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Review Article

ARTICLE ON "TISSUE CULTURE OF THE PLANTS"

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ABSTRACT

The term biotechnology is derived from a fusion of biology and technology. True to its name it concerns with the exploitation of biological agents or their components for generating useful products or services. A relatively simple and comprehensive definition due to B'ulock (1987) is as follows- Biotechnology comprises the controlled and deliberate application of simple biological agents- living or dead cells or cell components are technically useful operations, either of productive manufacture or as service operation[1].

Keywords: Tissue culture, Biotechnology,Ayurvedic point of view, Merrits & Demerrits.

INTRODUCTION

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 or service can be generated.

SCOPE AND IMPORTANCE:

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.

One of the perquisites 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. It is thus reasonable for decision maker to identify locally available plants or plant extracts that could be usefully added to the national list of drugs or that could even replace some pharmaceutical preparation that need to be purchased and imported[2].

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.

Definition:- Tissue + Culture → Tissue Culture

Tissue: Any of the coherent collections of specialized cells of which animals or plants are made.

Culture: Cultivation in an artificial nutrient medium in condition suitable for growth.

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. But in general, the word tissue culture is applied to both callus and suspension culture and cell culture is often used for callus culture as well. When organized structures like root tips, shoot tips, embryos etc, are cultured in-vitro to obtain development as organized structures. This is called organ culture.

AIMS AND OBJECTIVES:

1. Plant tissue culture studies are aimed to propagate, multiply and conserve rare, vulnerable, endangered and important medical taxa.
2. Clone multiplication of large number of plants within limited time and space without interruption of external climate.
3. To attain disease free and genetically identical plants.

REVIEW OF LITERATURE:

History:

Plant tissue culture encompasses culturing of plant parts on an artificial medium. The plant parts can be a single cell, tissue or an organ. It is also referred to as micro propagation. Plant

tissue culture was practically implemented for the first time by Haberlandt, a German scientist, in 1902.Later in 1934, Gautheret found successful results on in-vitro culture of plants. The basic key used in plant tissue is the totipotency of plant cells, meaning that each plant cell has the potency to generate into a complete plant. Within this characteristic, plant tissue culture is used to produce genetically identical plants (clones) in the absence of fertilization, pollen or seeds.

Totipotency is the ability of a plant cell to perform the functions of development which are characteristic of zygote i.e., ability to develop into a complete plant[3].

THE GENERAL TECHNIQUE:

- The technique of in-vitro cultivation of plant cells is primarily devoted to solve two basic problems:
- To keep the plant cells free from microbes to ensure the desired development in the cells by providing suitable nutrient media and other environmental conditions[4].

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:

Other equipments along with specifications regarded necessary for tissue culture work are given below:

pH meter – 230V 50Hz

MISCELLANEOUS ITEMS:

- Balances – for quick weighing -AC
- Electric oven-5˚C -Arrow heads
- Microscope (laboratory & inverted) -Bunsen burner
- Low speed centrifuge-230V 50Hz -Heaters
- Autoclaves -Hot plate with magnetic stirrer
- Steamers - Inoculation cabinets
- Filter sterilization equipment -Wooden/ metal racks
- Manestry stills -Deep freeze
- Environment growth cabinets
- Gyratory shakes
- Laminar air flow cabinets
- Fluorescent light
- Forceps

CULTURE MEDIUM:

CONSTITUENTS	AMOUNT
NH_4NO_3	13.2g
KNO_3	15.2g
$CaCl_2.2H_2O$	3.53g
$MgSO_4.7H_2O$	2.96g
$MnSO_4.4H_2O$	178.4mg
$ZnSO_4.7H_2O$	86.4mg
$CuSO_4.5H_2O$	0.2mg

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

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.

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[5].

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

$$\mathbf{N = P * 1000 * \frac{r}{0.1mm}}$$

Where N=total no of cells and small clumps

P =no of cells in the square of the haemocytometer

r =volume of the filtrate[6]

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[7].

AN AYURVEDIC POINT OF VIEW:

- In Atharva Veda (2000BC) medicinal plants and their uses were described.
- Parashara (1300BC) wrote a book on agriculture and weeds and the name of that book is Krishiparasharam.
- He also wrote another book describing 14 types of forests, the external and internal characters of plants and also medicinal plants – Vrikshayurveda. Vrikshayurveda, an ancient science of that time of the 10th century treatise of that title on the subject ascribed to Surupala.
- Vrikshayurveda mainly deals with various species of trees their healthy growth & productivity .The text mentions about 170 species of plants, including herbs shrubs & trees. There are 325 systematically arranged verses, beginning with the salutation of lord Ganesha, followed by glorification of trees, composition on tree

planting & production. Special references are made to procuring, preserving treatment of seeds & plant materials.

- Seed treatment,prior to sowing , to ensure successful & vigorous germination were given lot of importance
- The chapter on horticulture wonders for obtaining dwarf varieties of treesit is necessary to slit open & burn the portion of the trunk slightly.
- The most noteworthy fact in Vrikshayurveda is that it applies the tridhatu theory of ayurveda (the science of life) to plants. Kapha, pitta and vata are considered as the basic components of the plants, too, as of humans and the theory that a balance of the three indicates health and imbalance caused due to variation of anyone or more of them indicate disease is extended to plants too, justifying its title Vrikshayurveda. Even the treatment material prescribed in many cases is the same or similar to that of humans. Surupala, generally considered plants as equal and in some respect even superior to humans as stated above. But in this he treats them specifically equal to humans[8].

DISCUSSION

- In 1902, Haberlandt reported culture of isolated single palisade cells from leaves in Knops salt solution enriched with sucrose. The cell remained alive for up to one month, increased in size, accumulated starch but failed to divide. Efforts to demonstrate totipotency led to the development of techniques for cultivation of plants under defined conditions. This was laid possible by the brilliant contributions from R.J.Gautheret in France and P.R.White in USA during 3rd and 4th decades of 20th century.

SOME IMPORTANT POINTS ABOUT TISSUE CULTURE:

- When explants are cultured on a suitable combination many of its cell undergo division. Even mature and certain differentiated e.g., parenchyma and often collenchymas cells undergo changes to become meristematic. This is called dedifferentiation.
- Callus culture: Tissue and cells cultured on an agar gelled medium form an unorganized mass of cells called callus.
- Suspension culture: Tissues and cells culture in a liquid medium produce a suspension of single cells and cell clumps of few to many cells.
- Batch culture: The same medium and all the cells produce are retained in the culture vessel e.g., culture flask, fermenters etc.
- Continuous culture: The cell population is maintained in a steady state by regularly replacing a portion of the used or spent medium by fresh medium.
- Cloning: A clone of cells consists of all the cells derived through mitosis from a single cell and the process of obtaining a clone is called cloning.
- Cryopreservation: The preservation of cells, tissues and organs in liquid nitrogen (-196˚C).
- Thawning: Thawning of the frozen material is achieved by plunging the vials into water at 37˚C to 40˚C for 90 seconds. Etc are the examples

MERITS:

- ✓ To produce many copies of the same plant then which may be used to produce plants with better flowers, odors, fruits or any other properties of the plants that is beneficial to the human beings.
- ✓ To produce plants anytime we want, although the climate is not appropriate.
- ✓ If there is a plant with partially affected tissue, it is possible to produce new plant without infection.
- ✓ Very helpful in genetically modified organism studies.

DEMERITS:

- ❖ If large scale production is being thought, the cost of equipments is very expensive.
- ❖ The procedure needs special attention and diligently done observation.
- ❖ The infection may continue through generations easily if possible precautions are not taken.

CONCLUSION

- 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).

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