Chapter 7
Secondary Metabolite Production in Medicinal Plants Using Tissue Cultures

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Abstract Plants are an incredible treasure of lifesaving drugs and other products of diverse applications. Plant tissue cultures can be established routinely under sterile conditions from explants like plant leaves, stems, roots, meristems, etc. for both ways for multiplication and extraction of secondary metabolites. Strain improvement, methods for the selection of high-producing cell lines, and medium optimizations can lead to an enhancement in secondary metabolite production. Production of natural as well as recombinant bioactive products of commercial importance through the exploitation of plant cells has attracted substantial attention over the past few decades. Swift acceleration in the production of explicit secondary metabolism compounds at a rate similar or superior to the intact plants has been discovered through innovative plant cell cultures in the last few years. In view of obtaining optimum yields suitable for commercial exploitation, isolation of the biosynthetic activities of cultured cells has been focused upon, which is being achieved by the optimization of the cultural conditions, selection of high-yielding strains, and employment of transformation methods, precursor feeding, and immobilization techniques. Production of secondary metabolites through hairy root system is based on *Agrobacterium rhizogenes* inoculation and has grabbed substantial attention during the past few decades as an efficient method of secondary metabolite production in the plant roots. Due to certain reasons like very slow growth of root systems of higher plants and very difficult harvesting, alternative methods of bioactive compound production have been utilized and promising results have been obtained. Root cultures constitute a promising option for the production of medicinally

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important bioactive compounds. Organ cultures and in vitro biomass production often have sites of synthesis and storage of secondary metabolites in separate compartments. Elicitors, compounds triggering the formation of secondary metabolites, can be abiotic or biotic. Natural elicitors include polysaccharides such as pectin and chitosan, which are also used in the immobilization and permeabilization of plant cells. The present chapter reviews the secondary metabolite production through hairy root cultures, organ cultures, elicitation, and economically valuable secondary metabolites produced through tissue culture.

Keywords Plant cell cultures · Secondary metabolites · Elicitation

7.1 Introduction

Plants are an incredible treasure of lifesaving drugs and other products of diverse applications. Nowadays numerous distinct phytochemicals serve as imperative drugs, which are currently used across the globe to cure a variety of perilous diseases. Most of the medicinally important phytochemicals are the products of secondary metabolism, which, in addition to their pharmaceutical applications, find extensive applications in flavor and fragrances, food additives, pesticides, and dye and pigments. The chief role of these bioactive secondary products in plants is to help them in combating various types of biotic and abiotic stresses (Rao and Ravishankar 2002; Ahmad et al. 2019a, b; Naikoo et al. 2019). Production of natural as well as recombinant bioactive products of commercial importance through the exploitation of plant cells has attracted substantial attention over the past few decades (Canter et al. 2005). The mounting commercial importance of the secondary metabolism products has attracted significant interest in this subject in the recent past, particularly in the likelihood of alteration in the production of various bioactive plant metabolites with the help of tissue culture technology. Plant culture systems (cell and tissue cultures) signify a potential treasure of valued secondary metabolites and hold immense promise for the controlled production of such countless and valuable secondary metabolites on demand which find extensive applications in food additives, pharmaceuticals, and nutraceuticals (Zhong 2001). The synthesis of secondary metabolites with the help of the cell cultures is independent of environmental fluctuations as compared to their biosynthesis in plants. The chemical synthesis of various valuable metabolites is either not achievable or economically unfeasible. Furthermore, the natural bioactive phytoproducts used as food additives are better valued and accepted by consumers as compared to their synthetic counterparts. Swift acceleration in the production of explicit secondary metabolism compounds at a rate similar or superior to the intact plants has been discovered through innovative plant cell cultures in the last few years. In view of obtaining optimum yields suitable for commercial exploitation, isolation of the biosynthetic activities of cultured cells has been focused upon which is being achieved by the optimization of the cultural conditions, selection of high-yielding strains, and employment of transformation methods, precursor feeding, and immobilization

techniques (DiCosmo and Misawa 1995). The role of plant tissue culture in the production of secondary metabolites has been completely reformed by transgenic hairy root cultures. These are exceptional in their biosynthetic and genetic stability, swift in growth, and very easily maintained. With the help of this methodology, an extensive variety of phytochemical compounds of commercial value has been synthesized (Giri and Narasu 2000).

