

UNIT 11

PLANT TISSUE CULTURE |

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11.1 INTRODUCTION

Plant tissue culture is a technique for *in vitro* culturing of plant cells and tissues on synthetically defined media under controlled conditions of light, temperature and humidity. It is widely used for large scale multiplication of plants; generation of transgenic crops with improved qualities, production of secondary metabolites, conservation of endangered species coupled with assured availability of plant material / products all the year round.

The success of gene transfer techniques for plant improvement and introduction of novel genes from unrelated organisms is dependent on effective plant regeneration systems. This has extended the domain of plant tissue culture from basic research to an essential component of plant biotechnology.

In this unit we shall learn about early landmark discoveries; basic technique of plant tissue culture; types of culture; regeneration pathways and the applications of cell and tissue culture in agriculture and industry.

Expected Learning Outcomes

After studying this unit you should be able to:

- ❖ describe the basic technique of plant tissue culture;
- ❖ highlight landmark discoveries in the development of plant tissue culture techniques;
- ❖ differentiate between various types of culture;
- ❖ explain the pathways of *in vitro* plant regeneration;
- ❖ highlight the importance of plant regeneration systems in plant biotechnology;
- ❖ define the term 'Somaclonal variation' and its application in plant improvement;
- ❖ indicate applications of plant cell and tissue culture with specific reference to agriculture and industrially important secondary metabolites.

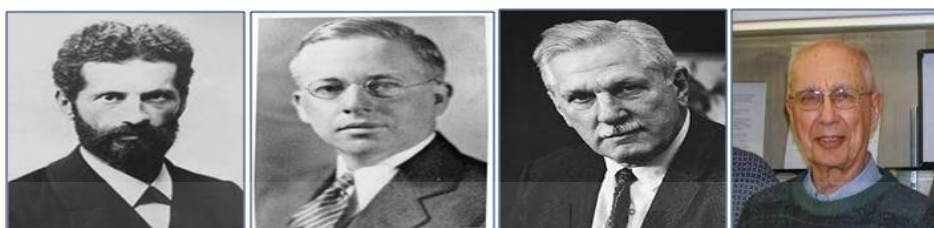
11.2 PLANT TISSUE CULTURE: A BRIEF HISTORY

With the recognition of the cell as the basic unit of life (Schleiden and Schwann; 1838 - 39), Austrian plant physiologist **Gottlieb Haberlandt** (1902) came up with the idea of 'totipotency' which meant that every plant cell has the genetic potential to form a complete plant. He attempted to culture mechanically isolated single palisade cells (fully differentiated) on a simple nutrient medium containing glucose, peptone and Knop's salt solution. The cells survived for several weeks, increased in size but failed to divide. Though unsuccessful, he laid the foundations of plant tissue culture and is regarded as the father of plant tissue culture. He also postulated the existence of plant hormones.

The first successful continuous cultures of tomato root tips were reported by Philip R. White (1934) using Knop's salt with sucrose and yeast extract. The latter component was subsequently replaced by three B-group vitamins. Then Johannes van Overbeek (1941) observed growth stimulation of *Datura* embryos by addition of coconut water. In 1953 W.H. Muir *et al* succeeded in isolating and culturing single cells from callus. This was followed by the discovery of a cell division factor, kinetin (Skoog *et al*, 1955) and the classic work of Skoog and Miller (1957) on the hormonal control of organ formation.

They demonstrated that the ratio of auxin to cytokinin influences morphogenesis of either roots or shoots in tobacco cultures.

An important advancement was the technique of enzymatic isolation and culture of protoplasts (Cocking, 1960). They are extremely useful in cell fusion and genetic transformation. Then Murashige and Skoog (1962) developed the most extensively used synthetic nutrient medium in plant tissue culture, popularly known as MS medium. The first haploid plants from pollen grains of *Datura* were produced by two Indian botanists, Guha and Maheshwari (1964). This set the phase for the development of suitable media to culture various plant tissues, pollen (haploid culture), protoplasts, etc and above all reproducible regeneration systems.



Gottlieb Haberlandt

Philip R. White

Folke K. Skoog

Carlos O. Miller



Toshio Murashige

Edward Cocking

S. Guha-Mukherjee

S.C. Maheshwari

A completely new dimension was added to plant tissue culture with successful introduction of genes of interest from different species into crop plants to produce genetically modified (GM; transgenic) plants with beneficial traits such as disease resistance, tolerance to abiotic stresses and improved nutritional quality.

11.3 CELL AND TISSUE CULTURE TECHNIQUES

Plant cell and tissue culture is based on 'cellular totipotency' which is the ability of a plant cell to regenerate into a complete plant from a single cell on an artificial nutrient media and suitable physical conditions. In this section we shall describe the basic technique of plant tissue culture (Fig.11.1) and core facilities required for sterilization, aseptic transfer and culture of plant parts and cells.

Plant tissue culture laboratory and equipments

Basic plant tissue culture setup requires maintenance of controlled laboratory conditions and a handful of instruments. The laboratory aims at fulfilling two main objectives - (i) to keep plant cells and organs free from contaminating microbes and (ii) to ensure the desired development of explants by providing suitable nutrient media and environmental conditions.

