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Review Article

PLANT TISSUE CULTURE TECHNIQUES: A REVIEW FOR FUTURE VIEW

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ABSTRACT

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of totipotency and the roles of hormones in cytodifferentiation and organogenesis. Currently, tissue-cultured plants that have been genetically engineered provide insight into plant molecular biology and gene regulation. Plant tissue culture techniques are also central to innovative areas of applied plant science, including plant biotechnology and agriculture. For example, select plants can be cloned and cultured as suspended cells from which plant products can be harvested. In addition, the management of genetically engineered cells to form transgenic whole plants requires tissue culture procedures; tissue culture methods are also required in the formation of somatic haploid embryos from which homozygous plants can be generated. Thus, tissue culture techniques have been, and still are, prominent in academic and applied plant science.

Keywords: Biotechnology, Genetically Engineered Cells, Haploid Embryos Somatic, Tissue Culture, Totipotency.

INTRODUCTION

The term plant tissue culture (Micro propagation) is generally used for the aseptic culture of cells, tissues, organs and their components under defined chemical and physical conditions in vitro. The basic concept of the plant body can be dissected into smaller part termed as “explants” and any explants can be developed into a whole plant. It is a central innovative areas of applied plant science, including agriculture and plant biotechnology. When the plant cells and tissues are cultured in vitro, most of them exhibit a very high degree of plasticity, which allows one type of organ or tissue to be initiated from another type. In this way, the whole plant can be subsequently regenerated. The plant tissue culture medium is an artificial nutrient supplement of organic and inorganic nutrients used for cultivation of plant tissue media. The culture media used for the in vitro cultivation of the plant cells are composed of three basic components- Essential elements (normal ions), an organic supplements and a source of fixed carbon. When cultured in an appropriate medium having auxin and cytokinin, explants gives rise to an unorganized, growing and dividing mass of cells called callus. Callus cultures are initiated from a small part of an organ or tissue segment called the explants on a growth supporting solidified nutrient medium under sterile conditions. Any part of the plant organ or tissues can be used as the explants. Callus varies considerably in appearance and texture, ranging from hard nodular cell masses to friable soft ones. They maybe white or creamish, orange, green either in whole or part as a result of

chloroplast development. The shape of individual cells within the callus mass ranges from the near spherical or markedly elongated. The applications of plant tissue culture can be divided into following areas; Cell behaviour, plant modification, germplasm storage and pathogen –free plants, clonal propagation and product formation. The techniques demonstrated in these exercises range from simple ones that can easily beperformed by beginning students to those done by botany or physiology students. [1]

THEORY

The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt, German Academy of science in1902 on his experiments on the culture of single cell. The first true cultures were obtained by Gautheret from cambial tissue of *Acer pseudoplatanus*. The term plant tissue culture (Micro propagation) is generally used for the aseptic culture of cells, tissues, organs and their components under defined chemical and physical conditions in vitro. The basic concept of the plant body can be dissected into smaller part termed as "explants" and any explants can be developed into a whole plant. It is a central innovative area of applied plant science, including agriculture and plant biotechnology. This technique is effective because almost all the plants cell is totipotent. In each cell possesses the genetic information and cellular machinery necessary to generate the whole organism. Since, this technique can be used to produce a higher number of plants that are genetically similar to a parent plant as well as to another. [2]

Two concepts, plasticity and totipotency, are the central processes to understand the regeneration and plant cell culture. Plants, due to its longer life span and sessile nature, have developed a greater ability to overcome the extreme conditions. Most of the processes inculed in plant development and the growth, adapt to environmental conditions. When the plant cells and tissues are cultured in vitro, most of them are generally exhibit a very high degree of plasticity, which allows one type of organ or tissue to be initiated from another type. Like this way, the whole plant can be subsequently regenerated. This maintenance of genetic potential is called totipotency. [3]

The plant tissue culture medium is an artificial nutrient supplement of organic and inorganic nutrients used for cultivation of plant tissue media. The appropriate composition of the medium largely determines the success of the culture. The culture media used for the in vitro cultivation of the plant cells are composed of three basic components.

