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PLANT TISSUE CULTURE - THE ALTERNATIVE AND EFFICIENT WAY TO EXTRACT PLANT SECONDARY METABOLITES

S Mukta[1], S R Ahmed[1], D Afrin[2]

[1]*Dept. of Plant and Environmental Biotechnology, Sylhet Agricultural University, Sylhet-3100*
[2]*Dept. of Animal and Fish Biotechnology, Sylhet Agricultural University, Sylhet-3100*

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Abstract

Plants are potent biochemical factories. Plant cell culture systems represent a potential renewable source of valuable medicinal compounds, flavors, fragrances and colorants. Biotechnological applications of plant cell cultures present the most updated reviews on current techniques in this field. Various types of cultures like organ culture, shoot culture, root culture, callus culture, suspension culture play the important roles in extraction of secondary metabolite and this methods can be used in large scale production. The principal advantage of this technology is that it may provide continuous, reliable source of plant pharmaceuticals and could be used for the large scale culture of plant cells from which these metabolites can be extracted. Additionally, in the discovery of new medicines, plant cell culture technology plays an even more significant role in solving world hunger by developing agricultural crops that provide both higher yield and more resistance to pathogens and adverse environmental and climatic conditions. This callus and suspension culture methods can be able to play an important role in large scale production of bioactive secondary metabolites from plants.

***Keywords*:** Secondary metabolite, tissue culture, suspension culture, callus.

Introduction

Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides (Balandrin and Klocke, 1988). Since the early days of mankind, plants with secondary metabolites have been used by humans to treat infections, health disorders and illness (Wyk and Wink, 2004). Many higher plants are major sources of useful secondary metabolites which are used in pharmaceutical, agrochemical, flavor and aroma industries. The search for new plant-derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity (Philipson, 1990). Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants (Rao and Ravishankar, 2002). Plant cell and tissue cultures hold great promise for controlled production of myriad of useful secondary metabolites on demand. In order to obtain high yields suitable for commercial exploitation, efforts have been focused on isolating the biosynthetic activities of cultured cells, achieved by optimizing the cultural conditions, selecting high-producing strains and employing precursor feeding, transformation methods, and immobilization techniques (DiCosmo and Misawa, 1995). Advances in tissue culture, combined with improvement in genetic engineering of pharmaceuticals, nutraceuticals, and other beneficial substances (Hansen and Wright, 1999). Recent advances in the molecular biology, enzymology and fermentation technology of plant cell cultures suggest that these systems will become a viable source of important secondary metabolites (Abdin, 2007). On a global scale, medicinal plants are mainly used as crude drugs and extracts. Several of the more potent and active substances are employed as isolated compounds, including many alkaloids such as morphine (pain killer), codeine (antitussive), papaverine (phosphordiesterase inhibitor), ephedrine (stimulant), ajmaline (antirrhythmic), quinine (antimalarial), reserpine (antihypertensive), galanthamine (acetylecholine esterase inhibitor), scopolamine (travel sickness), berberine (psoriasis), caffeine (stimulant), capsaicin (rheumatic pains), colchicines (gout), yohimbine (aphrodisiac), pilocarpine (glaucoma), and various types of cardiac glycosides (heart insufficiency) (Wink *et al*., 2005). The major advantages of cell culture systems over the conventional cultivation of whole plants are:

***Corresponding author: S Mukta,** Dept. of Plant and Environmental Biotechnology, Sylhet Agricultural University, Sylhet-3100, Bangladesh, Email: mukta.peb@sau.edu*

- Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions;
- Cultured cells would be free of microbes and insects;
- The cells of any plants, whether tropical or alpine, could be easily be multiplied to yield their specific metabolites;
- Automated control of cell growth and rational regulation of metabolite processes would contribute to reductions in labor costs and improvements in productivity; and
- Organic substances are easily extractable from callus cultures.

Plant secondary metabolites

The plant products of commercial interest are secondary metabolites, which in turn belong to three main categories: essential oils, glycosides and alkaloids. The essential oils consist of mixture of terpenoids, which are used as flavoring agents, perfumes and solvents. The glycosides include flavanoids, saponins, phenolics, tannins, cyanogenic glycosides and mustard oils, which are utilized as dyes, food colors and medicinals (e.g., steroid hormones, antibiotics and digitalis). The alkaloids are diverse group of compounds with over 4000 structures known; almost all naturally occurring alkaloids are of plant origin. Alkaloids are physiologically active in humans (e.g., cocaine, nicotine, morphine, strychnine) and therefore of a great interest for pharmaceutical industry. Secondary metabolites are chemicals produced by plants for which no role has yet been found in growth, photosynthesis, reproduction, or other "primary" functions. These chemicals are extremely diverse; many thousands have been identified in several major classes. Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants. Humans use some of these compounds as medicines, flavorings, or recreational drugs (Oksman-Caldentey and Inze, 2004).

Secondary metabolites can be classified on the basis of chemical structure (for example, having rings, containing a sugar), composition (containing nitrogen or not), their solubility in various solvents, or the pathway by which they are synthesized (e.g., phenylpropanoid, which produces tannins). A simple classification includes three main groups: the terpenes (made from mevalonic acid, composed almost entirely of carbon and hydrogen), phenolics (made from simple sugars, containing benzene rings, hydrogen, and oxygen), and nitrogen-containing compounds (extremely diverse, may also contain sulfur) (Rahini, 2014). Fig.1 showed a schematic presentation of obtaining plant secondary metabolite.