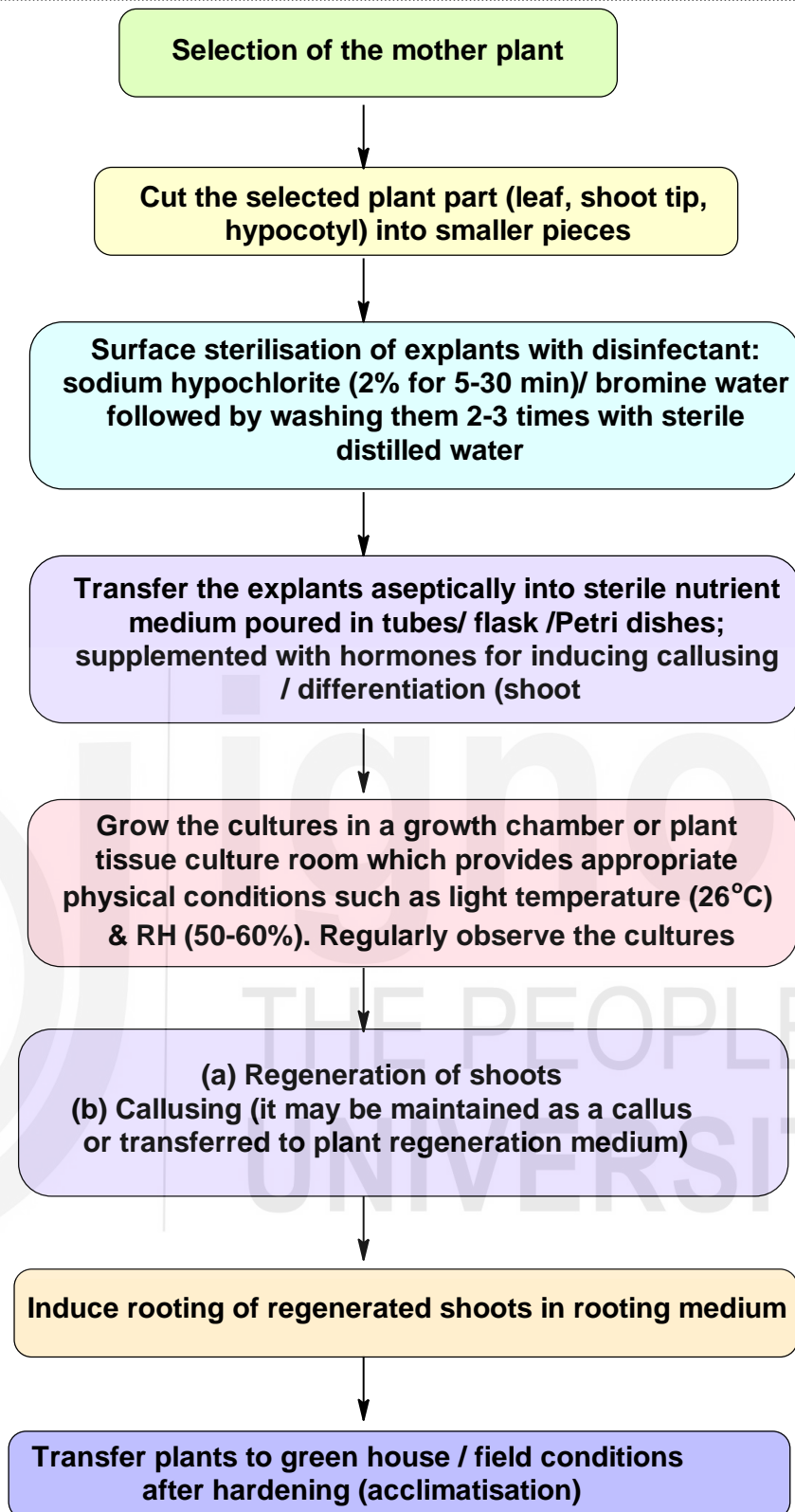


Fig. 11.1: Flow chart of basic plant tissue culture technique.

The first objective is achieved by adopting stringently aseptic culture techniques such as use of only sterilized glassware, media and other equipment like scalpel, forceps, etc. The culturing and maintenance of cells and tissues is done by choosing the best available nutrient medium / making changes in the selected media; addition of appropriate ratio of growth regulators; adjusting the duration and quality of light, humidity and temperature.

Minimal facilities required are:

- ❖ Media preparation facility, equipped with media preparation table(s), glassware; balance; autoclave for sterilization by moist heat and storage space. An oven can also be installed here or in the corridor for dry heat sterilization.
- ❖ A Laminar Flow clean air Bench (LFB) for safe / aseptic handling of explants and a set up for filter sterilization of heat labile substances (hormones, antibiotics). It may even be placed in a small partitioned area in the culture room.
- ❖ Culture rooms for maintenance of cultures must provide controlled, clean and aseptic conditions.
- ❖ A separate discard / wash area for washing, drying and safe keeping of glass ware and plastic ware.
- ❖ Microscopes – stereo and compound for routine observations.
- ❖ Incubators and deep freezers can be kept in corridor space.
- ❖ A greenhouse facility can be set up on terrace / any other available space; enclosed to save plantlets from rodents and birds. It is required for hardening of cultured plants prior to field transfer.

The organization of a plant tissue culture laboratory is an important factor. A tissue culture room may be replaced by growth chambers as per requirement. This facility aims at providing artificially controlled environmental conditions for optimal growth of plants. Temperature, humidity and light are the key environmental factors controlled. These days they are automated and rigidly controlled from outside, with LED display. In addition during culture, which usually takes a few weeks, maintenance of clean air is mandatory. HEPA (High Efficiency Particulate Air) filters takes care of this requirement and maintains positive air pressure inside the chamber with continuous but slow airflow. Nowadays light source are used that can provide photosynthetically active radiations (PAR) in growth chambers. Many types of growth chambers are available such as walk-in type and reach-in type moveable growth chambers.

