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Over Review on Plant Tissue Culture

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ABSTRACT

Plant tissue culture has been widely employed in area of agriculture, horticulture, forestry and plant breeding. It is an applied biotechnology used for mass propagation, virus elimination, secondary metabolite production and in-vitro cloning of plants. Recently, plant tissue culture has been used for the conservation of endangered plant species through short and medium term conservation also known as slow growth and cryopreservation also known as long term conservation. These methods had been effectively used to conserve plant species with recalcitrant seeds or dormant seeds and showed greater advantage over the conventional methods of conservation. At present plant cell culture has made great advances. Possibly the most significant role that plant cell culture has to play in the future will be in its association with transgenic plants. The ability to accelerate the conventional multiplication rate can be of great benefit to many crops/countries where a disease or some climatic disaster wipes out crops.

KEYWORDS: *agriculture, plants, conservation, species, transgenic*

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INRODUCTION

Concept of in-vitro cultivation of cell wall developed by German Botanist Gottlieb Haberlandt in 1902[1]. Different animal cells, isolated plant cells if grown aseptically in nutrient solution, have an intrinsic capacity to give rise to whole plant. The technique, commonly known as plant tissue-culture[2]. Plant tissue culture is an essential part of plant biotechnology. Apart from mass multiplication of elites, it also provides the means to grow and renew novel plants from genetically engineered cells. The promising plant thus produced may be readily cloned in cultures under aseptic conditions.

Tissue Culture is becoming as choice means to vegetative propagation of plants. In vitro growing plants are usually free from bacterial and fungal diseases. Virus eradication and maintenance of plants in virus free stage can also be rapidly achieved in cultures. Three main methods generally used in tissue culture are-

- Micro propagation through the enhanced multiplication of axillary Bud.
- Organogenesis.
- Somatic embryogenesis.

At current the most flourishing and commonly used method is enhanced shoot multiplication from axillary bud. Axillary buds are present in the axis of leaves. In tissue culture, by using optimum concentration of cytokinin or combination of cytokinin and Auxin the dormancy of the axillary buds can be broken. Once the dormancy is broken, they develop into shoots. By using media containing optimum concentrations of plant growth regulators, they can be made to multiply very rapidly. Modern plant tissue culture is performed under aseptic conditions under filtered air. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting materials (explants) in chemical solutions (usually alcohol) is required. Mercuric chloride is used as a plant sterilizing agent today, as it is dangerous to use, and is difficult to dispose off. Explants are then frequently placed on the surface of a solid culture medium, but 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. 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 explants. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots[3].

HISTORY OF TISSUE CULTURE

- 1838- Cell theory, representing towards totipotentiality of cells by Schleiden and Schwann.
- 1902- First but unproductive attempt of tissue culture using monocots by Haberlandt. He also explained the concept of cell totipotency.
- 1904- First attempt in embryo culture of selected Crucifers by Hannig.
- 1922- A symbiotic germination of orchid seeds by Knudson.
- 1934- In vitro culture of cambial tissues of different trees and shrubs failed by Guatheret.
- 1941- Coconut Milk used for growth and development of very young Datura embryos by Overbeek.
- 1942- Observation of secondary metabolites in plant callus cultures by Gautheret.
- 1943- Tumor-inducing principle of crown gall tumors identified by Braun.
- 1944- First In vitro culture of tobacco used to study adventitious shoot formation by Skoog.
- 1958- Regeneration of somatic embryos from nucleus of Citrus ovules by Maheshwari and Rangaswamy.
- 1962- Development of MS medium by Murashige and Skoog.
- 1964- First haploid plants from Datura androgenesis by Guha and Maheshwari.
- 1973- Cytokinins found to be able of breaking dormancy in Gerberas by Pierik
- 1978- Somatic hybridization of tomato and potato resulting pomato by Melchers.
- 1981- Isolation of auxotroph by cell colony screening in haploid protoplasts of Nicotiana plumbaginifolia treated with mutagens by Sidorov.
- 1985- Infection and transformation of leaf discs with Agrobacterium tumefaciens and renewal of transformed plants by Horsch.
- 1993- In vitro fertilization with isolated single gametes resulting in zygotic embryogenesis and healing of fertile maize plants by Kranz.
- 1996- Development of agrolistic method of plant transformation by Hansen[4].

IMPORTANCE OF TISSUE CULTURE

In a relatively short time period and space a large number of plantlets can be prepared starting from the single explant.

- Taking explants does not usually wipe out the mother plant, so rare and endangered plants can be cloned safely.
- It is easy to select popular traits directly from the culture setup (in-vitro) thereby decreasing the amount of space required, for field trials.
- Once established, a plant tissue culture line can give a continuous supply of young plants throughout the year.
- The time required is much condensed, no need to wait for the whole life cycle of seed development. For species that have long generation time, low level of seed production, or seeds that readily do not germinate, rapid propagation is possible.
- In vitro growing plants usually free, from the bacterial and fungal diseases. Virus eradication and maintenance of plants in virus free state. This facilitates movement of plant across international boundaries.
- Plant tissue banks can be frozen and then renewed through tissue culture. It preserves the pollen and cell collections from which plants may be propagated[5].