Plant tissue culture in secondary metabolite production

In plant cell culture, the isolated cells from the whole plant (or parts derived thereof) are cultivated under appropriate physiological conditions and the desired product is extracted from the cultured cells (Rahini, 2014). Optimization of secondary metabolite production is a sensitive process that depends on the dosage of environmental stress besides its stage of application during agriculture. Secondary metabolites produced by plants need to be further extracted and processed for specific use as pharmaceuticals, food additives and fine chemicals. Or else, they need to be manufactured by chemical synthesis. *In vitro* cultivation of plant cells in a bioreactor offers a controlled supply of secondary metabolites with consistent quality and yield independent of external factors (Fowler, 1985). Plant tissue culture refers to *in vitro* cultivation of any plant segment, whether a single cell, a tissue or an organ. There are five main types of plant tissue cultures: seedlings of plants (plant cultures), isolated embryos (embryo cultures), isolated plant organs (organ cultures), explant cultures (callus cultures), isolated cells or small aggregates dispersed in liquid media (cell suspension cultures).

Production of secondary metabolites using plant cell cultures has been a scientific challenge during the last fifty years due to low cell productivity, slow growth and genetic instability of productive cell-lines which makes the process infeasible. Most of the scientific studies on feasibility of the plant cell cultures have been directed (Goossens *et al.*, 2003; Sumner *et al*., 2003; Verpoorte and Memelink, 2002; Zhong, 2001; Memelink *et al*., 2001):

- To improve secondary metabolite yield by application of various chemical, physical and biological stresses as elicitors;
- To study the influences of these stresses on secondary metabolite production pathways;
- To study the effects of key intermediates of secondary metabolite pathways as a triggering mechanism for secondary metabolite biosynthesis;
- To manipulate regulator genes that are responsible from secondary metabolite biosynthesis;

- To clone genes that are responsible from the manufacture of secondary metabolites; and
- To analyze gene expression profiles that lead to the secondary metabolite biosynthesis.

So far, the only feasible industrial application of plant cell cultures has been shikonin, ginsenosides, berberine (Hara, 1996) and the anti-cancer drug taxol by Taxus cell cultures (Sajc *et al*., 2000). Table-1 showed production of bioactive secondary metabolites from plant cell cultures till now.

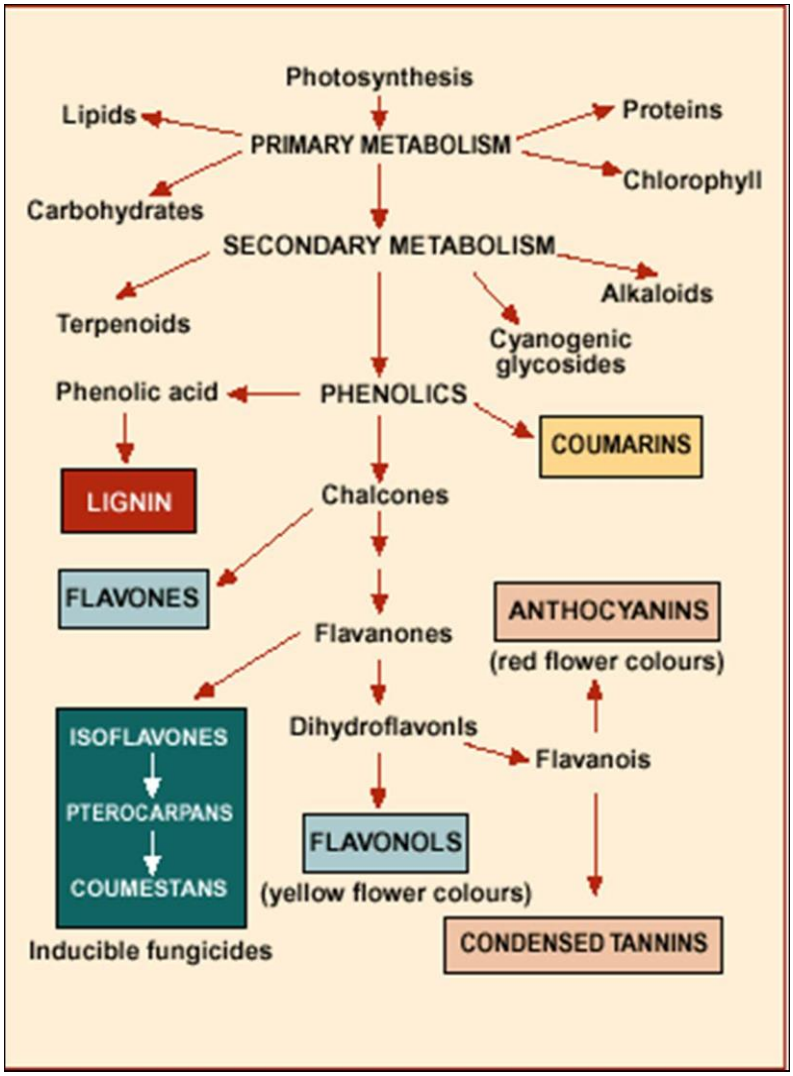

Fig. 1. Secondary metabolites (Rahini, 2014)

