**LECTURE 22: Callus culture and somatic embryogenesis**

**Objectives**

The main objectives of this chapter are to acquaint the students about the:

1. applications of callus induction and multiplication;
2. patterns of embryogenesis;
3. stages in development of somatic embryos; and
4. limitations and applications of somatic embryogenesis.

**Summary**

Callus in plant tissue culture is defined as an unorganised proliferative mass of cells produced from isolated plant cells, tissues or organs growing aseptically on solid media under controlled experimental conditions. When explants are cultured on medium supplemented with suitable growth regulators, the cultured cells, tissues or organs loses their original characteristics and undergoes dedifferentiation leading to changes in the morphology and metabolism to form an unorganised mass of actively dividing cells known as callus. Callus cultures are extremely important in plant biotechnology and development of shoots, roots or somatic embryos can be induced from the callus tissue by manipulating the ratio of auxin to cytokinin in the medium which can subsequently lead to the formation of whole plants. Callus cultures can also be used to initiate cell suspensions, which are used in a variety of ways in plant tissue culture studies.Somatic embryos are formed from a single somatic cell or group of somatic cells cultured *in vitro* on media containing plant growth regulators that induce somatic embryogenesis. Somatic embryos are bipolar structures with radical and plumule in contrast to shoot buds which are monopolar with only the plumular end. Somatic embryos can arise either by direct embryogenesis or indirect embryogenesis and several factors like genotype of explants and components of medium influence the success of somatic embryogeneis. Somatic embryos serve as a model to understand the event during plant embryogenesis and are also used as explants for synthetic seeds, protoplast isolation or for regeneration of plantlets. However, the main limitation of somatic embryogenesis is the low rate of embryo maturation and conversion.

**Introduction**

In plant tissue culture, callus is defined as an unorganised mass of actively proliferative cells produced from isolated plant cells, tissues or organs cultured *in vitro*. Callus -like tissue is also found to form naturally in various parts of intact plants stimulated due to deep wound or some diseases caused by *Agrobacterium tumefaciens, Synchytrium endobioticum,* some viruses, or insects etc. and such callus -like outgrowth is known as gall or tumour. Callus in plant tissue culture is produced experimentally when explants are cultured on medium supplemented with suitable growth regulators. Under the influence of growth regulators, the cultured cells, tissues or organs loses their original characteristics and undergoes dedifferentiation leading to changes in the morphology and metabolism to form an unorganised mass of actively dividing cells.

**Callus Induction and Multiplication**

**Callus Induction**

The most suitable explants for successful callus formation are often young tissues of one or a few cell types like the pith cells of young stem. Callus induction on explants *in vitro* generally initiate from peripheral layers as a result of wounding and in response to growth regulators, either endogenous or exogenously supplied in the medium. Callus formation from explants initially involves changes in the shape, size, symmetry, structural organization and cellular metabolism followed by division and expansion of the cells to form an unorganised mass of unspecialised parenchyma cells. The cells lose the ability to photosynthesise during the process of callus formation and as a result, callus culture requires addition of vitamins and a carbon source to the culture medium in addition to the mineral nutrients.

The callus growth from cultured explants is dependent on various factors such as the original position of the explant within the plant, the season of the year, growth conditions of the donor plant, the age and physiological state of the parent plant and the culture medium and conditions, etc. One of the most critical factors is the concentration of growth regulator in the culture medium. Auxin is the primary growth regulator used to induce callus and the presence of auxin at a moderate to high concentration or a high concentration of auxin with a low concentration of cytokinin in the medium promotes the formation of callus. Callus culture is most commonly performed in the dark as light can encourage differentiation of the callus. Callus tissue obtained from different plants species may be different in structure and growth habit and may be white or coloured, soft (watery) or hard, and friable (easy to separate into cells) or compact.