Nutrient media

Plant parts (explants) and cells can grow, divide and / differentiate under *in vitro* conditions, provided with the requisite nutrients, hormones and physical conditions. All tissue culture media are synthetic or chemically defined. In general these growth media consist of inorganic (salts) and organic nutrients essential for optimal plant growth. The former supplies the micro-and micro-nutrients while the latter includes few B group vitamins, myoinositol, amino acids (glycine), carbon source (2-5% sucrose) and growth regulators (auxin, cytokinins; natural / synthetic).

The synthetic auxin 2,4D (2,4-dichlorophenoxy acetic acid) and phenyl urea type cytokinin, thidiazuron (TDZ) are quite popular; for inducing callus / somatic embryogenesis and shoot regeneration, respectively. Often complex supplements (coconut milk, potato extract, yeast extract) of ill defined composition are added, especially when specific requirements for optimal results are not known. To eliminate microbial growth some investigators add antibiotics such as gentamycin, streptomycin, tetracycline (singly or in combination), especially for precious cultures. The pH of the medium is between 5.5-5.8.

Most frequently used culture media (Table1) includes White's (1963), MS media (Murashige and Skoog; 1962) and Gamborg's B5 media (1968). The choice of the media is dictated by the plant species and plant part used. Generally stock solutions of major and minor nutrients, vitamins, etc are prepared and frozen. Heat labile ingredients are filter sterilized and then aseptically added to the autoclaved medium. In case solid media is needed, agar (gelling agent) is added to the liquid medium, melted and autoclaved.

Table11.1: Composition of some plant tissue culture media

Components	Amount (mg l ⁻¹)				
	White's	Murashige and Skoog (MS)	Gamborg (B5)	Chu(N6)	Nitsch's
Macronutrients					
MgSO ₄ .7H ₂ O	750	370	250	185	185
KH ₂ PO ₄	-	170	-	400	68
NaH ₂ PO ₄ .H ₂ O	19	-	150	-	-
KNO ₃	80	1900	2500	2830	950
NH ₄ NO ₃	-	1650	-	-	720
CaCl ₂ .2H ₂ O	-	440	150	166	-
(NH ₄) ₂ .SO ₄	-	-	134	463	-
Micronutrients					
H ₃ BO ₃	1.5	6.2	3	1.6	-
MnSO ₄ .4H ₂ O	5	22.3	-	4.4	25
MnSO ₄ .H ₂ O	-	-	10	3.3	-
ZnSO ₄ .7H ₂ O	3	8.6	2	1.5	10
Na ₂ MoO ₄ .2H ₂ O	-	0.25	0.25	-	0.25
CuSO ₄ .5H ₂ O	0.01	0.025	0.025	-	0.025
CoCl ₂ .6H ₂ O	-	0.025	0.025	-	0.025
KI	0.75	0.83	0.75	0.8	-
FeSO ₄ .7H ₂ O	-	27.8	-	27.8	27.8
Na ₂ EDTA.2H ₂ O	-	37.3	-	37.3	37.3
Sucrose (g)	20	30	20	50	20
Organic supplements					
Vitamins					
Thiamine (HCl)	0.01	0.5	10	1	0.5
Pyridoxine (HCl)	0.01	0.5	1	0.5	0.5
Nicotinic acid	0.05	0.5	1	0.5	5
Myoinositol	-	100	100	-	100
Others					
Glycine	3	2	-	-	2
Folic acid	-	-	-	-	0.5
Biotin	-	-	-	-	0.05
pH	5.8	5.8	5.5	5.8	5.8

SAQ 1

Indicate whether the following statements are true or false:

- a) Tissue culture is a technique to transfer genomic DNA from one plant to another.
 - b) Plant tissue culture can be performed in fields and open spaces.
 - c) Auxin promotes rooting from explants in artificial culture conditions.
 - d) All media and glassware except heat labile components are sterilized by autoclaving.
 - e) Plant tissue culture media has only inorganic nutrients.
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11.4 TYPES OF CULTURE

As discussed in the preceding section, any plant part with living cells or tissues can be cultured using tissue culture techniques. It includes vegetative and reproductive cells as well as permanent and meristematic tissues. Cultures fall into two basic types; unorganized culture (callus and suspension culture) and organized culture (root tip culture, embryo culture, etc). In the following subsections you will learn about the various types of culture based on the plant organ / explants used or how they grow under a given set of conditions to form an unorganised callus / cell suspensions / plant organs / complete plants.

11.4.1 Organ Culture

An organ culture is initiated with isolated organs and is named depending on the organ used for culturing, for example, endosperm culture, root culture, meristem culture and another culture. They can be further subdivided into culture of indeterminate or determinate organs. The basic difference between them is in the presence / absence, respectively of active meristem in the mature organ. The embryonic cells in the meristem such as apical meristem are self perpetuating and divide to form cells that may remain meristematic or differentiate to become specialised and get incorporated into tissues / organs of the growing plant. Plant organs that stop growing once mature are determinate organs. They have defined size and shape such as leaf and flower. Indeterminate growth is observed in certain varieties of tomatoes, legume nodules and mature trees as they continue to grow.

11.4.2 Explant Culture

A culture initiated from plants parts (explants) is known as an explant culture. Any part of the plant could be cultured aseptically like small pieces of leaf, root tip, cotyledons, axillary buds, flowers and anthers. Prior to culturing, an explant is surface sterilized, cut into small pieces with a sterile blade / scalpel and then aseptically transferred to nutrient flasks or tubes. The size and choice of the explants are some of the parameters to be considered in obtaining the desired response. By manipulating the concentration and ratio of hormones it is possible to induce either callus formation or plant regeneration.

