

Plant Tissue Culture: A Manmade Manipulation of the Green World

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INTRODUCTION

Recent advances in the techniques and application of plant cell culture have created unprecedented opportunities for the genetic manipulation of plants. The potential impact of these novel and powerful techniques on the genetic improvement of crop plants has generated considerable interest, enthusiasm, and optimism in the scientific community. It is in part responsible for the rapidly expanding biotechnology industry (Vasil, 1985). Furthermore, plant tissue culture techniques have become a powerful tool for studying basic and applied problems in plant biology. Our intention is to draw the attention of school children to some physiological aspects underlying tissue culture techniques that affect the condition of explants and their proliferation and their re-organization into regenerated plantlets. We have included at the end of this paper a glossary of technical terms commonly used in plant tissue culture, for easy reference.

HISTORY OF PLANT TISSUE CULTURE

The regeneration of entire plants from detached parts is an ancient horticultural technique practiced through the ages. Although the idea of tissue culture emerged before the turn of the 20th century, the theoretical concept for tissue culture (discovery of callus formation) was conceived only in 1902 by the German botanist Gottlieb Haberlandt. New approaches to tissue culture were discovered by R.J. Gautheret (auxin utilization), A.J. Riker (success of callus culture), Albert Blakeslee (activity of coconut water), Folk Skoog (discovery of Cytokinins), Vasil and Hildebrandt (demonstration of totipotency in cell culture), and Guha and Maheswari (discovery of androgenesis).

TISSUE CULTURE AS A TOOL

The present uses and potential uses of tissue culture may be broadly categorized into six:

1. Rapid or large-scale plant propagation of existing clonal material.
 - Clonal micropropagation by using shoot tips, axillary buds, or meristem culture.

* Vasil, I.K. 1985. Cell culture and somatic cell genetics of plants. *Cell Growth, Nutrition, Cytodifferentiation and Cryopreservation*, Vol.2, p.330. Harcourt Brace Jovanovich, Academic Press Inc.

- *In vitro* micrografting.
2. Rapid or large-scale plant manipulation.
 - Adventitious bud proliferation.
 - Plant regeneration through callus culture.
 - Somatic embryogenesis through suspension culture.
 - Artificial seed production.
 3. Uses of increasing the genetic diversity.
 - Interspecific and intergeneric hybridization:
 - *in vitro* fertilization (pregametic incompatibility), and
 - *in vitro* embryo culture (postgametic incompatibility).
 - Production of haploid and homozygotic plants:
 - anther and pollen culture, and
 - unpollinated ovary culture/ovule culture.
 - Cell culture and somoclonal variation:
 - protoplast fusion and transfer of foreign DNA by genetic engineering methods, and
 - mutants and *in vitro* selection.
 4. Pathogen-free plant production.
 - Obtaining virus-free plants by using meristem culture or thermotherapy.
 - Implementation of plant quarantine and international shipment of *in vitro* plants.
 5. Cell culture for the production of useful secondary metabolites.

6. Germplasm storage.

- Cryopreservation of cultured cells and meristems (-196°C).
- Cold storage of plant material (5 to 9°C).

BASIC REQUIREMENTS IN TISSUE CULTURE

If relevant facts support the use of a tissue culture technique in attaining a certain objective, it is then necessary to follow a sequence of steps to conduct the process.

Establishment of Aseptic Culture

Explant Material

The following criteria should be considered in selecting a suitable explant:

- the organ that is to serve as tissue source,
- the physiological age of the organ,
- the season in which the explant is obtained,
- the size of the explant, and
- the overall quality of the plant from which explants are to be taken.

Virtually every organ/tissue can be used as a source of explant, but the degree of success can vary with each such source. Therefore, in developing a procedure for commercial use, the explants should be selected systematically. Sections of sterile seedlings, buds, stem or storage organs, leaf material, and embryos could be used as explants.

Sterilization

In a plant tissue culture all explant material, media and instruments should be free of contaminants. All activities must be carried out under aseptic conditions. This means that the cultures must be free of bacterial, algal or fungal contaminants. Contamination by such microorganisms is a problem in tissue culture because the media used to support higher plant cell growth also support the growth of these microorganisms. If their growth is not prevented, they may overgrow the plant tissues, inhibit their development, and interfere with the physiology and biochemistry of the system. Therefore, all nutrient media, culture vessels and instruments used in handling the plant material must be sterilized.

In tissue culture, all plant material should be regarded as contaminated because they harbour microorganisms. Plant material can be surface sterilized by a variety of sterilants. The type and concentration of sterilant to be used as well as the exposure time must be decided accordingly. Some of the sterilants that could be used are mercuric chloride, calcium or sodium hypochlorite, and silver nitrate. Some commonly used sterilants in plant tissue culture are given in Table 1.

Table 1. Commonly used disinfectants in plant tissue culture.