**Callus Multiplication**

Callus may be serially subcultured and grown for extended periods but the composition and structure of the callus tissue may change with time as certain cells are favoured by the medium and come to dominate the culture. Actively growing callus can be maintained on culture media with an even physiological balance of cytokinin and auxin. The callus biomass usually increases two to four times after 2-4 weeks of growth after which the callus can be divided and placed on fresh medium for callus multiplication and the multiplication process can be repeated several times (up to eight sequential transfers) before gross chromosome instability (or contamination) occurs. During long-term culture of callus cultures from some plant species, the culture may lose the requirement for auxin and/or cytokinin and this process is known as ‘habituation’.

**Differentiation and Plant Regeneration**

Usually, callus cells proliferate without differentiating but eventually differentiation occurs within the tissue mass and the extent of overall differentiation usually depends on the hormone balance of the support medium and the physiological state of the tissue. Multiplied callus can be stimulated to form shoots by increasing the cytokinin concentration and decreasing auxin content of culture media and the shoot masses can be cut apart and transferred to rooting medium to obtain complete plantlets. Callus cultures can also regenerate plants by somatic embryogenesis initiated with the differentiation of a single meristematic cell from the callus tissue.

**Application of Callus Culture**

Callus cultures are extremely important in plant biotechnology and can be used in a variety of ways in plant tissue culture studies. Some applications of callus culture are:

1. Callus tissue can be used to regenerate whole plants by inducing organogenesis or somatic embryogenesis through manipulation of the nutrient and hormonal constituents in the culture medium.
2. Callus can also be used to obtain transgenic crop plants with enhanced traits by inserting genes into callus cells and regenerating plants from the transformed cells.
3. Callus tissue can be induced to regenerate genetically variable plants since cells of the callus tissue are a good source of genetic or karyotypic variability.
4. Callus cultures are used to initiate cell suspension cultures.
5. Callus cultures may serve as useful alternative sources to obtain commercially important secondary metabolites, since the secondary metabolites of a medicinally important plant can be directly extracted from the callus tissues grown from explants of the plant without sacrificing the whole plant.
6. Several biochemical assays can also be performed from callus culture.

**Limitations of Callus Culture**

The most serious drawback in the use of callus cultures is in micropropagation due the genetic instability of the callus cells. Plantlets regenerated from calli either derived from cell suspension or isolated protoplasts constitute unique cases of cloning called as ‘calliclones’ and ‘protoclones’ and such clones commonly exhibit somaclonal variations. Moreover, cultures in which calli are produced are also not favoured as the initial plant regeneration capacity of the tissue may decline with the passage of time.

**Somatic Embryogenesis**

The process of embryo development from zygote is called embryogenesis and somatic embryogenesis is the development of embryo like structure in culture from the sporophytic or somatic cells of plants under suitable culture conditions *in vitro*. Somatic embryogenesis is the process of non-sexual development of bipolar embryo from somatic cells. Somatic embryos are different from shoot buds obtained from organogenesis since somatic embryos are bipolar structures with radical and plumule in contrast to shoot buds which are monopolar with only the plumular end.

Somatic embryogenesis involves three distinct steps:

1. **Induction:** It is the initiative phase where cells of callus are induced to divide and differentiate into groups of meristematic cells called embryogenic clumps.
2. **Maturation:** In this phase, somatic embryos develop into mature embryos by differentiating and the mature embryo here undergoes biochemical changes to acquire hardiness.
3. **Conversion:** Embryos germinate to produce seedlings.

**Patterns of embryogenesis**

Two general patterns of somatic embryogenesis or embryogenesis *in vitro* are identified. They are a)direct embryogenesis b) indirect embryogenesis.

**1) Direct embryogenesis:** It refers to the development of an embryo directly from the original explant tissue without an intervening callus phase. This occurs through “pre-embryogenic determined cells‟ (PEDC) where the cells are committed to embryonic development and such cells are commonly found in embryonic tissues.

**2) Indirect embryogenesis:** It is the formation of embryos from cells of callus tissue called the embryogenically determined cells which are induced to form embryos and are known as induced embryogenic determined cells (IEDC).

The somatic embryos regenerating from explants (or) callus are termed as primary somatic embryos. Somatic embryos may also regenerate from tissues of other somatic embryos (or) parts of germinating somatic embryos and are called secondary somatic embryos.