Research in the area of plant tissue culture technology has resulted in the production of many pharmaceutical substances for new therapeutics. Advances in the area of cell cultures for the production of medicinal compounds have made possible the production of a wide variety of pharmaceuticals such as alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids, and amino acids. Successful attempts to produce some of these valuable pharmaceuticals in relatively large quantities by cell cultures are illustrated (Jordon and Wilson, 1997). Taxol (paclitaxel), a complex diterpene alkaloid found in the bark of the *Taxus* tree, is one of the most promising anticancer agents known due to its unique mode of action on the microtubular cell system (Fig. 2A) (Cragg *et al*., 1993). Latex from the opium poppy, *Papaver somniferum* is a commercial source of the analgesics, morphine, and codeine. Callus and suspension cultures of *P. somniferum* are being investigated as an alternative means for the production of these compounds (Fig. 2B). Production of morphine and codeine in morphologically undifferentiated

cultures has been reported (Yoshikawa *et al*., 1985). L.3, 4-dihydroxyphenylalanine, is an important intermediate of secondary metabolism in higher plants and is known as a precursor of alkaloids, betalain, and melanine, isolated from *Vinca faba*, *Mucuna, Baptisia*, and *Lupinus* (Brain and Lockwood, 1976). It is also a precursor of catecholamines in animals and is being used as a potent drug for Parkinson's disease, a progressive disabling disorder associated with a deficiency of dopamine in the brain (Fig. 2C).Diosgenin is a precursor for the chemical synthesis of steroidal drugs and is tremendously important to the pharmaceutical industry (Tal *et al*., 1983). Zenk *et al.* (1978) reported on the use of cell cultures of *Dioscorea deltoidea* for the production of diosgenin (Fig. 2D).

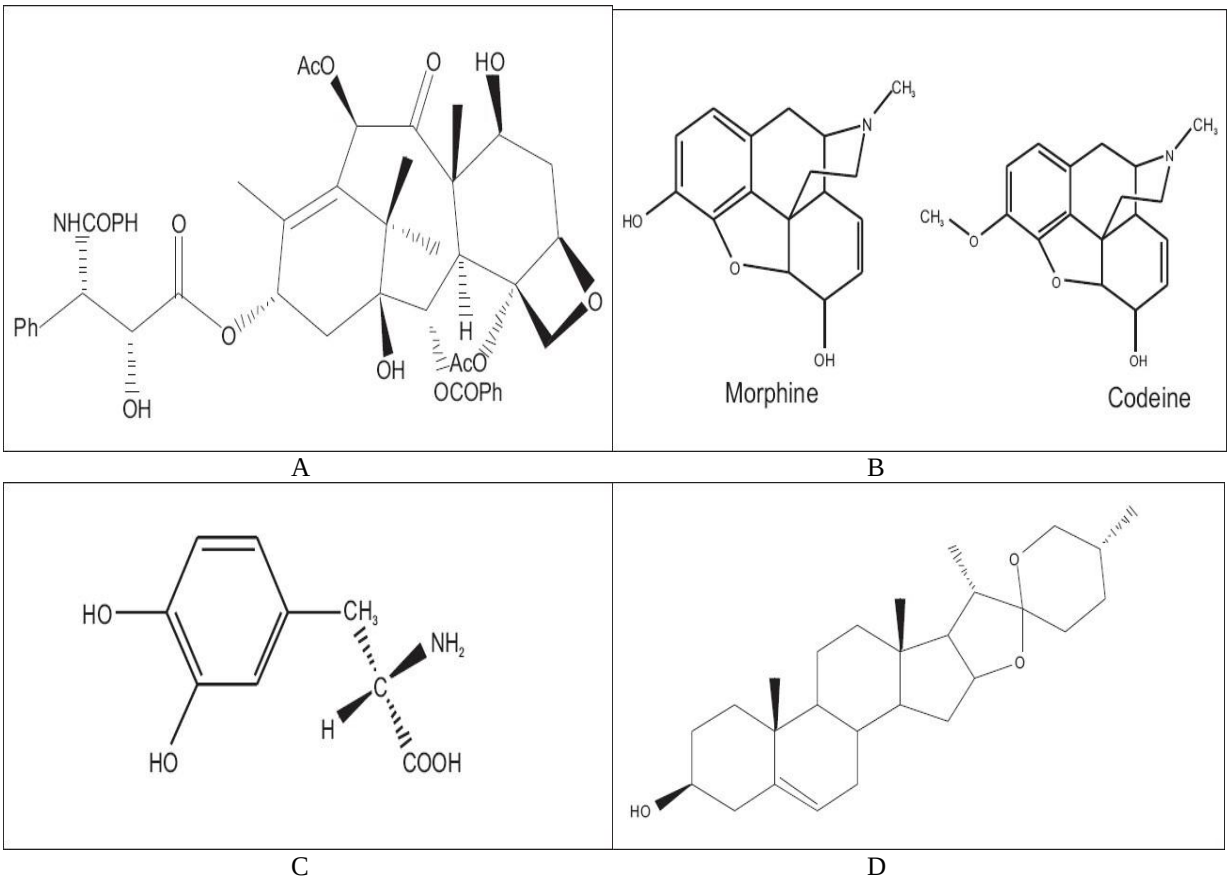

Fig. 2. Chemical structure of *A. Taxol, B. morphine, C. Dopamine* and *D. Diosgenin* (Cragg *et al*., 1993, Yoshikawa *et al*., 1985, Tal *et al*., 1983, Zenk *et al*.,1978)

Production of secondary metabolites in organ culture

The technique of *in vitro* organ culture of *Fritillaria unibracteata* has been established and chemical composition of the metabolites has been confirmed. *F. unibracteata* can be rapidly propagated, directly from small cuttings of the bulb by the technique of organ culture. The cultured bulb can be harvested after a 50-day culture period in MS media supplemented with 4.44M BA and 5.71M IAA. The growth rate was about 30–50 times higher than that under natural wild growth conditions. The content of alkaloid and beneficial microelements in the cultured bulbs was higher than found in the wild bulb (Gao *et al*., 2004).

