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# Cellular Totipotency and Crop Improvement Utilizing Plant Tissue Culture Techniques

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**Summary**

Plant tissue culture has become popular among horticulturists, plant breeders and industrialists because of its varied practical applications. It is also being applied to study basic aspects of plant growth and development. The discovery of the first cytokinin (kinetin) is based on plant tissue culture research. An important contribution made through tissue culture is the revelation of the unique property of plant cells, called "cellular totipotency". The technique has developed around the concept that a cell has the capacity and ability to develop into a whole organism irrespective of their nature of differentiation and ploidy level. The principles involved in plant tissue culture are very simple and primarily an attempt, whereby an explant can be to some extent freed from inter-organ, inter-tissue and inter-cellular interactions and subjected to direct experimental control.

The earliest application of plant tissue culture was to rescue hybrid embryos, and the technique became a routine aid with plant breeders to raise rare hybrids, which normally failed due to post-zygotic sexual incompatibility. Currently, the most popular commercial application of plant tissue culture is in clonal propagation of disease-free plants. *In vitro* clonal propagation,

popularly called micropropagation, offers many advantages over the conventional methods of vegetative propagation: (1) the rate of multiplication *in vitro* is extremely rapid and can continue round the year, independent of the season. Thus, over a million plants can be produced in a year starting from a small piece of tissue. (2) The enhanced rate of multiplication can considerably reduce the period between the selection of plus trees and raising enough planting material for field trials. The other application of tissue culture is the production of haploid plants, which are extremely valuable in plant breeding and genetics. With haploids, homozygosity can be achieved in a single step otherwise obtaining homozygous lines of woody perennials by the conventional method of recurrent inbreeding is impractical and time consuming because of highly heterozygous nature and long generation cycle of these plants as well as inherent inbreeding depression. Even the triploid cells of endosperm are totipotent, which provides a direct and easy approach to regenerate triploid plants difficult to raise *in vivo*.

## INTRODUCTION

Plant tissue culture has become popular among horticulturists, plant breeders and industrialists because of its varied practical applications. It is also being applied to study basic aspects of plant growth and development. The discovery of the first cytokinin (kinetin) is based on plant tissue culture research. The earliest application of plant tissue culture was to rescue hybrid embryos, and the technique became a routine aid with plant breeders to raise rare hybrids, which normally failed due to post-zygotic sexual incompatibility. Currently, the most popular commercial application of plant tissue culture is in clonal propagation of disease-free plants. *In vitro* clonal propagation, popularly called micropropagation, offers many advantages over the conventional methods of vegetative propagation: (1) many species (*e.g*. palms, papaya) which are not amenable to *in vivo* vegetative propagation are being multiplied in tissue cultures, (2) the rate of multiplication *in vitro* is extremely rapid and can continue round the year, independent of the season. Thus, over a million plants can be produced in a year starting from a small piece of tissue. The enhanced rate of multiplication can considerably reduce the period between the selection of plus trees and raising enough planting material for field trials. In tissue culture, propagation occurs under pathogen and pest-free conditions.

An important contribution made through tissue culture is the revelation of the unique property of plant cells, called “cellular totipotency”. The totipotency of plant cells was predicted in 1902 by Haberlandt and the first true plant tissue culture on agar was established. Since then plant tissue culture techniques have greatly evolved. The technique has developed around the concept that a cell has the capacity and ability to develop into a whole organism irrespective of their nature of differentiation and ploidy level. Therefore, it forms the backbone of the modern approach to crop improvement by genetic engineering. The principles involved in plant tissue

culture are very simple and primarily an attempt, whereby an explant can be to some extent freed from inter-organ, inter-tissue and inter-cellular interactions and subjected to direct experimental control.

