



REVIEW ON PLANT TISSUE CULTURE: A SOURCE OF PHYTO-PHARMACEUTICALS

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Research Paper

Received: **22.08.2022**

Revised: **05.09.2022**

Accepted: **13.09.2022**

ABSTRACT

Since ancient times, plants have been used in the form of medicines in various traditional systems like Ayurveda, Unani, Homeopathy, Chinese and Siddha. Plants have been a potential source of natural products from various plant parts like root, stem, leaf and bark. Plants usually produce primary and secondary metabolites and use of plant tissue culture for production of secondary metabolites is quite common. The plant cells offer a novel route for the synthesis of a wide array of secondary metabolites, many of which are used as valuable drugs. Some of these are difficult to synthesize by conventional methods. The morphology and chemical totipotency of plant cells enables any cell of plant for the synthesis of compounds produced by the intact plant. The secondary metabolites on plant cell cultures can be significantly enhanced by elicitation, precursor feeding, media modification, hairy root culture, immobilization of plant cells, biotransformation or by manipulating culture conditions.

Keywords: Secondary metabolite, precursor addition, elicitation, hairy root culture, biotransformation, immobilization.

INTRODUCTION

India has a rich heritage of traditional medicine and people have been using these medicines from the time immemorial. The traditional system of medicine mainly consists of three major systems namely Ayurveda, Unani and Siddha. In almost every system medicinal plants play a major role and constitute the backbone of traditional medicine. In Ayurveda system most of the medicines are polyherbal in nature. Many of the herbs used in the system have become endangered. In recent years, however, it has become difficult to maintain an ample supply of medicinal plants due to several factors, such as their ruthless exploitation, lack of conservation of the environment, increasing labour costs and economical or technical problems associated with the cultivation of medicinal plants. To overcome these problems plant tissue culture is the best alternative. The use of tissue culture technique holds promise for the controlled production of phytopharmaceuticals [1].

Plants are the source of a large variety of biochemicals, which are metabolites of both primary and secondary metabolism. But secondary metabolites are of much greater interest since they have impressive biological activity like

antimicrobial, antibiotics, anticancer, insecticides, hormonal properties and valuable pharmacological and pharmaceutical activity, and many are used as flavours, fragrance, colour etc. The term secondary metabolites is ill-defined but convenient, it is applicable to all those compounds which are not directly involved in the primary metabolic process e.g. photosynthesis, respiration, protein and lipid synthesis etc. Secondary metabolites includes a wide variety of compounds e.g. alkaloids (morphine, nicotine), terpenoids (menthol, camphor), phenylpropanoids (coumarins, flavonoids, stilbenes), quinines, steroids etc [2].

In modern time organic compounds isolated from cultures of micro-organism, as well as from plants have been used for the cure of diseases (e.g. penicillin and tetracycline antibiotics). These organic compounds from natural sources form a large group known as natural products or secondary metabolites. These are distinguished more precisely from the primary metabolite by the following criteria: they have a restricted distribution being found in plants and micro-organisms, and are often characteristic of individual genera, species, or strains; they are formed along specialized pathway from primary metabolites. Primary metabolites by contrast, have a

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broad distribution in all living things and are intimately involved in essential life process.

It is interesting to note that secondary metabolites are biosynthesized essentially from a handful of primary metabolites: alpha –amino acid, acetyl coenzyme-A, mevalonic acid and intermediates of the shikmic acid pathway[3].

METHODS OF PLANT TISSUE CULTURE IN SECONDARY METABOLITE PRODUCTION

Plant cells in culture offer many advantages over intact plant for secondary metabolites production and their biosynthetic studies. These are as follows:

1. Plant cells are relatively easy to grow and can be kept under strict controlled nutritional and environmental conditions. Hence the uncertainties of climate and soil can be avoided [4].
2. Cells are cultured aseptically, devoid of many microorganism or insects etc [5].
3. Suspension culture offers a very effective way of incorporating precursors which are often very difficult to administer to the plant growing in nature[6].
4. The technology is now available for relatively large scale production of plant suspensions, in batch cultures, closed continuous culture system, open continuous culture system etc. and may also provide an efficient means of producing commercially important plant products [7].