11.4.3 Callus Culture

A callus is an unorganized (dedifferentiated; totipotent) mass generally composed of unspecialized parenchymatous cells, on a solid media. It can be induced using a variety of plant parts; the actual response depends on the culture media, explant used; genotype, hormones and other ill defined factors. Initially callusing is confined to the cut ends but subsequently it extends to the whole explant. Generally relatively high concentration of auxin or a combination of auxin and cytokinin (in appropriate ratios) induces callus formation. Callus cultures can be maintained for prolonged periods by repeated sub-culturing. Over time the sub cultured callus loses sensitivity to exogenous auxin (habituated callus).

Callus cultures are used for:

- plant regeneration
- protoplast isolation and starting material for cell and suspension cultures
- production of industrially important secondary metabolites in high yield
- genetic transformation
- investigating morphogenetic potential
- generation of somaclonal variations

Calli differ considerably in shape, colour, degree of compactness and hydration. They are generally not homogeneous and include cells with different levels of differentiation and somaclonal variants. A friable (soft) callus has loosely associated cells that fall off easily as compared to a compact callus. After multiple sub-culturing a callus may become friable. Most often a callus has a creamish / white color; sometimes it becomes pigmented ranging from completely green / other colors (due to anthocyanins) or in patches. Monocots are less responsive than dicots to callus induction and woody explants take much longer time.

11.4.4 Cell Suspension Culture

A cell suspension culture is initiated with single isolated plant cells suspended in a liquid medium. Single cells can be obtained either from a friable callus or any part of the plant by mechanical or enzymatic methods. A callus mass / tissue may be ground to a fine suspension in a liquid medium and filtered or centrifuged to remove debris. The clear suspension may even be placed on a gyratory shaker at low speed to get separated cells. The enzymatic method uses a mix of enzymes (pectinase/ macerozyme) that dissolve the middle lamella, to release single cells. Generally suspension cultures grow much faster than callus and thus require more frequent sub culturing.

The isolated cells may be cultured in flasks as batch or continuous cultures. A batch culture is a closed system with limited nutrients. This type of culture shows a typical sigmoid kinetics with a lag, log, stationary and decline phases. The growing cells experience a changing environment with time due to consumption of nutrients and release of metabolic wastes. This method is useful for suspension initiation and cell selection for scaling up and isolation of intracellular metabolites.

A continuous culture is an open system that allows cells to be grown at a particular rate for an extended period (log phase). The growth medium has one of the nutrients in limited amount and before it gets exhausted it is replenished with fresh medium. At the same an equal volume is displaced from the culture vessel. In such a culture steady state can be reached eventually and the concentration of cells, metabolites and other nutrients remain constant.

Cell suspension cultures can be used for:

- Commercial production of phytochemicals such as alkaloids (for pharmaceuticals) and flavouring agents (used in food and cosmetic industry). This is carried out in specially designed plant bioreactors which are ideal for large scale culturing as well as economically viable.
- Biomass production.
- Genetic transformation.
- Induction of somatic embryos / shoots.
- *In vitro* mutagenesis.

11.4.5 Protoplast Culture

Protoplasts are living plant cells devoid of cell wall. They can be isolated from plant tissues usually leaves or single cells or cultures by mechanical (**Klercker, 1892**) or enzymatic method (**Cocking, 1960**). The latter method is preferred primarily due to the higher yield of protoplasts as compared to the former method.

The enzymatic method uses a mixture of cell wall digesting enzymes (pectinase, cellulase, hemicellulase) or ready to use cocktails. However, the choice of enzymes and treatment regimen depends on explant and structural composition of its cell wall. The yield and viability of protoplasts depends on age, physiological state, purity of enzymes; pH of the medium and incubation period. Utmost care is taken in handling the released protoplasts as they are extremely fragile.

Protoplasts are usually cultured at very low plating density (50-500 thousand protoplast / ml) by suspension culture in Petri plates, without agitating. The liquid medium initially has high osmoticum (13% or higher mannitol concentration) until cell wall synthesis is completed. In suitable medium protoplasts undergoes cell division and may form callus which can be shifted to a regeneration medium to obtain plantlets.

Before they regenerate their cell wall, protoplasts are suitable for:

- Genetic transformation.
- Somatic cell fusion to produce hybrids. It provides an efficient means of gene transfer with desirable traits from one species to another. The selected hybrid cell is then transferred to a regeneration system.
- fusion of protoplasts with anucleated cells (cybrids) in the presence of fusogenic agents like poly ethylene glycol PEG)
- Biochemical studies.

SAQ 2

- a) Ticks [✓] mark the best option:
- i) Which of the following is a type of organized culture practice?
 1. Single cell culture
 2. Root tip culture
 3. Callus culture
 4. Cell suspension culture
 - ii) Choose the correct statement about callus:
 1. It can be induced only by pathogen attack.
 2. Any explant can be used to induce callus.
 3. It requires sub culturing every day.
 4. It has uniform physical properties irrespective of explant source.
 - iii) Cell suspension culture can be readily made from.....
 1. Seeds
 2. Friable callus
 3. Shoot meristem
 4. Leaf
- b) What is the difference between organ culture and explant culture?
-

11.5 PLANT REGENERATION PATHWAYS

The unique ability of plant parts / protoplasts or suspension cells to dedifferentiate and redifferentiate into complete plants under the influence of hormones allows investigators to produce plants with novel / beneficial characteristics by genetic manipulation. Plant regeneration or organogenic differentiation can be defined as the development of organized plant organs like roots, shoots, floral buds and somatic embryos from cultured cells. There are two basic pathways of plant regeneration, namely organogenesis and somatic embryogenesis (Fig.11.4).