**Stages in Development of Somatic Embryos**

Somatic embryos generally originate from single cells and they develop into somatic embryos following four distinct stages:

1. Globular stage: Somatic embryos arise from a single cell which divides to form a group of meristematic cells and these cells becomes isolated by breaking cytoplasmic connections with the other cells around it and subsequently by cutinization of the outer walls of this differentiating cell mass. It increases in size by further division to form a spherical shaped structure known as the *globular* *stage*. At this stage, the primary meristem (protoderm, ground meristem and procambium) becomes visible.
2. Heart-shaped stage:The next stage in which the meristematic mass of cell continue to divide and differentiate with initiation of cotyledon primordia and transform into a heart-shaped embryo.
3. Torpedo stage:The embryo grows further developing the cotyledon and attains the torpedo-shaped stage.
4. Cotyledonary stages: The growth of the embryo in this stage involves the division of cells inside the cotyledonary ring to form shoot and root apical meristem and differentiation of procambium takes place leading to the production of a bipolar structure containing in root/shoot axis (radicle/plumule) with a closed independent vascular system.

**Factors Affecting Somatic Embryogenesis**

1. Growth regulators: The presence of auxin (generally 2,4-Dicholophenoxy acetic acid or 2,4-D) in the medium is essential for induction of embryogenic clumpsand maturation is achieved by culturing somatic embryos on medium containing some doses of cytokinin, high sucrose and ABA. Silver nitrate is sometimes added to the medium as inhibitor of ethylene for plant regeneration since ethylene inhibits both somatic embryogenesis and organogenesis.

2. Sucrose: The presence of high sucrose in the medium is required for maturation of somatic embryos to make them more sturdy and hardy.

3. Nitrogen source: NH4+ form of nitrogen is essential for induction of somatic embryogenesis while NO3- form is required during maturation phase.

4. Genotype of explant: Explant genotype may also determine the potential of regeneration of somatic embryos in culture.

5. Other factors: Some polyamines like putrescine, spermidine, spermine, etc. are required for somatic embryogenesis*.* High K+ levels and low dissolved O2 levels also promote somatic embryo regeneration in some species.

**Applications**

The main applications of somatic embryogenesis are:

1. Somatic embryos can be used for synthesis of artificially synthetic seeds or as the source of regenerable protoplast system.
2. Somaclonal variants can be obtained by regenerating plants by indirect organogenesis involving callus proliferation and differentiation of embryogenic cells from the callus.
3. Somatic embryogenesis may be used for the rapid propagation of economically important plants by multiplying germplasm that is initially present as embryonic material.
4. Somatic embryos have been used for elimination of viruses in certain plant species.
5. Somatic embryos may also serve as the source tissue for genetic transformation

**Limitations**

1) Somatic embryo maturation and conversion is difficult in many species and is critical to the practical utilization of somatic embryogenesis.

2) Occurrence of somaclonal variations in indirect somatic embryogenesis may limit its application in micropropagation.

3) Somatic embryo quality is often poor and field conversion frequencies are low.

4) Large scale production of somatic embryos is difficult.

**Conclusion**

Plants develop callus or undifferentiated cell mass after exposure to suitable growth conditions *in vitro*. Callus cultures are extremely important in plant biotechnology and development of shoots, roots or somatic embryos can be induced from the callus tissue by manipulating the ratio of auxin to cytokinin in the medium which can subsequently lead to the formation of whole plants. Callus cultures can also be used to initiate cell suspensions, which are used in a variety of ways in plant tissue culture studies.Somatic embryos are formed from a single somatic cell or group of somatic cells cultured *in vitro* on media containing plant growth regulators that induce somatic embryogenesis. Somatic embryogenesis has also served as a model to understand the event during plant embryogenesis processes as well as a component to important plant biotechnological advancement.