In order to carry out the efficient extraction and increased production of bioactive secondary metabolites, plant cell and tissue cultures can be constantly established from different explants (plant leaves, stems, roots, meristems) under sterile conditions (Vijaya et al. 2010). Optimization of the media, strain improvement, and selection of high-yielding cell lines can enrich the secondary metabolite production. These advances have enhanced the phytochemical production beyond expectations (Vijaya et al. 2010). The competence of plant cell cultures to produce and accumulate countless of the identical precious compounds as the parent plant finds recognition nearly since the commencement of in vitro technology. The persistently increasing demand for the natural products has attracted substantial attention toward the plant culture systems as potential biosynthetic machines for secondary metabolism products and has opened new doors of anticipation for novel research exploring expression of secondary products in vitro (Karuppusamy 2009). The most promising approach of large-scale sustainable production of secondary metabolites is with the help of the plant cell factories which offers an incessant supply with the help of large-scale culture (Rao and Ravishankar 2002).

Secondary metabolite production through cell cultures is advantageous over the conventional production because of its independence of the environmental factors and seasonal variations as the economically valuable bioactive secondary metabolite production is carried out in controlled conditions through the elimination of negative biological influences (microorganisms and insects) (Hussain et al. 2012; Canter et al. 2005; Rao and Ravishankar 2002). Moreover, selection of high-yielding cell lines and a defined production system ensuring uniform quality and continuous supply and yield is met through culture systems. Furthermore, production of novel compounds normally absent in the parent plant can be ensured through the tissue cultures (Hussain et al. 2012; Rao and Ravishankar 2002).

Production of precious secondary plant products with the help of plant cell cultures as compared to whole plant or in vivo production is followed by a series of distinct advantages (Vijaya et al. 2010). Some of these advantages include;

- Production of useful and valuable compounds independent of soil conditions or climatic changes.
- Cells cultured through varied culture systems would be microbe and insect-free.
- Plant cells of different origin (alpine or tropical) could be multiplied effortlessly to yield important and specific metabolites.
- Reduced labor expenses and improved productivity would result from coherent regulation of metabolite processes and programmed control of cell growth.
- Extraction of organic substances from callus cultures.

7.1.1 Secondary Metabolite Production Through Hairy Root Cultures

Production of secondary metabolites through hairy root system is based on *Agrobacterium rhizogenes* inoculation and has grabbed substantial attention during the past few decades as an efficient method of secondary metabolite production in the plant roots (Hussain et al. 2012; Karuppusamy 2009; Palazon et al. 1997). After the inoculation, the hairy root phenotype produced exemplifies swift hormone-independent growth, lateral branching, lack of geotropism, and genetic stability. Such a secondary metabolite production is edged as these secondary products produced are with similar or higher yields and identical to those produced by the intact roots of parent plants (Sevón and Oksman-Caldentey 2002). This attribute along with genetic stability as well as speedy growth in media lacking phytohormones makes them specifically appropriate for biochemical studies which usually are difficult to carry out in the root cultures of intact plants. A part of the DNA (T-DNA), located on the Ri plasmid, is transferred to the plant cells during the process of infection, and fascinatingly the transferred genes find expression in the same way as those of the endogenous plant cell genes. During the infection process, *A. rhizogenes* transfers a part of the DNA (T-DNA) located in the root-inducing plasmid Ri to plant cells, and the genes contained in this segment are expressed in the same way as the endogenous genes of the plant cells. Certain strains of *A. rhizogenes* have two sections in T-DNA, each finding its incorporation individually into the plant genome. The root induction process involves two sets of plasmid genes, the aux genes and the rol genes (Hussain et al. 2012). Usually the hairy roots are induced on the wounded plant parts after inoculating these with *A. rhizogenes*. Transformation mediated through *A. rhizogenes* is advantageous as any gene of interest can be transferred to the hairy root clone. This can prove very fruitful for secondary metabolite production. For example, 6-hydroxylase gene from *Hyoscyamus muticus* was introduced into *Atropa belladonna* using *A. rhizogenes*-mediated transformation (Hashimoto et al. 1993). Enhanced enzyme activity and about fivefold increase in the concentration of scopolamine in the engineered roots were observed.

