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PROPAGATION OF MEDICINAL PLANTS THROUGH TISSUE CULTURE-A REVIEW

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ABSTRACT

There is an ever increasing global inclination towards herbal medicine today, hence there is not only an obligatory demand for huge raw material of medicinal plants, but also of right stage when the active principles are available in its optimum quantities at the requisite time for the standardisation of herbal preparations. There are thousands of unexplored plants in Indigenous systems of medicine which should be brought forward integrating with modern principles of biotechnology and explored for its potential therapeutic benefits. Tissue culture is one such methodology in which identical clones of the target plant are produced in large quantities which can be used for therapeutic and research purposes. This technology is still in its developing stage and more researches on this should be conducted for medicinal plants especially for evaluating its potential benefits. This article aims at providing an in-depth literary analysis on tissue culture glancing through the evolution of the technology, the methodology and the potential advantages and disadvantages of the same.

Keywords : Tissue culture, Biotechnology, Indigenous system of medicine

INTRODUCTION

The modern world is facing an ever increasing demand for herbal raw materials 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[1].

The history of plant tissue culture begins with the concept of cell theory given independently by Schleiden and Schwann (1838 – 1839) that established the cell as a functional unit. The concept was tested experimentally by Haberlandt after 130 years, who conceived the idea of culturing plant cells. The developments in culturing of plant cells, tissues and organs are associated with developments in knowledge about nutritional requirements of plant cell, discovery of growth regulating factors, analytical tools and techniques, and development of microscopy. The work progressed rapidly after the discovery of auxine and cell division factor. With the better understanding of technique of plant tissue culture and nutritional requirements, of plant cell, it was possible to develop newer technologies by culturing plant organs (anther, ovary, ovule, petal etc.) leading to establishment of new research lines.[2]

MATERIALS AND METHODS

Plant tissue culture is defined as the *in vitro* and aseptic growth of any plant part on a nutrient medium. This technology is based on three fundamental objectives; the part or explant must be isolated from the rest of plant body,the explant must be maintained in controlled physically and chemically defined conditions,asepsis must be maintained.[3]

Principle[4]

The tissue culture technique has developed around the concept that a cell is totipotent, that is has the capacity and ability to develop into whole organism. In higher plants, embryos do develop *in situ* from appropriately stimulated somatic cells without any prior sexual act. Having established the genetic constitution of a given individual, the zygote really behaves developmentally as a very general kind of living cell- a cell with a built-in capacity to grow in an organ, the ovule that fosters that growth. Moreover, as will be shown, and in a satisfying number of cases, isolated somatic cells may return to a stimulated zygotic state and grow into embryos and to plants under the appropriately applied conditions which furnish the requisite nutrients and stimuli. Plants do all this by arranging, in their growing regions, living cells which retain the essential characteristics of the plant and which, during development, depart from, or return to, the situation to be seen in the primary growing apices of shoot and root by relatively small and often easily reversible steps.

REQUIREMENTS FOR PLANT TISSUE CULTURE[5]

The important requirements of plant tissue culture are summarized below:

1) Aseptic conditions

2) Optimum aeration

3) Suitable nutrient medium

4) Explants

Aseptic conditions

Complete aseptic conditions are required for tissue culture. The tissues, equipments, culture media, and the room should be free from micro-organisms. Usually dry heat, wet heat, ultrafiltration and chemicals are used for the sterilization process. The equipments are sterilized by the dry heat method in an autoclave. Wet heat sterilization is used for glass wares involves autoclaving at 121^0c and 15 lb pressure for 15 minutes. Ultrafiltration is used for the sterilization of liquid media which are unstable at high temperature. The working area and the instruments are sterilized by chemicals such as alcohol.

Optimum aeration

Adequate aeration is required for the cell to grow. Tissues which are cultured on semisolid media do not require any special method of aeration, but tissues which are grown in suspension cultures, require special devices for aeration. The aeration for submerged cultures can be provided by following methods:

1) By placing the culture vessel with the liquid medium on an orbital shaker.

2) Filter paper bridge method can also be used.

3) Aeration can also be provided by passing sterilized air through the medium and by stirring the medium.

The culture vessels are closed with non-absorbent cotton covered in cheese cloth. This process allows proper aeration but prevents the entry of microorganisms.

Nutrient medium

The isolated plant cells and tissues require a basal medium for their growth. Various media compositions which are frequently used for tissue culture. A nutrient medium generally contains inorganic salts of major and minor elements, carbon source, vitamins, growth hormones and gelling agent. Other components added for specific purposes include organic nitrogen compounds, hexitols, amino acids, antibiotics and plant extracts. The composition of nutrient media involves the following ingradients:

Inorganic salts

Macro elements (in mol/l) like Na, K, P, Ca, Mg and S

Micro elements (in mol/l) like Ba, Mn, Zn, Mo, Cl, Ni and Al

Ammonium nitrate or any ammonium salts serves as nitrogen source.

