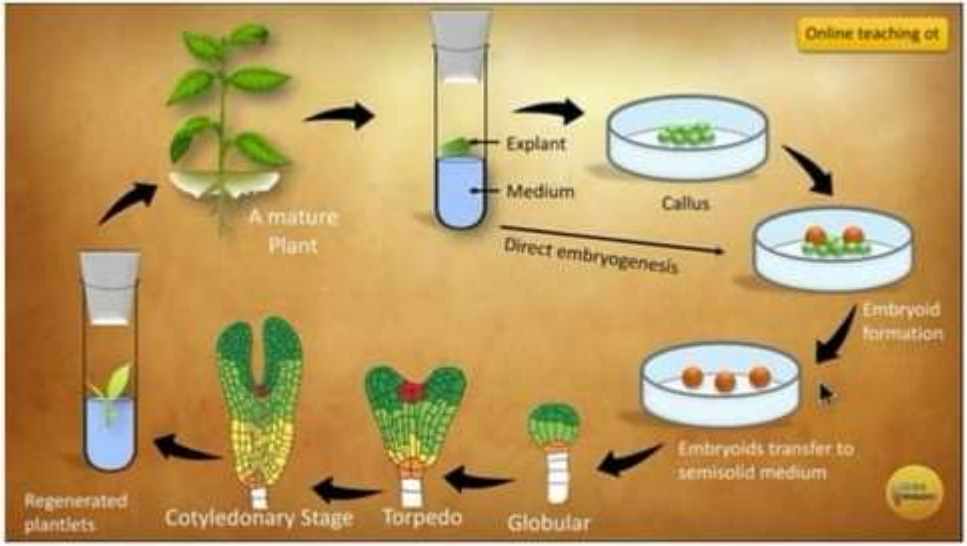
Globular stage:
Embryo is small and round multi-cellular structure

Heart stage
(bilateral symmetry)
Globular shape changes to heart

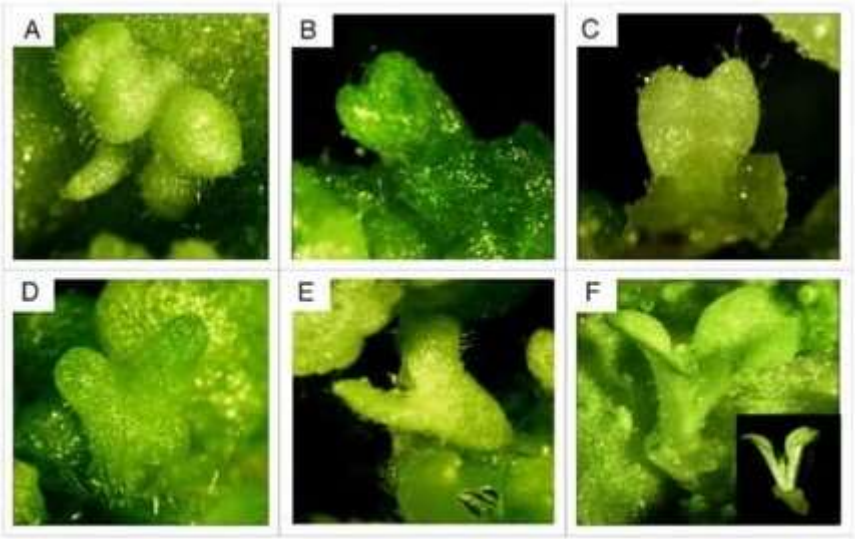
Torpedo – shaped stage
Initial cells for shoot/root meristem

Cotyledonary stage
Shape with more cotyledon development

Embryo becomes cylindrical and germinated



Somatic embryos have a **bipolar structure**, consisting of shoot apex and root apex, so they are able to develop into **shoot and root** respectively in one step, usually without any specific treatment


A
B
C
D
E
F

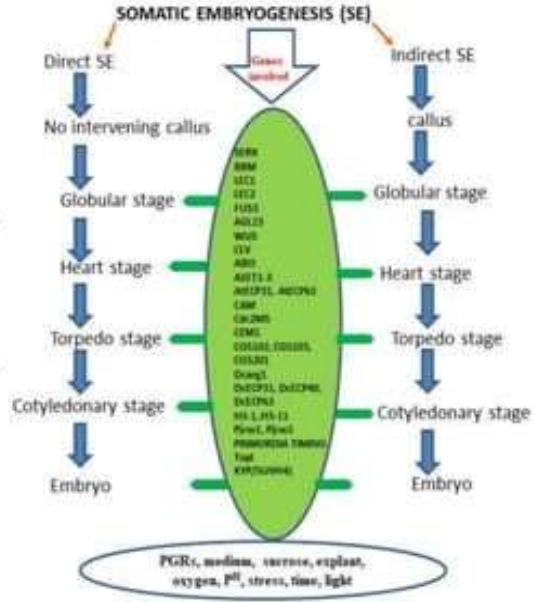
TYPES OF SOMATIC EMBRYOGENESIS :

1. Direct SE

When embryos are formed directly from explant tissue creating an identical clone **without** production of **intervening callus.**

2. Indirect SE

when explants produced undifferentiated mass of cells(**callus**) which is maintained or differentiated into embryo. Specific growth regulators and culture conditions are required for callus formation.



