PIJAR
Paryeshana International Journal of Ayuredic Reserach
www.pijar.org
ISSN:2456:4354

INTRODUCTION TO PLANT TISSUE CULTURE (PLANT PROPAGATION), ITS GENERAL TECHNIQUES & ESTABLISHMENT OF CULTURE

Archana S. Chougule [1], Shrikanth P [2]
[1]PhD Scholar, [2] Professor & Head, Department of PhD & PG Studies in Dravyaguna, Shri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi.
DOI: https://doi.org/10.47071/pijar.2021.v06i03.02

ABSTRACT:
For the success of primary health care is the availability and use of suitable drugs. Plants have always been a common source of medicaments, either in the form of traditional preparations or as a pure active principal. An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Plant propagation is the process of creating new plants from a variety of sources: seeds, cuttings and other plant parts. Plant propagation can also refer to the artificial or natural dispersal of plants.
KEYWORDS: Plant Tissue culture, Biotechnology, Methodology

INTRODUCTION:

Tissue culture consists of growing plant cells as relatively on organized masses of cells on an agar medium (callus culture) or as a suspension of free cells and small cell masses in a liquid medium. Tissue culture is used for vegetative multiplication of many species and in some cases for recovery of virus free plants.

Plant propagation is the process of creating new plants from a variety of sources: seeds, cuttings and other plant parts. Plant propagation can also refer to the artificial or natural dispersal of plants whereas cultivation means to grow them in a better manner.

Biotechnology has rapidly emerged as an area of activity having a marked, realized as well as potential impact on virtually on all domains of human welfare, ranging from food processing, protecting the environment to human health. As a result, it now plays a very

important role in employment, production and productivity, trade, economy, human health and quality of human life throughout the world.

THE GENERAL TECHNIQUE:

The technique of in-vitro cultivation of plant cells is primarily devoted to solve two basic problems:

1. To keep the plant cells free from microbes
2. To ensure the desired development in the cells by providing suitable nutrient media and other environmental conditions.

The first problem can be eliminated by using the modern equipments and careful handling during various operations. The second problem remains the area of active research and is likely to do so for quite some time in the future. At present it relies mainly on the manipulation of culture medium, especially growth regulators and to much less extent of other factors including environmental conditions.

TISSUE CULTURE LABORATORY:

The basic organization and facilities of most tissue culture labs are used as in common labs like incubation room, sterilization facility, incubators, gas and water supply, electricity, vacuum etc.

EQUIPMENTS:

pH meter – 230V 50Hz, Balances – for quick weighing, Electric oven-5˚C above the ambient environment to 250˚C± 2˚C, Microscope, laboratory microscope, inverted microscope, Low speed centrifuge-230V 50Hz, Autoclaves, Steamers, Filter sterilization equipment, Manestry stills , Environment growth cabinets, Gyratory shakes, Laminar air flow cabinets, Fluorescent light

CULTURE MEDIUM:

- Make volume to 1000 ml with double distilled water.
- Defined medium for growth of cell culture consists of inorganic salts, a carbon source, vitamins, growth regulators and some organic supplements.
- An ideal nutrient medium for plant tissue culture contains five classes of ingredients:
- Inorganic Salts: The concentration of potassium and of nitrate should be at least 20-25mM for each, whereas concentrations of 1-3mM of phosphate, sulphate and magnesium appear to be adequate. Ammonium is

essential although amounts in excess of 8mM could be deleterious. The recommended micronutrients are iodide, boric acid and salts of zinc, manganese, molybdenum, copper, cobalt and iron. The later is usually incorporated in the chelated form.

- Vitamin: Thiamin is the only vitamin which appears to be essential. Pyridoxine and nicotinic acid are frequently added to improve cell growth.
- Carbon Source: Sucrose or glucose at a concentration of 2-4% is the most suitable carbon source.
- Growth Regulators: These substances are needed to induce cell division. The compounds most frequently used are naphthalene acetic acid and 2,4-dichlorophenoxy acetic acid, in the molar concentrations of 10^{-7} to $5x10^{-5}$. Both 2,4-D and NAA are degraded very slowly by plant cells and are stable to autoclaving such as kinetin or benzyl adenine are sometimes required in conjugation with 2,4-D or NAA to obtain good callus formation.
- Organic Supplements: Protein hydrolyzates, yeast extracts, malt extracts and coconut milk (liquid endosperm) are used for enhancement in the growth rate of the cells in bio-mass.
- The chemicals are dissolved in glass distilled water, the stock solutions of vitamins, micronutrients and growth hormones are added and the pH of the medium is adjusted to 5.5 to 5.7. The solution is made to volume and then 50 and 100 ml quantities are distributed into 250 ml Erlenmeyer flask. The flask is stopped with cotton wool plugs and autoclaved at 120˚C for 15 minutes. The flasks are removed for cooling, as possible. The agar medium is autoclaved in lots of 500ml and subsequently poured into sterile containers. All media are stored 10˚C prior to use.
- Surface Sterilization of Explants: It is necessary to effect surface sterilization of the organ from which the tissue is to be aseptically excised or of the spore or seed whose germination shall yield the tissue explants. The commonly used surface sterilizing agents are sodium hydro chlorite (1-2%), bromine water (1-2%), hydrogen peroxide (10-12%), mercuric chloride (0.1-1%) and silver nitrate (1%).
- The seeds are treated with 70% ethanol for about 2 minutes, washed with sterile distilled water treated with

