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Brief Notes on Cellular culture of plants

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Abstract

The science or art of growing plant cells, tissues or organs isolated from the mother plant on artificial media is known as plant tissue culture. Plant tissue culture refers to growing and multiplication of cells, tissues and organs of plants on defined solid or liquid media under aseptic and controlled environment. The commercial technology is primarily based on micro propagation, in which rapid proliferation is achieved from in system cuttings, axillary buds, and to a limited extent from somatic embryos, cell clumps in suspension cultures and bioreactors. The merits of plant tissue culture in crop production is that it helps in disease elimination, quick international distribution of genetic resources, germplasm conservation and reduction in quarantine requirements, time and space of regeneration. This paper looks at the materials for in vitro plant tissue culture, different methods of plant tissue culture, media preparations, protocols of hygiene and sterilization in plant tissue, culture and techniques in plant tissue culture for crop improvement.

Keywords: Plant tissue; Culture; Propagation of plant tissues; Endangered and important plant species

Introduction

A whole plant can be regenerated from a small tissue or plant cells in a suitable culture medium under controlled environment. The plantlets so produced are called tissue-culture raised plants. Plant tissue culture is the technique of growing plant cells, Tissue and organism the artificial prepared nutrient medium static or liquid under aseptic conditions." Tissue culture plants are characterized by disease free growth, a more fibrous, healthier root system, a bushier branching habit, and a higher survival rate. Plant tissue culture technology is being widely used for large scale plant multiplication. Apart [1] from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis. Endangered, threatened and rare species have successfully been grown and conserved by micro propagation because of high coefficient of multiplication and small demands on number of initial plants and space.

History of Plant Tissue Culture

The science of plant tissue culture takes its roots from the discovery of cell followed by propounding of cell theory. In 1838, Schleiden and Schwann proposed that cell is the basic structural unit of all living organisms

1902 - Haberlandt proposed concept of *in vitro* cell culture

1904 - Hannig cultured embryos from several cruciferous species

1922 - Kolte and Robbins successfully cultured root and stem tips respectively

1926 - Went discovered first plant growth hormone –Indole acetic acid

1934 - White introduced vitamin B as growth supplement in tissue culture media for tomato root tip

1939 - Gautheret, White and Nobecourt established endless proliferation of callus cultures

1941 - Overbeek was first to add coconut milk for cell division in *Datura*

1946 - Ball raised whole plants of *Lupinus* by shoot tip culture

1954 - Muir was first to break callus tissues into single cells

1955 - Skoog and Miller discovered kinetin as cell division hormone

1957 - Skoog and Miller gave concept of hormonal control (auxin: cytokinin) of organ formation

1959 - Reinert and Steward regenerated embryos from callus clumps and cell suspension of carrot (*Daucus carota)*

1960 - Cocking was first to isolate protoplast by enzymatic degradation of cell wall

1960 - Bergmann filtered cell suspension and isolated single cells by plating

1960 - Kanta and Maheshwari developed test tube fertilization technique

1962 - Murashige and Skoog developed MS medium with higher salt concentration

1964 - Guha and Maheshwari produced first haploid plants from pollen grains of

Datura (Anther culture)

1966 - Steward demonstrated tot potency by regenerating carrot

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plants from single cells of tomato

1970 - Power et al. successfully achieved protoplast fusion

1971 - Takebe et al. regenerated first plants from protoplasts

1972 - Carlson produced first interspecific hybrid of *Nicotiana tabacum* by protoplast fusion

1974 - Reinhardintroduced biotransformation in plant tissue cultures

1977 - Chilton et al. successfully integrated Ti plasmid DNA from *Agrobacterium tumefaciens* in plants

1978- Melchers et al. carried out somatic hybridization of tomato and potato resulting in pomato

1981- Larkin and Scowcroft introduced the term somaclonal variation

1983 - Pelletier et al. conducted intergeneric cytoplasmic hybridization in Radish and Grape

1984 - Horsh et al. developed transgenic tobacco by transformation with *Agrobacterium*

