Discipline: Botany

Paper: Plant Biotechnology

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Lesson: Zygotic and Somatic Embryogenesis

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Introduction

Embryogenesis is the process of the development of an embryo from zygote. This process requires the fertilization of an egg cell with the sperm. In angiosperms, the product of double fertilization gives rise to the embryo and the endosperms.

The development of embryos can also take place apomictically. These may arise from an unfertilized egg, any other cell of embryo sac or from individual somatic cells of nucellus or integument. Like zygotic embryo, the apomictic embryo is also a new individual, although it does not contain a new set of genes. These embryos are called adventive embryos.

Isolated somatic or gametic cells can also give rise to embryos either naturally as is seen in *Kalanchoe* where somatic embryos develop from the edge of the leaves or *in vitro* after experimental induction.

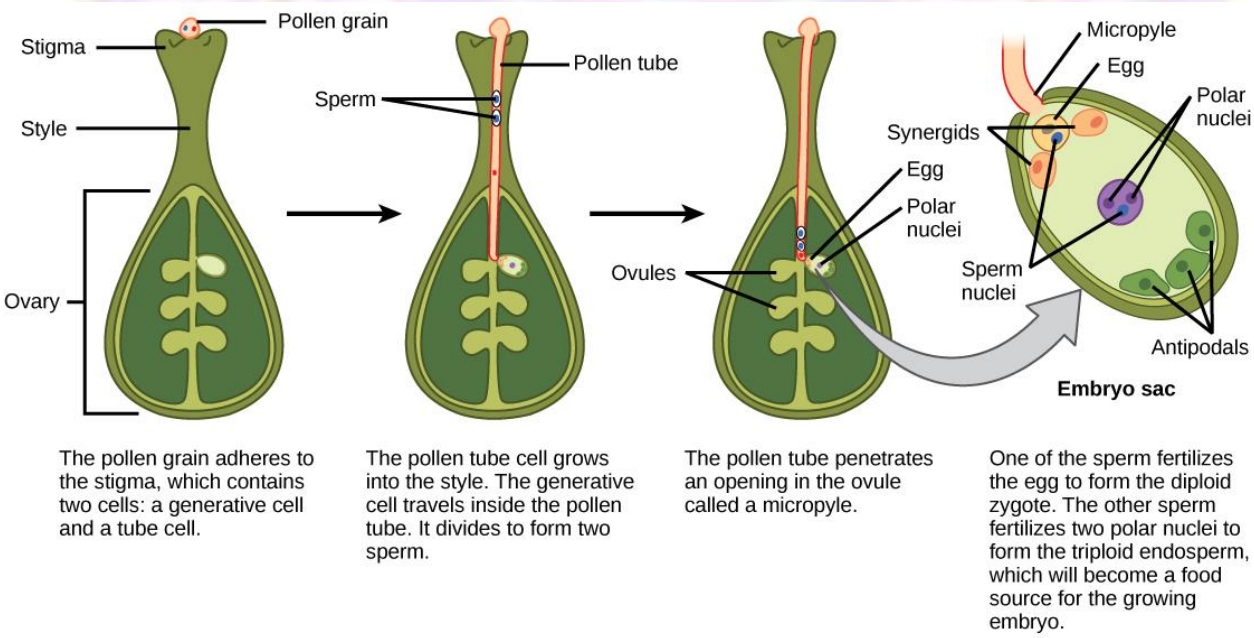

Figure: Fertilization in an angiosperm

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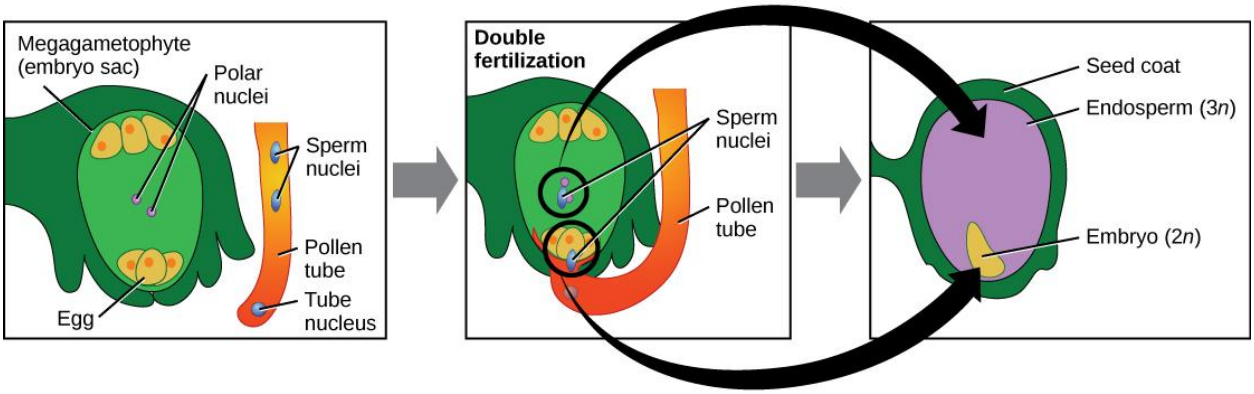

Figure: Embryogenesis in an angiosperm

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Morphological features of an embryo:

1. An embryo has a bipolar axis.
2. One end of the axis gives rise to shoot meristem
3. The other end of the axis develops into root meristem.
4. The middle portion of the embryo comprises the hypocotyl.