7.1.2 Secondary Metabolite Production Through Organ Cultures

Due to slow growth of root systems of higher plants and very difficult harvesting, alternative methods of bioactive compound production have been utilized, and promising results have been obtained (Pence 2011). Root cultures constitute a promising option for the production of medicinally important bioactive compounds (Pence 2011). Some of the noteworthy secondary metabolites that have been produced quite well in root cultures include the tropane alkaloids (hyoscyamine and scopolamine) (Fazilatun et al. 2004). Moreover, other aerial parts of the plants like

shoots can also be utilized for the production of important secondary metabolites (Nogueira and Romano 2002; Smith et al. 2002). Shoot cultures have been utilized for the commercial production of secondary metabolites so as to reduce or overcome the exploitation of natural plants (Karuppusamy 2009; Khanam et al. 2000). In addition, shoot cultures are aimed at inducing somaclonal variations and provide the chance for selecting clones capable of high secondary production (Dhawan et al. 2003). However, the organ cultures encounter some major problems when cultured at large scale (Kaimoyo et al. 2008). Different types of bioreactors have been used for the culture of plant roots and/or shoots (Kašparová et al. 2009; Kim et al. 2002). Compared to the cell suspension cultures, organ cultures generally display a lower sensitivity to shear stress, but they show a high degree of spatial heterogeneity in biomass production. Another problem is the quite high cost of these bioreactor systems for commercial large-scale production of plant secondary metabolites. As they have to compete with the cultivation of the whole plant, such a process in most cases is not economically viable (Zhao et al. 2010).

7.1.3 Economically Valuable Secondary Metabolites Produced Through Tissue Culture

Tissue culture holds immense potential of controlled production of myriad of economically valuable and pharmaceutically useful secondary products, and the field is quite intriguing. Swift acceleration has been witnessed in the discovery of cultures competent enough to produce explicit medicinal compounds at a similar or speedy rate to that observed in intact plants (Vijaya et al. 2010). Biosynthetic activities of cultured cells are of imperative importance in order to achieve significant yields appropriate for commercial production of the valuable pharmaceutical secondary metabolites, and isolation of such cultured cells is necessary for the optimization of the cultural conditions, selection of high-yielding strains, utilization of precursor feeding, and transformation and immobilization techniques (Vijaya et al. 2010; DiCosmo and Misawa 1995). Transgenic hairy root cultures have brought a new life to secondary metabolite production through plant tissue culture as such cultures are exceptional in their biosynthetic and genetic stability, swift in growth, and comparatively easier in maintenance. Utilizing such methodology, synthesis of a diverse range of chemical compounds has been achieved (Vijaya et al. 2010; Giri and Narasu 2000). Recent advances in the field of cell cultures have achieved significant success in the production of a diverse range of pharmaceuticals belonging to different classes of secondary metabolites including terpenoids, flavanoids, alkaloids, phenolics, saponins, steroids, and amino acids (Abdin and Kamaluddin 2006; Jordon and Wilson 1995). Some pharmaceutically imperative secondary metabolites are briefly discussed below.

Taxol (paclitaxel), an efficient and promising anticancer substance for its exceptional mode of action on cell cycle arrest by checking the microtubular assembly, is

a complex diterpene alkaloid obtained from the *Taxus* tree bark (Hussain et al. 2012; Cragg et al. 1993). Currently, taxol production by different *Taxus* species through cell cultures is an extensively explored area of tissue culture in the recent times owing to the colossal commercial importance of the alkaloid, the insufficiency of source tree, and the expensive chemical synthesis (Suffness 1995; Fett-Neto et al. 1994). Aiming at increased production of the alkaloid through the cultures owing to its pharmaceutical importance, cultures were supplemented with different amino acids, and the results revealed that phenylalanine supplementation had a profound effect on the production of taxol in *Taxus cuspidata* cultures (Ciddi et al. 1995). Moreover, the influence of different biotic and abiotic elicitors has also been studied to enhance the production of taxol through cultures (Hussain et al. 2012; Yukimune et al. 1996; Strobel et al. 1992; Tam et al. 1980).

Papaver somniferum, commonly known as opium poppy, is a rich treasure of commercial natural analgesics (morphine and codeine). These alkaloids are significantly valuable and of widespread use in different pharmaceutical preparations. Cell and suspension cultures of opium poppy are being envisaged as valuable and alternative means for the commercial production of these imperative alkaloids. Studies have revealed the production of codeine and morphine alkaloids through morphologically undifferentiated cultures (Yoshikawa and Furuya 1985). Application of growth regulators to the cultures results in the reduced biosynthesis of morphine and codeine as revealed from the study during which it evolved that highest morphine and codeine contents were 2.5 mg/g dry weight and 3.0 mg/g dry weight, respectively, which is about three times greater than the cultures supplemented with hormones (Hussain et al. 2012). Furthermore, Furuya et al. (1972) during the biotransformation studies of codeinone to codeine with the immobilized cells of *Papaver somniferum* reported the conversion yield of about 70.4%.