In vitro shoot multiplication of *Frangula alnus* was obtained on woody plant medium with indole-3-acetic acid and 6-benzylaminapurine, the highest metabolite production of total anthraquinone was in the shoots grown on the MS medium with addition of 1-naphthilaceneacetic acid (NAA) (0.1 mg^{-1}) and thidiazuron (TDZ) (0.1 mg^{-1}) (Namdeo, 2007).

Shoot cultures of *Gentianella austriaca* established from seedling epicotyls were maintained on MS medium. Shoot cultures contained the same types of secondary metabolites as plants from nature. Xanthones were the major constituents, with DMB (demethylbellidifolin), DGL (dimethyl bellidifolin8-*O*glucoside) and BGL (bellidifolin-8-*O*-glucoside) present at roughly two times lower concentrations than in samples from nature. Secondary metabolite production was strongly affected by the presence of BA in the medium (Vinterhalter *et al.*, 2008).

Production of secondary metabolites in callus culture

Callus culture is the culture of dedifferentiated plant cells induced on media usually containing relatively high auxin concentrations or a combination of auxin and cytokinin in *in vitro* conditions (Namdeo, 2007). Callus cultures can be embryogenic or non-embryogenic. Embryogenic calli contain differentiated embryogenically competent cells that can regenerate complete plants through the process called somatic embryogenesis (Ptak, 2013). Madhavi *et al*. (1998) studied the isolation of bioactive constituents from *Vaccinium myrtillus* fruits and cell cultures. Fruits and casus cultures were extracted and fractionated. Major fractions contained flavonoids, such as cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside and proanthocyanidins. Anthocyanin accumulation in callus was lower (0.08 mg^{-1} dry cell weight; DCW) than in the fruit (27.3 mg^{-1} DCW). Callus cultures accumulated both oligomeric (178 mg^{-1} DCW) and polymeric (436 mg^{-1} DCW) proanthocyanidins; proanthocyanidins were similarly present in fruit extracts (oligo- and polymeric, 202 and 1613 mg^{-1}DCW, respectively). Stable and optimized callus cultures are a logical step in the first phase of the cell culture production of plant secondary metabolites, i.e. preparing the inoculum for liquid suspension cultures. Production of secondary metabolities in cell suspension cultures have been widely published and it was proposed as a technology to overcome problems of variable product quantity and quality from whole plants due to the effects of different environmental factors, such as climate, diseases and pests (Yamamoto *et al*., 2002).

Establishment of cell suspension culture

To establish cell suspension culture of a medicinal herb, firstly the callus should be induced. During the callus induction, explants of plant origin should be surface sterilized and sliced into pieces about 0.5 cm^3 in clean bench, and inoculated on autoclaved solid basic media (MS, B5, N6, White etc.) supplemented with sucrose, hormones and agar. After the callus was induced, it should be sub-cultured. After sub-culture, usually various types of callus can be found with different texture and color. Callus of various types should be introduced into liquid media (most of the time, after removal of agar, the formula of solid media can be used for liquid media preparation). Contents determination of active compounds should be carried out for cell line selection. In this step, cultured cells of different types should be sampled at each growth stage, and the cell samples will be subjected to biomass measurement and content determination as shown in Fig. 3, because cell of different types might be growing at different speed, and the maximal yield of target compounds can also be different when collected at same culture time. Once the cell line is selected, investigation on cell growth, cell viability, pH changing, carbon source consumption, enzyme activity, gene expression, target compounds accumulation can be carried out (Hu and Jia, 2012).

Production of secondary metabolites in hairy root culture

For many medicinal herbs, roots are the medicinal part used for extraction, such as *Panaxgiseng*, *Panaxnotogiseng*, *Coptis chinensis*, *Saviamilti orrhiza* and so on. Furthermore, it has also been found that, in differentiated tissues, secondary metabolites accumulation is usually higher than callus and cells without differentiation. Therefore, hairy root can be an effective candidate culture form. Hairy roots grow rapidly, show plagiotropic growth, and are highly branched on phytohormone free medium (Hu and Du, 2006). The strong correlation between secondary metabolite production and morphological differentiation gives more impetus to the application of organized cell cultures for large scale production of phytochemicals. Intergeneric co-culture of genetically transformed hairy roots and shooty teratomas is effective for improving tissue specific secondary metabolites. This mimics the situation observed in the whole plant where a localized metabolite synthesis is translocated throughout the organs for further bioconversion (Giri and Narasu, 2000). Also, production of two different secondary metabolites is possible simultaneously by adventitious root co-cultures (Wu *et al.*, 2008). Hairy root culture of *Stizolobium hassjo* to yield 3,4-dihydroxyphenylalanine was reported using 9L mist bioreactor. Present scale-up technology dictates the use of stainless steel tanks for growth of plant cells on an industrial scale. The usage of bioreactors equipped with special hangers inside the vessel is reported. Hairy root cultures continue to attract interest as a potential resource for large-

scale production of commercially valuable compounds. Fig. 4 depicts a biotechnological application in hairy root for secondary metabolite.