**Transcript**

**Introduction**

Callus in plant tissue culture is defined as an unorganised proliferative mass of cells produced from isolated plant cells, tissues or organs growing aseptically on solid media under controlled experimental conditions. Callus - like tissue is also found to form naturally in various parts of intact plants stimulated due to deep wound or some diseases caused by *Agrobacterium tumefaciens, Synchytrium endobioticum,* some viruses or insects etc. and such callus-like outgrowth is known as gall or tumour. But the callus in tissue culture is produced experimentally when explants cultured under the influence of exogenously supplied hormones.

**Callus Induction and Multiplication**

**Callus Induction**

The most suitable explants for successful callus formation are often young tissues of one or a few cell types like the pith cells of young stem. Callus induction on explants *in vitro* generally initiate from peripheral layers as a result of wounding and in response to growth regulators, either endogenous or exogenously supplied in the medium. Callus formation from explants initially involves dedifferentiation both in morphology and metabolism and as a result callus is usually composed of unspecialised parenchyma cells and the cells lose the ability to photosynthesise.

The callus growth within a plant species is dependent on various factors such as the original position of the explant within the plant, the season of the year, growth conditions of the donor plant, the age and physiological state of the parent plant and the culture medium and conditions, etc.The presence of auxin at a moderate to high concentration and a low concentration of cytokinin in the medium promotes the formation of callus and callus culture is often performed in the dark as light can encourage differentiation of the callus. Callus tissue from different plants species may be different in structure and growth habit and may be white or coloured, soft (watery) or hard, and friable (easy to separate into cells) or compact.

**Callus Multiplication**

Callus may be serially subcultured and grown for extended periods but the composition and structure of the callus tissue may change with time as certain cells are favoured by the medium and come to dominate the culture. Actively growing callus can be maintained on culture media with an even physiological balance of cytokinin and auxin. The callus biomass usually increases two to four times after 2–4 weeks of growth after which the callus can be divided and placed on fresh medium for callus multiplication and the multiplication process can be repeated several times (up to eight sequential transfers) before gross chromosome instability (or contamination) occurs. During long-term culture of callus cultures from some plant species, the culture may lose the requirement for auxin and/or cytokinin and this process, known as ‘habituation’.

**Differentiation and Plant Regeneration**

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**Application of Callus Culture**

1.Callus tissue can be used to regenerate whole plants by inducing organogenesis or somatic embryogenesis through manipulation of the nutrient and hormonal constituents in the culture medium.

2. Callus tissue can be induced to regenerate genetically variable plants since cells of the callus tissue is a good source of genetic or karyotypic variability.

3. Callus cultures are used to initiate cell suspension cultures.

4. Callus cultures may serve as useful alternative sources to obtain commercially important secondary metabolites since the secondary metabolites of a medicinally important plant can be directly extracted from the callus tissues grown from explants of the plant without sacrificing the whole plant.

5. Several biochemical assays can also be performed from callus culture.

**Limitations of Callus Culture**

The most serious drawback in the use of callus cultures is in micropropagation due the genetic instability of the callus cells. Plantlets regenerated from calli either derived from cell suspension or isolated protoplasts constitute unique cases of cloning called as ‘calliclones’ and ‘protoclones’ and such clones commonly exhibit somaclonal variations. Moreover, cultures in which calli are produced are also not favoured as the initial plant regeneration capacity of the tissue may decline with the passage of time.

**Somatic Embryogenesis**

The process of embryo development is called embryogenesis and somatic embryogenesis is the development of embryo like structure in culture from the sporophytic or somatic cells of plants under suitable culture conditions in *vitro*. Somatic embryos are bipolar structures with radical and plumule in contrast to monopolar shoot bud obtained from organogenesis with only plumular end. Somatic embryogenesis involves three distinct steps which are:

* Induction: It is the initiative phase where cells of callus are induced to divide and differentiate into groups of meristematic cells called embryogenic clumps.
* Maturation: In this phase, somatic embryos develop into mature embryos by differentiating and the mature embryo here undergoes biochemical changes to acquire hardiness.
* Conversion: Embryos germinate to produce seedlings.