L-3,4-dihydroxyphenylalanine, a precursor of alkaloids, betalain, and melanin, is an imperative intermediate in the secondary metabolic pathway in higher plants and has been isolated from different plants (Brain and Lockwood 1976; Daxenbichler et al. 1971). Importantly, it is also a precursor of catecholamines which are involved in different signaling and metabolic phenomena in animals besides finding usage as an effective drug against a progressive immobilizing and disabling disorder resulting from the insufficiency of dopamine in the brain tissues called as Parkinson's disease. In view of this imperative pharmaceutical significance, a demand for hefty quantities was felt which led to the alternative ways of enhanced production of this alkaloid among which production through cell cultures has achieved significant success in this regard (Brain and Lockwood 1976). *Mucuna pruriens* has been reported to accumulate 25 mg/L DOPA in the medium under the influence of ample concentrations of 2,4-D. Among the induced callus cultures of three species of *Mucuna* (*M. hassjoo*, *M. pruriens*, *Mucuna deeringiana*), Teramoto and Komamine (1988) observed that the callus tissues of *M. hassjoo* accumulated the highest concentration of DOPA when the medium was supplemented with 10 mg/L kinetin and 0.025 mg/L 2.4-D.

Capsaicin, an alkaloid obtained from green pepper fruits, is used chiefly as a spicy food additive in various formulated foods (Ravishankar et al. 2003). In addi-

tion, it finds usage in various pharmaceutical preparations for treating rheumatic disorders besides acting as a digestive stimulant (Sharma et al. 2008). *Capsicum frutescens* suspension cultures are known to produce low capsaicin contents, but immobilization of the cells in reticulated polyurethane foam leads to100-fold increase in its production. Moreover, improvements in the yields can be obtained through the supplementation of isocaproic acid-like precursors. Lindsey (1985) reported that improved capsaicin synthesis can be obtained by the treatments which suppress primary metabolism and cell growth. Holden et al. (1988) have reported that spores of *Gliocladium deliquescens* can elicit capsaicin biosynthesis in the *C. frutescens* cell cultures. Detailed study on the influence of nutritional stress on capsaicin production of *Capsicum annuum* in immobilized cell cultures were carried out by Ravishankar and Ramachandra Rao (2000). Biotransformation of exogenously sourced caffeic acid and protocatechuic aldehyde to capsaicin in immobilized cells cultures and freely suspended cells of *Capsicum frutescens* has also been carried out (Sanatombi and Sharma 2007).

Diosgenin, a pharmaceutically valuable alkaloid which acts as a precursor for the synthesis of a variety of steroidal drugs, finds extensive appliance in the pharmaceutical industry because of which its demand is continuously mounting (Hussain et al. 2012; Tal et al. 1983). In 1983, culture experiments of Tal et al. (1983) revealed that carbon and nitrogen concentrations significantly influenced accumulation of diosgenin in one of the cell lines of *Dioscorea.* Furthermore, immobilized cell cultures were established by Ishida (1988), and it was observed that reticulated polyurethane foam stimulated diosgenin production, leading to an increase of 40% in the cellular concentration and an increase of 25% total yield. Moreover, 8% increase in the levels of diosgenin was reported in the batch-grown cell suspensions of *D. deltoidea* (Hussain et al. 2012; Tal et al. 1983).

7.1.4 Secondary Metabolite Production Through Elicitation

Secondary metabolite accumulation in plants is an important adaptive mechanism established by them during evolution as a part of defense system against pathogens, which is stimulated through elicitation by different elicitors, acting as signaling compounds during the defense responses in plants (Zhao et al. 2005). Elicitation has proven to be an efficient technique for enhancing the production of plant secondary metabolites through biotechnological approach. Elicitors are usually those compounds which stimulate plant defense response and promote production of secondary metabolism products in order to protect the plant (Klarzynski and Friting 2001; Baenas et al. 2014). Elicitors are of various types and varied nature and elicit secondary metabolite production through elicitation, a phenomenon during which induction or enhancement of secondary metabolites in plants is stimulated to ensure their survival, competitiveness, and persistence (Namdeo 2007). These elicitors can be categorized into different types on the basis of origin, viz., abiotic (elicitors of non-biological origin like physical factors and inorganic substances) and biotic