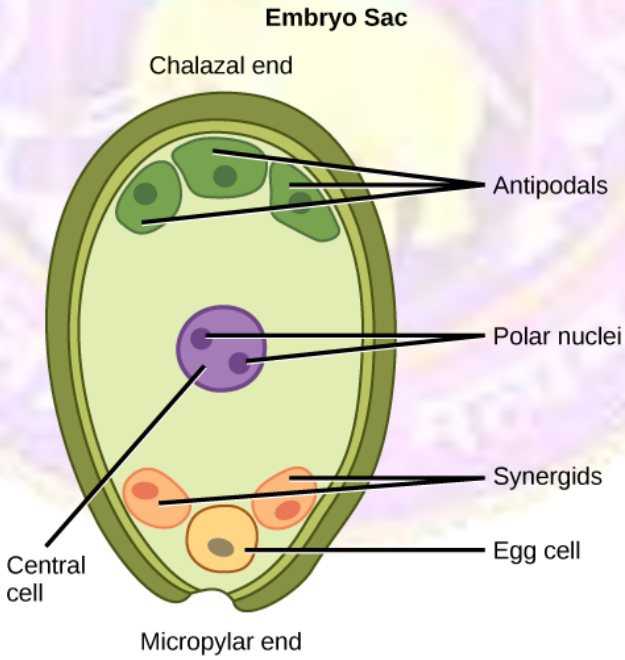


Figure: Embryo sac in angiosperm

Source: http://cnx.org/contents/b3798dae-10d6-426f-a82b-cdeaafbb6ec5@4

Types of embryo:

The following classification of embryos was proposed by Kohlenbach in 1978:

1. Zygotic embryos: Developed from zygote, the product of fertilization.

2. Non zygotic embryos: Developed from cells other than the zygote. It is further divided into the following types:

    a) Somatic embryos: Embryos formed from the sporophytic cells (except zygote).
    b) Parthenogenetic Embryos: embryos developed from the unfertilized egg.
    c) Androgenic embryos: Embryos developed from the male gametophyte (microspore pollen grain).

Zygotic embryogenesis

Zygotic embryogenesis is the process of development of embryo from the zygote, the product of fertilization.

Stages of embryogenesis

1. Asymmetric division

Embryogeny starts with an unequal division of the progenitor cell resulting in a smaller densely cytoplasmic terminal cell and a larger vacuolated basal cell. The embryo proper develops from the terminal cell, while the suspensor develops from the basal cell. The embryogenic developmental polarity is indicated by the asymmetric pattern of the first division.

2. Pattern formation

Axial and radial patterns develop to form a globular-stage embryo with tiers of cells. The emerging shape of the embryo depends on the patterns of cell division. The apical cell divides longitudinally to form two cells. Each of the two

cells divides again longitudinally in a plane perpendicular to the first division to form a quadrant or 4-celled filament. These four cells then divide transversely to form an octant. The transverse walls of these 4 cells divide the embryo and form a boundary between distinct domains of the embryo. All the cells then divide periclinally to form the first histologically distinct tissue, the protoderm. This stage is called the globular or dermatogen stage.

Based on the characteristic cell division patterns, the three basic tissue systems (dermal, ground, and vascular) can be recognized at this point. The dermal system, which is the outer protective layers of the plant, is the first tissue to differentiate from the protoderm. Subsequently the ground tissue system and vascular tissue systems differentiate from the embryo proper. In the center of the globular embryo, the procambial cells give rise to the the vascular system. The developing vascular tissues are surrounded by the ground tissue formed from the ground meristem. The differentiation of these three tissue layers give rise to globular embryo stage.

3. Establishment of shoot and root meristem

The actual plant arises from the clusters of embryogenic cells called the shoot apical meristem (SAM) and root apical meristem (RAM). The SAM and RAM persist in postembryonic development. The globular stage of the embryo changes to a heart-shaped stage as soon as the cotyledons begin to form, in dicots. Only a single cotyledon is formed in monocotyledons. The embryo proper is incorporated by the hypophysis, which is the apical-most suspensor cell. The root apical meristem comprises of both the hypophysis and apical cell. Continued cell division, growth and differentiation gives rise to the late heart stage and then to the torpedo stage which ultimately leads to the degeneration of the suspensor. Further growth and development of the cotyledons result in the walking stick stages. Embryogenesis is arrested at this point, and the mature seed desiccates and remains dormant until germination.