KCl, KNO3, or KH2PO4 is the potassium supplement

Magnesium and sulphur are added in the form of magnesium sulphate

Vitamins

These are usually added in traces. The following vitamins are usually used:

1) Thiamine hydrochloride (0.1-1mg/l)
2) Pyridoxine HCL (0.5mg/l)
3) Nicotinic acid (0.5mg/l)
4) Folic acid (0.5mg/l)
5) Pantothenic acid (0.1mg/l)

Since, the vitamins are heat sensitive, they are filter sterilized and added with a syringe or pipette to the warm unsolidified autoclaved medium.

Growth hormones

Growth hormones play a very important role in callus culture. Auxins and cytokinins promote cell division, cell elongation, cell differentiation, and organ formation.

IAA (indole-3-acetic acid, 1-50mg/l), NAA (naphthalene acetic acid, 0.1-10mg/l), and 2, 4-D (2, 4-dichlorophenoxyacetic acid, 0.05-0.5mg/l) are used as auxin source.

Kinetin (0.01-10mg/l), Zeatin and Benzyl adenine are used as cytokinins.

Carbon source

The carbohydrates in form of sucrose or glucose (2-5%), as a carbon source are essentially required in tissue culture as cells or tissues are generally not photosynthetically active. Lower levels of carbohydrate may be used in protoplast culture but higher levels are required for embryo culture or anther culture.

Gelling agent

In tissue culture, washed or purified agar is used as a gelling agent, usually at a concentration of 0.6-1%. The agar must be kept in motion while dissolving, otherwise it will burn on the bottom of the flask. The agar must be completely dissolved before it is dispensed in to culture vessels.

Explants

A piece of plant tissue taken out from original site of plant and transferred to an artificial tissue culture media for the growth or maintenance is called as explants. It includes nodal and inter nodal segments of stem, apical and axillary bud, leaf, leaf disc, petiole, anther, pollen, flower bud, petal, inflorescence, ovule, ovary etc have been used as an explant. The choice of tissue depends upon on ultimate goal of the tissue culture project. Any piece of plant tissue can be used as an explants material. Various factors of an explants tissue source influence the tissue culture media. These are,

1) Physiological and ontogenetic age of organ or tissue
2) Quality of source plant
3) Season in which explants tissue is obtained
4) Size of the explants
5) Aim of the culture

Pretreatment to explant tissues

Explants, when brought to laboratory, should preferably be brought under ice to minimize the catabolic activity. Material was washed thoroughly with tap water for 3-
4 times after removal of adhering particles, mud etc,. Then the explant was kept in distilled water with liquid detergent (Tween- 20) and washed thoroughly under running tap water up to the complete removal of liquid detergent, and followed by3-4 times in distilled water. After proper cleaning, explant is sterilized and used. The explants preparation and sterilization are carried out under laminar air flow bench. The explants were sterilized with 70% ethanol, 0.1% aqueous solution of mercuric chloride for few minutes and then rinsed thoroughly with sterilized distilled water.

Types of culture [6]

Tissue culture can be categorized into several types. They are callus culture, cell culture, bud culture, meristem culture, protoplast culture, seed culture, embryo culture, organ culture.

In callus culture, the culture have initiated by planting a sterilize tissue on an agar medium. Within 2-4 weeks, depending upon plant species, a mass of unorganized cells is produced, it is known as callus. Such a callus can be subcultured indefinitely by transferring a small piece on to the fresh agar medium. Callus cultures can be obtained from seedlings, young shoots, buds, root tips and developing embryos etc. Cell culture is a type of suspension culture. These are initiated by transferring friable callus to a liquid medium and they are agitated on a rotary shaker at 80-150rpm, with an orbital diameter of 2.5-5.0cm. Agitation causes the dispersion of cells and each cell can grow into a new plant.

Stages of Micropropagation[5]

Different stages of micropropagation are,

1) **Establishment:** Micropropagation begins with the selection of plant material to be propagated. Clean stock materials that are free of viruses and fungi are important in the production of the healthiest plants. Once the plant material is chosen for culture, the collection of explant(s) begins and is dependent on the type of tissue to be used; including stem tips, anthers, petals, pollen and other plant tissues. The explant material is then surface sterilized, usually in multiple courses of bleach and alcohol washes, and finally rinsed in sterilized water. This small portion of plant tissue, sometimes only a single cell, is placed on a growth medium, typically containing sucrose as an energy source and one or more plant growth regulators (plant hormones).