FACTORS AFFECTING SOMATIC EMBRYOGENESIS

1) Characters of explants:

- Even though a variety of explants can be utilized, the correct developmental stage of the explants are also crucial for the initiation of embryogenic callus.
- Young or juvenile explants produced more somatic embryos than older explants

Various types of explants used in SE

1. Immature zygotic embryos
2. Inflorescence
3. Petioles
4. Protoplasts
5. Leaves
6. Stems
7. Roots

2. Plant growth regulators:

i) Auxins

- 2, 4-D has been the best synthetic auxin used for inducing SEs.
- Continuous supply of auxin causes embryogenic cells to divide (Proliferation medium) without the appearance of embryos.
- **Witherell** (1971) have suggested that continuous supply of auxin induces endogenous **ethylene production** which suppresses embryo development.
- So, embryogenic cells after treatment with auxin must be transferred to auxin free medium that constitute the embryo development medium.

ii) Cytokinins:

Cytokinin produces globular embryo from initial embryos.

Zeatin is promotive when applied to embryogenic cells after days 3-4 transfer from the proliferation medium to ED medium whereas **BAP and kinetin** have inhibitory effect on embryogenesis.

High ratio of cytokinin than auxin induces shoot formation and reverse ratio favours rooting.

others include **Gibberllins,** inhibits SE.

ABA promote embryo maturation and prevent precocious germination and secondary embryogenesis.

3) Nitrogen source: reduced form of nitrogen is the sole source of embryo formation.

4) Others:

- ❖ Light generally promotes embryogenesis
- ❖ High temperature usually favorable for embryogenesis

Parameters:

1) pH: 5.6 to 5.8
2) Temperature: 26 °C to 28 °C
3) Humidity: 40 – 80%
4) a) light intensity: 5000 to 8000 lux
   b) light duration: 16hrs light and 8hrs darkness

Advandages:

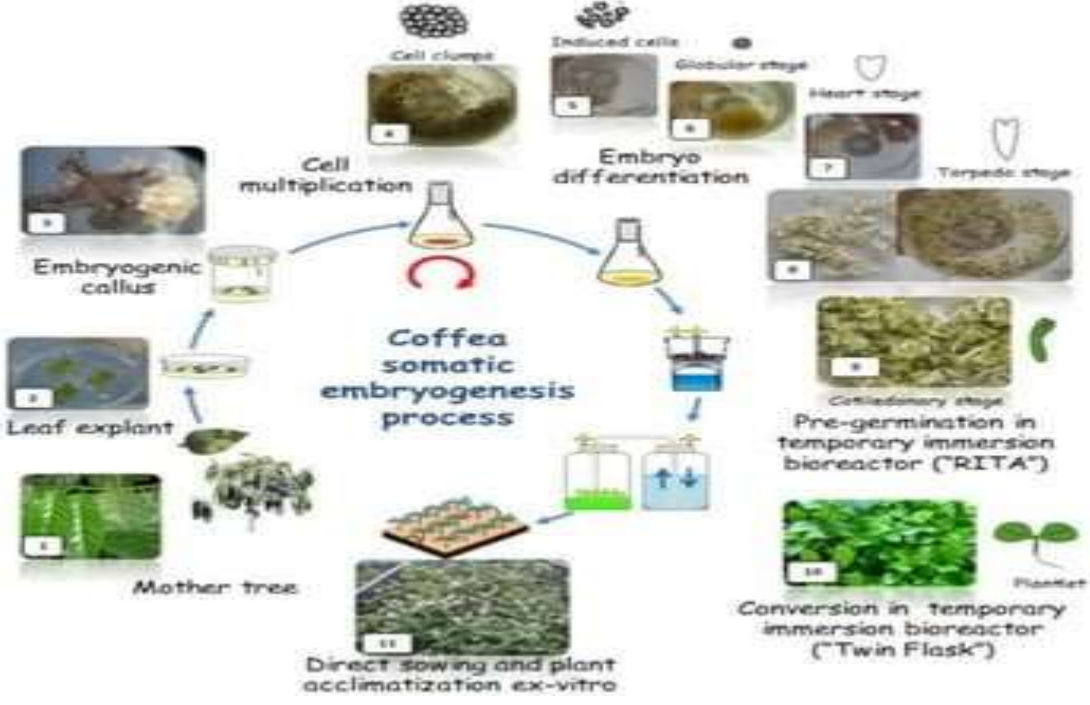
- ✓Can produce artificial seeds by encapsulation of agarose gel or sodium alginate.
- ✓Germplasm conservation.
- ✓Cryopreservation.
- ✓To study the biotechnological studies.
- ✓High propagation rate.
- ✓Somaclonal variations.
- ✓Elimination of diseases and viruses.
- ✓Suitable for suspension culture.