Conditions to realize the industrial applications of plant cell culture for medicinal compound production:

1. The rate of cell growth and biosynthesis should be high enough to result into a good production of the final product in short period of time.
2. The cultured cell should be genetically stable to give a constant yield of the product.
3. The metabolites should be accumulated in cells without reacting rapidly or preferably, they should be released in the liquid medium.
4. Production cost including the culture medium, precursor and chemical extraction should be low enough to be profitable to the industries.

Plant Tissue Culture techniques for the production of secondary metabolites

Recently few new techniques, which have enhanced the secondary metabolites in plant tissue culture, have been developed:

1. Hairy root culture.
2. Elicitation of product accumulation.

The simplicity of the procedure and the outstanding results made the technique most useful and have drawn attention of most of the tissue culturists. Plant cell cultures proved to be more complex and very often the rate of production of desired compounds is also very less. The less productivity in cell suspension culture could be overcome by:

- a) By manipulation in the composition of the medium
- b) By altering physical environment of cells, and
- c) By screening for clones of high producing cells.

The ability of selecting high producing cells from cell suspension culture is not an easy task. To maintain elevated productivity, it is necessary to screen repeatedly the desired clones. This inherent instability is associated with the changes at the genome level, both inter and intrachromosomal.

3. Precursor Addition
4. Biotransformation
5. Immobilization

1. Hairy root culture

Recently, highly productive and stable hairy root culture are obtained by the genetic transformation of plant tissue by the pathogenic soil bacterium, *Agrobacterium rhizogenes*. The infection of dicotyledonous plants by *A. rhizogenes* causes root to proliferate rapidly at the infection site. The insertion of t-DNA (transfer DNA) into the plant genome causes root proliferation, carried out on the bacterial Ri-plasmid (root inducing plasmid), coding for the auxin synthesis and other rhizogenic function.

The hairy roots can be removed from the parent plants and can be cultured indefinitely in simple defined media free of auxins and phytohormones. In this respect they differ from untransformed. Hairy root cultures are potentially capable for the production of all root-derived secondary metabolites from dicotyledonous plants. Recently reports conclude that now it is also possible in some monocotyledonous plants [8].

Many of the root synthesized compounds including tropane alkaloids atropine and hyocyamine, steroidal precursors such as solasidine and *Catharanthus* alkaloids are of sufficient high value (1000 US \$ /kg) to justify the exploitation of hairy root culture for their commercial production.

Characteristics and Genetics of hairy root formation:

1. High degree of lateral branching.
2. A profusion of root hairs
3. Absence of geotropism
4. Hairy root morphology is expressed
5. Amino acid metabolism alters to produce opines, and
6. Secondary metabolites synthesis is enhanced.

Hairy root cultures: Improvement of secondary metabolite formation

Every plant species has its biosynthetic capabilities depending on its genetic makeup and environmental condition of its habitat. Therefore, some species are considered as high-yielding for a particular fine chemical while others are made to be high yielding by selective breeding programs. For the production of fine chemicals only high-yielding varieties are selected. In culture there varieties are not very stable and thus their product formation is not always constant. Hairy root has abilities similar to the plants from which they were derived as these remain genetically stable for a long time.

As plasmid (gene) transfer is involved in the formation of hairy roots, the system is highly amenable to manipulate at the genetic level. Recently foreign genes have also been inserted into the plants during transformation with *Agrobacterium rhizogenes* by either insertion into the Ri-plasmid. Thus the manipulation of secondary metabolites by altering the expression of key genes involved in the pathway is now a reality in the present context.

Commercial exploitation of hairy roots for secondary metabolites formation in the high producing strains has two following advantages:

1. Regeneration of improved varieties of plants for growth in the fields, and
2. The exploitation of the secondary metabolites formation of the roots in bioreactor system.

2. Elicitation for enhanced synthesis of secondary metabolites

Elicitors are the compounds of biological origin involved in the plant-microbe interaction. Elicitation is a process of enhanced synthesis of secondary metabolites by plants which ensures the survival of plants in adverse conditions.