1. Essential elements (normal ions) supplied as a complex mixture of salts.
2. An organic supplements providing vitamins and amino acids.
3. A source of fixed carbon which is usually supplied as sucrose.

When cultured in an appropriate medium having auxin and cytokinin, explants will give rise to an unorganized, growing and dividing mass of cells called callus. Callus cultures are initiated from a small part of an organ or tissue segment called the explants on a growth supporting solidified nutrient medium under sterile conditions. Any part of the plant organ or tissues may be used as the explants. At the time of callus formation, there is some degree of dedifferentiation

happens both in morphology and metabolism. One of the major consequences of this dedifferentiation is that most plant cultures lose their ability to perform photosynthesis. The necessitates of the addition of other components such as carbon and vitamins source to the culture media, in addition to the unusual mineral nutrients.[4]

MORPHOLOGY OF CALLUS:

Callus varies considerably in appearance and texture, ranging from hard nodular cell masses to friable soft ones. They maybe white or creamish, orange, green either in whole or part as a result of chloroplast development. The shape of individual cells within the callus mass ranges from the near spherical or markedly elongated. A typical unorganized plant callus initiated from a new explants or piece of previously initiated calli has three stages of development.

1. The induction of cell division.
2. A period of active cell division during which differentiated cells lose specialized features they may have acquired and become de–differentiated. Cell division usually occurs in the outer layer of the explants.
3. Period when cell division slows down on ceases and when within the callus, there is increasing cellular differentiat

Callus culturing is performed in the dark while light can be encourage the differentiation of the callus. At the time of long term culture, the culture may loss the requirement for cytokinin and auxins. Manipulation of the auxins to cytokinin ratio in the medium can leads to the development of shoots, roots or somatic embryos from which the plant can be subsequently produced. [2,3]

Callus culture is useful for many purposes:

- Callus is the starting material for the suspension culture which cells are separated.
- It helps in the production of secondary plant products.
- It is useful for the synthesis of starting compounds that are subsequently modified to yield the desired product.
- It is the starting materials for vegetative propagation of plants.

Based on the availability of the various invitro techniques, the dramatic increase in their application to various problems in basic biology, agriculture, horticulture, and forestry. The applications can divide conveniently into five broad areas; [4,5]

- Cell behavior.
- Plant modification.
- Germplasm storage and pathogen free plants.
- Clonal propagation.
- Product formation.
- Improved varities.

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TECHNIQUES:

Modern plant tissue culture is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting material (explants) in chemical solutions (usually alcohol and sodium or calcium hypochlorite) is required. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar. [6] The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots. A balance of both auxin and cytokinin will often produce an unorganised growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and transferred to new media (subcultured) to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard. As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants. [7]

CHOICE OF EXPLANTS:

The tissue obtained from a plant to be cultured is called an explant. Based on work with certain plants, particularly tobacco, it has often been claimed that a totipotent explant can be taken from any part of a plant including portions of shoots, leaves, stems, flowers, roots and single, undifferentiated cells. However this is not true for all plants. In many species explants of various organs vary in their rates of growth and regeneration, while some do not grow at all. The choice of explant material also determines if the plantlets developed via tissue culture are haploid or diploid. Also the risk of microbial contamination is increased with inappropriate explants. [8]

The first method involving the meristems and induction of multiple shoots is the preferred method for the micropropagation industry since the risks of somaclonal variation (genetic variation induced in tissue culture) are minimal when compared to the other two methods. Somatic embryogenesis is a method that has the potential to be several times higher in multiplication rates and is amenable to handling in liquid culture systems like bioreactors.

