SOMATIC EMBRYOGENESIS AND ITS CONSEQUENCES IN CEREALS

NAME: PARDEEP KUMAR
ROLL NO. 21
CLASS: M.Sc. (Hons.) BOTANY 2nd Yr.
DEPARTMENT OF BOTANY, PANJAB UNIVERSITY, CHANDIGARH.

CONTENTS

- INTRODUCTION
- SOMATIC EMBRYOGENESIS (SE)
- FACTOR AFFECTING SE
- TYPES OF SE
- ROLE OF SE IN CEREALS
- CONCLUSION

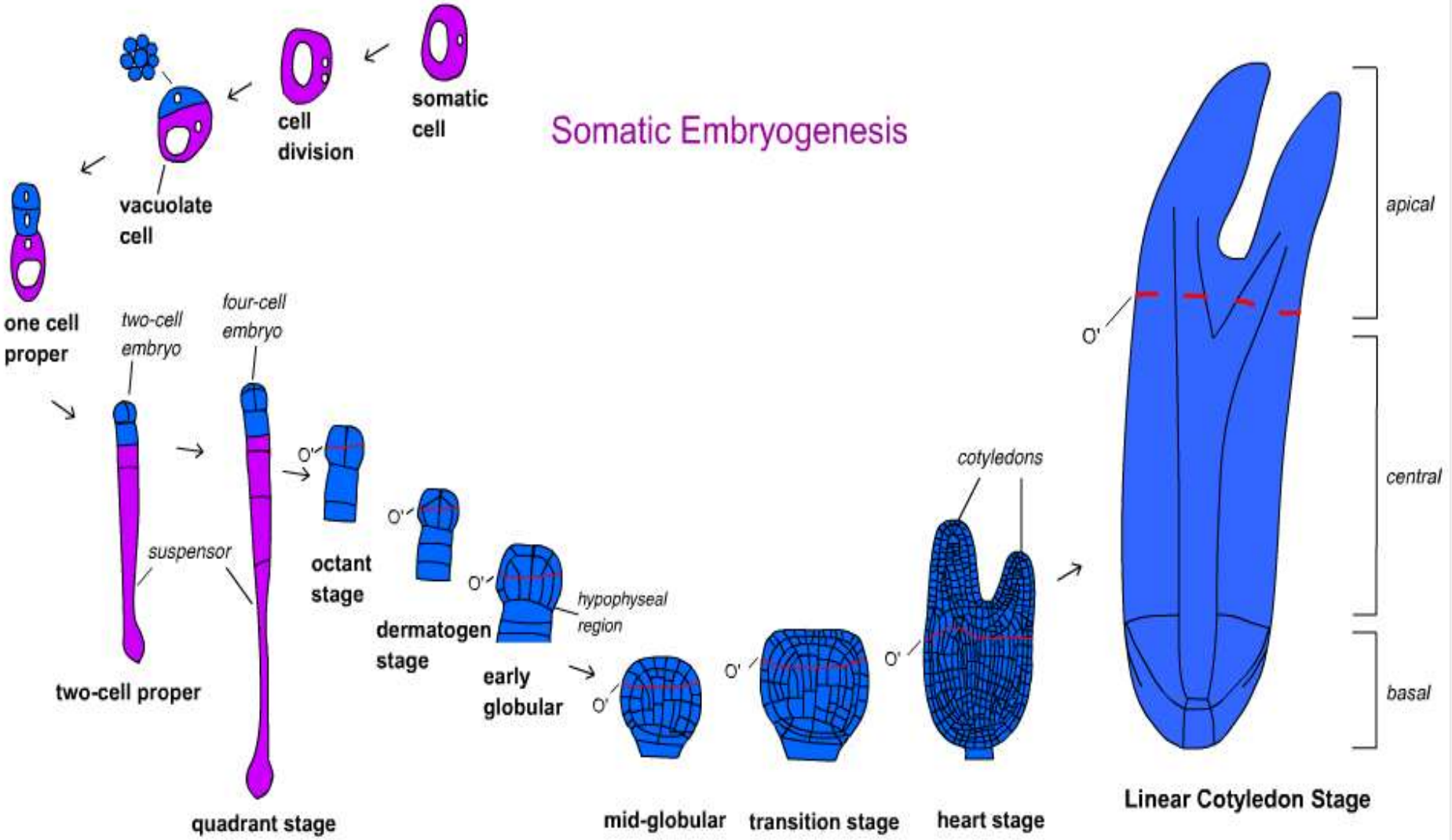
INTRODUCTION

Plant embryogenesis is the process that produces a embryo from a zygote by asymmetric cell division and differentiation of undifferentiated cells into mature tissues and organs.

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).