surface sterilizing agent for a specific period, once again rinsed with sterile distilled water and kept for germination under aseptic conditions. The seeds may be germinated aseptically by placing them on double layers of pre-sterilized filter paper in Petri-dishes moistened sufficiently with sterile distilled water or on moist cotton plugs in Petri- dishes or culture tubes. The seeds are germinated in dark at 26˚C to 28˚C and the small part of the seedlings is utilized for the initiation of callus culture.

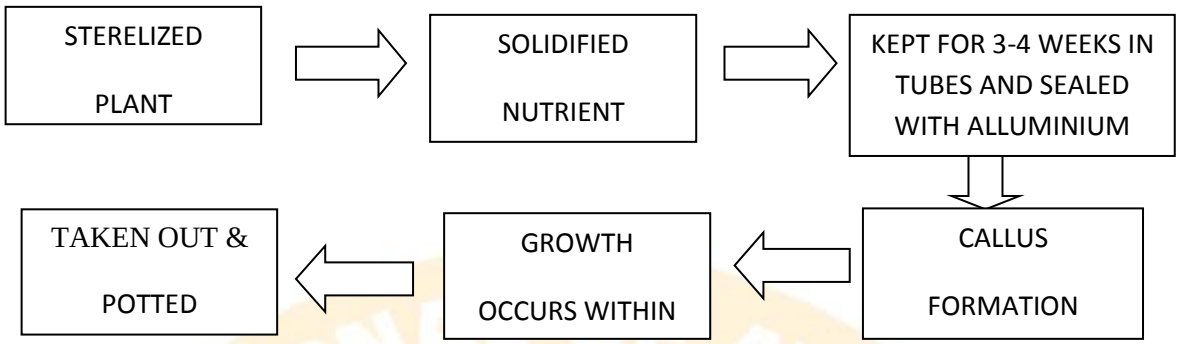
- The aerial portion of the plants such as bud, leaf, stem sections are sterilized by submerging for 2-3 minutes in 70% ethanol followed by 2-3 rinses in sterile distilled water.

Note: Besides the composition of the medium other factors such as light, temperature, pH and humidity influence the growth of the plant tissue in-vitro

- Light: Normally plant tissue culture does not require light for their growth. But for inducing the culture to differentiate light quite often plays a permanent role. The light intensity and the period of illumination may vary from one species to another.
- Temperature: Generally 25˚C to 27˚C is the most suitable temperature for callus growth. For differentiation, lower temperatures during the dark period should be maintained.
- pH: correct pH of the medium is important. Highly alkaline or acidic pH values affect the nutrient uptake in cultured tissues. Therefore the tissue culture medium is adjusted to a pH of 5.6 to 6.0 before autoclaving.
- Physical State of Media: Semi solid and liquid media are most commonly used for growing plant cells. A high concentration of gelling agents (agar, gelatin) makes the medium very hard and decreases the nutrient uptake by the tissues. Agar at 0.8% concentration is widely used.
- Humidity: Relative humidity is also an important factor. Less humidity (50-55%) results in the dehydration of the media whereas high humidity (85-90%) only promotes bacterial growth on cotton plug. A relative humidity between 70-75% is ideal for the growth of cultured plant tissue.

A viable tissue culture may be used for studying the nutrition, metabolism, morphogenesis, somatic hybridization and other processes of plants.

ESTABLISHMENT OF CULTURES:



The surface stabilized plant material is aseptically transferred on solidified nutrient medium in flask glass jars or culture tubes and allowed to incubate at 26˚C to 28˚C in dark. After 3-4 weeks, the callus should be about 5 times the size of the explants. The maintenance of growth callus tissue by sub culturing requires the transfer on each occasion of a piece of healthy tissue every 4 weeks in to the flasks containing fresh solidified nutrient medium.

Many culture shall remain healthy and continue slow rate of growth for much longer periods without sub culturing, if the standard incubation temperature of 26˚C is lowered to 5-10˚C. It has been observed that the growth of many cultures and particularly of those which form chlorophyll is stimulated by low-intensity illumination light on a 12b cycle or continuously.

The suspension cultures are generally initiated by transferring an established callus culture to a agitated liquid nutrient medium in Erlenmeyer culture vessels (30-60ml medium /250 ml flask). The releases of cell and tissue fragments from less friable callus masses and maintenance of good degree of cell separation may often be promoted by the presence in the liquid medium of high auxin concentration, an appropriate balance between yeast extract and auxin between auxin and kinetin. The suspension cultures are usually incubated at 25˚C in darkness or low intensity fluorescent light. Continuous agitation of flask culture is most commonly achieved by using horizontal shaker which rotates at between 100 and 200 rpm. The culture flask is sealed with double aluminum foils or paraffin to reduce evaporation during the process of culture growth.