2) **Multiplication**: Multiplication is the taking of tissue samples produced during the first stage and increasing their number. Following the successful introduction and growth of plant tissue, the establishment stage is followed by multiplication. Through repeated cycles of this process, a single explant sample may be increased from one to hundreds or thousands of plants. Depending on the type of tissue grown, multiplication can involve different methods and media.

3) Plantlet regeneration: Plantlets are produced through rooting of isolated shoots or germination of somatic embryos. For this purpose, shoots of proper length or age are required. Shoots are separated from clusters and transferred on a rooting medium containing an auxin. Cultures for rooting are placed in low light intensity or the lower portion of the culture tubes is covered with black paper to facilitate root induction. Low salt strength of rooting medium facilitates the rooting.

4) Preparation and Transfer to Field: This stage is concerned with transfer of plantlets in pots, their hardening and establishment in soil. Hardening refers to the preparation of the plants for a natural growth environment. Until this stage, the plantlets have been grown in "ideal" conditions, designed to encourage rapid growth. Due to the controlled nature of their maturation, the plantlets often do not have fully functional dermal coverings. This causes them to be highly susceptible to disease and inefficient in their use of water and energy. *In vitro* conditions are high in humidity, and plants grown under these conditions often do not form a working cuticle and stomata that keep the plant from drying out. When taken out of culture, the plantlets need time to adjust to more natural environmental conditions. Hardening typically involves slowly weaning the plantlets from a high-humidity and low light. When plantlets are taken out from the vessels, adhering agar is removed by careful washing with running tap water and plantlets are transferred in autoclaved soil or vermiculate and placed in mist-house under high humidity conditions. Plantlets are irrigated with dilute nutrient solution and they are covered with glass beakers. Plantlets are gradually exposed to high light intensity and decreased humidity.

Hardened plants are then transferred to a glass or poly houses with normal environmental conditions. Plants are irrigated frequently and their growth and variation are monitored regularly. Plants may be kept up to flowering or transferred to field after 4 to 6 weeks of acclimatization.

RESULTS AND DISCUSSION

Micropropagation holds the key for solving a myriad of issues that the pharmaceutical industry and the research area is facing today. It can provide large number of identical plants which can definitely reduce the stress on the existing resources and also preserve them for the future. For this, the intervention of tissue culture has to be precise; i.e. accelerating clonal multiplication of desired clones and strains (high-yielding) of medicinal plants through micropropagation and their conservation through establishing tissue culture banks or Gene banks. Thus, 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 fecundity 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 useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seed cannot 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 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.[7]

As every coin has two sides, Micropropagation also has its share of disadvantages. To mention a few it includes: It is very expensive, and can have a labour cost of more than 70% ; A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections; An infected plant sample can produce infected progeny. This is uncommon as the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.

Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant. Sometimes plants or cultivars do not come true to type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line. Some plants are very difficult to disinfect of fungal organisms.

The major limitation in the use of micropropagation for many plants is the cost of production; for many plants the use of seeds, which are normally disease free and produced in good numbers, readily produce plants in good numbers at a lower cost. For this reason, many plant breeders do not utilize micropropagation because the cost is prohibitive. Other breeders use it to produce stock plants that are then used for seed multiplication. Mechanisation of the process could reduce labour costs, but has proven difficult to achieve, despite active attempts to develop technological solutions.[8]

Tissue culture of medicinal plants has been looked upon with great interest. The application of tissue culture for large-scale plant production meant for commercial purposes is well demonstrated in the case of several crops and horticulture species.For example; solasudine content in berries of Solanum khasianum Clarke (night shade) is maximum when they are changing their colour from greenish to yellowish, while the diosgenin content in tubers of Dioscorea floribunda Mart & Gal.(medicinal yam) reaches to its maximum after three years of vegetative growth of leader shoots. Ideally the herbal plants should be grown under uniform environmental conditions and the planting material must have the same genetic make-up as of the selected high-yielding clones which is only possible only when they are cloned through an in-vitro strategy, i.e., micropropagation.(9)(8)

CONCLUSION

Its high time that the scientific world starts focusing on the system of Tissue culture for sustainable protection of the nature and also for providing optimum therapeutic benefits to the ailing society. This article has dealt in detail regarding the evolution of tissue culture, the standard methodology adopted for the process in general, the potential advantages and disadvantages of the same and the possible suggestions as to how to make this universal. Although there are a number of works of tissue culture are available on different sources the actual situation of the plants produced in this way, the difficulties faced by it while transferring it to normal conditions to the soil etc have not been practically discussed. This article also discusses on the importance of integration of various scientific disciplines like biotechnology, Indigenous systems of Medicine etc which can provide potential scientific leads for future research works.

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