11.5.1 Organogenesis

Organogenesis is the formation of organs such as shoots from cultured explants. They may arise from meristem or indirectly from callus. Plant organs are formed from vegetative tissues. It is a multistep process, each of which is influenced by hormones. Initially cells acquire competence to respond to hormones followed by induction of organ formation and finally organ formation even if hormones are withdrawn. The importance of the relative concentration of auxin to cytokinins in influencing organogenesis can be had from Fig. 11.2, although the actual ratio has to be experimentally worked out.

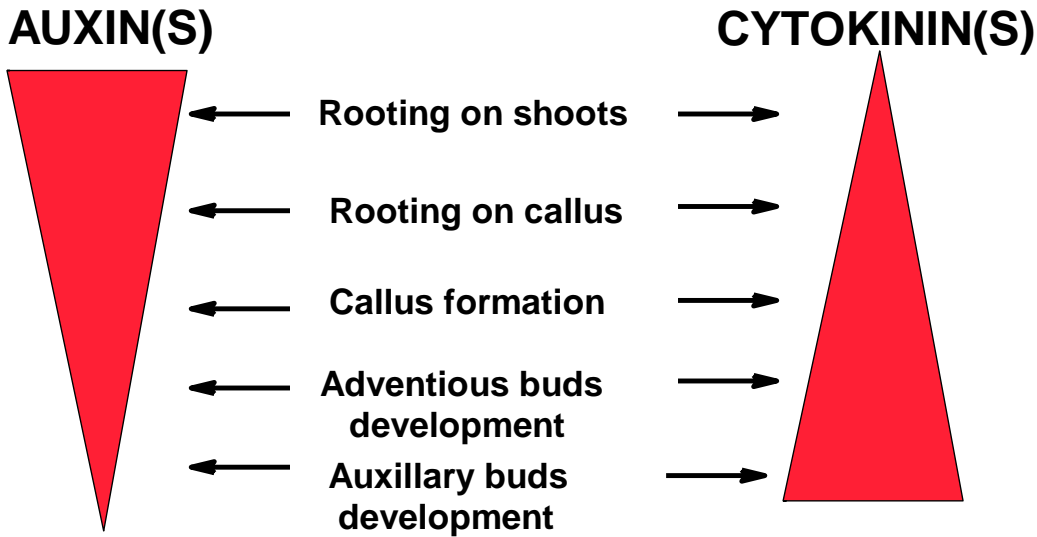


Fig. 11.2: The effect of auxin to cytokinin ratio on organogenesis.

11.5.2 Somatic Embryogenesis

In somatic embryogenesis, somatic totipotent plant cells / tissues can be coerced to enter the embryogenic pathway to form somatic embryos. They resemble zygotic embryos (seed embryos) formed by the fertilisation of haploid gametic cells. **F.C. Steward** (1958) and **J. Reinert** (1959) independently reported for the first time somatic embryogenesis in carrot (*Daucus carota*) callus and cell suspension cultures. Advancements in plant tissue culture technology have demonstrated that irrespective of ploidy and specialization, any plant cells can be induced to form viable embryos *in vitro*.

Somatic embryos are non-zygotic bipolar embryo-like structures derived from somatic cells.

Somatic embryo can be developed either directly from the explant without intervening callus stage (direct somatic embryogenesis) or through callus a phase (indirect somatic embryogenesis, Fig.11.3). A somatic embryo is easy to separate since it lacks vascular connections with the maternal tissue. It is observed that somatic embryos often have abnormal cotyledon numbers (three or more) and occur in variety of shapes and sizes. Various factors like (a) plant growth regulators; (b) form of nitrogen used; (c) type of explant and explant genotype affects somatic embryogenesis.

The somatic embryos can germinate *in situ* or upon transfer to a fresh medium. It is a widely used method for sustained clonal propagation and genetic manipulation. These embryos can also be encapsulated in a gel-matrix or desiccated with / without a protective coating and used as synthetic / somatic seeds. They are especially useful in the rapid and mass propagation of elite and hybrid varieties.

