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Botany

UG-SEM-VI Paper – C14T

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Somatic Embryogenesis

What is Somatic Embryogenesis?

In plant tissue culture, the developmental pathway of numerous well-organised, small embryoids resembling the zygotic embryos from the embryo genic potential somatic plant cell of the callus tissue or cells of suspension culture is known as somatic embryogenesis.

What is Embryo genic Potential?

The capability of the somatic plant cell of a culture to produce embryoids is known as embryo genic potential.

What is Embryoid?

Embryoid is a small, well-organised structure comparable to the sexual embryo, which is produced in tissue culture of dividing embryo genic potential somatic cells.

Brief Historical Background:

J. Reinert (1958-59):

Reported his first observations of in vitro somatic embryogenesis in Daucus carota.

F. C. Steward, M. O. Mapes and K. Mears (1958):

Also reported the somatic embryogenesis in carrot from freely suspended cells and emphasized the importance of coconut milk for in vitro somatic embryogenesis.

N. S. Rangaswamy (1961):

Studied in detail the somatic embryogenesis in Citrus sp.

R. N. Konar and K. Nataraja (1969):

Studied the somatic embryogenesis of Ranunculus sceleratus using various floral parts (including anthers) as well as somatic tissues in culture.

P. V. Ammirato (1974):

Reported the effect of abscisic acid on the development of somatic embryos from cells of Carum carvi.

H. Lang and H. W. Kohlenbach (1978):
Demonstrated the ability of mechanically isolated, fully differentiated mesophyll cells of Macleaya cordata to yield an embryogenic callus.

B. V. Conger, G. E. Hanning, D. J. Gray and J. K. McDaniel (1983):
Obtained direct embryogenesis from leaf mesophyll cells of orchard grass (Dactyhs glomerata L.) without an intervening callus tissue.

Types of Embryos:

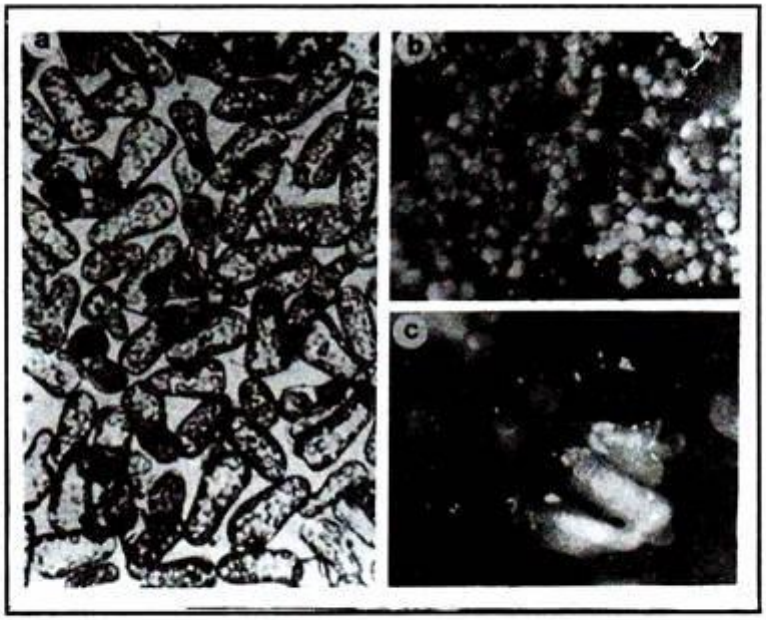
1. Zygotic Embryos: • These formed by fertilized egg or the zygote.

2. Non-Zygotic Embryos:

a) Somatic Embryos: • Those formed by Sporophytic cells in in-vitro condition. Such somatic embryos arsing directly from other embryos or organs are termed adventive embryos.

b) Parthenocapic Embryos: • Those formed by unfertilized egg. c) Androgenic Embryos: • Those formed by the male gametophyte.

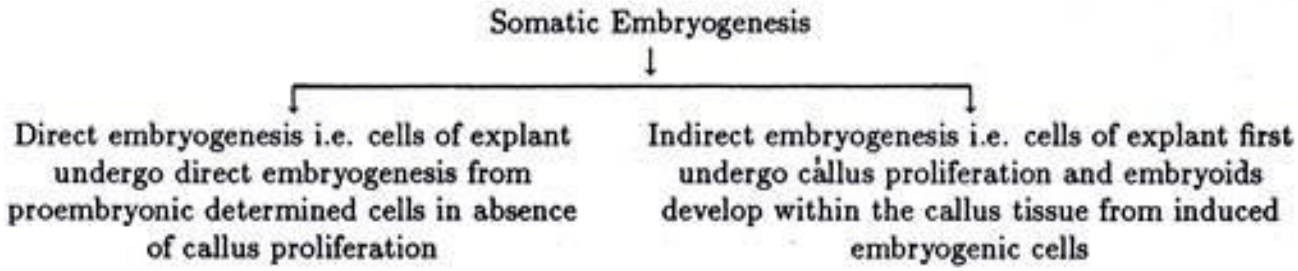
Principles of Somatic Embryogenesis:
Somatic embryogenesis may be initiated in two different ways:
1. In some cultures somatic embryogenesis occurs directly in absence of any callus production from "pro-embryo genic determined cells" that are already programmed for embryo differentiation (Fig 8.1). For instance, somatic embryos has been developed directly from leaf mesophyll cells of orchard grass (Dactyhs glomerata L.) without an intervening callus tissue.



❒ Fig 8.1

Photograph showing direct embryogenesis. A. A suspension of mechanically isolated mesophyll cells. B. Embryogenesis. C. A portion enlarged from B.

