Aim: To Study the Plant Tissue Culture Technique: Somatic Embryogenesis

Introduction:

Somatic embryogenesis (SE) is an artificial process in which a plant or embryo is derived from a single somatic cell. Somatic embryos are formed from plant cells that are not normally involved in the development of embryos, i.e. ordinary plant tissue. No endosperm or seed coat is formed around a somatic embryo. **The first documentation of somatic embryogenesis was done by Steward et al. in 1958 and Reinert in 1959 in *Daucas carota* L. (carrot)** cell suspension cultures. Von Arnold and co-workers (2002) further defined somatic embryogenesis as a process in which a bipolar structure resembling a zygotic embryo develops from a non-zygotic cell without vascular connection with the original tissue. Embryos formed by somatic embryogenesis are called embryoids.

Part of plant used as Explant for SE:

- Stem
- Root
- Leaves
- Inflorescence
- Petiole
- Protoplast
- Cell suspension
- Protoplast

Types of SE: Somatic embryogenesis has been described to occur in two ways: directly or indirectly.

1. **Direct Embryogenesis:**

   The embryos initiate directly from the explant without callus formation and here some cells which are called as 'Pre-embryonic determined cells' (PEDC) initiates embryonic development, only those cells need to be released. Such cells are found mostly in embryonic tissues, certain tissues of young in vitro grown plants, hypocotyl, nucellus, embryo-sac, etc.

2. **Indirect Embryogenesis:**

   Here, the embryos are developed through cell proliferation i.e., callus formation. The cells from which embryos arise are called as 'Induced

embryogenic determined cells' (IEDC). Here growth regulators with specific cultural conditions are required for initiation of callus and then redetermination of those cells into the embryo development for Eg. 2,4-Dichlorophenoxyacetic acid (2,4-D), 6-Benzylaminopurine (BAP) and Gibberellic acid (GA) has been used for development of indirect somatic embryos in strawberry (*Fragaria ananassa*)

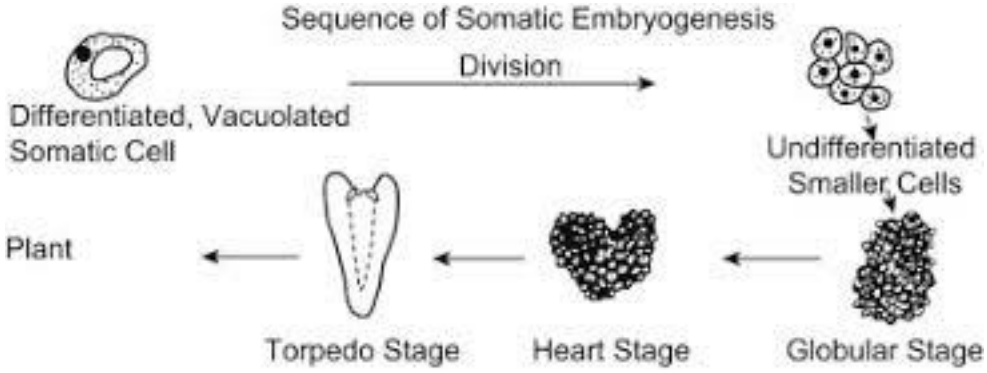
Stages in Somatic Embryogenesis:

Explant is the source to initiate the process of Somatic embryos and uses an exogenous supply of carbohydrates and their morphological stages occur without vascular tissue connection. Somatic embryos are mainly produced in vitro and for laboratory purposes, using either solid or liquid nutrient media which contain plant growth regulators (PGR's). The main PGRs used are auxins but can contain cytokinin in a smaller amount. Cells derived from competent source tissue are cultured to form an undifferentiated mass of cells called a callus. Plant growth regulators in the tissue culture medium can be manipulated to induce callus formation and subsequently changed to induce embryos to form the callus. The ratio of different plant growth regulators required to induce callus or embryo formation varies with the type of plant. Most somatic embryos pass through the same stages of development as a zygotic embryo; such as the globular-shaped, heart-shaped, torpedo-shaped, and cotyledonal stages (in dicotyledonous species) and globular, scutellar, and coleoptile stages in the case of monocotyledonous species (Winkelmann, 2016, Zhao et al., 2017). Once the somatic embryos reach the cotyledonary stage, they initiate a shoot meristem, and seedling growth begins (Yang and Zhang, 2010).

1. **Induction:** Auxin particularly 2,4-D, is utilized to induce embryogenesis (concentration depends on type of plant)
2. **Development:** After initiation of cell division and proliferation, cells are transferred to auxin free medium. Group of cells now known as Proembyonic mass of cells (PEMs). Cytokinins are used to form globular stage from the initial embryonic cells.

3. **Electrical stimulation:** In some plants like alfalfa and tobacco mild electric current (0.02 V DC for 20 h) promote embryogenesis through promoting ploliferation and polarity.
4. Maturation: Abscisic acid (ABA) is used in this stage.

