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Module: 11 Somatic Embryogenesis

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Somatic Embryogenesis

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Somatic Embryogenesis

Introduction
You have learnt earlier, that callus mass can either give rise to shoot buds (organogenesis or somatic embryos). Somatic embryogenesis (SE) involves the development of somatic embryos from the somatic cells irrespective of their ploidy levels and of their specializations. SE has enormous applications in basic and applied sciences of Plant Biotechnology.

History of Somatic Embryogenesis
In early 1900s, Haberlandt has proposed that a single vegetative cell could develop into a complete embryo. Later, in 1958 two scientists Steward from USA (Steward et al., 1958) and Reinert from Germany (Reinert, 1958) succeeded in developing carrot plants through somatic embryos. This discovery opened new doors for the studies on SE and since then more than 500 species of monocots and dicots were explored (Thorpe and Stasolla, 2001). Reports are on different genus of Daucus sp., Citrus sp., Macleaya sp., Coffea sp. and Medicago sp. (Rangaswamy, 1986). The remarkable contribution in the field of molecular cell biology and genetics was in deducing the complete genome sequence by the understanding the system associated with SE in Arabidopsis sp (Raghavan, 2006). Furthermore, different experiments were carried out by using different media to produce efficient varieties. For example callus formation was observed by culturing the root segments of seedlings from Daucus carota in 2, 4-D medium (McWilliam, 1974). Medium enriched with coconut milk and IAA promoted somatic embryo formation from the callus of Ranunculus sceleratus (Dorion et al., 1975). The production of somatic embryos from single cells was observed in the mesophyll tissues of Macleaya cordata (Bhojwani and Dantu, 2013).

Mode of Direct and Indirect Somatic Embryogenesis
Somatic embryogenesis ways in two different manners:
1. Direct SE
2. Indirect SE
In case of Direct SE, it is expected that plantlets produced will be mirror image of the mother plant (except in case of chimeras, where cells of different types occur in the tissue) and depending on the cell which differentiates pure / solid lines will be produced. In case of Indirect SE, explants first produce undifferentiated cells called callus, which then differentiates into somatic embryos. The callus cells may show variability and so will be the plantlets originating from them.

**Steps for Plant regeneration by Somatic Embryogenesis**

There are five major steps for plant regeneration by Somatic Embryogenesis:

1. Initiation
2. Proliferation
3. Prematuration
4. Maturation
5. Plant Development

**Factors affecting Somatic Embryogenesis (SE)**

Before the development of somatic embryos, the plant cells are subjected to biological pattern of re-differentiation and thus, there are many associated factors affecting somatic embryogenesis and development of somatic embryos. Somatic embryogenesis involves a through selection of explants, careful selection of nutrient media (such as MS, B5, N6 and White media), plant growth regulators and the physical environment during incubation in the growth rooms (including light and temperature). The experimentation underlying the development involves a series of processes from stimulation of embryogenesis, development of embryonic cells, and their transformation through germination into complete plants. Usually shoot tips, immature embryos and young floral parts have been used as explants. In monocots (palms and cereals), and many dicots the most common explant is immature zygotic embryos. In case of mature embryos, proto dermic cells of cotyledon forms SE. The
growth regulators provide stimulus for embryogenesis. Auxins occupy the most prominent position regarding tissue regeneration. Different concentration of synthetic auxin 2, 4-D is used for callus induction. Its concentration from 0.5-1.0 mg/L is used for callus induction and proliferation. While callus proliferates on auxin supplemented media, to induct embryogenesis, it must be transferred to cytokinin rich media. Zeatin, at concentration of 0.1µM promoted regeneration in carrot. Other cytokinins such as BAP with IAA has led to the development of globular embryos in *Podophyllum* sp. Furthermore, combinations of various growth regulators are known to effectively promote SE. A proportion of ABA, gibberellic acid and zeatin and that of 2iP and GA3 are required for subsequent growth in grape vine (Ammirato, 1977). pH modification and high sucrose concentration can also promote SE without the use of plant growth regulators.