Genetic manipulation in hairy root culture for secondary metabolite production

Transformed roots provide a promising alternative for the biotechnological exploitation of plant cells. *A. rhizogenes* mediated transformation of plants may be used in a manner analogous to the well-known procedure employing *Agrobacterium tumifaciens*. *A. rhizogenes*-mediated transformation has also been used to produce transgenic hairy root cultures and plantlets have been regenerated (Karuppusamy, 2009). None of the other T-DNA sequences are required for the transfer with the exception of the border sequences. The rest of the T-DNA can be replaced with the foreign DNA and introduced into cells from which whole plants can be regenerated. These foreign DNA sequences are stably inherited in a Mendelian manner (Zambryski *et al.,* 1989). The *A. rhizogenes*-mediated transformation has the advantage of being able to transfer any foreign gene of interest placed in binary vector to the transformed hairy root clone. An example of a gene of interest with regard to secondary metabolism that was introduced into hairy roots is the 6-hydroxylase gene of *Hyoscyamus muticus* which was introduced to hyocyamin-rich *Atropa belladonna* by a binary vector system using *A. rhizogenes (*Hashimoto *et al.,*1993*).*

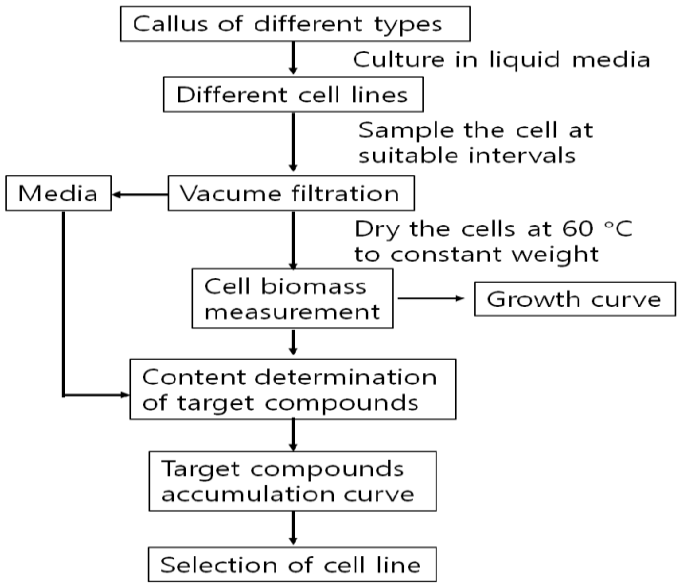

Fig. 3. Flow chart of cell line selection (Hu and Jia, 2012)

Bioreactors scaling up of production of secondary metabolites

In vitro manufacture of secondary metabolites is an interdisciplinary field which requires collaboration between plant physiologists, cell and molecular biologists, pharmacologists, chemists, and chemical engineers to assess:

- Tissue morphology and composition
- Flow and mass transfer conditions in the bioreactor
- Kinetics of cell growth and product formation
- Genetic stability of productive cell lines
- Control of micro and macro environment in the bioreactor
- Implications of bioreactor design on downstream processing
- The potential for process scale up.

Bioreactor operation can be batch, fed-batch or continuous. Batch bioreactors are used to determine optimum production conditions upon scale-up from small-scale fermentations in a flask. If secondary metabolite biosynthesis is growth-related, a single-step bioreactor is sufficient. Otherwise, stage-wise fermentation is recommended where the first bioreactor is used for culture growth and the second one is used for secondary metabolite biosynthesis (Payne *et al.*, 1991).

In air-lift bioreactors, aeration also provides mixing. Secondary metabolite biosynthesis is sensitive to aeration because:

- Secondary metabolite biosynthesis increases with increasing diameter of cell aggregates starting at a range of several millimeters.
- High mass transfer resistance caused by large aggregate size induces secondary metabolite biosynthesis due to lack of mass transfer towards the center of the cell aggregates.
- Cell aggregate size causes diffusion resistance hindering diffusion of intracellular substrates (Payne *et al*., 1991).