Some explants, like the root tip, are hard to isolate and are contaminated with soil microflora that become problematic during the tissue culture process. Certain soil microflora can form tight associations with the root systems, or even grow within the root. Soil particles bound to roots are difficult to remove without injury to the roots that then allows microbial attack. These associated microflora will generally overgrow the tissue culture medium before there is significant growth of plant tissue. [9]

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Some cultured tissues are slow in their growth. For them there would be two options: (i) Optimizing the culture medium; (ii) Culturing highly responsive tissues or varieties. Necrosis can spoil cultured tissues. Generally, plant varieties differ in susceptibility to tissue culture necrosis. Thus, by culturing highly responsive varieties (or tissues) it can be managed. [10]

PROCEDURE: [7,8,9,10]

1. Wipe down and turn on the laminar air flow 15 minute before doing work in the hood. Flames sterilize the instruments.
2. Cut the carrot root into 3-6 cm long, discarding both ends of the root. Remove the epidermis and any blemishes with scalpel.
3. Put the tap root section in to a sterile jar having chlorate bleach solution (approximately 1.4% available chlorine) and shake it for few seconds.
4. Remove the bleach solution into the waste beaker.
5. Cut 1cm of the carrot root section from each end and discard this end portions.
6. Cut 3-5 transverse section (1-5mm thick) across the tap root and transfer each to a fresh sterile Petri dish.
7. Cut the smaller sections, explants (approximately 5mm square) from each of the transverse sections by cutting across the cambium. The following method is recommended.
   a. Trim the cortex and some of the phloem from each transverse section
   b. Cut off 5mm – wide strips containing the cambium
   c. Each strip can then be subdivided to produce 5mm square explants, each containing parts of the phloem, xylem and cambium.
8. Measure the weight of the explants.
9. Put each explants sections into culture tubes containing the carrot callus initiation medium (one explants per tube).
10. Seal all the tubes with parafilm to reduce dehydration of the medium.
11. Incubate the culture tubes in the dark at 25°C. Examine at weekly intervals and record the changes observed.
12. Callus formed is removed from the primary explants after 45 days and it is weighed.
13. The calli is subculture into the same medium for further callus growth or to the carrot shoot / root initiation medium.
14. Seal all the tubes having calli with parafilm to reduce dehydration of the medium.
15. Incubate all the tubes in the dark at 25 °C.
16. Measuring the efficacy of roots and shoots.