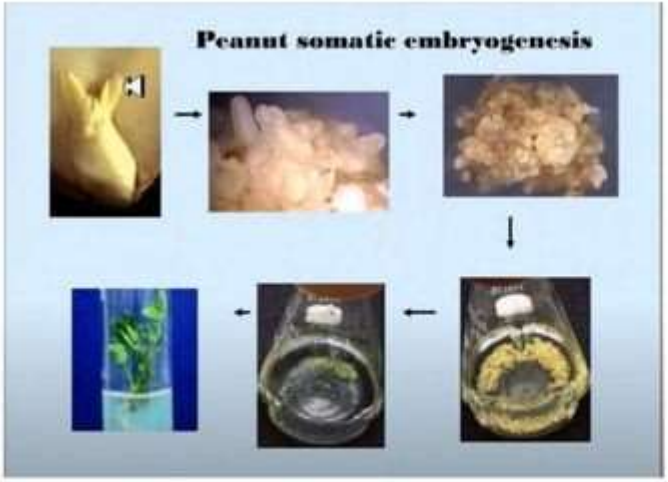
Disadvandages:

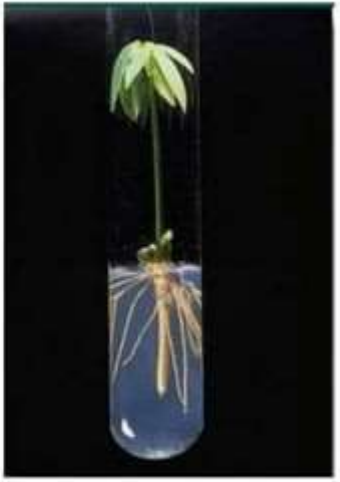
- Confined to few species only.
- Response tissue specific (Explants).
- Show very poor germination because of their physiological and biochemical immaturity.
- Requires skilled labour.
- Time consuming.

Difference between zygotic and somatic embryo:

Variables	Somatic embryo	Zygotic embryo
Formed by	Somatic cells	Fertilized egg or zygote
Covered by	No covering	seed coat
Output	Only form embryo	Seed
Nature of plantlet	Weak	Healthy
Alike	Mother plant	Not like mother plant
Propagation rate	High	Comparatively low


Cell clumps
Induced cells
Globular stage
Heart stage
Torpedo stage
Cell multiplication
Embryo differentiation
Embryogenic callus
Coffea somatic embryogenesis process
Leaf explant
Cotiledonary stage
Pre-germination in temporary immersion bioreactor ("RITA")
Mother tree
Conversion in temporary immersion bioreactor ("Twin Flask")
Direct sowing and plant acclimatization ex-vitro





Rubber tree from somatic embryogenesis

References:

- Bhojwani S. S. and Razdan M. K. 1983. Plant Tissue Culture: Theory and Practice. Elsevier publications. pp: 236-250
- Bajaj, Y. P. S. (1995). Somatic Embryogenesis and Its Applications for Crop Improvement. Somatic Embryogenesis and Synthetic Seed I, pp: 105–125.
- Alemanno, L., Devic, M., Niemenak, N., Sanier, C., Guilleminot, J., Rio, M., et al. 2008. Characterization of leafy cotyledon1-like during embryogenesis in *Theobroma cacao* L. *Planta.* pp: 227, 853–866
- Chalupa, V. 1985. Somatic embryogenesis and plantlet regeneration from cultured immature and mature embryos of *Picea abies* (L.) Karst. *Commun. Inst. For. Cech* 14.pp: 57–63.
- Joshi, R., Kumar, P., 2013, Regulation of Somatic Embryogenesis in Crops: A Review, Agri. Reviews, 34 (1): 1- 21, 2013.
- Warren, G.S., Fowler, M.W. 1981. Physiological interactions during the initial stages of embryogenesis in cultures of *Daucus carota* L. New Phytol. pp: 481-486.