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Plant Cell and Organ Culture Methods for Production of High Value Phytochemicals from Plants

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Abstract—*Plants are rich source of beneficial phytochemicals like alkaloids, steroids, terpenoids, phenolics, sweetners, bittering agents, pigments and perfumes. These plant-derived chemicals are used in large number of industrial products, including agrochemicals, pharmaceuticals and food additives. There is a continued commercial demand for these naturally available chemicals in food and pharmaceutical industries. In vivo production of phytochemicals is mainly achieved by field cultivation of plants. It is difficult to cultivate some plants outside of their original ecosystems. The isolation of these high value compounds through the extraction of organs and seeds of whole plants is difficult and costly. Low yield of secondary compounds from field grown plants, slow growth and variability in accumulation of product have made in vitro culture methods preferred choice for secondary metabolite production. Many studies have reported in vitro production of these bioactive compounds from plants using cell, tissue and organ culture methods. Various methods like free cell suspension culture, immobilization, elicitation, biotransformation and hairy root culture has been developed to produce and accumulate these compounds under in vitro conditions. Most of the studies are focused on development of cost effective and efficient methods for large-scale culturing of plant cells, tissues or organs and harvesting of commercially important phytochemicals. The purpose of this paper is to provide brief overview of plant cell and organ culture methods used in literature for enhanced production of high value plants derived compounds.*

Keywords*: Plant cell cultures; Phytochemicals; Elicitation; Biotransformation; Hairy root cultures; Immobilization.*

1. INTRODUCTION

Plants are valuable sources of industrially important natural products, which include pigments, essential oils, flavours, fragrances, sweeteners, feed-stocks, antimicrobials and pharmaceuticals. Most of the high value bioactive compounds are obtained by extraction from the naturally grown whole plants. Destruction of plants from their natural resources for harvesting of phytochemicals is expensive, time-consuming and has caused a major threat to the plant species by over harvesting [1].

The preference of consumers for natural food colors and flavors over synthetic counterparts increases our dependence on plants. About 25% prescription medicines and various raw materials used in the industries are obtained from plants. Many high value phytochemcials are synthesized by plants. Some of the important plant-derived chemicals, their cost (US $/Kg) and health benefits is listed in Table 1. Considering the high economical and pharmacological importance of phytochemicals, industries are deeply interested in utilizing plant tissue culture technology for large-scale production of these substances [2]. Production of these secondary metabolites is very low under *in vivo* conditions and depends greatly on the physiological and developmental stage of the plant [3]. Natural sources for the manufacture of these products are not enough to meet the consumers' demand and efforts have been made by various researchers to develop technology for their production at an industrial level.

Increased demand of plant derived high value biologically active chemicals in agriculture, food and pharmaceutical industry has increased interest in plant tissue culture technology for secondary metabolite production. Plant cell, tissue and organ culture is an attractive alternative for the large-scale production of valuable phytochemicals. It is a flexible system that can easily be manipulated to increase the secondary metabolite production. Plant tissue culture offers well defined production system with controlled supply of products independent of plant availability. However, poor control of cellular differentiation, slow growth of plant cells, intracellular storage of products and low productivity have restricted wider application of plant cell cultures. Plant cell metabolism involved in biosynthesis of secondary metabolites needs be realized fully for understanding the mechanism involved in production of these phytochemicals. Plant cell culture and organ culture system requires physical and chemical environment like that of intact plant for efficient production of high value phytochemicals. In this paper, different *in vitro* culture methods used for enhanced

production of phytochemicals form plants have been discussed and highlighted.

Table 1: List of important phytochemicals their cost and health benefits.

Phytochemical	Health benefits	Plant species	Approximate cost (US $/kilogram)
Ajmalicine	Circulatory problems	*Catharanthus roseus*	3,215
Artemisinin	Antimalarial	*Artemisia annua*	400
Azadirachtin	Antimicrobial	*Azadirachta indica*	330
Berberine	Antibiotic	*Coptis japonica*	3,250
Cardamon	Flavor	*Elettaria Cardamomum*	100
Cinnamon	Flavor	*Cinnamomum camphora*	200
Caffeine	Stimulant	*Coffea arabica*	300
Camptothecin	Anticancer	*Camptotheca acuminata*	25,000
Capsaicin	Counterirritant	*Capsicum frutescens*	500
Codeine	Pain reliever	*Papaver somniferum*	18,000
Colchicine	Antigout, Antitumour	*Colchium autumnale*	10,000
Digoxin	Heart stimulant	*Datura lanata*	3,000
Diosgenin	Steroidal precursor	*Dioscorea deltoidea*	800
Forskolin	Antiallergic and heart problems	*Coleus forskohli*	200
Morphine	Analgesic	*Papaver somniferum*	3,40,000
Paclitaxel	Anticancer	*Taxus brevifolia*	35,000
Quinine	Antimalarial	*Cinchona ledgeriana*	1,000
Rosmarinic acid	Anti-inflammatory	*Coleus blumei*	300
Sanguinarine	Antiplaque	*Sanguinaria canadensis*	1,000
Shikonin	Antimicrobial	*Lithospermum erythrorhizon*	4,500
Vincristine	Antileukemic	*Catharanthus roseus*	2,000,000
Vinblastine	Antileukemic	*Catharanthus roseus*	1,000,000