elicitors (plant hormones like methyl jasmonate, salicylic acid, brassinosteroids, bacterial- and fungal-derived proteins, and peptides) (Gorelick and Bernstein 2014; Namdeo 2007). Inorganic elicitors like metal ions or salts have been utilized for increased bioactive compound production by eliciting or stimulating secondary metabolism. Zinc ions and salts like $AlCl_3$, $AgNO_3$, $CaCl_2$, and $MgSO_4$ have been used in cell suspensions, hairy roots, and adventitious roots for secondary metabolism elicitation (Verpoorte et al. 2002). Bulk of the biotic elicitors is recognized and bound by specific cell membrane receptors. After its stimulation, the cell surface receptor transfers the stimulus to the cell leading to a signal transduction cascade (Baenas et al. 2014). Once the signal transduction cascade is stimulated, different signaling molecules are produced which lead to the biosynthesis of products of secondary origin like phytoalexins; such a signaling response is determined by various factors, predominantly physiological state and genetic characteristics.

Studies have revealed that exposure of plant cell cultures to different elicitors can lead to increased production of secondary metabolites (Staniszewska et al. 2003). Elicitors of frequent usage in culture systems include fungal carbohydrates, yeast extract, methyl jasmonate (MJ), and chitosan. MJ, a phytohormone and an imperative signal compound, has proven as an effective elicitor for the production of taxol (Wu and Lin 2002) and ginsenoside (Yu et al. 2002; Kim et al. 2004; Thanh et al. 2005) in the cell/organ culture. MJ has also proven promising in the production of secondary metabolites in cell/adventitious root cultures of *Bupleurum falcatum* L. (Aoyagi et al. 2006) and *Taxus* spp. (Yukimune et al. 1996; Ketchum et al. 1999). Additionally, MJ-induced elicitation led to significant increase in the eleutheroside content after *Eleutherococcus senticosus* embryo culture was supplemented with this elicitor (Shohael et al. 2007).

The possible mechanism of elicitor signaling may involve stimulation of elicitor-induced DNA synthesis via G-protein-coupled receptor (GPCR) and phosphoinositide-specific phospholipase C (PI-PLC) pathways (Boland et al. 2003a, b, 2006), and the elicitor-induced methyl jasmonate biosynthesis (Doares et al. 1995) might give indication regarding the activation of methyl jasmonate response elements in DNA due to elicitors (Fig. 7.1). Enhanced methyl jasmonate biosynthesis may have been induced by elicitors, which led to increased secondary metabolite production, thereby increasing active constituents and the yield of the plant. Increase in the yield of the active constituents, such as citral, is in line with other studies (Adams 2007; Dar et al. 2015), which report enhancement in citronellal and trigonelline contents in eucalyptus and fenugreek, respectively, in response to elicitors.

7.2 Conclusion

The advances in modern technology, especially protocols for plant tissue cultures, paved a way for the commercial production of even rare plants and the chemicals they provide. The main advantage of plant tissue culture is that it can ultimately