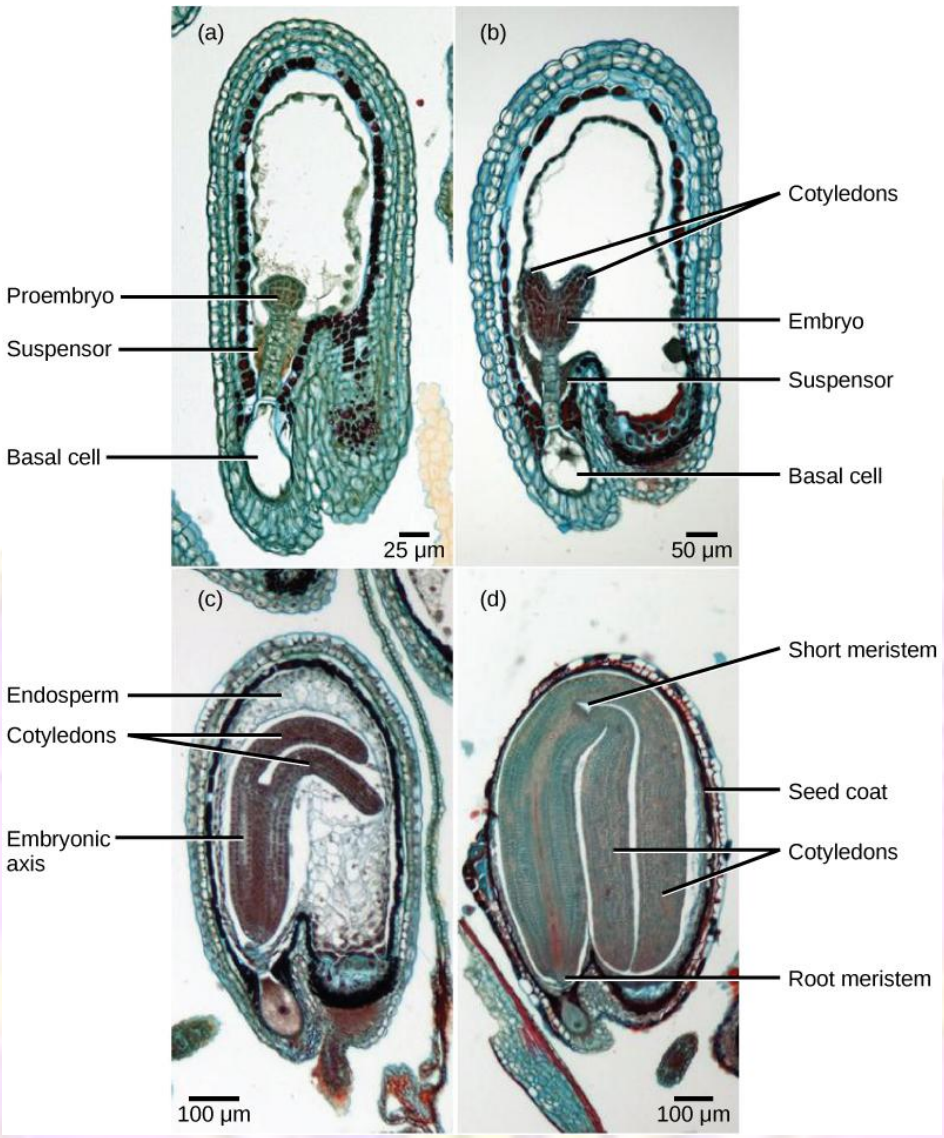


Figure: Embryo development in *Capsella bursa*

Source: http://cnx.org/contents/cef4ebc4-36e1-4364-b0e0-4d3f176443f3@1.13:40

4. Maturation and germination

The developmental program switches from pattern formation to storage product accumulation during the period of embryo maturation. The young sporophyte undergoes dormancy and postembryonic development before germination.

Somatic embryogenesis

Somatic embryogenesis refers to the process of the development of embryos in culture. Embryos formed in cultures have been variously designated as accessory embryos, adventive embryos, embryoids and supernumerary embryos.

Somatic embryogenesis may be of two types:

1. Direct somatic embryogenesis: The embryo may arise directly from the cell of the explants.
2. Indirect somatic embryogenesis: It involves dedifferentiation of organized tissue into callus prior to embryo production.

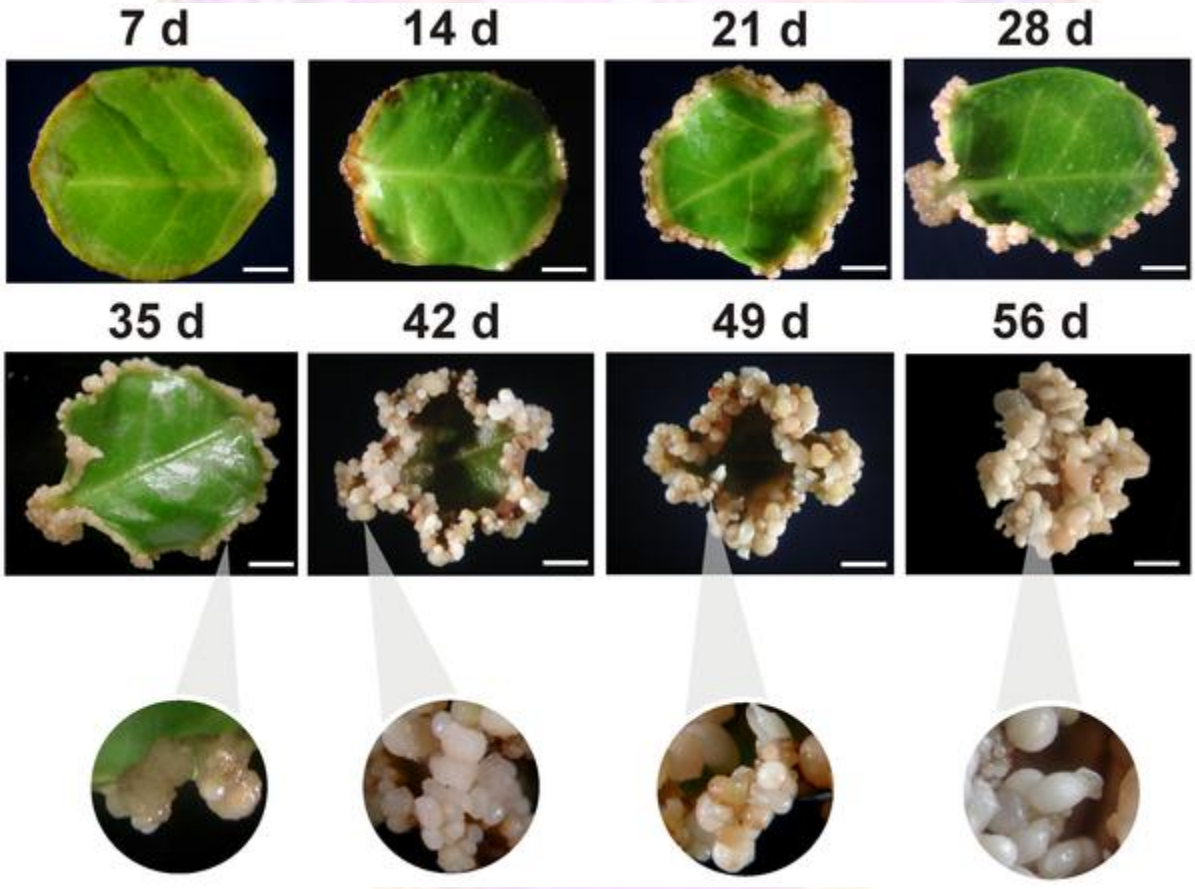


Figure: Development of direct somatic embryos from leaf explants of *Coffea canephora.*

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0072160

Figure: Callus induction from walnut leaf explants for indirect somatic embryogenesis using four different media.

