Somatic Embryogenesis

The process of embryogenesis includes the fusion between the male and female gamete during sexual reproduction and the zygote or fused structure gives rise to multicellular embryo after germination period. The embryo thus produced is known as zygotic embryo and the process is known as embryogenesis.

Sometimes the process is observed without the formation of zygote or not undergoing sexual union. By the process of parthenogenesis, the embryo thus formed is known as parthenogenetic embryo.

In culture the embryo is formed from isolated plant organ and this embryo is non-zygotic embryo. This process of embryo formation is referred as somatic embryogenesis. It may be defined as, in plant tissue culture, the developmental pathway of numerous well organised small embryoids resembling the zygotic embryos from the embryogenic potential of somatic plant cell of the callus tissue or cells of suspension culture is known as somatic embryogenesis.

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

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

Historical Background

- J. Reinert (1958 - 59) - Reported his first observations of in vitro somatic embryogenesis of Daucus carota.
- F.C Steward, M.O Mapes & 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 sps.
- B.V. Conger, G.E. Hanning, D.J. Gray (1983) - obtained direct embryogenesis from leaf mesophyll cells of orchard gras without an intervening callus tissue.

Principles of Somatic Embryogenesis

Somatic embryogenesis may be initiated in two different ways —

Direct Embryogenesis - i.e cells of explant undergo direct embryogenesis from proembryonic determined cells in absence of callus proliferation.

Indirect embryogenesis - i.e cells of explant first undergo callus proliferation and embryoids develop within the callus tissue from induced embryonic cells.

- In most of cases indirect embryogenesis occurs.

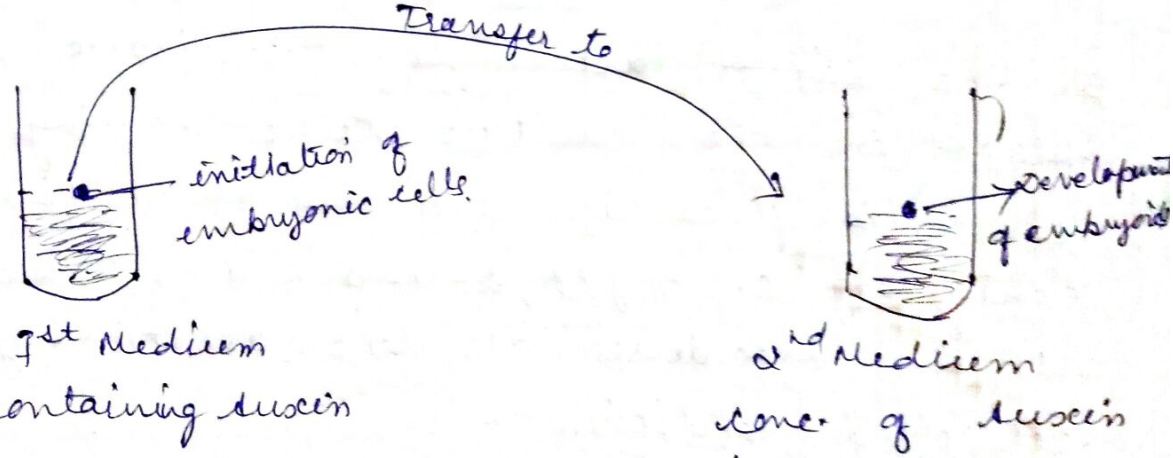
- Embryo formation is more common in liquid grain culture.
- For embryogenesis, two distinctly different types of medium is required.

Structure and stages of Embryo development

The excised somatic cell in culture medium initiates the formation of callus tissue from which embryoids are differentiated.

Two types of Medium is used in Embryo development. One medium for the initiation of the embryonic cells. The first or induction medium must contain auxin (in case of carrot tissue). Another medium for subsequent development of these cells into embryoids.

Embryonic cells can be initiated in the second medium by removing the hormone or lowering its concentration.



Embryoids of (carrot tissue) passes through 3 developmental stages of embryo formation — such as,

1. Globular stage
2. Heart shaped stage
3. Torpedo stage (gives rise to complete plantlet)

- somatic embryogenesis occurs in short term culture and their ability decreases with increasing duration.
- changes in ploidy of the cultured cells may lead to loss of embryogenic potential in long term culture.