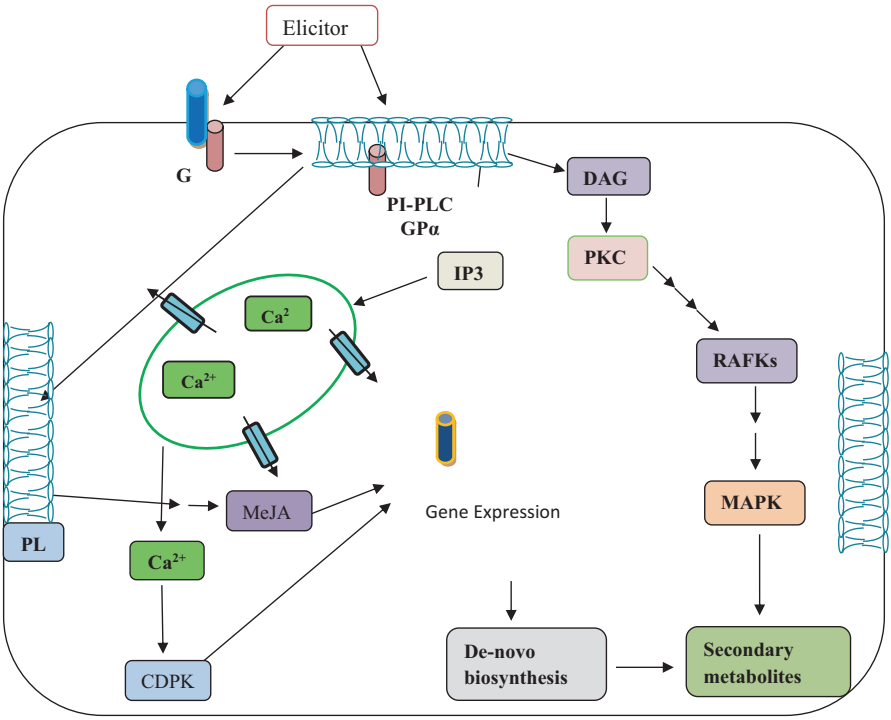

Fig. 7.1 A possible signaling mechanism of in vitro secondary metabolite production through elicitation. Elicitors perhaps activate G-protein-coupled receptor (GPCR) pathway. In the activated form, the Gα subunit of the GPCR binds the induced phosphoinositide-specific phospholipase C (PI-PLC) complex, which may lead to the activation of two different signaling molecules, inositol triphosphate (IP3) and diacylglycerol (DAG). Elicitors have earlier been known to generate PI-PLC signaling independent of the GPCR pathway as well as IP3, in turn, activating cyclin-dependent protein kinase (CDPK), which leads to the nucleus activating de novo DNA synthesis of enzymes involved in metabolic pathways. Protein kinase C (PKC), after activation, leads to nucleus activating gene expression through phosphorylation of transcription factors and DNA synthesis. It might also lead to activation of the MAP-kinase (MAPK) pathway, leading to increased biosynthesis of monoterpenes in lemongrass

provide a continuous, reliable source of natural products. The synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions, is the main benefit of this method. This has a great strides building on advances in plant science has been accomplished by the use of in vitro plant cell culture for the production of secondary metabolites. Knowledge of biosynthetic pathways of desired phytochemicals in plants as well as in cultures is often still in its infancy, and consequently strategies are needed to develop an information based on a cellular and molecular level. 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 toward making cell cultures more applicable to the commercial production of secondary metabolites (Table 7.1).

Table 7.1 Secondary metabolite production through tissue culture

Active ingredient	Plant	Culture type	References
Flavonolignan	*Silybum marianum*	Root	Alikaridis et al. (2000)
		(LS + TDZ)	
Saikosaponins	*Bupleurum falcatum*	Root	Kusakari et al. (2000)
		(B5 + IBA)	
Anthraquinones	*Cassia acutifolia*	Suspension	Nazif et al. (2000)
		(MS + 2,4-D + kinetin)	
Gallotannins	*Rhus javanica*	Root	Taniguchi et al. (2000)
		(LS + IAA +Kinetin)	
Capsiacin	*Capsicum annuum*	Callus	Varindra et al. (2000)
		(MS + 2,4-D+ GA_3)	
Reserpine	*Rauvolfia serpentina*	Callus	Gerasimenko et al. (2001)
		(LS)	
Ramiflorin alkaloid	*Aspidosperma ramiflorum*	Callus	Olivira et al. (2001)
		(MS + 2-4,D + BAP + Sucrose)	
Withaferin A	*Withania somnifera*	Shoot	Ray and Jha (2001)
		(MS + BA)	
Indole alkaloids	*Catharanthus roseus*	Suspension	Zhao et al. (2001)
		(MS + NAA + Kinetin)	
Diterpenoids	*Torreya nucifera*	Suspension	Orihara and Furuya (1990)
		(MS + 2,5-D)	
Terpenoids	*Salvia officinalis*	Callus	Santos-Gomes et al. (2002)
		(MS + 2,4-D + BA)	
Plumbagin	*Plumbago zeylanica*	Hairy root	Verma et al. (2002)
		(MS + BAP + IBA)	
Plumbagin	*Plumbago rosea*	Callus	Komaraiah et al. (2003)
		(MS + $CaCl_2$)	
Hypericin	*Hypericum perforatum*	Multiple shoot	Santarem and Astarita (2003)
		(MS + BA + TDZ)	
Triterpenes	*Hyssopus officinalis*	Suspension	Skrzypek and Wysokinsku (2003)
		(G5 + 2,4-D + IAA)	
Triterpenoid	*Ammi majus*	Suspension	Staniszewska et al. (2003)
		(MS + 2,4-D + BA)	
Alkaloids	*Fritillaria unibracteata*	Multiple shoot	Gao et al. (2004)
		(MS + 2,4-D + Kin)	
Corydaline	*Corydalis ambigua*	Embryo	Hiraoka et al. (2004)
		(MS + IAA + sucrose)	
Asiaticoside	*Centella asiatica*	Shoot	Kim et al. (2004)
		(MS + BAP + IAA)	
Berberine	*Coscinium fenestratum*	Suspension	Narasimhan and Nair (2004)
		(MS + 2,4-D + GA3)	