ABA, which prevent precocious germination and promotes normal development of embryogenesis by triggering expression of genes which normally express during drying-down stage of seeds (Dure *et al*,1981).



Stages during the process of Somatic Emryogenesis

(Note: Draw the above diagram in your file)

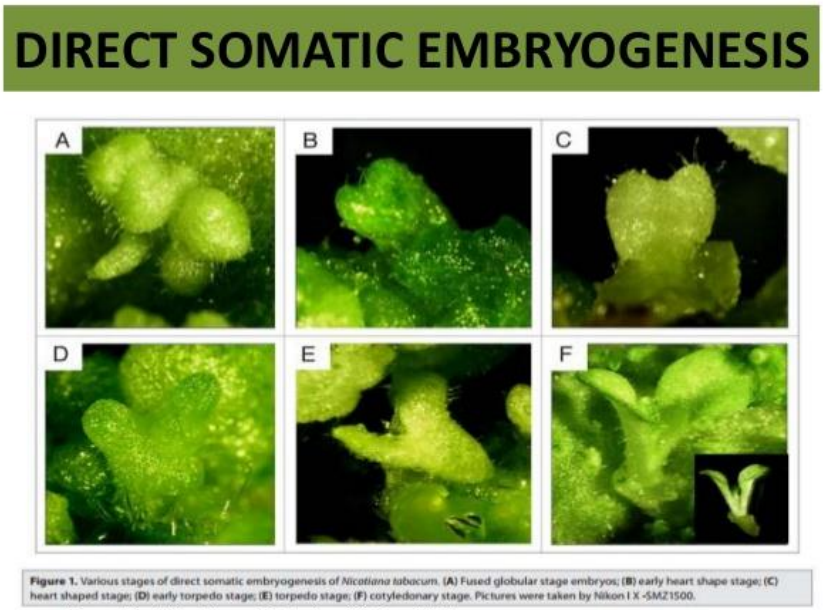


Figure 1. Various stages of direct somatic embryogenesis of *Nicotiana tabacum*. (A) Fused globular stage embryos; (B) early heart shape stage; (C) heart shaped stage; (D) early torpedo stage; (E) torpedo stage; (F) cotyledonary stage. Pictures were taken by Nikon I X -SMZ1500.

Source: Plant Signaling and Behaviour 8:6, June, 2013.

Paste the print out of this picture (u can get same pic in better resolution from internet)

Applications of Somatic Embryogenesis:

(i) Large Scale Propagation Compared to Zygotic Embryos:
Induction of somatic embryogenesis forms the ultimate goal in free cell suspension cultures relying on the totipotency of the cell and could reasonably be exploited for micro-propagation. Somatic embryogenesis is highly desirable and holds out promise for rapid multiplication in a shorter time, with a shoot-root axis.

ii) Cryopreservation

Plant somatic embryos can be preserved in liquid nitrogen, keeping them in a physical state at -196°C (Kartha et al. 1988). Cryopreservation is an effective technique for long-term conservation of woody plant somatic embryos.

(ii) More Useful than Organogenesis:
The adventitious embryo is a bipolar structure that develops directly into a complete plantlet and there is no need for a separate rooting phase as with shoot culture.

(iii) Useful for Mutagenic Studies and Mutant Production:
The somatic embryos generally arise from single cells, so it may be advantageous for mutagenic studies. Also the plantlets arising from such somatic embryos are more homogeneous in nature, so the mutant gene expression can be studied well.

(iv) Useful for Genetic Manipulation Technique:
In plant biotechnological application, during foreign gene transfer if the transformed cell gives rise to plantlet via somatic embryogenesis then there is least possibility of chimera formation. So for transgenic plant production this method of multiplication system is very much useful.

(v) Useful for Pathogen-Free Plant Production:
Plants derived from this kind of somatic embryos may be free from viral or other pathogens. So it may be an alternative approach of disease free plant production.

(vi) A Good Source of Protoplast Culture:
Embryogenic cultures are specially valuable in providing a source of regenerable protoplasts in the graminaceous and coniferous plants. Protoplasts from these cultures were induced to divide to form a cell mass from which the embryoids, even plantlets are regenerated on a suitable nutrient medium.

(vii) Conservation of Genetic Resources/ Clonal Propogation:

Somatic embryos which originate from single cells and subsequently regenerate mostly genetically uniform plants are good materials for genetic resource conservation. Embryogenic cultures as well as somatic embryos remain viable upon storage at ambient temperature, cold storage or cryostorage.

viii) **Synthetic/ Artificial Seeds:** The synthetic or artificial seed was defined as an encapsulated single somatic embryo inside a matrix covering. The concept of synthetic seeds was first mentioned by Murashige (1977). Synthetic seeds could, therefore, be easily handled for storage, transport, and sowing, the same as a zygotic seed.

Disadvantages of Somatic Embryogenesis:

- Confined to few species.
- The somatic embryos show very poor germination because of their physiological and biochemical immaturity.
- Instability of cultured cells in long-term cultures is a major limitation in commercial exploitation and mass propagation of SEs.