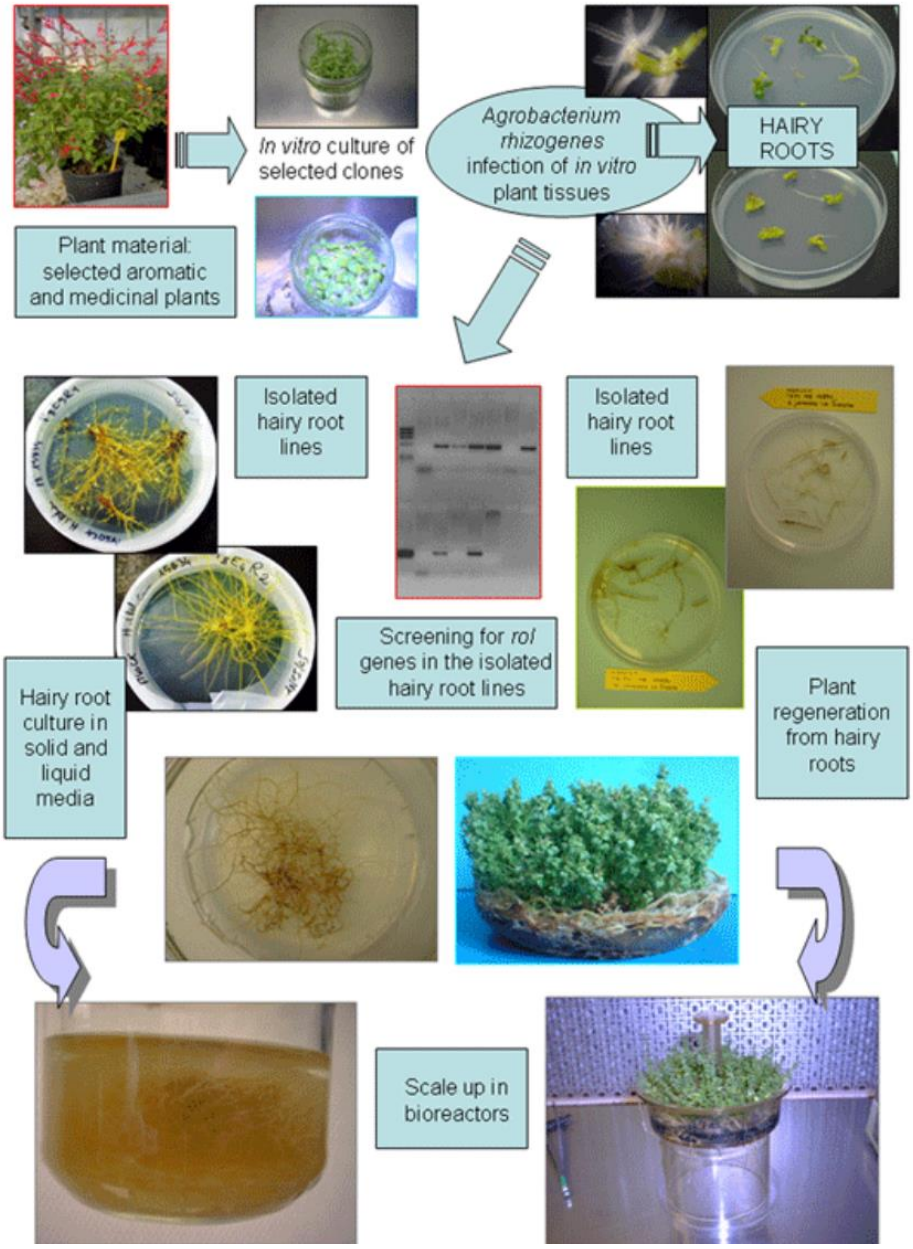

Fig. 4. Biotechnological applications of hairy roots research (Wu *et al.*, 2008)

Table 1: Secondary metabolites from plant cell cultures

Plant name	Active ingredient	Culture type	Reference
Agave amaniensis	Saponins	Callus	Vanisree and Hsin-Sheng, 2004
Ailanthus altissima	Alkaloids	Suspension	Karuppusamy, 2009
Ailanthus altissima	Canthinone alkaloids	Suspension	
Allium sativum L.	Alliin	Callus	Vanisree and Hsin-Sheng, 2004
Aloe saponaria	Tetrahydroanthraceneglucosides	Suspension	Vanisree and Hsin-Sheng, 2004
Ambrosia tenuifolia	Altamisine	Callus	Karuppusamy, 2009