Explants, made from the basal portions of two innermost leaves of orchard grass were cultured on a Schenk and Hildebrandt medium supplemented with 30 µM 3, 6-dichloro-O-anisic acid (dicamba). Plant formation occurred after sub culturing the embryos on the same medium without dicamba (Conger et al., 1983).



2. The second type of somatic embryo development needs some prior callus formation and embryoids originate from "induced embryo genic cells" within the callus tissue.

In most of the cases, indirect embryogenesis occurs. For indirect somatic embryogenesis where it has been induced under in vitro condition, two distinctly different types of media may be required—One medium for the initiation, of the embryonic cells and another for the subsequent development of these cells into embryoids.

The protocol is described below:

1. Leaf petiole (0.5-1 cm) or root segments from seven-day old seedlings (1 cm) or cambium tissue (0.5 cm^3) from storage root can be used as explant. Leaf petiole and root segment can be obtained from aseptically grown seedlings (Cambium tissue can be obtained from surface

sterilized storage tap root 2. Following aseptic technique, explants are placed individually on a semi-solid Murashige and Skoog's medium containing 0.1 mg/L 2, 4-D and 2% sucrose. Cultures are incubated in the dark. In this medium the explant will produce sufficient callus tissue.

3. After 4 weeks of callus growth, cell suspension culture is to be initiated by transferring 0.2 gm. of callus tissue to a 250 ml of Erlenmeyer flask containing 20-25 ml of liquid medium of the same composition as used for callus growth (without agar). Flasks are placed on a horizontal gyratory shaker with 125-160 rpm at 25°C. The presence or absence of light is not critical at this stage.

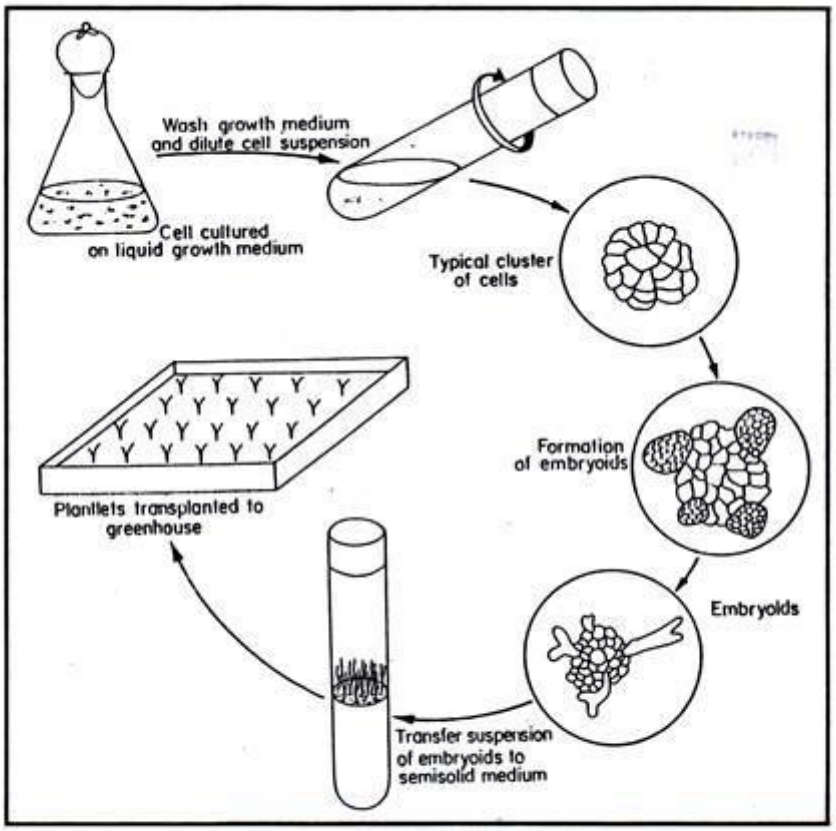
4. Cell suspensions are sub-cultured every 4 weeks by transferring 5 ml to 65 ml of fresh liquid medium.

5. To induce a more uniform embryo population, cell suspension is passed through a series of stainless steel mesh sieves. For carrot, the 74 µ sieve produces a fairly dense suspension of single cell and small multiple clumps. To induce somatic embryogenesis, portions of sieved cell suspension are transferred to 2, 4-D free liquid medium or cell suspension can be planted in semi-solid MS medium devoid of 2, 4-D. For normal embryo development and to inhibit precocious germination especially root elongation, 0.1- 1 µM ABA can be added to the culture medium. Cultures are incubated in dark.

6. After 3-4 weeks, the culture would contain numerous embryos in different stages of development.

7. Somatic embryos can be placed on agar medium devoid of 2, 4-D for plantlet development.

8. Plantlets are finally transferred to Jiffy pots or vermiculite for subsequent development.



❒ Fig 8.4

Flow diagram illustrating the protocol for inducing somatic embryogenesis in culture

Importance of Somatic Embryogenesis:

- The potential applications and importance of in vitro somatic embryogenesis and organogenesis are more or less similar. The mass production of adventitious embryos in cell culture is still regarded by many as the ideal propagation system. 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.

- Somatic embryo has no food reserves, but suitable nutrients could be packaged by coating or encapsulation to form some kind of artificial seeds. Such artificial seeds produce the plantlets directly into the field. Unlike organogenesis, somatic embryos may arise from single cells and so it is of special significance in mutagenic studies.

- Plants derived from asexual embryos may in some cases be free of viral and other pathogens. For an example, Citrus plant propagation from embryo genic callus of nuclear origin are free of Virus. So it is an alternative approach for the production of disease-free plants.