(continued)

Table 7.1 (continued)

Active ingredient	Plant	Culture type	References
Vincamine	*Vinca major*	Hairy root	Tanaka et al. (2004)
		(MS + BAP)	
Catechin	*Rheum ribes*	Callus	Farzami and Ghorbant (2005)
		(MS + IBA + BA)	
Flavonoids	*Vaccinium myrtillus*	Callus culture	Hohtola et al. (2005)
		(MS + BAP + NAA)	
Asiaticoside	*Centella asiatica*	Callus	Kiong et al. (2005)
		(MS + 2,4-D + Kin)	
Hypericin	*Hypericum perforatum*	Suspension	Hohtola et al. (2005)
		(Liquid MS + NAA + GA_3)	
7-Methyljuglone	*Drosera rotundifolia*	Shoot culture	Hohtola et al. (2005)
		(MS + BAP + NAA)	
Lupeol, rutin	*Hemidesmus indicus*	Shoot culture	Misra et al. (2005)
		(MS + BAP + NAA)	
Reserpine	*Rauvolfia tetraphylla*	Callus	Anitha and Kumari (2006)
		(MS + 2,4-D + Tryptophan)	
Gymnemic acid	*Gymnema sylvestre*	Callus	Devi et al. (2006)
		(MS + IAA + BA)	
Gymnemic acid	*Gymnema sylvestre*	Callus	Gopi and Vatsala (2006)
		(MS + 2,4-D + IAA)	
Umbelliferone	*Ammi majus*	Shootlet	Krolicka et al. (2006)
		(MS + BAP)	
Vincristine	*Catharanthus roseus*	Suspension	Lee-Parsons and Rogce (2006)
		(MS + 2,4-D + GA3)	
Anthocyanin	*Vitis vinifera*	Suspension	Qu et al. (2006)
		(MS + BAP + NAA)	
Essential oil	*Cymbopogon citratus*	Shoot	Quiala et al. (2006)
		(MS + IAA + GA3)	
Sennosides	*Cassia senna*	Callus	Shrivastava et al. (2006)
		(MS + NAA + Kin)	
Anthraquinone	*Saprosma fragrans*	Callus	Singh et al. (2006)
		(MS + 2,4-D + NAA)	
Silymarin	*Silybum marianum*	Callus	Tumova et al. (2006)
		(MS + IAA + BA)	
Flavonoid	*Momordica charantia*	Callus	Agarwal and Kamal (2007)
		(MS + BAP + NAA)	
Rosmarinic acid	*Zataria multiflora*	Callus	Francoise et al. (2007)
		(MS + IAA + Kin)	
Glycoside	*Panax ginseng*	Hairy root	Jeong and Park (2007)
		(MS + NAA + Kin)	

(continued)

Table 7.1 (continued)