Adapted from [13]

2. METHODS FOR PRODUCTION OF PHYTOCHEMICALS

2.1. Cell suspension culture

Cell suspension culture is the most convenient mode of mass cultivation of plant cells and large-scale production of bioactive compounds. Suspension cultures of plant cells by providing necessary culture media requirements results into adequate growth and efficient product formation. In order to make effective use of plant cell suspension in large-scale cultivation as an alternative source of natural compounds, the retention of both adequate growth rates and efficient product formation is necessary. Production of secondary metabolites by plant cell suspension culture is generally low because of poor growth rates and low densities of cultures resulting from low concentrations of the nutrients present in the medium. Plant cell suspension grows slowly in a normal culture vessel because of the limited space, inadequate supply of oxygen and improper available of nutrients from the medium. Many research studies have been focused on commercialization of plant cell suspension culture for production of phytochemicals [4]. Some of the standardized protocols of plant cell suspension culture have already been introduced in the market.

Specially designed culture vessels called as bioreactors are available for large scale plant cell suspension culture. The main problems that are encountered in plant cell suspension culture at high density are cell aggregation and hydrodynamic stresses generated by the aeration-agitation system used for supplying oxygen. There are some reports available in literature on the development of different types of plant bioreactors to overcome these problems in cell suspension cultures. Plant bioreactors provide controlled environmental conditions and are easy to scale up. Many important phytochemicals are currently being produced in plant bioreactors [5]. Plant cell suspension technology has emerged as new area of research for industrial level production of secondary metabolites [4, 6].

2.2. Plant cell immobilization

Immobilization of plant cells on or within polymer matrices offers the potential of overcoming many current obstacles in secondary metabolite production. It is recognized as a mean to manipulate environment around cell by which the activities of particular enzymes of secondary pathways have been found to be enhanced as compared with liquid-suspended cells to overproduce secondary metabolites [7]. Problem associated with free cell suspension culture like cell aggregation, slow growth, low shear resistance, accumulation of products within the cell, low yield of product and genetic instability of the cell line could be reduced or over come by immobilization [8]. Immobilization methods commonly used are gel encapsulation, surface immobilization and entrapment by membrane barriers. Immobilization forms diffusion gradients around and between the cells. Immobilization of plant cells changes the physiology of the cells as matrix it self can act as an inducer or a repressor for certain metabolic processes and physical barrier for the formation of plasmodesmata between cells. Immobilized plant cell suffer from compressive stresses due to restricted space in matrix that could influence the biochemical differentiation and enhances secondary metabolite production. Immobilization promotes spontananeous release of secondary metabolites that are normally stored in cytoplasm and vacuoles of cell in free cell suspension culture [9]. Problem associated with free cell suspension culture like cell slow growth, accumulation of products within the cell, low shear resistance, low yield of

product and genetic instability of the cell lines and cell aggregation could be reduced or over come by plant cell immobilization [10]. Although plant cell immobilization represents an important alternative, a very few commercial successes has been obtained for the production of valuable secondary compounds at industrially useful levels. The disadvantage of immobilization is that it is only of use with those plant cell lines, which excrete secondary metabolite into the culture medium. Cost of immobilized plant cell culture for secondary metabolite production was found to be higher than other *in vitro* culture methods.

2.3. Plant cell elicitation

Elicitation is the induced or enhanced biosynthesis of metabolites due to addition of trace amounts of elicitors. An 'elicitor' may be defined as a substance which, when introduced in small concentrations to a plant cell system, initiates or improves the biosynthesis of specific compounds. 'Elicitor' for a plant refers to chemical from various sources that can trigger physiological and morphological responses and phytoalexin accumulation. Elicitors induce product accumulation not only in intact plants or plant organs but also in plant cell cultures as a result of their defensive, protective or offensive reactions [11-12]. The effectiveness of elicitation for enhanced production of phytochemicals depends on interaction between the elicitor and the plant cell. The treatment of the plant cells with elicitors like polysaccharides, glycoproteins, chitosan, xanthan, methyljasmonate, salicylic acid, and heavy metals were found to have enhanced the production of secondary metabolites [13-14]. Elicitors may increase the production of secondary metabolites by modulating the rates of biosynthesis and accumulation. Though, elicitation enhances secondary metabolism in plants or plant cells *in vitro* but the exact mechanism of elicitation is not exactly understood. Elicitation provides an opportunity for intensive research in the field of biosciences for exploitation of plant cells for the production of secondary metabolites. Employment of elicitors in plant cell immobilization to stimulate secondary metabolism have helped in releasing higher quantity of secondary products. Elicitation proved beneficial in many plant species for enhanced production of bioactive compounds of medical and industrial interest [13].