Requirement of nitrogen is considered as the second major requirement for induction of embryo formation. It is required for initiation and maturation of embryos. Ammonia (a reduced form of nitrogen) acts as triggering factor for rapid embryogenesis. Addition of reduced form of nitrogen (casein hydrolysate/ NH₄Cl/ or NH₄NO₃/ or amino acid etc.) into the media helps in induction of embryogenesis once the callus is shifted from auxin to auxin free media or media enriched with cytokinin. It has been observed that the most frequent source of carbon is sucrose.

Cell density, ethylene concentration and light are other factors affecting SE. It is observed that the initial cell density is the critical factor for differentiation of somatic embryos. In carrot, high density culture on auxin free medium resulted in inhibition of somatic embryo formation. The cells which are cultured at high density have the tendency to release high amount of phenolic compounds such as pHBA, benzoic acid, 4-hydroxy benzoic acid etc (De Vries, 1988) which inhibit embryo formation. The ethylene biosynthesis is able to increase SE in *Daucus carota* and *Hevea brasiliensis*. Appropriate oxygen concentration has significant role in the development of somatic embryos. Six-fold improvement was observed in SE by the reduction of dissolved oxygen in wheat cultures. Enhanced somatic embryo development was seen when co-culturing of soyabean was done with *Pseudomonas maltophilia*, similarly extracts of blue green algae *Anabena* in cultures of *Daucus carota* promoted embryogenesis. Different plant species respond differently towards light intensity. In *Solanum melongena*, the somatic embryos grew well under light whereas in *Populus* SE was promoted in complete darkness. Additionally, quality of light has strong impact for
development of somatic embryos for example in *Daucus carota*, high intensified white and blue light has inhibitory effects.

**Induction**
The most important prerequisite for acquiring embryonic development is the dedifferentiation of cells. Various PGRs, factors such as pH and the use of heavy metals are known to promote dedifferentiation of cells and elicit embryonic responses. Moreover, cellular polarity is remarkably associated with the induction of SE. The cellular polarity is highly altered by PGRs as well as other treatments used for the induction of SE and promote asymmetric division. The single cell suspension in *Daucus carota*, the first cell division is asymmetric in nature and it is the smaller daughter cell that develops into somatic embryo. The polarity of complete SE is predefined much before the first embryonic cell division because the root pole of somatic embryo is towards the larger cell.

**Development**
Usually after the reinitiation of cell divisions as well as cell proliferation the embryogenic cells are released into the medium. The suspended culture contains the following two types of cells:

1. Proembryogenic masses (PEMs)
2. Cytoplasmically rich cells

The PEMs comprise of embryogenic cells that are 400–800 µm, angular and are connected to adjacent cells with plasmodesmatas, have small vacuoles, large starch grains (5-25%), high density ribosomes, ER etc. The embryogenic cells are held by non-embryogenic cells which are 1000–3000 µm³; rounded; large vacuoles (80% of cell volume); few starch grains (1-2%); low population of ribosomes, etc. The further availability addition of auxin after induction of embryogenesis leads to cell elongation. The enlarged cells permanently lose ability to form somatic embryos.

One of the most studied system for SE in culture is *Daucus carota*. Steps for inducing somatic embryogenesis in culture are as follows:
1. Explant such as leaf petiole or cambium tissue is sterilized and placed on semi-solid Murashige and Skoog’s medium and allowed to produce sufficient amount of callus tissue.

2. This callus tissue is transferred to Erlenmeyer flask. These flasks are placed horizontally on the gyratory shaker for initiation of cell suspension culture.

3. Sub culturing is done after every 4 weeks by transferring a small amount of cell suspension to the fresh liquid medium.

4. For uniformity in embryo formation, the cell suspensions may be passed through stainless steel mesh sieves.

5. Within four weeks, numerous embryos develop.

6. Somatic embryos are placed on agar medium for further development.

7. Small plantlets are then transferred to Jiffy pots for further growth and development.

![Figure 1: Steps for Inducing SE in Culture](image-url)
Figure 2: Developmental stages of in vitro somatic embryogenesis

Physiological and Biochemical aspects of SE

Research has proved that cell acquires embryogenic competence because of physiological and biochemical changes that occur much before the morphological differentiation of somatic embryo. The most remarkable changes that are observed are in the endogenous levels of various phytohormones and amino-acid metabolism.

