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(*Review paper*)

Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures

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Abstract. Plants are a tremendous source for the discovery of new products of medicinal value for drug development. Today several distinct chemicals derived from plants are important drugs currently used in one or more countries in the world. Many of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances. The evolving commercial importance of secondary metabolites has in recent years resulted in a great interest in secondary metabolism, particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology. Plant cell culture technologies were introduced at the end of the 1960's as a possible tool for both studying and producing plant secondary metabolites. Different strategies, using an *in vitro* system, have been extensively studied to improve the production of plant chemicals. The focus of the present review is the application of tissue culture technology for the production of some important plant pharmaceuticals. Also, we describe the results of in vitro cultures and production of some important secondary metabolites obtained in our laboratory.

Keywords: Biotransformations; Cell suspension cultures; Hairy root cultures; Pharmaceuticals; Secondary metabolites.

Contents

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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). 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 (Phillipson, 1990). In the search for alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Ramachandra Rao and Ravishankar, 2002). Cell suspension culture systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products.

Discoveries of cell cultures capable of producing specific medicinal compounds (Table 1) at a rate similar or superior to that of intact plants have accelerated in the last few years. New physiologically active substances of medicinal interest have been found by bioassay. It has been demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformations of natural compounds has been demonstrated (Cheetham, 1995; Scragg, 1997; Krings and Berger, 1998; Ravishankar and Ramachandra Rao, 2000). Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations.

The major advantages of a cell culture system over the conventional cultivation of whole plants are: (1) Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions; (2) Cultured cells would be free of microbes and insects; (3) The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites; (4) Automated control of cell growth and rational regulation of metabolite processes would reduce of labor costs and improve productivity; (5) Organic substances are extractable from callus cultures.

In order to obtain high yields suitable for commercial exploitation, efforts have 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). Transgenic hairy root cultures have revolutionized the role of plant tissue culture in secondary metabolite production. They are unique in their genetic and biosynthetic stability, faster in growth, and more easily maintained. Using this methodology a wide range of chemical compounds have been synthesized (Shanks and Morgan, 1999; Giri and Narasu, 2000). Advances in tissue culture, combined with improvement in genetic engineering, specifically transformation technology, has opened new avenues for high volume production 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. Genome manipulation is resulting in relatively large amounts of desired compounds produced by plants infected with an engineered virus, whereas transgenic plants can maintain constant levels of production of proteins without additional intervention (Sajc et al., 2000). Large-scale plant tissue culture is found to be an attractive alternative approach to traditional methods of plantation as it offers a controlled supply of biochemicals independent of plant availability (Sajc et al., 2000). Kieran et al. (1997) detailed the impact of specific engineering-related factors on cell suspension cultures. Current developments in tissue culture technology indicate that transcription factors are efficient new molecular tools for plant metabolic engineering to increase the production of valuable compounds (Gantet and Memelink, 2002). In vitro cell culture offers an intrinsic advantage for foreign protein synthesis in certain situations since they can be designed to produce therapeutic proteins, including monoclonal antibodies, antigenic proteins that act as immunogenes, human serum albumin, interferon, immuno-contraceptive protein, ribosome unactivator trichosantin, antihypersensitive drug angiotensin, leu-enkephalin neuropeptide, and human hemoglobin (Hiatt et al., 1989; Manson and Arntzen, 1995; Wahl et al., 1995; Arntzen, 1997; Hahn et al., 1997; La Count et al., 1997; Marden et al., 1997; Wongsamuth and Doran, 1997; Doran, 2000). The appeal of using natural products for medicinal purposes is increasing, and metabolic engineering can alter the production of pharmaceuticals and help to design new therapies. At present, researchers aim

Table 1. Bioactive secondary metabolites from plant tissue cultures.