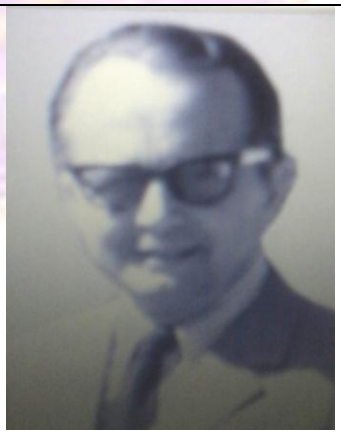
Source: http://www.scielo.cl/scielo.php?pid=S071858392009000300020&script=sci_arttext

Historical events

The concept of the cultivation of artificial embryos (today's somatic embryos) from vegetative cells was predicted by Gottlieb Haberlandt, in his famous publication in 1902. Experimental production of somatic embryos was first attained by Jakob Reinert and by Frederick C. Steward in callus and cell suspension culture of carrot root tissue in 1958. Although different developmental stages of somatic embryogenesis were identified, Reinert could not prove that a single cell can be converted into plantlets. Steward achieved almost similar results in the same year. His experiments proved that carrot root tissues cultured in the presence of 2, 4-D

and coconut milk gave rise to embryogenic culture and that somatic embryos formed in such cultures germinated to form carrot plants.

Gottlieb Haberlandt (1854-1945) was a great German botanist. Haberlandt laid the foundation of plant cell culture by giving the concept of cell totipotency in plants. The result of his work was made known to the scientific world in a meeting in Vienna Academy of sciences in Berlin (1902). Unfortunately, he could not prove the concept of cell totipotency propounded by him. He recommended the use of coconut milk which could cause cell division. This paved the way for experimentation on culture of embryos. In view of his great contribution, Haberlandt is considered the father of plant cell culture.	 Source: http://en.wikipedia.org/wiki/Gottlieb_Haberlandt

Jakob Reinert was quite an eminent researcher who published most of his work in German. He was very poor in spoken English. He reported somatic embryogenesis using carrot tissues in 1958.	

Frederick C. Steward (1904-1993) was a British Botanist. He was an eminent plant physiologist who impressed audience by his eloquent speeches. He had a great command over English language. People remember his famous lecture "Carrots and Coconuts". He wrote more than 100 scientific journal articles and several books and was an editor and contributor to the 10 volumes and 15 books of "Plant Physiology". The demonstration of cellular totipotency in carrot made him known worldwide.	

Factors affecting somatic embryogenesis

Various factors controlling *in vitro* somatic embryogenesis include explants, genotype, medium, growth regulator, physical and culture environment, etc.

a. Explant

Various explants including anthers, pollen, ovaries, immature and mature embryos, mature cotyledon, leaves, petioles, stems etc. have been utilized for the initiation of *in vitro* somatic embryogenesis. The choice of explants for the production of SE is generally limited to immature or less differentiated parts, although success has been achieved with fully differentiated explants as well. In majority of cases, zygotic embryos in the early stages of development have been used to initiate cultures, as its cells already possess embryogenic competence and are termed pre-embryogenic determined cells (PEDCs). Quite often, hypocotyls and leaf explants have also been used as

explants. Other more differentiated explants have cells that must be induced to become embryogenic and are termed Induced embryogenic determined cells (IEDCs).

b. Genotype

Regeneration *via* shoot bud differentiation during somatic embryogenesis is affected by the genotype of the species. Even though the explants and media used are same, different genotypes of a species respond differently. Striking examples are found in rice, alfalfa, groundnut, soybean, tea, red clover and maize. Difference in endogenous levels of plant growth regulators (PGR) could be the cause of genotypic variations.

c. Growth regulators

i. **Auxins**

The induction of somatic embryogenesis requires synthetic auxin. Embryo differentiation occurs on transfer to auxin free medium. The most commonly used auxin for the induction of somatic embryogenesis is the synthetic auxin, 2, 4-D. The other auxins which have been used include Naphthalene acetic acid (NAA), Indole acetic acid (IAA), Indole butyric acid (IBA), dicamba, picloram, and others. The role of auxin in the formation of embryonic cells could possibly be the initiation of differential gene activation and promotion of embryonic cell population through repetitive cell division by simultaneously suppressing cell differentiation and growth into embryo. However, in an instance where explants consist of pre-existing embryogenic cells possibly does not require application of auxin because a discrete induction step is not required.

ii. **Cytokinins**

Cytokinins such as BAP (6-Benzylaminopurine) are reported to inhibit embryogenic potential but promote cell division in carrot cultures. The

non-embryogenic cell components may be selectively stimulated for cell division by the action of cytokinins and thus inhibit the potential of embryogenic component of the culture. However, the promotive effect of cytokinins on embryogenesis is reported by some workers. Zeatin promotes embryogenesis in carrot. Kinetin in addition to 2, 4-D was used in the induction medium for alfalfa. The type of morphogenetic differentiation after transfer to hormone free medium was determined by the relative concentrations of the two growth regulators in the induction medium.

d. Concentration of oxygen

Embryogenic development in cultures has been shown to be promoted by oxygen tension. Reducing dissolved oxygen in carrot and wheat cultures improved somatic embryogenesis. However there are contradictory reports of the affectivity of reducing oxygen on embryogenesis in carrot.

e. Light and humidity

Light is a major factor of the culture environment and has been shown to have definite effect on embryogenesis of some plants. Somatic embryo induction in *Solanum melongena* has an absolute requirement for light. In contrast, poplar has an absolute requirement for darkness. For induction of somatic embryos in carrot, green light, red light or darkness was equally effective, while high intensity of white light and blue light were inhibitory. It has been suggested that *in vitro* cultured immature embryos of wheat respond to 2, 4 D and to light signals by modifying expression of G-proteins, NDP kinases and arrestin proteins.