TYPES OF TISSUE CULTURE

A. Callus culture

Callus culture may be defined as manufacture and preservation 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.

B. Organ culture

That may allow separation and conservation 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.

C. Single cell culture

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

D. Anther culture

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

E. Pollen culture

Pollen culture is the in vitro technique by which the pollen grains (preferably at the microscope stages) are squeezed from the intact anther and then cultured on nutrient medium where the microspores without producing male gametes.

F. Somatic Embryogenesis

Somatic embryogenesis is the process of a single or group of cells initiating the development pathway that leads to reproducible renewal of non zygotic embryos capable of germinating to form complete plants.

G. Protoplast Culture

It is the culture of isolated protoplasts which are naked plant cells surrounded by plasma membrane which is potentially able of cell wall renewal, cell division, growth and plant renewal on suitable medium under aseptic condition.

H. Shoot tip and Meristem culture

The tips of shoots (which contain the shoot apical meristem) can be cultured in vitro producing clumps of shoots from either axillary or adventitious buds. This method can, be used for clonal propagation.

I. Explant Culture

There are diversity of forms of seed plants viz., trees, herbs, grasses, which exhibit the basic morphological units i.e. root, stem and leaves. Parenchyma is the most adaptable of all types of tissues. They are capable of division and growth[6].

MEDIA CONSTITUENTS

Inorganic Nutrients

- Mineral elements are very significant in the life of a plant.
- Mg is a part of chlorophyll molecules
- Ca is a component of cell wall
- N is an essential part of amino acids, vitamins, proteins and nucleic acid.
- Fe, Zn, and Mo are part of certain enzymes.
- Besides C, H, and O there are 12 elements, known to be essential for plant growth viz. N, P, S, K, Ca, Mg, Fe, Mn, Cu, Zn, B and Mo[7].

Macro Elements

- **C**- Carbon forms the backbone of many plants Bio-molecules, including starches and cellulose. It is fixed through photosynthesis from the carbon synthesis in the air and is a part of the carbohydrates that store energy in the plant.
- **H-** Hydrogen also is necessary for structure the plant and it is obtained almost entirely from water.
- **O-** Oxygen is essential for cellular respiration. Cellular respiration is the process of generating energy rich adenosine tri phosphate (ATP) via the use of sugars made in photosynthesis. Plants manufacture oxygen gas during photosynthesis to manufacture glucose but then require oxygen to undergo aerobic cellular respiration and break down this glucose and produce ATP.
- **N**- Nitrogen is necessary component of all proteins. Nitrogen insufficiency most often results in stunted growth.
- **Ca**- Calcium regulates move of other nutrients into the plant. It is also involved in the activation of certain plant enzymes. Calcium deficiency results in stunting.
- **Mg**- Magnesium is significant part of chlorophyll, a critical plant pigment vital in photosynthesis. It is important in the production of ATP through its role as an enzyme cofactor. There are many other biological roles for magnesium in biological system for more information. Magnesium deficiency can result in intervenial chlorosis.
- **S**- Sulphur is a structural component of some amino acids and vitamins. It is necessary in the production of chloroplasts.
- **Microelements**- These are necessary as catalysts for many biochemical reactions; microelement deficiency symptoms include Leaf chlorosis (Fe, Zn, and Mn) Shoot tip necrosis (B, Co, Ni) inhibits ethylene synthesis.
- **Fe**- Iron is necessary for photosynthesis and is present as an enzyme cofactor in plants. Iron deficiency can result in intervenial chlorosis and necrosis.
- **Zn**- Zinc is required in a large number of enzymes and plays an essential role in DNA transcription. A typical symptom of zinc deficiency is the stunted growth of leaves, commonly known as "little leaf" and is caused by the oxidative degradation of the growth hormone auxin.
- **Mn**- Manganese is essential for building the chloroplasts. Manganese deficiency may result in coloration abnormalities, such as discolored spots on the foliage.
- **B**- Boron is important for binding of pectin in the RG II region of primary cell wall; secondary roles may be in sugar transport, cell division and synthesizing certain enzymes. Boron deficiency causes necrosis in young leaves and stunting.
- **V**- Vanadium may be required by some plants, but at very minimum concentrations. It may also substitute for Molybdenum.
- **Se-** Selenium and Sodium may also be beneficial. Sodium can substitute potassium's regulation of stomatal opening and closing[8].

Organic Nutrients

- Vitamins: Plants can create their requirements of vitamins. However, plant cell cultures need to be supplemented with certain vitamins like Thiamine (vit B1), Niacin S (vit. B3), Pyridoxine (vit B6), and Myo-inositol (Member of the vit. B complex).
- Thiamine – Involved in the direct biosynthesis of certain amino acids and essential co-factor of carbohydrates metabolism.
- Vit E-Antioxidants.
- Vit C- To prevent blacking during explants isolation.
- Vit D- Growth regulatory effect.