Anchusa officinalis	Rosmarinic acid	Suspension	Hu and Jia, 2012
Brucea javanica (L.) Merr.	Canthinone alkaloids	Suspension	Hu and Jia, 2012
Bupleurum falcatum	Saikosaponins	Callus	Yamamoto *et al*., 2002
Camellia sinensis	Theamine, γ-glutamyl derivatives	Suspension	Vanisree and Hsin-Sheng, 2004
Canavalia ensiformis	L-Canavanine	Callus	Karuppusamy, 2009
Capsicum annuum L.	Capsaicin	Suspension	Karuppusamy, 2009
Cassia acutifolia	Anthraquinones	Suspension	Karuppusamy, 2009
Catharanthus roseus	Indole alkaloids	Suspension	Karuppusamy, 2009
Catharanthus roseus	Catharanthine	Suspension	Hu and Jia, 2012
Choisya ternata	Furoquinoline alkaloids	Suspension	Hu and Jia, 2012
Chrysanthemum cinerariaefolium	Pyrethrins	Callus	Karuppusamy, 2009
Chrysanthemum cinerariaefolium	Chrysanthemic acid and pyrethrins	Suspension	Hu and Jia, 2012
Cinchona L.	Alkaloids	Suspension	Vanisree and Hsin-Sheng, 2004
Cinchona robusta	Robustaquinones	Suspension	Hu and Jia, 2012
Cinchona spe.	Anthraquinones	Suspension	Hu and Jia, 2012
Cinchona succirubra	Anthraquinones	Suspension	Hu and Jia, 2012
Coffea arabica L.	Caffeine	Callus	Yamamoto *et al*., 2002
Citrus sp.	Naringin, Limonin	Callus	Karuppusamy, 2009
Cornuskousa	Polyphenols	Suspension	
Corydalis ophiocarpa	Isoquinoline alkaloids	Callus	Yamamoto *et al*., 2002
Croton sublyratus Kurz	Plaunotol	Callus	Yamamoto *et al*., 2002
Cruciata glabra	Anthraquinones	Suspension	
Cryptolepis buchanani	Cryptosin	Callus	Vanisree and Hsin-Sheng, 2004
Digitalis purpurea L.	Cardenolides	suspension	Hu and Jia, 2012
Dioscorea deltoidea	Diosgenin	Suspension	Hu and Jia, 2012
Dioscorea doryophora Hance	Diosgenin	Suspension	Karuppusamy, 2009
Duboisial eichhardtii	Tropane alkaloids	Callus	Yamamoto *et al*., 2002
Ephedra spp.	L- Ephedrine, D-pseudoephedrine	Suspension	Hu and Jia, 2012
Eriobotrya japonica	Triterpenes	Callus	Vanisree and Hsin-Sheng, 2004
Eucalyptus tereticornis	Sterols and Phenolic compounds	Callus	Yamamoto *et al*., 2002
Eucommia ulmoides	Chlorogenic acid	Suspension	Karuppusamy, 2009
Fumaria capreolata	Isoquinoline alkaloids	Suspension	Karuppusamy, 2009
Gentiana spp.	Secoiridoidglucosides	Callus	Yamamoto *et al*., 2002
Ginkgo biloba	Ginkgolide A	Suspension	Hu and Jia, 2012
Glehnia littoralis	Furanocoumarin	Suspension	Hu and Jia, 2012
Glycyrrhiza echinata	Flavanoids	Callus	Yamamoto *et al*., 2002
Glycyrrhiza glabra var. *glandulifera*	Triterpenes	Callus	Yamamoto *et al*., 2002
Hyoscya musniger	Tropane alkaloids	Callus	Karuppusamy, 2009
Isoplexis isabellina	Anthraquinones	Suspension	Karuppusamy, 2009
Linum flavum L.	5-Methoxypodophyllotoxin	Suspension	Karuppusamy, 2009
Lithospermume rythrorhizon	Shikonin derivatives	Suspension	Karuppusamy, 2009
Lycium chinense	Cerebroside	Suspension	Hu and Jia, 2012
Morinda citrifolia	Anthraquinones	Suspension	Hu and Jia, 2012
Nicotiana tabacum L.	Nicotine	Suspension	Vanisree and Hsin-Sheng, 2004
Panaxnoto ginseng	Ginsenosides	Suspension	Vanisree and Hsin-Sheng, 2004
Papaversom niferum	Morphine, Codeine	Suspension	Vanisree and Hsin-Sheng, 2004
Mucuna pruriens	L-DOPA	Callus	Karuppusamy, 2009
Nandina domestica	Alkaloids	Callus	Karuppusamy, 2009

Nicotiana rustica	Alkaloids	Callus	Yamamoto *et al*., 2002
Nothapodytes foetida	Camptothecin	Callus	Yamamoto *et al*., 2002
Ophiorrhiza pumila	Camptothecin related alkaloids	Callus	Vanisree and Hsin-Sheng, 2004
Panax ginseng	Saponins and Sapogenins	Callus	Vanisree and Hsin-Sheng, 2004
Papaver bracteatum	Thebaine	Callus	Yamamoto *et al*., 2002
Papaver somniferum L.	Alkaloids	Callus	Yamamoto *et al*., 2002
Peganum harmala L.	β-Carboline alkaloids	Suspension	Karuppusamy, 2009
Phytolacca americana	Betacyanin	Suspension	Hu and Jia, 2012
Picrasma quassioides Bennett	Quassin	Suspension	Hu and Jia, 2012
Podophyllum hexandrumroyle	Podophyllotoxin	Suspension	Hu and Jia, 2012
Polygonum hydropiper	Flavanoids	Suspension	Hu and Jia, 2012
Portulaca grandiflora	Betacyanin	Callus	Yamamoto *et al*., 2002
Pteleatri foliata L.	Dihydrofuro [2,3-b] quinoliniumalkaloids	Callus	Yamamoto *et al*., 2002
Rauwol fiasellowii	Alkaloids	Suspension	Karuppusamy, 2009
Rauwol fiaserpentina Benth.	Reserpine	Suspension	Karuppusamy, 2009
Trigonella foenumgraecum	Saponins	Suspension	Karuppusamy, 2009
Torreya nucifera var. *radicans*	Diterpenoids	Suspension	Vanisree and Hsin-Sheng, 2004
Thalictrum minus	Berberin	Suspension	Hu and Jia, 2012
Taxuscus pidata	Taxoids	Suspension	Hu and Jia, 2012
Tecomasam bucifolium	Phenylpropanoid glycosides	Callus	Vanisree and Hsin-Sheng, 2004
Taxus baccata	Taxol, baccatin III	Suspension	Vanisree and Hsin-Sheng, 2004
Taxus spp.	Taxol	Suspension	Hu and Jia, 2012
Tabernaemon tanadivaricata	Alkaloids	Suspension	Hu and Jia, 2012
Stizolobium hassjoo	L DOPA	Suspension	Hu and Jia, 2012
Solanum paludosum	Solamargine	Suspension	Hu and Jia, 2012
Solanuml aciniatum Ait	Solasodine	Suspension	Hu and Jia, 2012
Solanum chrysotrichum (Schldl.)	Spirostanolsaponin	Suspension	Vanisree and Hsin-Sheng, 2004
Scutellaria columnae	Phenolics	Callus	Yamamoto *et al*., 2002
Scopolia parviflora	Alkaloids	Callus	Yamamoto *et al*., 2002
Salvia miltiorrhiza	Lithospermic acid B and Rosmarinic acid	Callus	Yamamoto *et al*., 2002
*Ruta s*p.	Acridone and Furoquinoline, alkaloids, coumarins	Callus	Yamamoto *et al*., 2002
*Rauvolfias erpentina*x *Rhazyastricta (*Hybrid plant)	3-Oxo-rhazinilam	Callus	Vanisree and Hsin-Sheng, 2004
Salvia miltiorrhiza	Cryptotanshinone	Suspension	
Sapium sebiferum	Tannin	Callus and Suspension	Vanisree and Hsin-Sheng, 2004
Salvia fruticosa	Rosmarinic acid	Callus and Suspension	Vanisree and Hsin-Sheng, 2004
Aconitum heterophyllum	Aconites	Hairy root	Namdeo, 2007
Adhatod avasica	Vasine	Shoot Culture	Vanisree and Hsin-Sheng, 2004
Agasta cherugosa	Rosmarinic acid	Hairy root	Karuppusamy, 2009
Ajuga reptans	Phytoecdysteroids	Hairy root	Namdeo, 2007
Ammim ajus	Umbelliferone	Shootlet	Namdeo, 2007
Corydalis ambigua	Corydaline	Embryo	Karuppusamy, 2009
Fritillaria unibracteata	Alkaloids	Multiple Shoot	Vanisree and Hsin-Sheng, 2004
Gypsophila paniculata	Saponin	Root	Karuppusamy, 2009