Disinfectant	Concentration (%)	Exposure time (minutes)
Calcium hypochlorite	9.0-10.0	5.0-30.0
Sodium hypochlorite	0.5-5.0	5.0-30.0
Hydrogen peroxide	3.0-12.0	5.0-30.0
Ethyl alcohol	70.0-95.0	5.0-15.0
Silver nitrate	5.0	0.1-5.0
Mercuric chloride	0.01-1.0	2.0-10.0
Benzalkonium chloride	0.01-0.1	5.0-20.0

Normally, the microflora is restricted to the epidermis of the plant. But there will be instances where the internal tissues harbour organisms and nothing can be done other than to culture the tissues on a broad spectrum of antibiotics. Instruments are generally sterilized by flaming after dipping in 70 percent ethyl alcohol. Culture vessels and nutrient media are autoclaved at a temperature of 121⁰C and a pressure of 1.06 Kg/cm². There are some growth factors such as zeatin, abscisic acid and certain vitamins which are heat-sensitive and which cannot be autoclaved with the rest of the nutrient medium. In such instances, the technique of filter sterilization is applied.

All aseptic manipulations are carried out within a laminar flow hood where a stream of sterile air is flowing. When a large number of cultures must be routinely handled, a walk-in transfer room can be useful.

Culture Medium

Plant cell culture media generally comprise a mixture of inorganic salts consisting of essential elements, organic substances, and vitamins in an aqueous solution. The success in the technology and the application of the *in vitro* method is largely due to the better understanding of the nutritional requirements of cultured cells and tissues.

Inorganic salts. Nitrogen (N), phosphorus (P), calcium (Ca), sulphur (S) and magnesium (Mg) are required in millimolar quantities. The optimum concentration of each nutrient for achieving the maximum growth rate varies considerably. The essential nutrients required in micromolar concentrations include iron (Fe), zinc (Zn), manganese (Mn), boron (B), copper (Cu), and molybdenum (Mo).

Carbon and energy source. The standard carbon source used is sucrose or glucose.

Vitamins. Normal plants synthesize the vitamins required for growth and development. When cells of higher plants are grown in culture, some vitamins may become limited. Among the vitamins required are thiamines (vitamin B₁), pyridoxine (vitamin B₆), biotin (vitamin H), and riboflavin (vitamin B₂). The pH of the medium is adjusted to range from 5.6 to 5.8 before it is autoclaved. This pH range has been reported as the optimum for plant cell growth.

Phytohormones. Cytokinins and auxins are two classes of compounds which are of special importance in plant tissue culture.

Environmental Factors

The growth rate is closely related to the prevailing temperature. A temperature between 23 to 25⁰C is considered optimum for achieving maximum growth. Plant cells in culture are aerobic. Aeration is an essential requirement for suspension cultures. Therefore, rotary shakers are operated at rates which vary from 100 to 150 rpm.

Generally, light is provided by white fluorescent lamps at intensities of 300 to 10,000 lux at the culture level. Light may be given continuously or in a photoperiod (usually for 8 to 16 hours).

Transplanting of *In Vitro* Material to Soil

A critical stage of *in vitro* developed plant is the transfer of the plant to the soil. This step is often unsuccessful. It is important, therefore, to gradually prepare the plants for their new environment. Some essential points concerning these preparations are:

- There should be a reasonable balance in growth between roots and shoots prior to transplanting.
- The plants must undergo gradual transition (over 2 to 3 weeks) from a constantly high humidity regime to one of varying and low humidity in the nursery or the field, by misting in a greenhouse, or by other methods.
- All traces of agar should be removed as agar provides a substrate for pathogen growth.
- Some shading is necessary to prevent leaf burn during the transition to full sunlight. Containers in which plants are rooted and grown must also be carefully selected.

If the plants die after all these precautions, as they do in some cases, it is because the vascular connection between the shoot and the roots has not been completed.

Tissue culture techniques have been widely used in commercial plant production in the last 10 years. The potential of these techniques for plant improvement is accepted worldwide as can be seen in the growing research interest in these techniques. Several tissue culture laboratories are found in Sri Lanka's universities and public- and private-sector organizations. The following organizations in Sri Lanka have tissue culture laboratories:

1. The Agricultural Research Station, Bandarawela
2. The Central Agricultural Research Institute, Gannoruwa
3. The Ceylon Institute of Scientific and Industrial Research Institute, Colombo
4. The Ceylon Tobacco Company Limited, Kalagedihena
5. The Coconut Research Institute, Lunuwila
6. The Institute of Fundamental Studies, Kandy
7. The Plant Genetic Resources Centre, Gannoruwa
8. The Rubber Research Institute, Agalawatta
9. Research Center for Export Crops, Matale
10. The Sugarcane Research Institute, Udawalawe
11. The Tea Research Institute, Talawakale
12. The University of Colombo, Colombo
13. The University of Peradeniya, Peradeniya
14. The University of Ruhuna, Kamburupitiya
15. The University of Sri Jayawardenapura, Nugegoda

CONCLUSION

It may be said that Sri Lanka is at a stage when the benefits of tissue culture techniques are beginning to have some effect on both theoretical and applied research. However, we must remember that this technology has its limitations. Genetic instability has been a problem associated with the micropropagation industry. Initial cost of setting up tissue culture laboratories and the use of expensive chemicals are other draw backs. Another difficulty is that when perennials are propagated by this method, we are unable to predict the output for several years. Therefore, before any research on tissue culture is initiated or any tissue culture products are introduced, systematic planning covering all aspects is essential in order to achieve the final goals.