Growth Regulators

- Auxin – Auxin are involved in cell division and elongation and in cell wall synthesis. IAA, IBA, NAA, 2, 4-D are the most regularly used auxin in plant tissue culture. The principal naturally occurring auxin, the IAA is not often used in the tissue culture, because it is unstable. IBA is slightly more potent than IAA and is not easily broken down. Hormones of this group are involved with elongation of stems and inter nodes, tropism, apical dominance abscission, rooting etc.
- Cytokinin –These hormones, are concerned with cell division, change of apical dominance, shoot differentiation etc. Most commonly used cytokinins are BAP, BA, Kinetin, 2 ip and Zeatin. They usually promote cell division if added together with an auxin.
- Gibberellins – There are over 20 known gibberellins. Of these, generally, GA3 is used. They are rarely used and reported to stimulate normal development of plantlets from in vitro formed adventives embryos.
- Others – Abscisic acid is most frequently required for normal growth and development of somatic embryos and only in its presence they resemble zygotic embryos[9].

Tissue culture media

Generally all culture media are made up of:

- Macronutrients
- Micronutrients
- Vitamins
- Growth regulations
- Carbohydrates (Sucrose)

Special plant groups like conifers have nutritional requirements, which appear, not to meet by standard media, and then some additional nutrients are required in media[10].

STAGES OF TISSUE CULTURE

A. First stage

This first stage is also known as growth stage. In this stage cytokine is present. Due to the presence of the cytokine there is increase in the shoot of plant. So there is growth in the plant. It around requires 2 to 3 months for the whole process[11].

B. Second stage

This secondary stage is also known as reproduction stage. In this stage also there is presence of cytokine. In this stage the cytokine level is increased. This increased cytokine helps to the multiplication of the plant shoots. It around requires three and half month for the whole process[11].

C. Third stage

This third stage is also known as rooting stage. In this stage auxin is added. This auxin is useful for the rooting reason of the plant. It approximately requires 2 months for the whole process[11].

PREPARATION OF THE STOCKS SOLUTIONS

It is hard to weigh and mix all the constituents just before preparation of medium. It is time consuming and a boring job. Again if 100 ml medium is to be prepared, then it is very hard to weigh some constituents that are used in very low quantity for 1 liter medium. So it is suitable to prepare concentrated stock solution of macro salts, micro salts, Vitamins, amino acids, hormones etc. and all stocks solution should be preserved in a refrigerator and should be checked visually for contamination with microorganism or precipitation of ingredients[12].

STERILIZATION

It is very significant to maintain aseptic environment during the in vitro culture of plant cells and tissues. Following are some of the methods adopted for sterilization.

1. Sterilization of Glassware- The glassware can be sterilized in a hot air oven at 160-1800C for 2-4 hours.
2. Sterilization of instruments- The metallic instruments are incinerated by dipping them in 75% ethanol followed by flaming and cooling.
3. Sterilization of nutrient media- The culture media are transferred into glass container, plugged with cotton or sealed with plastic closures and sterilized by autoclaving at 15 psi for 30 min. The autoclaving denatures the vitamins, plant extracts, amino acids and hormones therefore the solution of these compounds are sterilized by using Millipore filter paper with pore size of 0.2 micrometer diameter. Surface sterilization of juvenile material is generally not difficult. However, if older trees are used, as it is the initial and basic material for tree breeders when selection is done contamination of explants is sometimes a serious problem, unless the tree produce juvenile sprouts.

   Ethyl and isopropyl alcohol have also been used to surface sterilize of plant tissues. Explants were washed in distilled water to remove dust particles, then they were washed in detergent solution and surface sterilized in 0.1% solution of HgCl2, NaCl for 5 minutes. To eliminate the sterilant nodal segments were again washed with sterile distilled water[13].

Flow chart showing complete explants sterilization process:

Excised explants from selected plant

↓

Wash in running tap water (5-7 min.)

↓

Explants treated with detergent (Tween-80) for 5 min.
Rinsed thrice with distilled water to remove extra detergent

↓

To prevent bacterial & fungal contamination treated with Streptomycin & Bavistin (100mg/100ml) for (15-20 min.)

↓

Wash with distill water thrice or more to remove disinfectants properly

↓

Surface sterilization the explants with 0.1% of HgCl2 for 7 min (at LAF Bench)

↓

Washed with autoclaved distill water to remove extra disinfectant and inoculate the explant on sterilized medium[14]

GENERAL PROCEDURE

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 to decrease dehydration of the medium.
11 Incubate the culture tubes in the dark at 25°C. Inspect 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 call is subculture into the same medium for further callus growth or to the carrot shoot / root initiation medium.
14 Seal all the tubes having call 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[15, 16.]

APPLICATION OF PLANT TISSUE CULTURE

1 To study the respiration and metabolism of plants.
2 For the evaluation of organ functions in plants.
3 To study the different plant diseases and work out methods for their elimination.
4 Single cell clones are helpful for genetic, morphological and pathological studies.
5 Embryonic cell suspensions can be used for large scale clonal propagation.

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