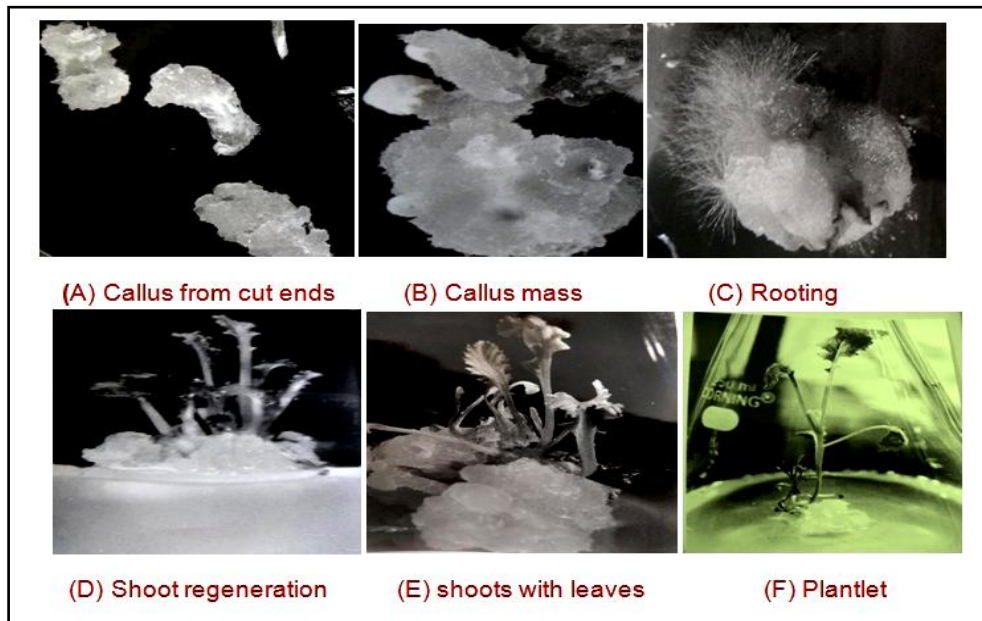


Fig.11.3: Plant regeneration by indirect organogenesis. / Courtesy Sunita Joshi

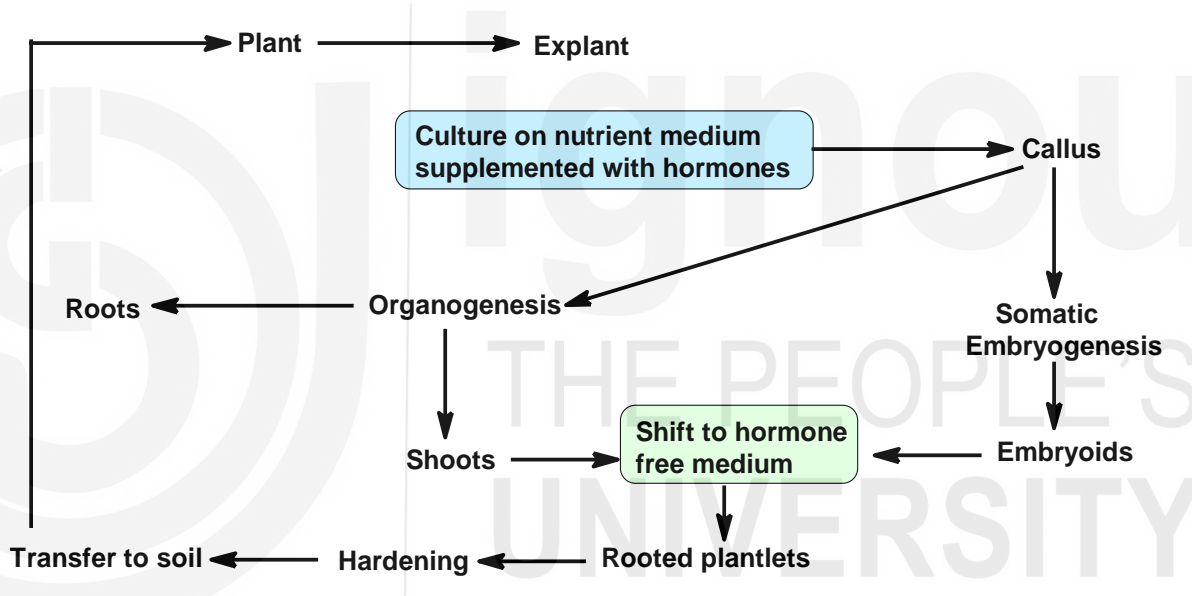


Fig. 11.4: An overview of plant regeneration pathways. (Adapted from Hussain *et al*, 2012 /Creative Commons Attribution License)

We end this section with a comparison of plant regeneration pathways (Table 2); some of the characteristics enlisted here are not mentioned in this section earlier.

Table 11.2: Comparison of plant regeneration pathways

Characteristics	Organogenesis	Somatic Embryogenesis
Origin	Group of cells	Single cell; usually superficial
Polarity	Unipolar (ex. shoot buds)	Bipolar (ex. somatic embryo); have both plumule and radicle.

Vascular connection with maternal tissue	Present	Absent
Separation from explant	Difficult	Easy
Viability	Very high (almost 100%)	Few may not germinate
Can be maintained for	Definite period	Indefinite period

SAQ 3

Indicate whether the following statements are true or false:

- Direct somatic embryogenesis may / may not involve an intervening callus phase.
- Organogenesis develops bipolar propagules (part of an organism used for propagation).
- A somatic embryo can be separated with ease from the maternal tissue.
- Unlike zygotic / seed embryos, somatic embryos may be unipolar or bipolar.

11.6 SOMACLONAL VARIATION

Somaclonal ('soma'= somatic cells; clonal = generations) variation refers to genetic variation encountered in the progeny of plants regenerated *in vitro* from somatic cells. This term was introduced by **P.J. Larkin and W.R. Scowcroft (1981)** for the novel source of variability arising in cell cultures. It occurs to varying extent in all plant regeneration systems that go via a callus phase for several cycles. The plants derived from such cells or progeny of such plants are called somaclones. Those generated directly from tissue culture are R₀ / R₁ (or SC1) plants and subsequent generations are numbered R₂, R₃ and so on.

The source of somaclonal variations includes both heritable and non heritable changes. The heritable changes are due to variations in chromosome number and / or structure, generally at high frequency. Selected variants arise randomly and often genetically unstable. The other changes are non hereditary epigenetic modifications (altered DNA methylation) as in 'bal variant' of *Arabidopsis thaliana* or mitotic crossing over that brings about temporary phenotypic changes, at low frequency. These variations can be detected by morphological (phenotypic) traits where possible or with cytological, biochemical and molecular tools.

Somaclonal variations are like a double edged sword; on one hand they ruin the genetic homogeneity (true-to-type clones) of valuable genetic stocks maintained *in vitro* and on the other hand they are source new variability which may be exploited in breeding programs for generating crop plants with

improved seed quality and / yield; increased production of secondary metabolites; resistance to biotic and abiotic stresses. Such variations are regularly screened using molecular tools. An example of somaclonal variation is flower colour in *Torenia* (Fig.11.5).