Regeneration of plants from cultured cells has many other applications. Plant regeneration from cultured cells is proving to be a rich source of genetic variability, called "somaclonal variation". Several somaclones have been processed into new cultivars. Regeneration of plants from microspore/pollen provides the most reliable and rapid method to produce haploids, which are extremely valuable in plant breeding and genetics. With haploids, homozygosity can be achieved in a single step, cutting down the breeding period to almost half. This is particularly important for highly heterozygous, long-generation tree species. Pollen raised plants also provide a unique opportunity to screen gametic variation at sporophytic level, This approach has enabled selection of several gametoclones, which could be developed into new cultivars. Even the triploid cells of endosperm are totipotent, which provides a direct and easy approach to regenerate triploid plants difficult to raise *in vivo.*

The entire plant tissue culture techniques can be largely divided into two categories based on to establish a particular objective in the plant species:

**I. Quantitative Improvement (Micropropagation)**

- Adventitious shoot proliferation (leaf-, root-, bulb-, corm-, seedling- explants etc.)
- Nodal segment culture
- Meristem/Shoot tip culture
- Somatic embryogenesis
- Callus culture

**II. Qualitative Improvement**

- Anther/ Microspore culture
- Ovary/ Ovule culture
- Endosperm culture
- Cell culture
- Protoplast culture

The above techniques are discussed in detail in subsequent chapters.

## MICROPROPAGATION

Growing any part of the plant (explants) such as cells, tissues and organs, in an artificial medium under controlled conditions (aseptic conditions) for obtaining large scale plant propagation is called micropropagation. The basic concept of micropropagation is the plasticity, totipotency, differentiation, dedifferentiation and redifferentiation, which provide the better understanding of the plant cell culture and regeneration. Plants, due to their long-life span, have the ability to withhold the extremes of conditions unlike animals. The plasticity allows plants to alter their metabolism, growth and development to best suit their environment. When plant cells and tissues are cultured *in vitro*, they generally exhibit a very high degree **of plasticity**, which allows one type of tissue or organ to be initiated from another type. Hence, whole plants can be subsequently regenerated and this regenerated whole plant has the capability to express the total genetic potential of the parent plant. This is unique feature of plant cells and is not seen in animals. Unlike animals, where differentiation is generally irreversible, in plants even highly mature and differentiated cells retain the ability to regress to a meristematic state as long as they have an intact membrane system and a viable nucleus. However, sieve tube elements and xylem elements do not divide any more

where the nuclei have started to disintegrate, According to Gautheret (1966) the degree of regression a cell can undergo would depend on the cytological and physiological state of the cell. The meristematic tissues are differentiated into simple or complex tissues called **differentiation**. Reversion of mature tissues into meristematic state leading to the formation of callus is called **dedifferentiation**. The ability of callus to develop into shoots or roots or embryoid is called **redifferentiation**. The inherent potentiality of a plant cell to give rise to entire plant and its capacity is often retained even after the cell has undergone final differentiation in the plant system is described as cellular **totipotency**.

**Micropropagation vs. Conventional Methods of Propagation**

All living plant cells, irrespective of their nature of specialization and ploidy level, have been shown to regenerate plants *via* organogenesis or embryogenesis. The latter involves a highly specialized mode of development that normally occurs only inside the seed, under the cover of several layers of parental tissues. Consequently, the observation of developing embryos and their isolation in intact and living conditions for experimental studies have been extremely difficult. *In vitro* production of embryos from somatic and gametic cells has opened up the possibility of obtaining large numbers of embryos of different stages, enabling investigations on cellular, genetic and physiological control of embryogenesis (induction, pattern formation, organ differentiation and maturation). *In vitro* expression of cellular totipotency and other techniques of plant tissue culture have also facilitated and/ or accelerated the traditional methods of plant improvement, propagation and conservation.

**Micropropagation vs. Vegetative Propagation**

The vegetative propagation has been conventionally used to raise genetically uniform large scale plants for thousands of years. However, this technique is applicable to only limited number of species. In contrast to this, micropropagation has several advantages which are summarized here:

i. The rapid multiplication of species difficult to multiply by conventional vegetative means. The technique permits the production of elite clones of selected plants.
ii. The technique is independent of seasonal and geographical constraints.
iii. It enable large numbers of plants to be brought to the market place in lesser time which results in faster return on the investment that went into the breeding work.
iv. To generate disease-free (particularly virus-free) parental plant stock.
v. To raise pure breeding lines by in vitro haploid and triploid plant development in lesser time.
vi. It can be utilized to raise new varieties and preservation of germplasm
vii. It offers constant production of secondary medicinal metabolites.