- a) Abiotic elicitors
- b) Biotic elicitors

Abiotic elicitors are classified as physical or chemical and hormonal e.g. UV radiation, stress, alkalinity, Jasmonates, heavy metal ions, or osmotic pressure etc.

Biotic elicitors are complex culture homogenates of fungal or bacterial origin or fractions thereof, both pathogenic (*Phytophthora*, *Botrytis*, *Verticillium* etc.) as well as non-pathogenic (*Aspergillus*, *Micromucor*, *Rhodotorula* etc.) microbes have been employed for this purpose. The chemical nature of the elicitors is oligosaccharides, polysaccharides, glycoproteins and low molecular weight compounds like arachidonic acid [9].

3. Precursor addition

When precursors or intermediate precursors are fed in culture

medium they increase the synthesis of various phytochemicals in in-vitro conditions. They are considered to be the limiting factors such as Cinnamic acid or Phenylalanine which result in increased synthesis of Flavonoids and tropic acid for tropane alkaloids. High doses are proved to be lethal to tissue growth.

Shikonin production increases by three folds when *Lithospermum* sp. cultured cells are fed with L-phenylalanine, however, p-hydroxybenzoic acid is found ineffective in case of Shikonin. When *Datura* sp. cell suspension cultures are supplemented with hydroquinone in trace amounts, the Arbutin synthesis increases considerably from economic point of view because it is necessary that the precursor must be of low price than the ultimate product [10].

4. Biotransformation

One of the advantageous techniques is the biotechnological application of plant cell culture is the biotransformation of some less important substrates to medicinally useful products that could be used for therapeutic purposes. Otherwise, the synthesis of these chemicals is very difficult and not feasible economically. Their synthesis by plant cell culture method is easier than that done by the microorganism. Several biotechnologists have therefore realized the importance of biotransformation technology recently. It has been observed that anaerobic conditions of cell physiology favor the biotransformation rate.

Biosynthesis can be defined as chemical transformation which is catalyzed by microorganism or their enzymes. Enzymatically catalyzed biotransformation is superior to chemically catalyzed reaction because of:

1. **Reaction specificity:** Only one type of reaction takes place, hence no side reaction occurs.
2. **Regiospecificity:** specific in relation to the position of the reaction in substrate molecules.
3. **Stereospecificity:** only one enantiomer can be selected or a racemic mixture.
4. **Mild reaction condition:** can be formed under mild condition of temp (less than 40°C) pH (in vicinity of 7), and in aqueous solution.

Lowering of activation Energy: It is important for transforming the specific molecule at the specific position. Biotransformation can be carried out by growing cells, spores immobilized cells or enzymes. Cell culture can successfully be applied in biotransformation in two ways.

- a) Growing cells
- b) Stationary cells

5. Plant cell Immobilization

The plant cells are immobilized on an inert support by simple manipulation of the cell environment to enhance the yields of

secondary metabolites. Initial techniques of immobilisation were done using enzymes or microbes using some kind of gels [11,12]. The idea is to create a direct contact between the gel matrix and the cells and then the cells are subjected to high concentration of ions and organic compounds. Although the immobilisation procedure could lead to undesirable effects on cell metabolism. The use of metal biomass support particles which could entrap fungi and yeast in passive manner [13].

Secondary metabolite production has both medicinal and industrial applications. Though problems such as requirement for dedifferentiation of plant cell for the production of certain compounds is difficult when using cell immobilisation techniques [14].

Biological control on production of secondary metabolites

Active production of pharmacologically important compounds by plant cell cultures is mainly dependent upon following factors:

Growth Factor:

Biosynthetic activity of cells in a batch culture depends on cell growth and substrate utilization. Not much is known about the correlation between the rate of secondary metabolites formation and age of individual cell in culture. However production growth pattern can be categorized in three major types:

First type: product production proceed parallel with cell growth for e.g. anthraquinone, nicotine

Second type: product productions delayed until cell growth declines or stops, e.g. shikonin,

Third type: product production declines as the cell growth increases for e.g. diosgenin, ascorbic acid, etc.