Plant name	Active ingredient	Culture medium	Culture type	Reference
Agave amaniensis	Saponins	MS + Kinetin (23.2 µM), 2,4-D (2.26 µM), KH_2PO_4 (2.50 µM), Sucrose (87.64 mM)	Callus	Andrijany et al., 1999
Ailanthus altissima	Alkaloids	MS + 2,4-D (1 mg/l), Kinetin (0.1 mg/l), Sucrose (5%)	Suspension	Anderson et al., 1987
Ailanthus altissima	Canthinone alkaloids	MS + 2,4-D (1 mg/l), Kinetin (0.1 mg/l), Sucrose (5%)	Suspension	Anderson et al., 1986
Allium sativum L.	Alliin	MS + IAA (11.4 µM), NAA (10.8 µM), Kinetin (9.3 µM), Coconut water (15%)	Callus	Malpathak and David, 1986
Aloe saponaria	Tetrahydroanthracene glucosides	MS + 2,4-D (1 ppm), Kinetin (2 ppm)	Suspension	Yagi et al., 1983
Ambrosia tenuifolia	Altamisine	MS + Kinetin (10 µM), 2,4-D (1 µM), Ascorbic acid and Cystine (10 µM)	Callus	Goleniowski and Trippi, 1999
Anchusa officinalis	Rosmarinic acid	B5 + 2,4-D (1.0 mg/l), Kinetin (0.1 mg/l)	Suspension	De-Eknamkul and Ellis, 1985
Brucea javanica (L.) Merr.	Canthinone alkaloids	MS + 2,4-D (1 mg/l), Kinetin (0.1 mg/l), Sucrose (5%)	Suspension	Liu et al., 1990
Bupleurum falcatum	Saikosaponins	LS + 2,4-D (2 mg/l)	Callus	Wang and Huang, 1982
Bupleurum falcatum L.	Saikosaponins	B5 + IBA (8 mg/l), Sucrose (1-8%)	Root	Kusakari et al., 2000
Camellia sinensis	Theamine, γ-glutamyl derivatives	MS + IBA (2 mg/l), Kinetin (0.1 mg/l), Sucrose (3%), Agar (9 g/l)	Suspension	Orihara and Furuya, 1990
Canavalia ensiformis	L-Canavanine	LS + NAA (1.8 mg/l), 2,4-D (0.05 mg/l), BA (4.5 mg/l), Picloram (0.05 mg/l)	Callus	Ramirez et al., 1992
Capsicum annuum L.	Capsaicin	MS + 2,4-D (2 mg/l), Kinetin (0.5 mg/l), Sucrose (3%)	Suspension	Johnson et al., 1990
Cassia acutifolia	Anthraquinones	MS + 2,4-D (1.0 mg/l), Kinetin (0.1 mg/l), Sucrose (3%), Myo-inositol (100 mg/l)	Suspension	Nazif et al., 2000
Catharanthus roseus	Indole alkaloids	MS + Sucrose (3%)	Suspension	Moreno et al., 1993
Catharanthus roseus	Catharanthine	MS + NAA (2 mg/l), IAA (2 mg/l), Kinetin (0.1 mg/l), Sucrose (3%)	Suspension	Zhao et al., 2001b
Cephaelis ipecacuanha A. Richard	Emetic alkaloids	MS + NAA (1 mg/l) or IAA (3 mg/l)	Root	Teshima et al., 1988
Chrysanthemum cinerariaefolium	Pyrethrins	MS + 2,4-D (2.0 mg/l), Kinetin (5.0 mg/l), Sucrose (3%)	Callus	Rajasekaran et al., 1991
Chrysanthemum cinerariaefolium	Chrysanthemic acid and pyrethrins	MS + Casein hydrolysate (1 g/l), 2,4-D (0.5 mg/l), Kinetin (0.75 mg/l)	Suspension	Kueh et al., 1985
Cinchona L.	Alkaloids	MS + Koblitz and Hagen vitamins and amino acids, 2,4-D (4.52 µmol/l), Kinetin (1 µmol/l), GA_3 (0.3 µmol/l), Sucrose (0.09 mol/l)	Suspension	Koblitz et al., 1983
Cinchona robusta	Robustaquinones	B5 + 2,4-D (2 mg/l), Kinetin (0.2 mg/l), Cystine (50 mg/l), Sucrose (2%)	Suspension	Schripsema et al., 1999
Cinchona spec.	Anthraquinones	B5 + 2,4-D (1.0 mg/l), Kinetin (0.2 mg/l)	Suspension	Wijnsma et al., 1985
Cinchona succirubra	Anthraquinones	MS + 2,4-D (1 ppm), Kinetin (0.1 ppm), Myoinositol (100 ppm), Coconut milk (5%), Sucrose (2%)	Suspension	Khouri et al., 1986
Citrus sp.	Naringin, Limonin	MS + 2,4-D (0.66 mg/l), Kinetin (1.32 mg/l), Coconut milk (100 ml)	Callus	Barthe et al., 1987
Coffea arabica L.	Caffeine	MS + Thiamine. HCl (0.9×10^3), Cysteine. Hcl (10.0×10^2), Kinetin (0.1×10^3), 2,4-D (0.1×10^3), Sucrose (30×10^3)	Callus	Waller et al., 1983
Corydalis ophiocarpa	Isoquinoline alkaloids	MS + 2,4-D (1 mg/l), Kinetin (0.1 mg/l)	Callus	Iwasa and Takao, 1982
Croton sublyratus Kurz	Plaunotol	MS + NAA (2 mg/l), BA (0.2 mg/l), Sucrose (2%)	Callus	Morimoto and Murai, 1989