**Patterns of Embryogenesis**

Two general patterns of somatic embryogenesis or embryogenesis *in vitro* are identified: Direct embryogenesis and indirect embryogenesis.

**1) Direct embryogenesis:** Refers to the development of an embryo directly from the original explant tissue without an intervening callus phase. This occurs through “pre-embryogenic determined cells‟ (PEDC) where the cells are committed to embryonic development and such cells are commonly found in embryonic tissues.

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**Factors Affecting Somatic Embryogenesis**

1. Growth regulators: The presence of auxin (generally 2,4-Dicholophenoxy acetic acid or 2,4-D) in the medium is essential for induction of embryogenic clumpsand maturation is achieved by culturing somatic embryos on medium containing some doses of cytokinin, high sucrose and ABA. Silver nitrate is sometimes added to the medium as inhibitor of ethylene for plant regeneration since ethylene inhibits both somatic embryogenesis and organogenesis.

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3. Nitrogen source: NH4+ form of nitrogen is essential for induction of somatic embryogenesis while NO3- form is required during maturation phase.

4. Genotype of explant: Explant genotype may also determine the potential of regeneration of somatic embryos in culture.

5. Other factors: Some polyamines like putrescine, spermidine, spermine, etc. are required for somatic embryogenesis*.* High K+ levels and low dissolved O2 levels also promote somatic embryo regeneration in some species.

**Applications**

The main applications of somatic embryogenesis are:

* Somatic embryos can be used for synthesis of artificially synthetic seeds or as the source of regenerable protoplast system.
* Somaclonal variants can be obtained by regenerating plants by indirect organogenesis involving callus proliferation and differentiation of embryogenic cells from the callus.
* Somatic embryogenesis may be used for the rapid propagation of economically important plants by multiplying germplasm that is initially present as embryonic material.
* Somatic embryos have been used for elimination of viruses in certain plant species.
* Somatic embryos may also serve as the source tissue for genetic transformation.

**Limitations**

1) Somatic embryo maturation and conversion is difficult in many species and is critical to the practical utilization of somatic embryogenesis.

2) Occurrence of somaclonal variations in indirect somatic embryogenesis may limit ita application in micropropagation.

3) Somatic embryo quality is often poor and field conversion frequencies are low.

4) Large scale production of somatic embryos is difficult.

**conclusion**

Plants develop callus or undifferentiated cell mass after exposure to suitable growth conditions *in vitro*. Callus cultures are extremely important in plant biotechnology and development of shoots, roots or somatic embryos can be induced from the callus tissue by manipulating the ratio of auxin to cytokinin in the medium which can subsequently lead to the formation of whole plants. Callus cultures can also be used to initiate cell suspensions, which are used in a variety of ways in plant tissue culture studies.Somatic embryos are formed from a single somatic cell or group of somatic cells cultured *in vitro* on media containing plant growth regulators that induce somatic embryogenesis. Somatic embryogenesis has also served as a model to understand the event during plant embryogenesis processes as well as a component to important plant biotechnological advancement.

**Glossary**

**1. Callus:** An unorganized mass of differentiated plant cells.

**2. Cell culture:** Culture of cells or their maintenance *in vitro* including the culture of single cells.

**3. Culture:** Plant growing *in vitro*.

**4. Embryogenesis:** *De novo* formation of somatic embryos.

**5. Embryoid:** Embryo-like structure formed under *in vitro* conditions, this structure has the potential for further development into a plantlet.

**6. Explant:** An excised piece or part of a plant used to initiate a tissue culture.

**7. Genotype:** Plants with the same genetic information (DNA)

***8. In Vitro*:** To be grown in glass.

**9. Medium:** A solid or liquid nutritive solution used for culturing cells.

**10. Subculture:** The aseptic division and transfer of a culture or portion of that culture to a fresh synthetic media.

**11. Tissue culture:** *In vitro* culture of cells, tissues, organs and plants under aseptic conditions on synthetic media.