- The most basic requirement for embryo development is the physical and chemical environment which is available only inside the '**Magic Bath**' of embryo sac.
- *In vitro* embryo can be developed if we provide the nutritional conditions same as in magic bath.



Source: Plant and soil sciences eLibrary

FACTORS AFFECTING SOMATIC EMBRYOGENESIS

1. EXPLANT

- Explant as source material to induce SEs are very diverse. There are very responsive plants such as carrot in which any part of the plant can be use to induce embryogenic cultures.

- There are some very recalcitrant plants such as cereals and legumes in which explant varies even within the genetically identical species.

Various types of explants used in SE

1. Immature zygotic embryos
2. Inflorescence
3. Cell suspension cultures
4. Petioles
5. Protoplasts
6. Leaves
7. Stems
8. 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 foramtion and reverse ratio favours rooting.**
- others include **Gibberllins,** inhibits SE.
- **ABA** promote embryo maturation and prevent precocious germination and secondary embryogenesis.

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

iv) Polyamines

- Involved *in vitro* and *in vivo* SEs.
- Involved in cell growth, proliferation and aging.
- Interact with negatively charged molecules DNA, RNA, Proteins.
- Of the three polyamines studied (putrescine, spermidine, spermine) putrescine showed the most drastic increase in SEs (Altman *et al*.,1990).

v) Genotype

- Genotypic effect on somatic embryogenesis occurs as for regeneration via shoot bud differentiation.
- Genotypic variations could be due to endogenous levels of hormones.

vi) Electrical stimulation

- Exposure of explant to mild electric current of 0.02 V DC for 20 h promoted embryogenesis in alfalfa and tobacco (Rathore and Goldsworthy 1985).

- The electric stimulus seems to promote the differentiation of organised shoot/embryo by affecting cell polarity through changes in organization of microtubules and induction of asymmetric first division.

STAGES OF SOMATIC EMBRYOGENESIS

1. Induction

- An auxin, particularly 2, 4-D, is generally necessary to induce embryogenesis.
- Requirement of exogenous auxin for induction of SEs depends on nature of explants used with relative concentration of auxin.

2. Development

- After reinitiation of cell division and a period of cell proliferation in presence of auxin embryogenic cells are released into auxin free medium. These cells are in the clusters of cytoplasmic cells called Proembryonic mass of cells(PEMs).

3. Maturation

- The quality of SEs with regard to their germinability or conversion into plants is very poor. This is because the apparently normal looking SEs are actually incomplete in their development.
- Unlike seed embryos, SEs do not go through the final phase of embryogenesis, called **embryo maturation** which is characterised by accumulation of embryo specific reserve food materials and proteins which impart desiccation tolerance to the embryos; embryo size does not increase during this phase.
- **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).

TYPES OF SOMATIC EMBRYOGENESIS

1. Direct SE

when embryos are formed directly from explant tissue creating an identical clone without production of intervening callus. The explants capable of direct embryogenesis seem to carry competent or "**pre-embryonic determined cells**"(PEDCs).These cells are committed to Embryo development and need only to be released.

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 and the redetermination of embryogenic development pattern called "**induced embryogenic determined cells**"(IEDCs).