Immobilization scaling up of secondary metabolite accumulation

Advances in scale-up approaches and immobilization techniques contribute to a considerable increase in the number of applications of plant cell cultures for the production of compounds with a high added value. Plant-derived compounds with cancer chemotherapeutic or antioxidant properties use rosmarinic acid and taxol as representative examples. Cell cultures of *Plumba gorosea* were immobilized in calcium alginate and cultured in Murashige and Skoog's basal medium containing 10 mM $CaCl_2$ for the production of plumbagin, an important medicinal compound. Studies were carried to find out the impact of immobilization on the increased accumulation of this secondary metabolite. Immobilization in calcium alginate enhanced the production of plumbagin by three, two, and one folds compared with that of control uncrosslinked alginate and $CaCl_2$ treated cells, respectively (Vanisree *et al.*, 2004).

Role of endophytes in *in vitro* production of secondary metabolites

There is the argument that both plants and endophytic microbesco-evolved with pathways to produce these natural products. Another thought is that an ancient horizontal gene transfer took place between plants and microbes. The third suggests that either plants or endophytic fungi produce these secondary metabolites and transfer them to the other symbiont. Biosynthetic pathway studies using radiolabeled precursor amino acids reveal that plants and endophytic fungi have similar, but distinct metabolic pathways for production of secondary metabolites (Jennewein *et al.*, 2001). Evidence to support the independent production of Taxol by endophytic fungi is the isolation of the gene 10-deacetylbaccatin-III-10-Oacetyl transferase from the endophytic fungus *Clasdosporium cladosporiodes* MD213 isolated from *Taxus media*. Recently, it was reported that plants also have endophytic fungi associated with them that make Taxol. This suggests that plants and fungi are independently capable of producing these important secondary metabolites. The fact that a combination of inducing factors from both plants and endophytic fungi increased the accumulation of secondary metabolites in plants and fungi respectively (Li *et al*., 2009)

Conclusions

This review briefly summarized the possible sources of secondary metabolities for their perspective biotechnological production. Plant tissue culture techniques have been tried for large-scale production of secondary metabolites in plant species that have medicinal importance or those that is generally difficult to cultivate. The entire biotechnological potential of secondary metabolites has not yet been exploited. Production of secondary metabolites using plant tissue cultures is an attractive alternative to classical agriculture and chemical synthesis. However, it involves many challenges due to difficulties in scale-up, sustainability of the culture and its rate of secondary metabolite biosynthesis and also product recovery due to the fact that secondary metabolites are usually intracellular. The major advantage of the cell cultures include synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions. The use of *in vitro* plant cell culture for the production of chemicals and pharmaceuticals has made great strides building on advances in plant science. The increased use of genetic tools and an emerging picture of the structure and regulation of pathways for secondary metabolism will provide the basis for the production of commercially acceptable levels of product. The increased level of natural products for medicinal purposes coupled with the low product yields and supply concerns of plant harvest has renewed interest in large-scale plant cell culture technology. Knowledge of biosynthetic pathways of desired phytochemicals in plants as well as in cultures is often still in its infancy, and consequently strategies needed to develop an information based on a cellular and molecular level. These results show that *in vitro* plant cell cultures have potential for commercial production of secondary metabolites. The introduction of newer techniques of molecular biology, so as to produce transgenic cultures and to effect the expression and regulation of biosynthetic pathways, is also likely to be a significant step towards making cell cultures more generally applicable to the commercial production of secondary metabolites. Further studies can be carried out to find more suitable way of culture for secondary metabolite and for increasing the amount of secondary metabolite in existing process.

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