Protocols for Somatic Embryogenesis

Material → Daucus carota

Culture Medium → Murashige and Skoog's medium.

1. Carrot (Daucus carota) root of seven day old seedling as storage root is taken after surface sterilization. The material is washed again in double distilled water.

2. Root segments or cambial tissue of the storage root is cut aseptically by sharpen scalpel.

3. The ~~excised~~ excised somatic tissue is placed in the culture tube having semisolid Murashige and Skoog's medium, containing 0.1 mg/l, 2-4-D and 2% sucrose. All the procedures are done aseptically in controlled condition.

4. The culture tubes with excised plant materials are incubated in dark.

5. After 4 weeks the ~~explo~~ excised plant material shows callus tissue.

6. This tissue is transferred to another culture flask containing 25 ml liquid medium (M/S) without agar and cell suspension culture is made there by placing the flask on a horizontal gyratory shaker with 125 rpm at 25°C.

7. When the cell suspension is clearly made, it is (3ml) transferred again in another culture flask with fresh liquid medium.

8. After 4 weeks the subcultures are again subcultured and after 4-5 subcultures and after 4 weeks duration the cell suspension shows the development of embryo of different stages.

9. Somatic embryos can be placed on agar medium for plantlet development, (medium without 2, 4-D).

10. The plantlet may be transferred to soil for further growth.

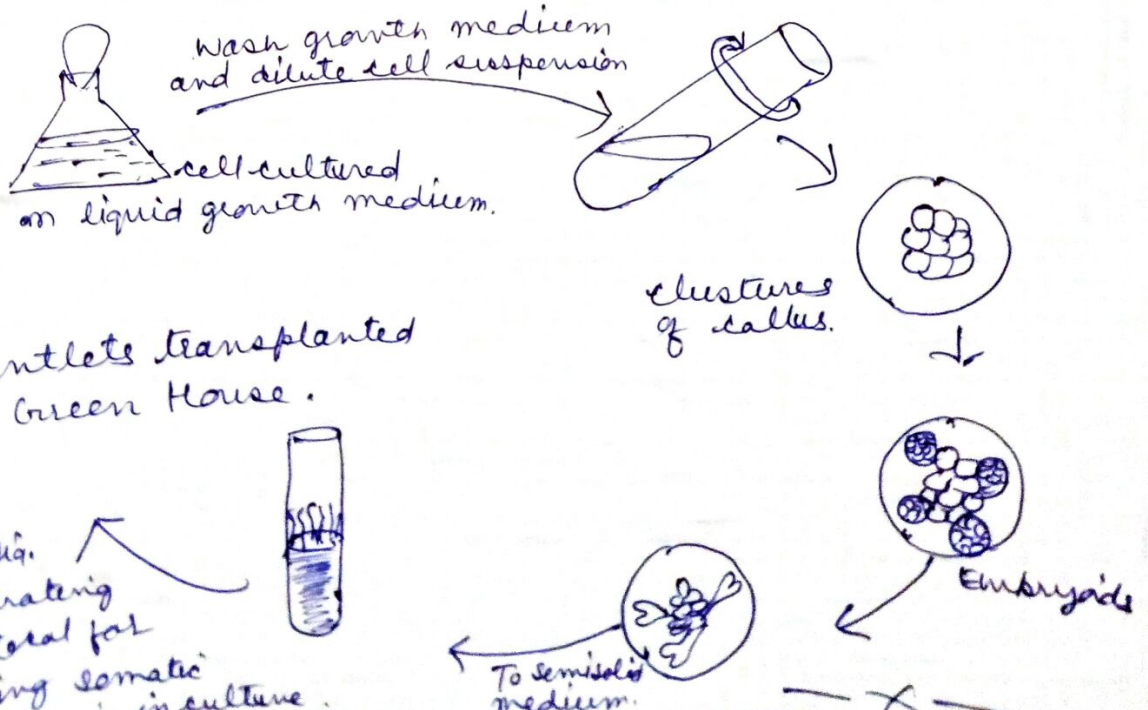


Fig- Fig. illustrating the Protocol for inducing somatic [illegible] in culture.