DIRECT SOMATIC EMBRYOGENESIS

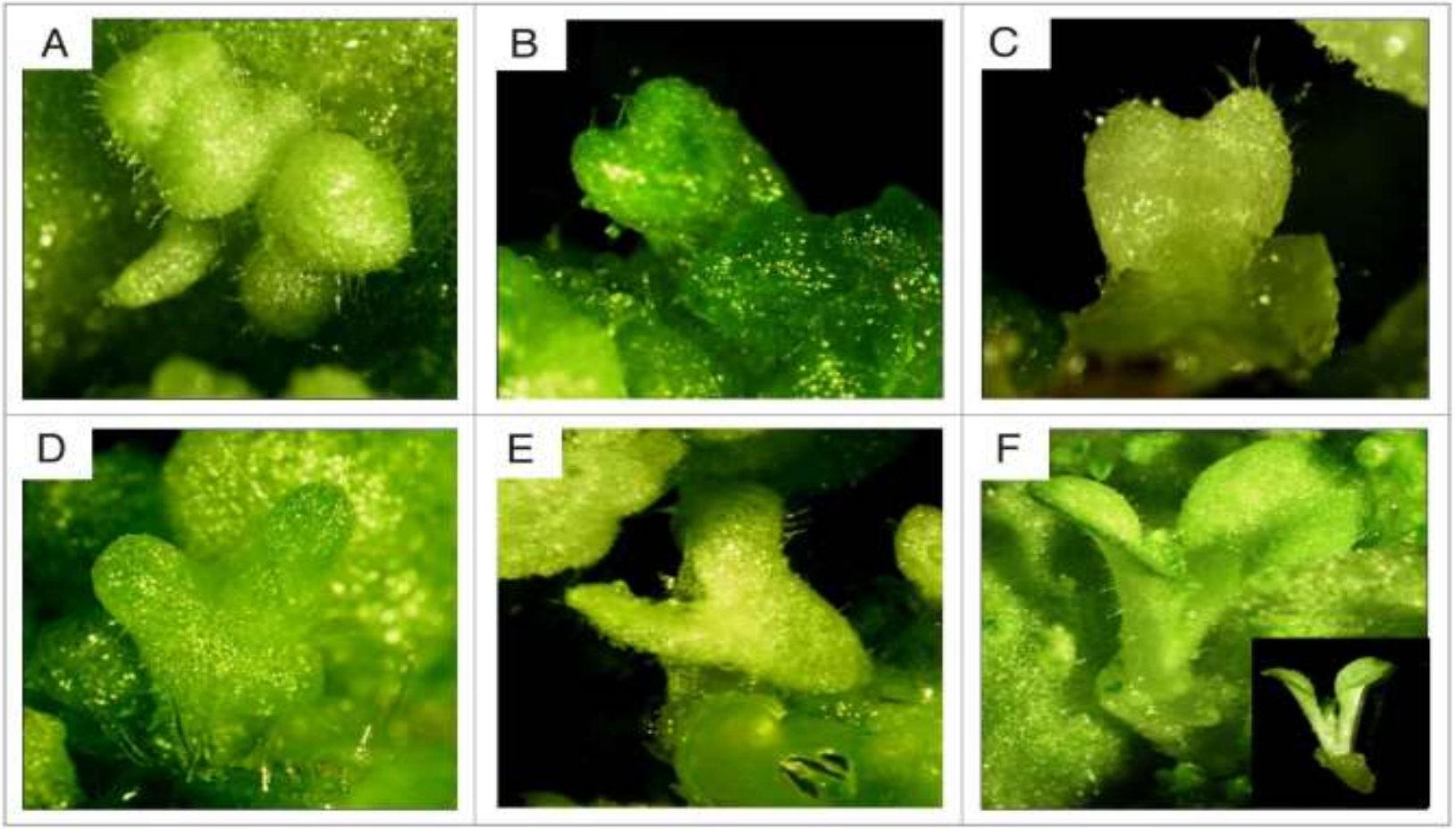


Figure 1. Various stages of direct somatic embryogenesis of *Nicotiana tabacum*. (**A**) Fused globular stage embryos; (**B**) early heart shape stage; (**C**) heart shaped stage; (**D**) early torpedo stage; (**E**) torpedo stage; (**F**) cotyledonary stage. Pictures were taken by Nikon I X -SMZ1500.