Humidity stress and starvation of cultures has recently been shown to improve embryogenesis in carrot. Production of somatic embryos increased when the embryogenic callus was cultured on half strength MS medium or MS medium without sucrose or cultured under conditions of reduced humidity (69.3%).Increase in somatic embryo production was also observed in

embryogenic callus cultured on MS medium after starvation by placing in empty culture plates.

f. Medium

Murashige and Skoog (1962) basal medium or its modification is the most widely used medium for somatic embryogenesis. Very rarely White's, Gamborg, SH basal medium have been used albeit with suitable supplements. The most commonly used carbon source is although sucrose. In scarlet runner bean, glucose was found to be superior for embryogenic cell induction. Similarly, for citrus nucellus cultures, galactose and lactose were found to be superior.

In vitro embryogenesis is significantly affected by the form of nitrogen in the medium. The two main source of nitrogen in the medium for induction and differentiation of somatic embryos are KNO_3 and NH_4Cl. Other nitrogen sources such as casein hydrolysate and amino acids have also been found to increase somatic embryo formation.

g. Selective subculture

The morphogenetic potential of multicellular explants is generally heterogeneous. Under a set of culture condition only a small portion of these cells are able to express their cellular totipotency. Therefore, such explants produced calli which are heterogeneous. The morphological appearance of embryogenic/organogenic portions of the callus is distinct from the non-embryogenic portion of the callus. To establish a regeneration tissue culture, it is essential to make selective subcultures of the calli.

Induction and Development

The process of somatic embryogenesis induction and development could be summarized in the following diagram:

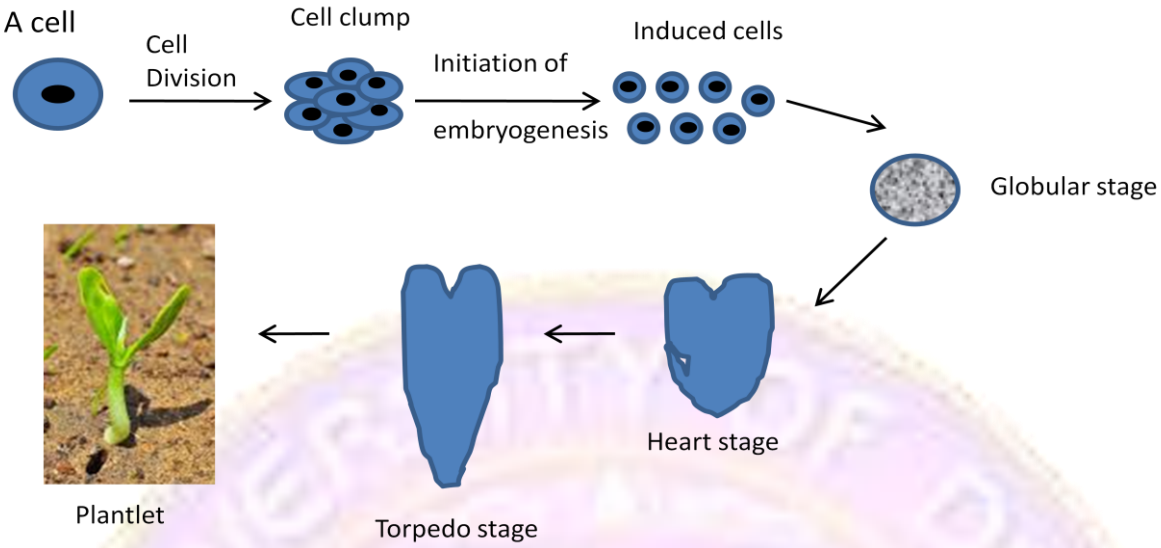


Figure: The pathway of somatic embryogenesis in tissue culture.

Source: Developed by Vinee Khanna, Dept of Genetics, UDSC

Different stages of somatic embryogenesis and plantlet regeneration in grape are shown in the following figure.