The cell suspension should be formed within 4-6weeks. The suspension cultures are sub cultured by the transfer at regular intervals of untreated or fractioned aliquots of the suspension to fresh medium.

There are several parameters for measuring growth of cultured cells such as measurement of cell number, pcv, fresh and dry weight, total nitrogen etc. None of these methods reflects growth in all its facts which include cell division, elongation and differentiation.

The method of measuring growth of cultured cells by dry weight determination has the advantage of being a method which is simple used quite commonly and gives an acceptable assessment of overall synthetic activity of cells.

The balance between formation of root or shoot is governed by ratio of auxin to cytokinin (auxin to cytokinin in 4:1 proportion cause root formation). High auxin concentrations cause the formation of meristem like cells.

PROCEDURE:

a) Initiation of callus and suspension culture: Once the suspension cultures are initiated, the growth of cells in suspension should be determined at regular intervals to standardize the period between subcultures.

b) Determination of cell number: Take an aliquot of the suspension and filter of big clumps using a wire mesh (300µm). Note the volume of the filtrate (r) containing the single cell and small clumps. Transfer a drop of this suspension to a haemocytometer and determine the number of cells by equation : $N = P * 1000 * \frac{r}{0.1mm}$

Where N=total no of cells and small clumps

P=no of cells in the square of the haemocytometer r=volume of the filtrate.

Isolation of the desired variant cells callus and suspension cultures can be used for isolation of NaCl tolerant variants.

TRANSFER OF PLANTS TO SOIL:

1. Autoclave soil in covered enamel container for 4 hrs at 6.8kg.
2. Distribute sterilized soil to clean small plastic pots (size 65mm)
3. Soak the soil with just enough amount of non sterile water. The soil for salt tolerant plants is soaked with NaCl solution, the concentration of which should be the same as the

concentration in the regeneration media.

4. Remove the rooted plantlets from the tubes using a pair of forceps taking extreme care, not to damage the roots. Roots are fragile at this stage. Wash the root system with tap water carefully for 10-15 minutes and transfer the rooted plantlets to soil in the pots.
5. Transfer the pots to a chamber with a light intensity of 1000-1200 flux and 60% humidity for 46 weeks before transferring to the glass house.
6. Water the plants in control with clean tap water. Use NaCl solution to water the salt tolerant plants. The concentration of NaCl should be the same as used in regeneration medium.
7. After 6 weeks in the glass house transfer the plantlets to pots of bigger size (250mm) water the controlled plants with tap water and the salt tolerant plants with NaCl solution.

DISCUSSION:

Biotechnology comprises the controlled and deliberate application of simple biological agents- living or dead cells or cell components- in technically useful operations, either of productive manufacture or as service operation. Thus the main features of biotechnology are the utilization of biological entities (micro-organisms, cells of higher organisms - either living or dead), their components or constituents (e.g. Enzymes) in such a way that some product can be generated. The term tissue culture is commonly used in a very wide sense to include in-vitro culture of plant cells, tissues as well as organs. But in a strict sense, tissue culture denotes the in-vitro cultivation of the plant cells in an unorganized mass e.g., Callus culture. Another term, cell culture is used for in-vitro culture of single or relatively small groups of plants cells, e.g., suspension culture. Clone multiplication of large number of plants within limited time and space without interruption of external climate.

CONCLUSIONS:

- Through plant tissue culture we can conserve the extinct medicinal plants.
- This also helps for the good qualitative and quantitative production of medicinal plants.
- Drugs which are distributed at a particular region can be cultivated and made easily available, which are rarely available (irrespective of climatic conditions).

REFERENCES:

1. Peter Martin Roy; The Living Plant; Library of Congress Catalogue, USA; 1972:170p
2. Endangered medicinal plants , The free Encyclopedia through, www.google.com
3. Dr. B.P.Pandey; A text book of Botany Angiosperms; I ed; S.Chand & Company Pvt Ltd, New- Delhi; 2014:698p
4. Hill, Poop Grove; Botany; IV ed; McRaw Hill Book Company,USA;1967:206p
5. T.Elliot Weir, C.Ralph & Michel George Barbour; Botany- An Introduction to Plant Biology; IV ed; John Wiley & Sons. Inc, Newyork; 1970:399,401p
6. F.C.Steward; Plant Physiology; I ed; Academic press, Newyork; 1969:359p
7. U. Satyanarayan; Text Book of Biotechnology; IV ed; Books & Allied (P) Ltd, Kolkata; 2007:497p

Corresponding author:
Archana S. Chougule
PhD Scholar, Department of PhD & PG Studies in Dravyaguna, Shri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi
E-mail: archanachougule7@gmail.com

Published BY:
Shri Prasanna Vitthala Education and Charitable Trust (Reg)

Source of Support: NIL
Conflict of Interest : None declared