Basics of Plant Cell and Tissue Culture

In plant cell, culture, plant tissues and organs are grown *in vitro* on artificial media, under aspect and controlled environment. The technique depends mainly on the concept of tot potentiality of plant cells which refers to the ability of a single cell to express the full genome by cell division. Along with the totipotent potential of plant cell, the capacity of cells to alter their metabolism, growth and development is also equally important and crucial to regenerate the entire plant. Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon source and some gelling agents in case of solid medium. Murashige and Skoog medium (MS medium) is most [1] extensively used for the vegetative propagation of many plant species *in vitro*. The pH of the media is also important that affects both the growth of plants and activity of plant growth regulators. It is adjusted to the value between 5.4 - 5.8. Both the solid and liquid medium can be used for culturing. The composition of the medium, particularly the plant hormones and the nitrogen source has profound effects on the response of the initial explants. Plant growth regulators (PGR's) play an essential role in determining the development pathway of plant cells and tissues in culture medium. The auxins, cytokinins and gibberellins are most commonly used plant growth regulators. The type and the [2] concentration of hormones used depend mainly on the species of the plant, the tissue or organ cultured and the objective of the experiment. Auxins and cytokinins are most widely used plant growth regulators in plant tissue, culture and their amount determined the type of culture established or regenerated. The high concentration of auxins generally favors root formation, whereas the high concentration of Figure 1 cytokinins promotes shoot regeneration. A balance of both auxin and cytokine leads to the development of mass of undifferentiated cells known as callus. Maximum root induction and proliferation was found in *Stevia rebaudiana*, the medium is supplemented with 0.5 mg/l NAA. Cytokinins generally promote cell division and induce shoot formation and axillary shoot proliferation. High cytokinin to auxin ratio promotes shoot proliferation while high auxin to cytokinins ratio results in root formation. Shoot initiation and proliferation was found maximum, when the callus of black pepper was shifted to medium supplemented with BA at the concentration of 0.5 mg/l.

Figure 1: Roots are fully developed prior to moving plants to pots of soil.

Importance

A single explants can be multiple into several plants. Plant cultures in approved media are easier to export than soil- grown plants.

Tissue culture allows fast selection for crop improvement. Virus free [3] ex plant scan be frozen. Tissue culture clones are true or type as compared with seedlings. Tissue culture allows fast selection for crop improvement. Virus free ex plant scan be frozen.

Tissue culture clones are true or type as compared with seedlings.

Fundamental Principles

Totipotency

It is the Ability of plant cells to regenerate into a whole plant.

Plasticity

It is the ability of plants to alter their metabolism; growth and development to best suit their environment.

Growing the plants

The tubes containing plant sections may be placed in a well-lit area of the classroom although not in direct sunlight. The shoots will probably grow more quickly if the explants are placed under fluorescent or grow lights to provide at least 12 hours of light per day. The aquarium can be used as a growth chamber with the lighting about 8-10" overhead. This will also help maintain a more regular and warm temperature. Ensure that the temperature does not goover 28o C. New shoots should develop within 2 weeks, and should be well a vanced in 3 to 4weeks. Check the tubes daily and discard any that show signs of infection (before discarding first sterilize in the pressure cooker or add bleach into the tube).

- Roots can appear within 6 weeks on cauliflowers. The roses, African violet and other cuttings will need to be moved into rooting media for roots to properly develop. This transfer [4] to the second, rooting media must be conducted under the same sterile conditions as at the initiation of the culture. All necessary equipment and the aquarium should be set up as before and properly sterilized.

- Working inside the sterile aquarium chamber, remove the cap from the culture tube.

There will usually be several shoots that have arisen from each explant. These shoots should be carefully separated by gently removing the whole explant from the media with sterile forceps and then

separating the shoots by gently pulling them apart using two pairs of forceps. Each shoot should then be placed into a tube of rooting media and the bottom of the shoot pushed into the media so that good contact is made. The cap is replaced and the shoots are then allowed to grow as in step 1 until roots are formed, usually within 2-3 weeks.

Potting the clones:

Once roots are well formed the plants are ready to be transferred into soil

- Each plant should be carefully removed from its tube of media and planted into a smallpot containing a clean light potting mix. Gently wash off the entire agar medium prior toplanting. The plants will still need to be protected at this stage since they are not accustomed to the drier air of the classroom when compared to the moist environment of the tube of media.

- Place all of the pots onto a tray and cover lightly with a plastic dome or tent. Place the plants in an area with 12-16 hours of light (either natural or artificial) but not direct sunlight.

- After a week the cover can be gradually removed and the plants acclimated to stronger light and drier atmospheric conditions.

- You now have a collection of plants in your classroom that are genetically exactly the same. You could use these plants to carry out other experimental tests knowing that one of the main variables in the experiment has been eliminated. Some of these tests could include looking at plant responses to low light levels, to drought or to saline soil conditions.

Materials Plant Tissue Culture

Plants

Any part obtained from any plant species can be employed to induce callus tissue. Younger and fresh explants are preferable as explants materials.