**Cell Differentiation**

During in-vitro and in vivo cytodifferentiation (cell differentiation), the main emphasis has been on vascular differentiation, especially tracheary elements (TEs). These can be easily observed by staining and can be scored in macerated preparations of the tissues. Tissue differentiation goes on in a

fixed manner and is the characteristic of the species and the organs

**Factors Affecting Vascular Tissue Differentiation**

Vascular differentiation is majorly affected qualitatively and quantitatively by two factors, auxin and sucrose. Cytokinins and gibberellins also play an important role in the process of xylogenesis.

Depending upon the characteristics of different species, concentration of phytohormones, sucrose and other salt level varies and accordingly it leads to the vascular tissue differentiation.

## MICROPROPAGATION TECHNIQUES

**Strategies for Propagation In Vitro**

Typical micropropagation systems can be broadly divided into five distinct stages (**Fig. 1**).

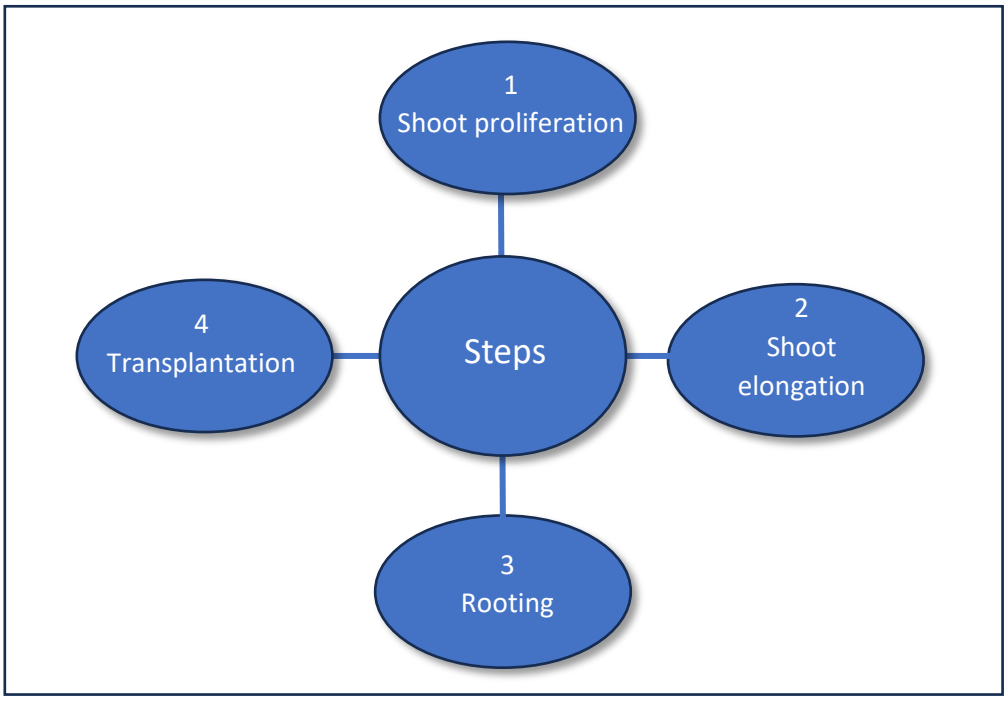


**Figure 1.** Micropropagation stages.

The stage zero is the selection of mother plant and preparation of explant.

The First stage is the initiation of a sterile culture of the explant in a particular enriched media for specific species. The second stage includes initiation of cell division from almost any part of the plant system to initiate regeneration or multiplication of shoots or other propagules from the explant. Adventitious shoot proliferation is the most frequently used multiplication technique in micropropagation systems. The culture media and growth conditions used in second stage need to be optimized for maximum rate of multiplication. The third stage is the development of roots on the shoots to produce plantlets. Specialized media may or may not be required to induce roots, depending upon the species. The final or the fourth stage is to produce self-sufficient plants. This stage usually involves a hardening-off process and acclimatization of plants in soil under green-house conditions for later transplanting to the field.