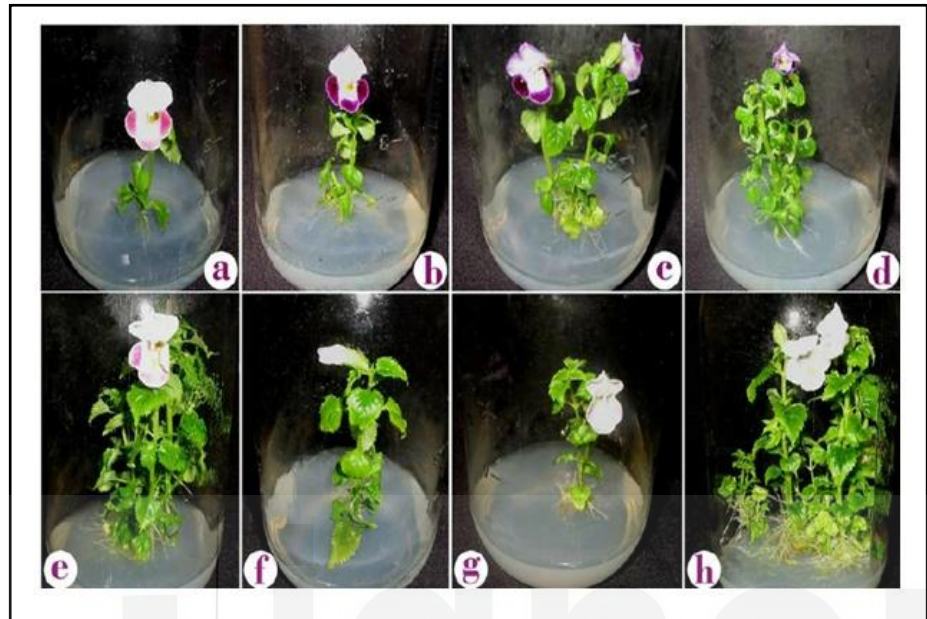


Fig. 11.5: Somaclonal variation in *Torenia* flower color (control: a and e)

Source: researchgate.net.

SAQ 4

Indicate whether the following statements are true or false:

- Somaclonal variation can be used for crop improvement.
- Somaclones are ideal for maintaining valuable genetic stocks.
- The term 'somaclonal variations' include all variation in a given species in natural populations.
- Somaclonal variations are always heritable.

11.7 APPLICATIONS OF CELL AND TISSUE CULTURE

Since the first successful culture of plant parts, tissue culture techniques are no longer restricted to research labs but finds application in almost every aspect of crop plant improvement, horticulture, forestry, conservation of plant species, large scale clonal multiplication and industrial production of metabolites. We shall now elaborate some of the applications of plant cell and tissue culture.

❖ Micropropagation

Many important plants in agriculture, forestry and horticulture are multiplied by vegetative propagation to obtain genetically homogenous (clones) material for

planting. Earlier this was done using cuttings, budding, grafting and other vegetative propagules. These methods apart from being labor intensive could be performed only at particular times of the year and have low productivity.

The tissue culture technique of clonal multiplication (micropropagation) is free from the above mentioned problems. The culture is initiated with apical shoots, axillary buds or meristem on suitable nutrient media. Next the shoots are multiplied and subsequently transferred to rooting medium. The tissue culture raised plants are hardened before transplantation to the green house / field.

Micropropagation is the technique of rapid clonal propagation of plants by tissue culture.

Plants propagated *in vitro* are smaller than *in vivo* plants. Micropropagation has been adopted for many plants such as banana, strawberry, pear, papaya; floricultural plants- Dahlia, Gerbera, Orchids, Carnations, Rose; trees- Poplar, Eucalyptus, Teak. The technique is also useful for seed production in certain crops.

❖ Meristem culture and virus free plants

Many crop plants are systemically infected with viruses that affect both quality and yield. In case of vegetatively propagated plants if the stock is infected all clonal descendants raised from it will also be infected. The trick to obtain virus free plants is based on the unequal distribution of viruses in infected plants. The apical or axillary meristems are generally virus free and culturing meristems (<1mm long) from infected plants is used to get virus free plants.

The excised meristem includes the apical dome and limited number of youngest leaf primordia. The plantlets produced are genetically stable as it does not go through callus formation and adventitious organogenesis (formation of adventitious shoots and roots). Somaclonal variants are also reduced. Remember these plants are not virus resistant. It has been successful with clonal plants like sugarcane, banana, potato and Dahlia.

❖ Embryo rescue

Attempts to produce inter-specific or inter-generic hybrids are often unsuccessful due to failure of pollen germination / pollen tube growth or abnormal development of the endosperm which results in loss of the hybrid embryo and produces sterile seeds. The embryo can be rescued by transferring it at an immature stage aseptically to a suitable nutrient medium. The novel hybrids can then be selected for desirable characteristics. Many times the plants may be haploids due to lack of pairing partners. This can be overcome by diploidisation.

❖ Plant improvement

The availability of tools for isolation of novel genes and their transfer to heterologous plants has led to the successful development of genetically

modified (GM) or transgenic crops with improved and new characteristics. The plants may become resistant to herbicides, pests and abiotic stresses; improved nutritional quality; free from undesirable toxic metabolites; etc. These genes are often linked to strong promoters and other cis elements that permit regulated expression in a tissue specific / only at certain stages of development.

❖ **Production of secondary metabolites**

Plants are known to produce thousands of secondary metabolites whose biological role we are just beginning to unravel (Refer to unit 10). From prehistoric times plant parts (leaves, bark) and extracts have been used against various diseases affecting mankind. These compounds also have wide ranging industrial applications. They are generally produced in plants in low amounts and therefore expensive. Many important secondary metabolites are now produced in large quantities in bioreactors using cell suspension (Berberine, Artemisinin, and Capsaicin) or hairy root (Resveratrol, Shikonin) culture techniques.