Media

Inorganic salts: These are carriers of nutrients such as nitrate, potash and ammonium. A common inorganic salts medium is the Murashige and Skoog media and B5 medium

Carbon sources: Sucrose, fructose, maltose and [4,5] other sugars are suitable carbon sources which are added to the basal medium to support the growth of various plant cells and serve as energy supplier for physiological activities in cell.

Stages of tissue culture process

Preparation of nutrient medium: A semi-solid medium is prepared in double distilled water containing macro elements, micro elements, amino acids, vitamins, iron source, carbon source like sucrose and phyto-hormones.

Establishment of Aseptic Culture

The starting material for the process is normally an actively growing shoot tip of axiliary or terminal bud or shoot tip of a plant.

Inoculation

Inoculation is carried out under aseptic conditions. In this process explants or micro shoots are transferred on to the sterilized nutrient medium

Development of Plant in Growth Room

After the inoculation of the plant tissue, the bottles are sealed and transfer red into growth room to trigger developmental process under diffused light (fluorescent light of 1000-2000 lux) at 25 ± 2 o Cand 50 to 60% relative humidity.

Hardening of micro plants: Due to very high humidity inside the culture vessel and artificial conditions of development, the plantlets re tender and are therefore are not ready for coping up with the field conditions

Types of Tissue Culture

Callus culture: Callus culture may be defined as production and maintenance of an unorganized mass of proliferative cell from isolated plant cell, tissue or organ by growing them on artificial nutrient medium in glass vials under controlled aseptic conditions

Organ culture: That may allow differentiation and preservation of the architecture. The organ culture refers to the *in vitro* culture and maintenance of an excised organ primordial or whole or part of an organ in way and function.

Single cell culture: Single cell culture is a method of growing isolated single cell aseptically on nutrient medium under controlled condition.

Suspension culture: Suspension culture is a type of culture in which single cell or small aggregates of cell multiply while suspended in agitated liquid medium. Suspension cultures are used in induction of somatic embryos and shoots, production of secondary metabolites, *in vitro* mutagenesis, selection of mutants and genetic transformation studies.

Embryo culture: Embryo culture may be defined as aseptic isolation of embryo (of different developmental stages) from the bulk of maternal tissue of mature seed or capsule and *in vitro* culture under aseptic and [6] controlled physical condition in glass vials containing nutrient semisolid or liquid medium to grow directly into plantlet.

Anther culture: Androgenesis is the *in vitro* development of haploid plants originating from potent pollen grains through a series of cell division and differentiation.

Techniques in Plant Tissue Culture for Crop Improvement

Meristem culture: Meristem is the region of active cell division in plant. This region occurs at the tip of shoots, root and leaves of plants. In this practice, very small shoot apices, each consisting of the apical meristem tic dome with or without leaf primordium is inoculated. For most dicotyledonous plants, the size [2, 5] depends on plant species and is usually less than 1 cm. This technique is use in the disease elimination and quick distribution of genetic materials.

Shoot tip or shoot culture: Shoot tip culture consist of shoot tips or buds having several leaf primodia and are larger than shoot meristems which are inoculated on culture medium. This is appliedin horticultural plants like *Musa* germplasm. The constituents of the medium are designed to induce lateral buds and the shoot growth.

Micropropagation methods: Among the various applications of plant tissue culture, micropropagation of plant species has attained the status of large plant based study. The development in the study of various aspects of plant growth and differentiation were rapid during 1960s and 70s. The technique of culturing plants becomes a wide

subject embracing morphology, physiology, biochemistry, molecular biology and genetic engineering.

- Somatic Embryogenesis
- Axillary Bud
- Adventitious Budding

General Technique of Micropropagation

The process of plant micropropagation aims to produce clones (true copies of a plant in large numbers). The process is usually divided into the following stages:

Stage 0: pre-propagation stage

The pre-propagation stage requires proper maintenance of the mother plants in the greenhouse under disease and insect free conditions with minimal dust. Clean enclosed areas, glasshouses, plastic tunnels and net covered tunnels, provide high quality explant source plants with minimal infection. Collection of explants for clonal propagation should be done after appropriate pre-treatment of the mother plants with fungicides and pesticides to minimize contamination in the *in vitro* cultures. This improves growth and multiplication rates of *in vitro* cultures. The control of contamination begins with the pretreatment of the donor plants. The choice of explant depends on the methods of shoot multiplication to be followed. All plant organs viz. nodal segment, inter-nodal segments, shoot tip, root tip. For axillary bud induction, callus culture, somatic embryogenesis explants nodal segments, internodes and leaves are collected.