**Mode of Differentiation**

Regenerants may differentiate either directly from the explants or indirectly via callusing. Dedifferentiation favours unorganized cell growth and the resultant developed callus has meristems randomly distributed in the callus (**Fig. 2**). Most of these meristems, if provided appropriate in vitro conditions, would differentiate shoot-buds, roots or embryos.

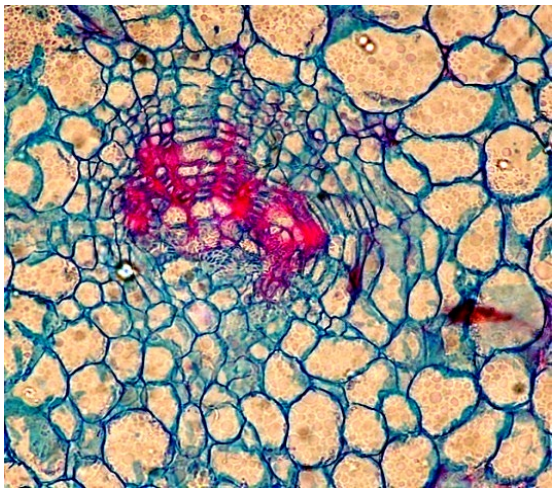

**Figure 2.** Meristem formation in callus. Thick tracheidal cells (stained red) are surrounded by cambium-like cells (stained blue).

**TROUBLE SHOOTING**

- Few explants exude dark colored compounds, like phenols, pigments etc which leach into the medium from the cut ends of the explant. It results in the browning of tissues and the medium as well. The browning of medium is associated with poor culture establishment and low regeneration capacity of the explants. This can be overcome by:
  - minimizing the wounding of explants during isolation and surface disinfection to reduce this browning response.
  - washing or incubation of explants for 3-5 hrs in sterile distilled water to remove phenolics responsible for browning of medium or explants.
  - frequent subculture of explants with excision to fresh medium at regular intervals.
  - initial establishment of cultures in liquid medium and later transfer to the semi-solid medium.
  - culture of explants on porous substrate or paper bridges.
  - addition of activated charcoal (AC) or polyvinylpyrrolidone (PVP) for adsorbtion of phenolics.
  - Antioxidants like ascorbic acid, citric acid etc. can also be used to prevent browning of tissues in culture.
- Appearance of vitrified tissues (hyperhydricity), a physiological disorder occurring in the in vitro cultures due to which the tissues look transparent and fluffy resulting from excessive intake of water. Hyperhydricity can be caused by a high concentration of cytokinin or low concentration of gelling agent or high water retention capacity of explants if the container is tightly closed.
- Loss of regeneration ability in long-term cultures due to epigenetic variations (temporary variations) and culture aging, including transition from juvenile to mature stage. Epigenetic variation are phenotypic temporary variations which disappear as soon as the culture conditions are removed.
- Genotypic variations are also seen in the cultures, therefore, cytological, biochemical and molecular analyses are required to confirm clonal fidelity of in vitro regenerants. Besides, morphological and physiological testing is also required to remove undesired genetic variability.

## ADVANTAGES OF PLANT TISSUE CULTURE OVER CONVENTIONAL PRODUCTION

The most important advantage of in vitro grown plants is that they are independent of geographical variations, seasonal variations and also environmental factors. It offers a defined production system, continuous supply of products with uniform quality and yield. Novel compounds which are not generally found in the parent plants can be produced in the in vitro grown plants through plant tissue culture. In addition, stereo- and regiospecific biotransformations of the plant cells can be performed for the production of bioactive compounds from economical precursors. It is also independent of any political interference. Efficient downstream recovery of products and rapidity of production are their added advantages (**Fig. 3**).