❖ **Haploids and triploids**

The technique of haploid production through anther and pollen culture allows us to get plants expressing recessive traits. Haploids can be easily diploidised using colchicine. These diploids are genetically homozygous.

The *in vitro* culture of endosperm (triploid tissue) offers an effortless method of producing triploid seedless fruits in Kiwi, Custard apple, Pear and lemon.

SAQ 5

Choose the best option:

- a) Which *in vitro* culture method is used for getting virus free plants?
- i) Meristem culture
 - ii) Embryo culture
 - iii) Leaf culture
 - iv) Anther culture
- b) Genetically modified (GM) crops are:
- i) Plants with genes from heterologous sources
 - ii) Not ecofriendly
 - iii) Spontaneously generated under lab conditions
 - iv) Haploid

- c) Virus free plants produced *in vitro* from virus infected plants are / acquire:
- Virus resistant
 - Virus sensitive
 - Genetically stable
 - Both (ii) and (iii)
- d) The tissue culture technique of clonal multiplication (micropropagation) is:
- Labor intensive
 - Undertaken only in spring
 - Initiated with root tips
 - Adopted for many trees & floricultural plants
-

11.8 SUMMARY

- Plant tissue culture is a technique for *in vitro* culturing and manipulating of plant cells and tissues on synthetically defined media under controlled physical conditions.
- It is widely used for large scale multiplication of plants; generation of disease resistant varieties, production of secondary metabolites, conservation of endangered species coupled with assured availability of plant material / products all the year round.
- Cultures fall into two basic types; unorganized (callus and suspension culture) and organized culture (root tip culture; embryo culture,). An organised culture is initiated with isolated organs and is named depending on the organ used for culturing. A culture initiated from plant parts (explants) is known as explant culture.
- A callus is an unorganized (dedifferentiated; totipotent) mass generally composed of unspecialized parenchymatous cells, on a solid media that can be induced from any plant part. Similar to animal cell culture, plants can also be used to produce suspension culture from a friable callus.
- Plant cells can be converted to protoplasts (devoid of cell wall) by mechanical or enzymatic methods. They are suitable for genetic transformation and generation of somatic hybrids. In a suitable medium the protoplasts undergoes cell division and may form a callus which can be shifted to a regeneration medium to obtain plantlets with novel characteristics.

- There are two basic pathways of plant regeneration, namely organogenesis and somatic embryogenesis. Organogenesis is the formation of organs such as shoots from cultured explants. In somatic embryogenesis, the somatic totipotent plant cells / tissues can be coerced to enter embryogenic pathway to form somatic embryos that may germinate *in situ* or when transferred to a fresh medium.
- Micropropagation is the technique of rapid clonal propagation of plants by tissue culture.
- Somatic (somaclonal) variations are always found to varying extent when plants are regenerated *in vitro* from callus. Though somaclonal variations ruin the homogeneity of genetic stocks, it is also a source of new variability.

10.9 TERMINAL QUESTIONS

1. Describe the basic steps of plant tissue culture.
2. What is a callus? How can it be induced in the lab? Give two applications of callus culture.
3. What is somaclonal variation? Indicate the limitations and applications of somaclonal variations.
4. Compare the following:
 - a) Organogenesis and somatic embryogenesis
 - b) Batch and continuous cultures.
5. Give any five applications of plant cell and tissue culture.

11.10 ANSWERS

Self Assessment Questions

1.
 - a) False
 - b) False
 - c) True
 - d) True
 - e) False
2.
 - a) i) ---- 1.; ii) --- 2.; iii) ---- 2.
 - b) Organ culture is the technique of culturing isolated organs under laboratory conditions. They are named depending on the organ used, for example, root culture, endosperm culture and ovary culture.

Explant culture: The culture of plant parts is known as explant culture such as a piece of stem or leaf.

3. a) False
b) False
c) True
d) False
4. a) True
b) False
c) False
d) False
5. a) i)
b) i)
c) iv)
d) iv)

Terminal Questions

1. Refer to Fig.11.1.
2. A callus is an unorganized (dedifferentiated; totipotent) mass generally composed of unspecialized parenchymatous cells, on a solid media. It can be induced from any plant part / organ. Generally relatively high auxins or a combination of auxins and cytokinins induces callus formation. Refer to subsection 11.4.3 for applications.

3. Somaclonal variation refers to genetic variation encountered in the progeny of plants regenerated *in vitro* from somatic cells.

Limitations: They ruin the genetic homogeneity of valuable genetic stocks maintained *in vitro*.

Applications: They are source new variability which may be exploited in breeding programs for generating crop plants with improved seed quality and / yield; increased production of secondary metabolites; etc.

4. (a) Refer to subsections 11.5.1 and 11.5.2
(b) Refer to subsection 11.4.4
5. Refer to section 11.7

11.11 FURTHER READINGS

1. Thorpe, T. History of plant tissue culture, *Mol. Biotechnol* (2007) 37: 169-180.
2. Glick, B.R, Pasternak, J.J and Patten, C.L, *Molecular Biotechnology: Principles and applications of recombinant DNA*, 4th Ed, ASM Press, Washington, DC.
3. Hussain, A; Qarshi, I.A; Nazir, H and Ullah, I. *Plant tissue culture: Current status and opportunities* (2012), Chapter 1, pg 1-28 This is an open access chapter distributed under the terms of the Creative Commons Attribution License
4. A textbook of biotechnology (CBSE, Class XII), Unit 6, Chapter II: Plant cell culture and applications and references therein.



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