2.4. Biotransformation

Biotransformations are the bio-chemical reactions catalyzed by cells, organs or enzymes. Many important bioactive compounds which can not be produced by synthetic routes are easily synthesized in plants due to their biochemical reactions and enzyme system. Plant cell cultures have ability to transform precursors into product of interest by using suitable biocatalysts in complex biochemical reactions. The main classes of biochemical reactions carried out by plant cell cultures in biotransformation are hydroxylation, glucosylation, oxido-reduction between alcohols and ketones, hydrolysis, epoxidation, reduction of the carbonyl groups, reduction of carbon-carbon double bond and nitroreduction [15-16]. Biotransformation using plant cell, organ and enzyme system was successfully used by many researchers to produce high value phytochemicals [15,17,18]. Large scale plant cell cultures in bioreactors are used for transforming cheap and plentiful substances into rare and expensive substances. Biotransformation studies by plant cell culture have great potential to generate phytochemicals using known precursors. Bioconversion is considered as an important tool for the structural modification of molecules to get compounds possessing useful properties. The success rate in biotransformation studies depends on many factors like solubility of precursors, localization of enzymes, enzyme activity, presence of side reactions producing undesired byproduct and presence of enzymes degrading the desired product [15]. Many studies have focused on the ability of plant cell cultures to transform exogenous substrates in to important bioactive compounds [15-19].

2.5. Hairy root culture

Roots of many important medicinal plants are source of high value phytochemicals. *In vitro* cultures of roots are used for production of these metabolites. Establishment of hairy root cultures by transformation of plant tissues with *Agrobacterium rhizogenes* has been shown to be an efficient organ culture system for large-scale production of secondary metabolites. Root loci (rol) genes of Ri (root inducing) plasmid present in *A. rhizogenes* get incorporated into the nuclear genome of the host plant during infection. Expression of these genes causes excessive growth and proliferation of hairy roots. Hairy root cultures are characterized by auxin independent growth and stable metabolite production. The product content in transformed root cultures is higher than in parent plants [20]. Hairy root cultures are especially useful for the production of root-associated metabolites because of their high-growth rate and genetic stability. They are unique in their genetic and biosynthetic stability, faster in growth, and more easily maintained. The hairy root cultures have been exploited as an alternative source of phytochemicals and were mainly used for production of terpenoids, alkaloids and phenolics [20]. Hairy roots accumulate secondary metabolites like that of naturally growing plant and remain stable. Hairy root cultures have been established for many plant species and using this method a wide range of chemical compounds has been synthesized [21]. Transgenic hairy root cultures have now revolutionized the role of plant tissue culture in secondary metabolite production. Genetic modification of hairy root cultures using genes encoding the enzymes involved in secondary metabolism of high value compounds needs to be explored further for production of many high value plant derived compounds.

3. CONCLUSION AND FUTURE PERSPECTIVES

Plant cell and organ culture represents an important alternative for the production of valuable phytochemicals at industrially

useful levels. *In vitro* production of secondary metabolites continues to attract considerable attention. Many plant species are being studied using the technique, and novel strategies are being employed with the aim of augmenting the accumulation of high value bioactive compounds. There is a need for further research in this field for better understanding of the regulation of specific secondary metabolism so that it can be manipulated as desired. There is a need to undertake systematic studies on cell suspension cultures and transformed root cultures to enhance the production of therapeutically important secondary compounds by employing a variety of elicitors, optimization of microenvironment and somaclonal variations coupled with *in vitro* induced mutagenesis. New tools of functional genomics combined with metabolomics and proteomics will revolutionize our knowledge on pathways and enzymes involved in the synthesis of natural products. The increasing demands of secondary metabolites from plants for different purposes have necessitated our immediate concern for development of highly efficient tissue culture methods for production of these important compounds.

The increased use of molecular level tools and more in depth understanding of the secondary mechanisms for these compounds in plants would enable researchers to get more benefit by further improvement in these *in vitro* culture methods. At present, many *in vitro* methods are available but the new challenges that should be addressed include cost efficiency, automation and optimization of microenvironment for continuous production.

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