Active ingredient	Plant	Culture type	References
Hyperforin	*Hypericum perforatum*	Multiple shoot (MS + 2,4-D + Leusine)	Karppinen et al. (2007)
Asiaticoside	*Centella asiatica*	Hairy root (MS + 2,4-D)	Kim et al. (2007)
Hypericins	*Hypericum perforatum*	Multiple shoot (MS + BA + IAA)	Kornfeld et al. (2007)
Rosmarinic acid	*Agastache rugosa*	Hairy root (MS + 2,4-D + Kin + 3% sucrose)	Lee et al. (2007a)
Rutin	*Fagopyrum esculentum*	Hairy root (MS + NAA)	Lee et al. (2007b)
Saponins	*Primula veris*	Shoot (MS + BAP + GA_3)	Okrslar et al. (2007)
Eleutherosides	*Eleutherococcus senticosus*	Suspension (MS + 2,4-D)	Shohael et al. (2007)
Glucoside	*Gentiana macrophylla*	Hairy root (MS + IAA + Kin)	Tiwari et al. (2007)
Camptothecin	*Ophiorrhiza rugosa*	Shoot (MS + BA + Kin)	Vineesh et al. (2007)
Fixed oil	*Simmondsia chinensis*	Callus (MS + TDZ + GA_3)	Aftab et al. (2008)
Quercetin	*Pluchea lanceolata*	Callus (MS + NAA + BAP)	Arya et al. (2008)
Artemisinin	*Artemisia annua*	Callus (MS + NAA + Kinetin)	Baldi and Dixit (2008)
Flavonoid	*Salvia officinalis*	Multiple shoot (LMS + IAA + BAP)	Grzegorczyk and Wysokinska (2008)
Berberine	*Coscinium fenestratum*	Callus (MS + 2,4-D + BAP)	Khan et al. (2008)
Resveratrol	*Arachis hypogaea*	Hairy root (G5 + 2,4-D + Kin.)	Kim et al. (2008)
Tropane	*Brugmansia candida*	Hairy root (MS + 2,4-D + IAA)	Marconi et al. (2008)
Glycyrrhizin	*Glycyrrhiza glabra*	Hairy root (MS + 2,4-D + GA3)	Mehrotra et al. (2008)
Withanolide A	*Withania somnifera*	Hairy root (MS + IAA + Kin)	Murthy et al. (2008)
Reserpine	*Rauvolfia serpentina*	Callus (MS + IAA + Cu^{2+})	Nurchgani et al. (2008)
Azadirachtin	*Azadirachta indica*	Suspension (MS + 2,4-D + Cyanobacterial elicitor)	Poornasri Devi et al. (2008)

(continued)

Table 7.1 (continued)

Active ingredient	Plant	Culture type	References
Berberine	*Tinospora cordifolia*	Suspension	Rama Rao et al. (2008)
		(MS + IAA + GA_3)	
Silymarin	*Silybum marianum*	Hairy root	Rahnama et al. (2008)
		(MS + IAA + GA3)	
Catharanthine	*Catharanthus roseus*	Suspension	Ramani and Jayabaskaran (2008)
		(MS + 2,4-D + UV-B)	
Serpentine	*Rauvolfia serpentina*	Callus	Salma et al. (2008)
		(MS + BAP + IAA)	
Azadirachtin	*Azadirachta indica*	Suspension	Sujanya et al. (2008)
		(MS + 2,4-D)	
Corydalin	*Cordyline terminalis*	Callus	Taha et al. (2008)
		(MS + 2,4-D + BAP)	
Xanthone	*Gentianella austriaca*	Multiple shoot	Vinterhalter et al. (2008)
		(MS + BAP)	
Cathin	*Brucea javanica*	Suspension	Wagiah et al. (2008)
		(MS + IAA + GA3)	
Deoursin	*Angelica gigas*	Hairy root	Xu et al. (2008)
		(MS (Liq.) + 2,4-D + GA_3)	
Piperine	*Piper solmsianum*	Suspension	Balbuena et al. (2009)
		(MS + 2,4-D + BA)	
Myristin	*Myristica fragrans*	Shoot	Indira Iyer et al. (2009)
		(MS + NAA + TDZ)	
Resveratrol	*Vitis vinifera*	Callus	Kin and Kunter (2009)
		(MS + IAA + GA3 + UV)	
Podophyllotaxin	*Podophyllum hexandrum*	Shoot	Li et al. (2009)
		(MS + BAP + GA_3)	
Flavonoid	*Crataegus sinaica*	Callus	Maharik et al. (2009)
		(MS + 2,4-D + NAA + BAP)	
Steroidal lactone	*Withania somnifera*	Callus	Mirjalili et al. (2009)
		(MS + 2,4-D + BA)	
Flavones	*Camellia chinensis*	Callus	Nikolaeva et al. (2009)
		(MS + 2,4-D + NAA)	
Anthraquinone	*Rubia akane*	Hairy root	Park and Lee (2009)
		(B5 + NAA + Kin)	
Stilbenes	*Cayratia trifoliata*	Suspension	Roat and Ramawat (2009)
		(MS + IAA + GA_3)	
Isoflavones	*Psoralea cordifolia*	Multiple shoot	Shinde et al. (2009)
		(MS + TDZ + BAP)	
Vasine	*Adhatoda vasica*	Shoot culture	Shalaka and Sandhya (2009)
		(MS + BAP + IAA)	

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