Synchronization of SE development

It is worth mentioning that while somatic embryos are of single cell origin, the cell population either in liquid medium or semi-solid medium exhibits embryos at different stages of development (asynchronous development of embryos). It is, however, desirable to synchronize embryo development as to optimize the number of embryos, it is essential to have them at the same stage as different stages have different hormonal requirements. This is achieved by

i) Physical separation of embryos at different stages of development
ii) Through the application of growth regulators, control the growth stage physiologically
Molecular makers and somatic embryogenesis

During the development of somatic embryos Transcriptomics of several species is carried out. Different genes such as LEC, WUS, FUS; auxins and transcriptional factors have a predominant role during the induction of SE i.e. transfer of a somatic cell into an embryogenic cell. Epigenetics controlling the expression of genes has significant role and research proves that auxins in addition to modification of DNA methylation patterns influence the embryogenic cells. Other factors that regulate proteins are post translation modifications, protein turnover and protein-protein interactions.

As the somatic cells move towards embryogenic development, changes in gene expression occur. Molecular markers indicating this transition have been recognized and also genes that enhance embryogenic response have been cloned.

The position of callus and appearance of calcium in the vacuoles are the first signs of recognition of embryogenesis cells. Similarly cell wall associated proteins also participate in embryogenic process. It is well documented that somatic embryogenesis in fresh cultures can be induced by addition of cell free conditioned medium. This is achieved due to the presence of extra cellular proteins (ECP) released by embryogenic cells that are present in the conditioned media.

Maturation
The zygotic embryos typically undergo a maturation phase during which they acquire tolerance towards desiccation; store food and sometimes synthesis ABA to induce dormancy. Somatic embryos, however, do not have the stage of embryo maturation and germinate immediately resulting in weak seedlings. Thus, morphologically mature embryos of many species require additional stage something similar to zygotic embryo formation. For example gradual desiccation increase in sucrose concentration and ABA treatment during maturation stage helps in better survival of plantlets developed from somatic embryos upon transplantation. To improve germination of somatic embryos, reducing humidity inside the culture vial mimicking embryo sac environment also favours survival of embryos in vivo.
Applications of SE

- **Micro-Propagation Industries:**
  Somatic embryos are formed in large numbers & thus if efficient method for cloning is developed, large number of embryos can be developed at low cost. Further, SE can be carried out in bioreactors, thus reducing manpower & space requirement.

- **For the Production of Synthetic Seeds:**
  Artificial seeds or the synthetic seeds are encapsulated somatic embryos. Plants species exhibiting sterility in seeds usually produce synthetic seeds, used for germplasm conservation. You will be learning more about it in the module on somatic embryogenesis.

- **Embryo Cloning:**
  Large numbers of somatic embryos are produced because of recurrent embryogenesis. These embryos produce metabolites such as proteins and lipids (α-linolenic acid) which have high industrial applications.

- **Use of Somatic Embryos in Genetic Engineering:**
  The most idealistic approach for gene transfer technology is the use of somatic embryos. This remarkable approach is more advantageous than many other methods due to ease of transformationi & its genetic stability. The process involves incubation of somatic embryos on Agrobacterium solution or by subjecting these cells to particle bombardment.

**Conclusion**

Somatic embryogenesis has been observed in a large number of species while a large number of explants have been used, zygotic embryos have proved to be the most potent explants. Somatic embryogeneseis has proved to a cost effective method for the clonal propagation of plants. Somatic embryogenesis has proved to a cost effective method for the clonal propagation of plants. Use of bioreactors has further enhanced application of SE for micropropagation. Somatic embryogenesis has further added to the understanding of physiological, biochemical and molecular events occurring at various stages of transition from somatic cells to embryogenic cells. Also, modecular studies have contributed to the
identification of genes responsible for SE. While possibility of encapsulating somatic embryos in nutrient media has been well documented, more research is needed to use the SE as synthetic seeds and the possibility of storing germplasm as synthetic seeds.