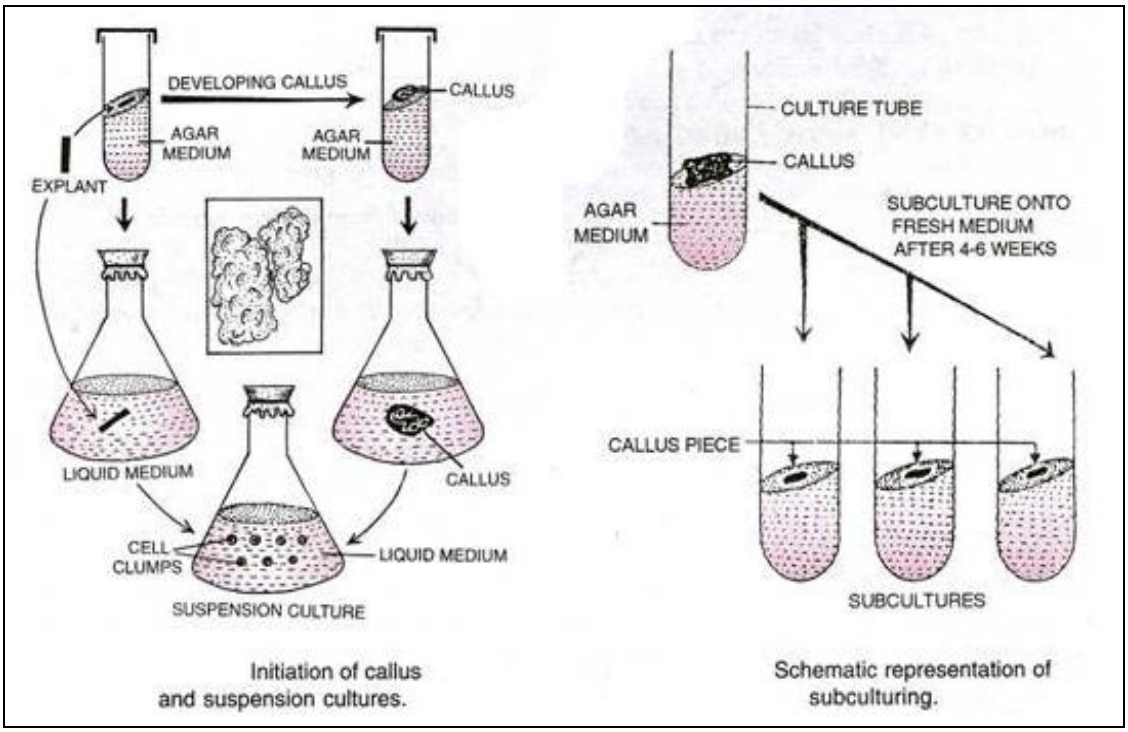

Fig. 1 Diagrammatic representation of tissue culture technique.

Difference Encountered in a Real Laboratory [11]

In an actual laboratory setting, there are certain important steps that are not necessarily applicable in a virtual lab:

- Properly adjust the flame of the Bunsen burner. The proper flame is a small blue cone; it is not a large plume, nor is it orange.
- Always label the tubes and plates with:
  - ✓ The type of media
  - ✓ Your initials
  - ✓ The date and time
- Seal the tissue culture container to keep it sterile.
- Always disinfect your work area when you are finished.

APPLICATIONS OF PLANT TISSUE CULTURE: [12,13]

The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.

- To conserve rare or endangered plant species.

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- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants, and in vitro flowering studies.
- To cross-pollinate distantly related species and then tissue culture the resulting embryo this would otherwise normally die (Embryo Rescue).
- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.

Alternative avenues for plant products have gained prominence during the past few years and the production of secondary metabolites using plant cells has been the subject of extended research. Hence, plant biotechnology has a major role to play in plant based industries. (Table no. 1) [13,14,15]

Table no. 1: Bioactive Secondary metabolites from Plant Tissue Culture

Secondary metabolite	Plant	Culture
Baccatin–III, Deacetyl baccatin-III	*Taxus wallichiana, T. Canadensis*	callus, cell suspension
Camptothecin	*Nothapodytes foetida*	callus, cell suspension
Forskolin	*Coleus forskohlii*	hairy root
Hypercin, Pseudohypercin	*Hypericum perforatum*	shoot cultures
Ajmalicine and Serpentine	*Catharanthus roseus*	hairy root

SUMMERY:

Several reports are demonstrating the production of wide array of secondary metabolites of plants and some being produced on commercial scale. Tissue culture protocols have been developed for several plants but there are many other species, which are over exploited in pharmaceutical industries and other fields and do need conservation. Tissue culture technique is useful not only for production of phytoconstituents but also for multiplying and conserving the species, which are difficult to regenerate by conventional methods and save them from extinction. Still the word "potential" is used in connection with tissue culture technology. Hence, there is much scope to develop and bring it to commercial scale for the benefit of human welfare and to preserve biodiversity.

Research on tissue culture of trees was initiated in Late 1970s with emphasis on Teak and Eucalyptus for which protocols were developed. The institutes under Indian Council of Forestry Research and Education (ICFRE) have taken up a number of species for mass multiplication through tissue culture, to produce adequate number of good quality planting stock. Good numbers of studies were also taken on mass multiplication of different Bamboos, including edible Bamboos, and several thousands of plantlets have been transferred to the field. The technique is used in conjunction with selection strategies so that the material produced is of high quality. Also, the plant tissue culture technique such as hairy root culture can lead to secondary metabolite production in short period.

Plant *in vitro* techniques, in which plant cells, tissues and organs are cultivated under aseptic conditions totally independent of geographical and climatic factors, offers alternatives for producing important metabolites, and several technologies based on them have been developed. Plant tissue culture technology could be a potential alternative approach for bio production of phytoconstituents of therapeutic value and might be attractive under certain conditions if, for example, the source plant is difficult to cultivate, has a long cultivation period or has a low metabolite yield; if chemical synthesis has not been achieved or if it is technically problematic.

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