Morphogenic differentiation:

In nature, certain compounds are synthesized and stored up only in some specific plant parts such as essential oils in certain sex glands or ducts; tropane alkaloids in roots of tobacco;

And latex in laticifer ducts. The cells in suspension culture can not synthesize such compounds reported in intact plants but if organogenesis is induced in cell cultures, these are synthesized in vitro.

In *Scopolia parviflora* suspension culture, root initiation coupled with normal production of tropane alkaloids; alkaloids contents increases many folds when organogenesis is induced in suspension culture of *D. innoxia*.

Shikonin, a derivative, found localized in cork cell only, has been produced experimentally in suspension cultures.

Inducing morphological differentiation in culture can have two effects:

- To enable the accumulation of a group of compounds
- To alter the qualitative composition of the product accumulation

Other examples are mint oil production in *Mentha* culture, *Ruta graveolens*, etc.

Chromosomal stability in cultures

Transformed roots usually show the normal level of ploidy for the species from which they are derived and retain their chromosomal complement over numerous year of culture. In contrast, dispersed culture or callus culture shows extensive aneuploidy and polyploidy, and the degree of genetic heterogeneity varies with time. When transformed tissue is grown as a dispersed culture, chromosomal aberration is introduced leading to extensive aneuploidy in the population.

Genetic manipulation of secondary metabolite formation

For the genetic manipulation of secondary metabolite product pathway it is essential to have:

- The gene or the genes to be manipulated
- A delivery system that will introduce the DNA into the genome of the desired plant species
- An expression cassette tailored to give expression of the gene in the light tissue and compartment
- A tissue culture and/or regeneration method in order that cultures and plants of the engineered strain may be obtained.

DNA can now be delivered to plant in a variety of ways as:-

- ❖ *Agrobacterium*-mediated plasmid based integration
- ❖ Biolistic bombardment
- ❖ Uptake of naked DNA by protoplasts
- ❖ Ultrasonication
- ❖ Microinjection

Environmental control on production of secondary metabolites

There are a number of environmental factors which affect the growth of the culture as well as the metabolites. Light is considered as important environmental factor which controls the synthesis of many secondary metabolites in vitro. An appreciable quantitative change is observed in volatile oil content in cell culture of *Ruta graveolens* when grown in light and dark. Stimulatory effect of light on the production of medicinal compounds has also been reported by many workers.

Inhibitory effect of light is also observed specially with white and blue light on shikonin derivative formation when cell cultures of *Lithospermum* sp. however, in other cases no

significance changes in the content of secondary metabolite production could be observed when cell cultures were grown in light and dark.

Effect of nutrients on production of secondary metabolite

It has been reported that certain nutrients in culture media increase some secondary metabolites while other shows inhibitory effect. In addition to nutrients, growth hormones, vitamins etc. in culture media is primarily aimed to increase cell growth in cultured condition.

e.g. Addition of sucrose in culture media above its ordinary level increases Shikonin in cultured cells, lower concentration of sugar increases production of ubiquinone-10 in tobacco cell culture, carbon-nitrogen ratio (C : N) plays a vital role in the increase [production of catechol tannins in *Scynamore* sp. Suspension culture unfortunately, not much more study has been made focus in this field.

CONCLUSION

The secondary metabolites have high economical and pharmacological importance. Industries are deeply interested in large variety of chemical substances being produced by plants due to their lesser toxicity. Though these substances are greatly extracted from the plant parts, the plant tissue culture technology has widened the scope and opened new vistas for the production of secondary metabolites. Thus many drugs like Taxol, Shikonin and Forskolin have been produced through plant cell culture commercially on large scale. Certainly, efforts expanded in biotechnology will prove more successful as research continues in this area.

Role of new technologies and future perspectives

Due to the immense importance of secondary metabolites to mankind, the introduction and optimization of plant tissue culture techniques in their production is inevitable. These techniques not only enhance the secondary metabolite production but also result into large scale production of these compounds in quick and cost effective manner. Moreover, these techniques are environment friendly. Hence, the production of secondary metabolites need to be elevated by incorporation of new biotechnological techniques.

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