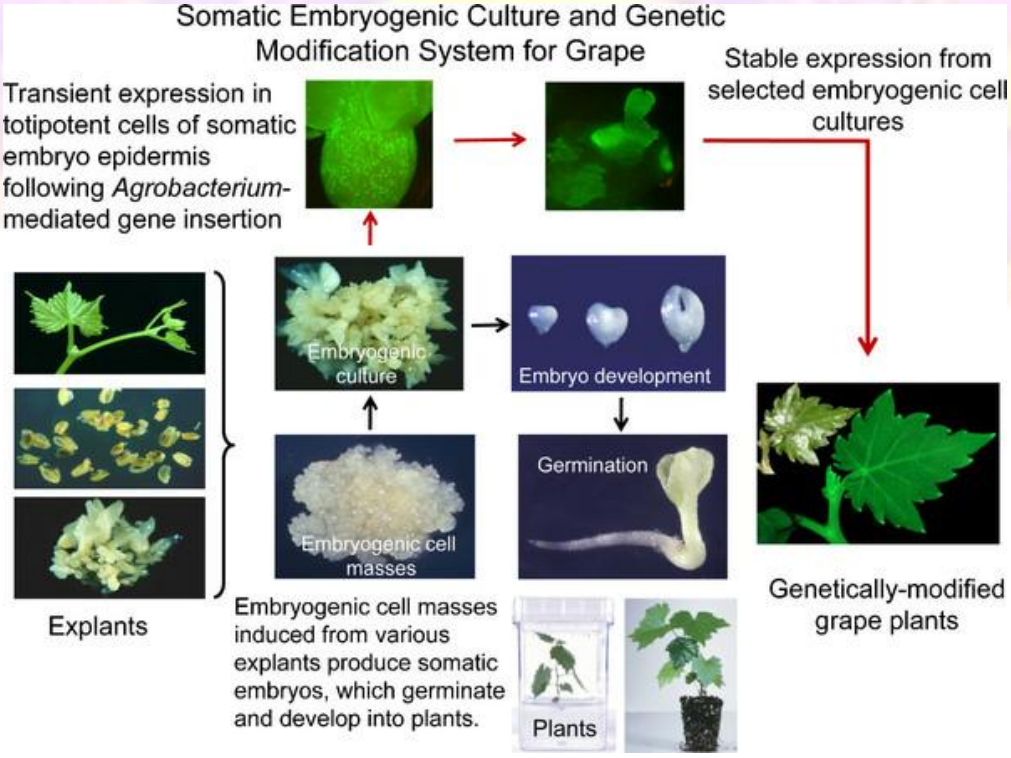


Figure: Somatic embryogenic culture in grape.

Zygotic and Somatic embryogenesis

Source: http://www.nature.com/articles/hortres201427

Plant regeneration via somatic embryogenesis can be summarized by the following five steps.

1. **Induction**

   Induction of somatic embryogenesis in plants generally requires auxin. However, for the induction of somatic embryogenesis, the requirement of exogenous auxin depends on the nature of the explants used. The change of cellular polarity is an important phenomenon associated with the induction of somatic embryogenesis.

   Plant growth regulators employed for the induction of embryogenesis alter the cell polarity and promote subsequent asymmetric division. A shift from the normal anticlinal divisions in the epidermis to periclinal oblique divisions is the first cytological sign of the induction of embryogenic cells. The polarity of the entire somatic embryo is already determined prior to the first division of an embryogenic cell because the root pole of the somatic embryo is always oriented towards the larger cell.

   All the genetic information necessary to create a complete and functional plant is contained in the somatic cells. The termination of the current gene expression in the explants tissue is a must for the induction of somatic embryogenesis and its replacement with an embryogenic gene expression program is necessary. An important mechanism for down regulation of current gene expression is DNA methylation, which is influenced by auxin. It has also been proposed that PGRs and stress play a central role in mediating the signal transduction cascade leading to the reprogramming of gene expression. This results in a series of cell divisions that induced either unorganized callus growth or polarized growth leading to somatic embryogenesis.

2. Proliferation

The embryogenic cells, once formed, continue to proliferate in the form of clusters of cytoplasmic cells called PEMs (proembryonic masses). The PEMs are small, angular embryogenic cells, connected with the adjacent cells by many plasmodesmata. The PEMs are held together by non-embryogenic cells which are larger, rounded, with fewer plasmodesmata.

Auxins cause both induction of somatic embryogenesis as well as elongation of cell which leads to disruption of formerly adhering cells. Therefore, continued presence of auxin in the medium may cause the elongation of non-embryogenic cells of the PEMs leading to the breakdown of the PEMs and release the embryogenic cells. Pre-globular embryos or globules formed from the released embryogenic cell cluster develop into new PEMs and the non-embryogenic cells, elongate to form non embryogenic component of the suspension. Thus this cycle of PEM or pro embryo proliferation continues in the presence of auxin.

3. Pre-maturation

It is important for the PEMs to reach the appropriate developmental stage before exposing them to maturation treatments. For this, the embryogenic cultures need to be transferred to medium lacking auxin. When cultures containing PEMs are transferred to auxin-free medium, the globules develop into globular embryos as there is no disruption of cells from each other in the absence of auxin. The first differentiation step in this process is the formation of a protoderm outside the globule. The globular embryos then continue further development and subsequently form heart shaped embryo through early torpedo stage, then cotyledon and ultimately proper embryo.

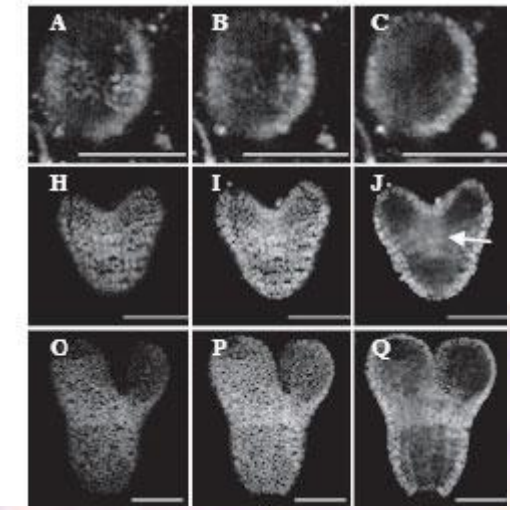


Figure: A-C represents globular embryo, H-J represents heart shaped embryo, O-Q represents torpedo shaped embryo in *Arabidopsis thaliana*

Source: http://www.scielo.cl/scielo.php?pid=S0716-97602010000100012&script=sci_arttext

4. Maturation

Unlike seed embryos, the SEs are actually incomplete in their development. Maturation is the terminal event of somatic embryogenesis. Somatic embryos undergo morphological and biochemical changes during the maturation stage. Maturation of somatic embryos is characterized by accumulation of storage products similar as those of the zygotic embryos, although in lesser amount. Reduction in water contents and often a gradual decline or cessations of metabolism are other characteristic features of embryo maturation.