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

INDIRECT SOMATIC EMBRYOGENESIS

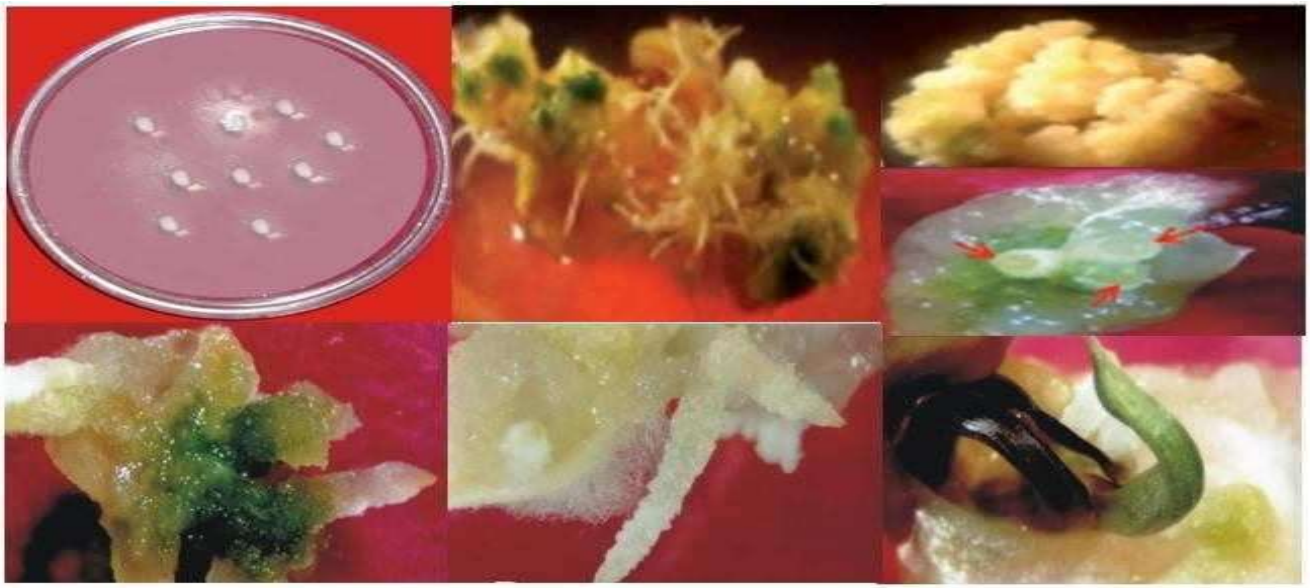


Figure 1 - Primary callus induction, somatic embryogenesis and plant regeneration from immature embryos of maize cv. Gaurav (A) Inoculation of immature embryos in MS with 5 mg/L 2,4-D and 2 mg/l NAA + 1 mg/l BAP, (B) Callus proliferation in MS with 5 mg/L 2,4-D and 2 mg/l NAA + 1 mg/l BAP, (C-D) Globular shape observed during somatic embryogenesis (arrows) (E) Regenerating calli in MS medium with 5.0 mg/L 2,4-D. Culture showing mature green somatic embryos (arrow) (F) Root induction in MS medium with 5.0 mg/L 2,4-D (Joshi et al., 2010).

Somatic embryos	Zygotic embryos
1. SEs are formed by sporophytic cells. **2**. SEs store less amount of embryo specific reserves. **3**. A distinct suspensor is absent in SEs even if it is present it may not be functional as in seed embryos. **4**. Embryos have no vascular connections with the cultured explant. **5**. SEs generally lack a dormant phase and often show secondary embryogenesis and pluricotyledony. **6**. SEs show high rate of propagation .	**1**. Formed by fusion of gametic cells. **2**. Seed storage proteins, carbohydrates are the characterstic features. **3**. A well developed distinct suspensor is present. **4.** Embryos have vascular connections with the explant. **5**. They do not show secondary embryogenesis and pluricotyledony. **6**. Low rate of propagation than SEs.

ADVANTAGES AND DISADVANTAGES OF SOMATIC EMBRYOGENESIS

ADVANTAGES

- It is observable, as its various culture conditions can be controlled.
- Lack of material is not a limiting factor for experimentation.
- High propagation rate.
- Somaclonal variations.
- Germplasm conservation.
- Labour saving.
- Elimination of diseases and viruses.

DISADVANTAGES

- 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.

ROLE OF SOMATIC EMBRYOGENESIS IN CEREALS

- Due to change of emphasis from medium manipulation to explant and genotype selection, several species of cereals have been regenerated which were once regarded recalcitrant.
- High frequency of somatic embryos were obtained in Indian bread wheat cultivar HD 2967 by using mature embryos and increasing concentration of Agar gel in the medium (Gill *et al*, 2015).
- Use of somaclonal variation in somatic embryogenesis has broaden the genetic variation in crop plants.
- Several lines of disease resistant wheat, rice, barley have been isolated from somaclones (Jain *et al*, 1998).
- By using a Cephalosporin antibiotic, Cefotaxime several varieties of rice have been developed by PAU, Ludhiana in 2009.

CONCLUSION

- somatic embryogenesis is a model system for the conventional plant breeding, mass propagation and the rapid genetic improvement of the commercially important crops.
- The induction of somatic embryogenesis is being examined at the molecular level.
- Several ‘embryo specific’ genes have been isolated from embryogenic cultures of cereals, and several molecular markers to distinguish between embryogenic and non-embryogenic cultures have been identified.
- So future research for molecular analysis in somatic embryogenesis must focus not simply on isolating genes during embryo development but also on their biological significance.

REFERENCES

- Bhojwani, S. S., and Razdan, M. K. 2013. Plant Tissue Culture: Theory and Practice, a Revised Edition. pp.125-167. Elsevier India Pvt. Ltd., New Delhi.
- Kakkar, R. K., Nagar, P. K. and Ahuja, P. S. 2000. Polyamines and Plant Morphogenesis. Biologia Plantarum 43(1): 1-11.
- Marilyn, A. L., West, L. and Harada, J. 1993. Embryogenesis in Higher Plants: An Overview. The Plant Cell 5: 1361-1369.
- Vasil, I. K. 1986. Somatic Embryogenesis and its Consequences in Gramineae. Springer 32: 31-47
- Victor, M. and Jimenez, H. 2005. Involvement of Plant Hormones and Plant growth Regulators on *in vitro* Somatic Embryogenesis. Plant Growth Regulation 47: 91-110.
- Zimmerman, J. L. 1993. Somatic Embryogenesis: A Model for Early Development in Higher Plants. The Plant Cell, 5: 1411-1423.

Contd....

- Gill, A.K., Gosal, S.S. 2015. Improved somatic embryogenesis in Indian Bread Wheat (*Triticum aestivum* L.). Journal of Cell and Tissue Research, 7: 19-26.

THANK YOU