**Frequently Asked Questions (FAQs)**

1. What is callus in plant tissue culture?

Ans.: Callus, in plant tissue culture is defined as an unorganised mass of actively proliferative cells produced from isolated plant cells, tissues or organs cultured *in vitro*. Callus in plant tissue culture is produced experimentally when explants are cultured on medium supplemented with suitable growth regulators. Under the influence of growth regulators, the cultured cells, tissues or organs loses their original characteristics and undergoes dedifferentiation leading to changes in the morphology and metabolism to form an unorganised mass of actively dividing cells.

1. What are the factors affecting callus induction from explants?

Ans.: Callus induction from cultured explants is dependent on various factors such as the original position of the explant within the plant, the season of the year, growth conditions of the donor plant, the age and physiological state of the parent plant and the culture medium and conditions, etc. One of the most critical factors is the concentration of growth regulator in the culture medium. Auxin is the primary growth regulator used to induce callus and the presence of auxin at a moderate to high concentration or a high concentration of auxin with a low concentration of cytokinin in the medium promotes the formation of callus. Light also affects callus induction and callus culture is most commonly performed in the dark as light can encourage differentiation of the callus.

1. What are the applications of callus culture?

Ans.: The main applications of callus cultures are:

1. Callus tissue can be used to regenerate whole plants by inducing organogenesis or somatic embryogenesis through manipulation of the nutrient and hormonal constituents in the culture medium.
2. Callus can also be used to obtain transgenic crop plants with enhanced traits by inserting genes into callus cells and regenerating plants from the transformed cells.
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5. Callus cultures may serve as useful alternative sources to obtain commercially important secondary metabolites since the secondary metabolites of a medicinally important plant can be directly extracted from the callus tissues grown from explants of the plant without sacrificing the whole plant.
6. Several biochemical assays can also be performed from callus culture.
7. What is somatic embryogenesis?

Ans.: Somatic embryogenesis is the development of embryo like structure in culture from the sporophytic or somatic cells of plants under suitable culture conditions in *vitro*. It is the process of non-sexual development of bipolar embryo from somatic cells and is different from shoot buds obtained from organogenesis which are monopolar with only the plumular end. Somatic embryogenesis involves three distinct steps-Inductions or the initiative phase where cells of callus are induced to divide and differentiate into groups of meristematic cells called embryogenic clumps, maturation or development of mature embryos by acquiring hardiness, and conversion where embryos germinate to produce seedlings.

1. What are the factors affecting somatic embryogenesis in plant tissue culture?

Ans.: The main factors affecting somatic embryogenesis are growth hormones, genotype of explants, the form of nitrogen source and concentration of some other substances like sucrose, ethanol and maltose.

#### What are the applications of somatic embryogenesis.

Ans.: The main applications of somatic embryogenesis are:

1. Somatic embryos can be used for synthesis of artificially synthetic seeds or as the source of regenerable protoplast system.
2. Somaclonal variants can be obtained by regenerating plants by indirect organogenesis involving callus proliferation and differentiation of embryogenic cells from the callus.
3. Somatic embryogenesis may be used for the rapid propagation of economically important plants by multiplying germplasm that is initially present as embryonic material.
4. Somatic embryos have been used for elimination of viruses in certain plant species.
5. Somatic embryos may also serve as the source tissue for genetic transformation.

## **References**

1. Bhojwani, S.S. and Rajdan, 1996: *Plant Tissue Culture: Theory and Practice*, Amsterdam, Elsevier.
2. Chawla, H.S. 2002: *Introduction to Plant Biotechnology* (2nd ed. ed.). Enfield, N.H.: Science Publishers. ISBN 1-57808-228-5.
3. Razdan, M. K. 2003: *Introduction to Plant Tissue Culture* (2. ed. ed.). Enfield, NH [u.a.]: Oxford Publishers. ISBN 1-57808-237-4.
4. Smith, R. 2013: *Plant Tissue Culture:Techniques and Experiments,* Academic Press, Withers, L. A.

## **Links**

1. http://www.biobasics.gc.ca
2. http://theagricos.com
3. [http://agriinfo.in](http://agriinfo.in/default.aspx?page=topic&superid=3&topicid=1897)