5. Plant regeneration

The conditions provided at earlier stages of somatic embryogenesis greatly determine the survival and growth of regenerated plant through somatic embryo. Therefore, dissection of critical factors that might contribute to ex vitro performance of plants is required for mass propagation of somatic embryo plants. The quality of somatic embryos with regard to their germinating ability and plant regeneration has been generally very poor even though there are several reports on the success of somatic embryogenesis in crop species. The development of mature embryos into normal plants is only

those which have accumulated enough storage materials and acquired desiccation tolerance at the end of maturation.

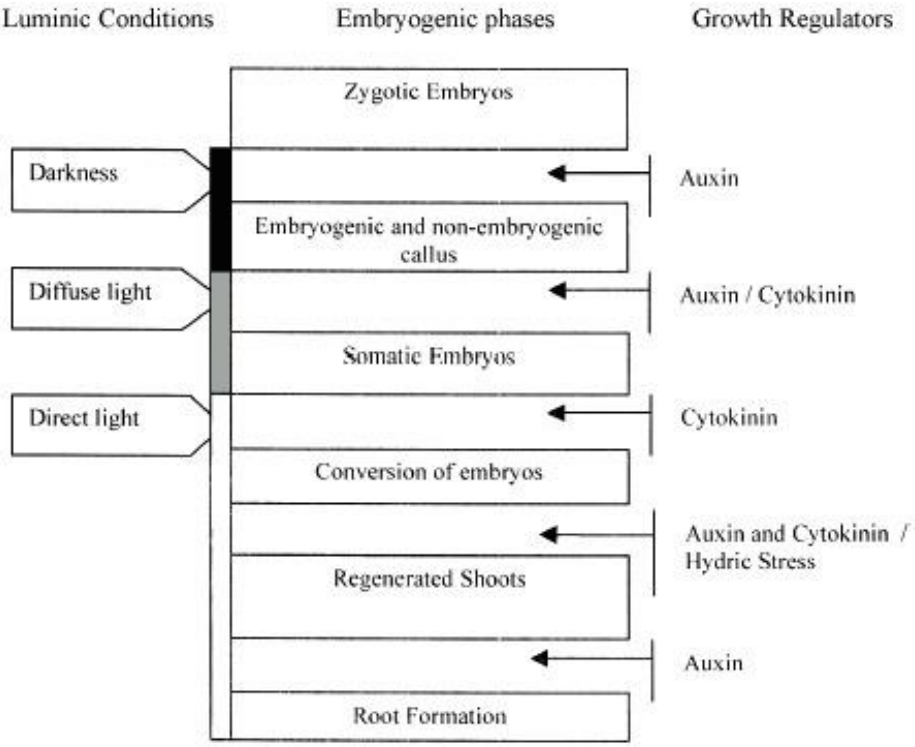


Figure: Factors involved in indirect somatic embryogenesis in rice.

Source: http://www.scielo.sa.cr/scielo.php?pid=S0034-77442005000200006&script=sci_arttext

Application of somatic embryogenesis:

1. Biotechnological tool for genetic improvement and mass propagation.
2. Crop improvement through clonal propagation
3. Serve as a useful tool for study of plant development
4. Provision of source tissue for genetic transformation.
5. Synthetic seed production.

Synthetic seeds are the artificially encapsulated somatic embryos which functionally mimic seeds and can develop into seedlings under *in vivo* and *ex vivo* condition. There are two types of synthetic seeds:

i. Desiccated synthetic seeds: These are polyoxyethylene glycol encapsulated embryos. This type of synthetic seeds is produced in desiccation tolerance species of plant.

ii. Hydrated synthetic seeds: These are produced by encapsulating the embryos in hydrogels like sodium alginate, calcium alginate, potassium alginate, sodium pectate etc.

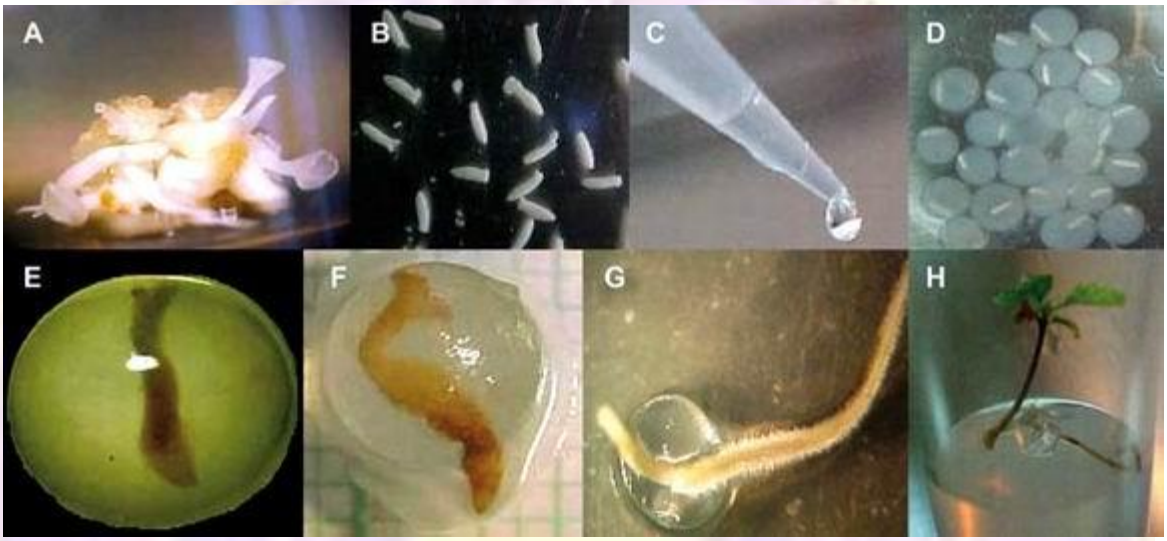


Figure: Encapsulated somatic and zygotic embryos

Source: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0718-58392009000100014