GLOSSARY OF COMMONLY USED TERMS IN TISSUE CULTURE

ADVENTITIOUS. Developing from usual points of origin, such as shoots or root tissues; developing from the callus or embryos; and developing from sources other than zygotes.

ANDROGENESIS. Development of plants from male gametophytes.

ASEPTIC CONDITIONS. Absence of fungi, bacteria, viruses, mycoplasma, or other microorganisms in cultures.

CALLUS. An unorganized proliferative mass of undifferentiated plant cells; a wound response.

CLONAL PROPAGATION. Asexual reproduction of plants that are considered to be genetically uniform and that have originated from an individual plant or an explant.

EMBRYO CULTURE. *In vitro* development or maintenance of isolated embryos that may be either mature or immature.

EMBRYOGENESIS. The process of embryo indication and development.

EXPLANT. Tissue taken from its original site and transferred to an artificial medium for growth or maintenance.

EXPLANT CULTURE. The maintenance or growth of an explant in culture.

IN VITRO. Literally means 'in glass.' This term is now applied to any process carried out in sterile cultures.

IN VITRO PROPAGATION. Propagation of plants in a controlled and artificial environment, using plastic or glass culture vessels, aseptic techniques, and a defined growing medium.

JUVENILE. A condition of a plant before it has reached the ability to flower. A phase in the sexual cycle of a plant characterized by differences in appearance from the adult and which lacks the ability to respond to flower-inducing stimuli.

MERISTEM CULTURE. *In vitro* culture of a generally shiny dome-like structure measuring less than 0.1 mm in length when excised (i.e., cut, often from the shoot apex).

MICROPROPAGATION. This term is synonymous with *in vitro* propagation.

Stage I. A step in *in vitro* propagation characterized by the establishment of an aseptic tissue culture of a plant cell or tissue.

Stage II. A step in *in vitro* plant propagation characterized by the rapid numerical increase of organs or other structures.

Stage III. A step in *in vitro* plant propagation characterized by propagation of propagule, involving rooting of shoot cuttings, hardening of plants, and initiating the change from the heterotrophic to the autotrophic state.

Stage IV. A step in *in vitro* plant propagation characterized by the establishment in soil of a plant grown by tissue culture, either after undergoing a Stage III pretransplant treatment or, in certain species, after the direct transfer of plants from Stage II into soil.

MORPHOGENESIS. 1).The process of growth and development of differentiated structures. 2). The evolution of a structure from an undifferentiated to a differentiated state.

MUTANT. A phenotype variant resulting from a changed or new gene.

ORGANOGENESIS. The evolution from dissociated cells of a structure which shows natural organ form or function or both.

PATHOGEN FREE. Free from specific organisms based on specific test for the designated organisms.

PRIMARY CULTURE. A culture started from cells, tissues or organs taken directly from organisms. A primary culture may be regarded as such until it is successfully subcultured for the first time. It then becomes a 'cell line.'

PROTOPLAST. A plant cell without its outer retaining cell wall.

PROTOPLAST FUSION. Techniques in which protoplast are fused with a single cell.

SHOOT APICAL MERISTEM. A group of dividing and undifferentiated cells, located within the shoot tip, generally appearing as a shiny dome-like structure distal to the youngest leaf primordium, and measuring less than 0.1 mm in length when excised.

SHOOT TIP (APEX) CULTURE. A structure consisting of the shoot apical meristem plus one to several primordial leaves, usually measuring from 0.1 to 1.0 mm in length; in instances where more mature leaves are included, the structure can measure up to several centimeters in length.

SOMATIC CELL. A vegetatively functioning cell which does not take part in sexual reproduction.

SOMATIC CELL HYBRID. The cell or plant resulting from the fusion of animal cells or plant protoplast respectively, derived from somatic cells which differ genetically.

SOMATIC CELL HYBRIDIZATION. The *in vitro* fusion of animal cells or plant protoplasts derived from somatic cells which are different genetically.

SUBCULTURE. In plant cultures, this is the process by which the tissue or explant is first subdivided and then transferred into fresh culture medium.

SUSPENSION CULTURE. A type of culture in which cells or aggregates of cells multiply suspended in liquid medium.

TISSUE CULTURE. A general term that encompasses (and is concerned with) the study of cells, tissues, and organs maintained or grown *in vitro* for more than 24 hours.

TOTIPOTENCY. A cell characteristic in which the potential for forming all the cell types in the adult organism is retained.

VEGETATIVE PROPAGATION. The reproduction of plants by a nonsexual process involving the culture of vegetative plant parts such as stem and leaf cuttings.