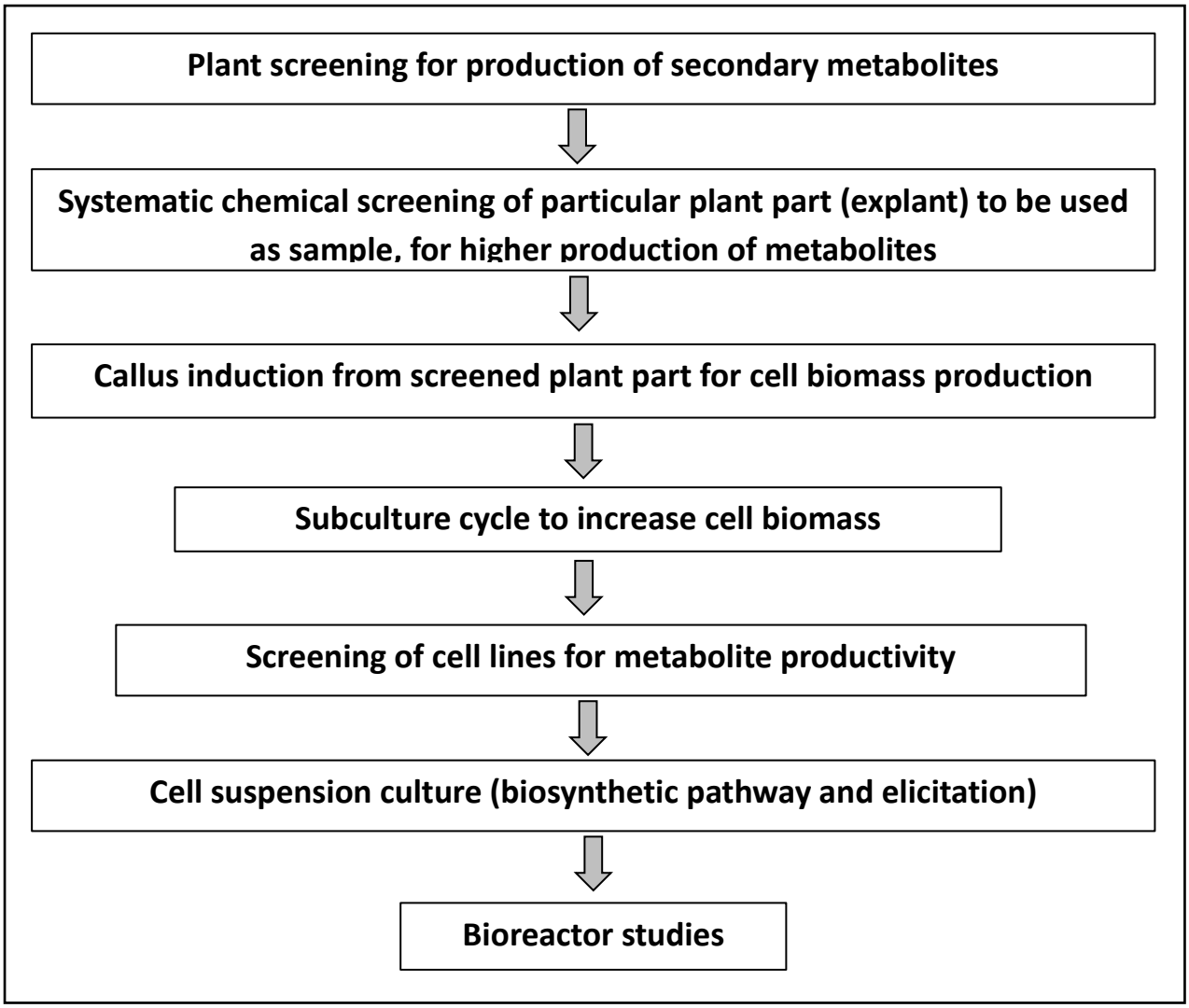


**Figure 3.** Steps involved in the production of secondary metabolites from plant cells.

## CONCLUSIONS

The plants are conventionally propagated by seeds. However, poor seed set and poor seed germination in its natural habitat poses limitations on plant multiplications. Moreover, propagation by seeds is undesirable in some plants because of their highly heterozygous nature due to strict cross pollination, which results in genetic variation within the population. This genetic variation may result into high heterogeneity in quality and quantity of chemical make-up of the plant.

Stem cuttings, though useful for propagation, are dependent upon weather conditions for proper growth. In order to produce large numbers of quality planting material and high value therapeutic compounds, a constant source of genetically uniform planting material is required, which could be utilized as a ready stock to meet the demand of the pharmaceutical industries. In this context, in vitro approaches have been proved handy in establishing true-to-type plants that are enriched in selected parental characters.