Difference between zygotic and non-zygotic embryos

Zygotic embryos	Non zygotic embryo
1. Embryos are compressed, flattened and are of distinct size and shape because of physical constraint opposed by seed coat	1. Non zygotic embryos are mostly larger in size and have wider hypocotyls and fleshier cotyledons.
2. Generally normal embryos develop.	2. Abnormal embryos develop in *in vitro* culture for example, zygotic embryos with suspensor produce cluster of non-zygotic embryos
3. Embryo development is synchronous.	3. Non zygotic embryos in culture contain several stages at a given time as they are produced asynchronously.
4. Uniform conditions during embryogenesis result into normal embryo development and its maturation and abnormalities are rare in zygote embryogenesis.	4. Changing *in vitro* environment i.e. depletion of medium nutrients etc results into bypassing of embryo maturation and cause disorganization forming new embryogenic cells resulting into asynchrony.
5. Zygotic embryos have quiescent phase during maturation	5. Non zygotic embryo do not have quiescent resting period.
6. Seed can be stored due to provision of protective and nutritive tissues.	6. Non zygotic embryo cannot be stored due to lack of protective seed coat.

Summary

- Plant embryogenesis can be broadly divided into two phases: the morphogenesis phase and the maturation phase. The basic plan of embryo formation is established during morphogenesis phase and in maturation phase; the embryo becomes tolerant to desiccation and accumulates storage macromolecules such as starch, proteins and lipids.
- Somatic embryos follow similar pattern of embryo development as their zygotic counterpart and they can be induced in culture.
- The major factor for success in the induction of somatic embryogenesis in many plant species has been the careful selection of explants and genotype instead of medium manipulation.
- Plant regeneration in tissue culture is a five step process: a) Induction b) proliferation c) Pre-maturation d) Maturation and e) Plant development.
- Somatic embryos formed in cultures are genetically/physiologically unstable and their morphogenic potential is lost in long term culture, which is the major handicap in commercial exploitation for mass scale propagation of plants.

Exercises

Questions

1. Briefly describe the stages of development in plant embryogenesis.
2. Describe the morphogenesis phase and maturation phase of zygotic embryogenesis.
3. What is somatic embryogenesis? Describe the production of somatic embryo in culture.
4. Differentiate between direct and indirect somatic embryogenesis.
5. What are the factors affecting somatic embryogenesis?
6. Describe the application of somatic embryogenesis.
7. What are the advantages and disadvantages of somatic embryogenesis?
8. Describe the process of plant regeneration through somatic embryogenesis.
9. What is somaclonal variation? Describe its mechanism of production.
10. Compare and contrast between somatic and zygotic embryogenesis.

Define the following terms

1. Embryogenesis
2. Cellular totipotency
3. Explants
4. Suspension culture
5. Organ culture
6. Embryo
7. Embryoid
8. Zygote
9. Plant growth regulators
10. Medium
11. Subculture
12. Axenic culture
13. Callus
14. Differentiation
15. Dedifferentiation

Multiple choice questions

1. For induction of somatic embryogenesis plant growth regulator that is used is

a) Gibberellic acid
b) Absicissic acid
c) PEG
d) 2,4-D

2. Somatic embryo is a

a) Unipolar structure
b) Multipolar structure
c) Bipolar structure
d) Has no polarity

3. Synthetic seeds are

a) Encapsulated somatic embryos
b) Quality somatic embryos
c) Mature and desiccated SEs
d) all of the above

4. Somatic embryogenesis was first time reported in which plant species

a) Nicotiana tabacum
b) Ranunculus sceleratusinert
c) Albizialebbeck
d) Daucus carrotus

5. Who reported in vitro somatic embryogenesis for the first time?

a) K. Redenbaugh
b) J. Reinert
c) E.C. Cocking
d) O.L.Gamborg

Answers

1. d	**2. c**	**3. a**	**4. d**	**5. b**

Glossary

Anther: Pollen producing sac like structure of the terminal portion of a stamen.

Auxins: A plant growth hormone that cause apical dominance, cell and root elongation etc.

C**allus**: A disorganized mass of tissue proliferating from cells in cultures.

Cell culture: Culture of single cells or small groups of similar cells.

Culture: growing cells, tissues, plant organs or whole plants in nutrient medium, under aseptic conditions.

Cytokinins: A class of plant growth hormones which cause cell division, cell differentiation, shoot differentiation, breaking apical dominance etc.

Dedifferentiation: Reversal of mature cell into meristematic state forming undifferentiated callus tissue.

Differentiation: The process by which a less specialized cell develop into a more specialized cell type.

Embryo: A multicellular organized structure formed inside the female gametophyte with or without fertilization

Embryo culture: Culture of embryos excised from immature or mature seeds.

Embryogenesis: The process of initiation and development of embryo either in the ovule or tissue culture.

Embryoid: Somatic embryo formed in tissue cultures.

Explants: Any portion of plant (organ or tissue) used to initiate a culture.

Gibberellins: A class of plant growth hormones that stimulates cell enlargement, seed germination and flowering

In vitro: Literally "in glass" now applied to any process carried out in sterile cultures.

Induction: The process of initiation of a structure.

Proliferation: Rapid multiplication of new units (cell, embryos, shoot etc).

Regeneration: In tissue culture, a morphogenetic response that results in the formation of new plants from cultured explants or calli.

Somatic: Referring to vegetative or non-reproductive part.

Somatic embryogenesis: The process of development of embryo from vegetative or non-gametic cells.

Synthetic seeds: Artificial seeds formed by encapsulating somatic embryos in hydrated or desiccated coating.

Totipotency: Potential or ability of a cell to differentiate into a whole plant.

Zygote: Product of fertilization between male and female gametes.

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Suggested reading

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