Advantages of micropropagation

Micropropagation has a number of advantages over traditional plant propagation techniques:

The main advantage of micropropagation is the production of many plants that are clones of each other. Micropropagation can be used to produce disease-free plants. Micropropagation produces rooted plantlets ready for growth, saving time for the grower when seeds or cuttings are slow to establish or grow. It can have an extraordinarily high frequency rate, producing thousands of propagules while conventional techniques might only produce a fraction of this a number. It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion. It is Figure 2 useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seed can't be stored. Micropropagation often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods - like seeds or cuttings. Some plants with very small seeds, including most orchids, are [6] most reliably grown from seed in sterile culture.

A greater number of plants can be produced per square meter and the propagules can be stored longer and in a smaller area.

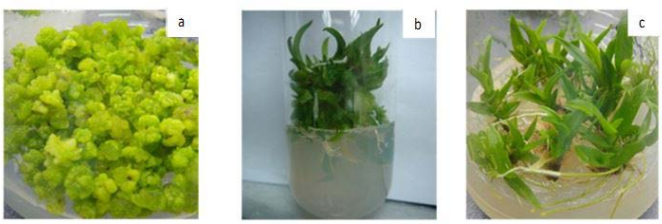

Figure 2: Micro propagation of Orchids (a) callus culture (b) shoot regeneration (c) rooted plantlets.

Disadvantages of micropropagation

Micropropagation is not always the perfect means of multiplying plants, conditions that limits its use include:

It is very expensive, and can have a labour cost of more than 70%.

A monoculture is produced after micro propagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.

Commonly Used Terms in Tissue Culture

Adventitious: development of organs such as buds, leaves, roots, shoots and somatic embryos from shoot and root tissues and callus.

Agar: Natural gelling agent made from algae

Aseptic technique: procedures used to prevent the introduction of microorganisms such as fungi, bacteria, viruses and phytoplasmas into cell, tissue and organ cultures, and cross contamination of cultures.

Autoclave: A machine capable of sterilizing by steam under pressure

Axenic culture: a culture without foreign or undesired life forms but may include the deliberate co-culture with different types of cells, tissues or organisms.

Callus: an unorganized mass of differentiated plant cells.

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

Chemically defined medium: a nutritive solution or substrate for culturing cells in which each component is specified.

Clonal propagation: asexual multiplication of plants from a single individual or explant.

Clones: a group of plants propagated from vegetative parts, which have been derived by repeated propagation from a single individual. Clones are considered to be genetically uniform.

Contamination: infected by unwanted microorganisms in controlled environment **Cryopreservation:** ultra-low temperature storage of cells, tissues, embryos and seeds. **Culture**: A plant growing *in vitro* in a sterile environment

Differentiated: cultured cells that maintain all or much of the specialized structure and function typical of the cell type *in vivo*.

Embryo culture: *In vitro* culture of isolated mature or immature embryos.

Explant: an excised piece or part of a plant used to initiate a tissue culture.

***Ex vitro*:** Organisms removed from tissue culture and transplanted; generally plants to soil or potting mixture.

Hormone: Generally naturally occurring chemicals that strongly affect plant growth

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

***In Vivo*:** To be grown naturally

Laminar Flow Hood: An enclosed work area where the air is cleaned using HEPA filters

Medium: a solid or liquid nutritive solution used for culturing cells

Meristem: a group of undifferentiated cells situated at the tips of shoots, buds and roots, which divide actively and give rise to tissue and organs.

Micropropagation: multiplication of plants from vegetative parts by using tissue culture nutrient medium.

Propagule: a portion of an organism (shoot, leaf, callus, etc.) used for propagation.

Somatic embryos: non-zygotic bipolar embryo-like structures obtained from somatic cells.

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

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

Totipotency: capacity of plant cells to regenerate whole plants when cultured on appropriate media.

Transgenic: plants that have a piece of foreign DNA

Undifferentiated: cells that have not transformed into specialized tissues.

Conclusion

Tissue culture is one of the most important part of applied biotechnology. In the coming decades the world's population will increases more and accommodation space, agricultural lands will decrease significantly global climate change is also another consideration .keeping these in mind we have to ensure a peaceful, healthy and hunger free greener world for our next generation. For doing this there is no alternate of plant tissue culture.

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Conflict of Interest

The authors declared no potential conflicts of interest for the research, authorship, and/or publication of this article

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