Table 1. (Continued)

Plant name	Active ingredient	Culture medium	Culture type	Reference
Cruciata glabra	Anthraquinones	LS + NAA (2 mg/l) , Kinetin (0.2 mg/l), Casein hydrolysate (1 g/l)	Suspension	Dornenburg and Knorr, 1996
Cryptolepis buchanani Roem. & Schult	Cryptosin	B5 + 2,4-D (2 mg/l), Kinetin (0.5 mg/l)	Callus	Venkateswara et al., 1987
Digitalis purpurea L.	Cardenolides	MS + BA (1 mg/l), IAA (1 mg/l), Thiamine. HCl (1 mg/l)	Suspension	Hagimori et al., 1982
Dioscorea deltoidea	Diosgenin	MS + 2,4-D (0.1 ppm)	Suspension	Heble and Staba, 1980
Dioscorea doryophora Hance	Diosgenin	MS + 2,4-D (2 mg/l), BA (0.2 mg/l)	Suspension	Huang et al., 1993
Duboisia leichhardtii	Tropane alkaloids	LS or B5 or White + NAA (5×10^{-5} M), BA (5×10^{-6} M)	Callus	Yamada and Endo, 1984
Ephedra spp.	L- Ephedrine D-pseudoephedrine	MS + Kinetin (0.25 µM), 2,4-D or NAA (5.0 µM), Sucrose (3%)	Suspension	O'Dowd et al., 1993
Eriobotrya japonica	Triterpenes	LS + NAA (10 µM), BA (10 µM)	Callus	Taniguchi et al., 2002
Eucalyptus tereticornis SM.	Sterols and Phenolic compounds	MS + 2,4-D (2 mg/l)	Callus	Venkateswara et al., 1986
Fumaria capreolata	Isoquinoline alkaloids	LS medium	Suspension	Tanahashi and Zenk, 1985
Gentiana sp.	Secoiridoid glucosides	B5 + Kinetin (1 mg/l), 2,4-D (0.5 mg/l)	Callus	Skrzypczak et al., 1993
Ginkgo biloba	Ginkgolide A	MS + NAA (1 mg/l), Kinetin (0.1 mg/l), Sucrose (3%)	Suspension	Carrier et al., 1991
Glehnia littoralis	Furanocoumarin	LS + 2,4-D (1 µM), Kinetin (1 µM)	Suspension	Kitamura et al., 1998
Glycyrrhiza echinata	Flavanoids	MS + IAA (1 mg/l), Kinetin (0.1 mg/l)	Callus	Ayabe et al., 1986
Glycyrrhiza glabra var. *glandulifera*	Triterpenes	MS + IAA (5 ppm), or 2,4-D (1 ppm), Kinetin (0.1 ppm)	Callus	Ayabe et al., 1990
Hyoscyamus niger	Tropane alkaloids	LS + NAA (10^{-5} M), BA (5×10^{-6} M)	Callus	Yamada and Hashimoto, 1982
Isoplexis isabellina	Anthraquinones	MS + 2,4-D (5 µM), Kinetin (10 µM)	Suspension	Arrebola et al., 1999
Linum flavum L.	5-Methoxypodophyllotoxin	MS salts+ B_5 vitamins, Folic acid (0.88 mg/l), Glycine (2 mg/l), Sucrose (2%)	Suspension	Uden et al., 1990
Lithospermum erythrorhizon	Shikonin derivatives	LS + IAA (10^{-6} M), Kinetin (10^{-5} M)	Suspension	Fujita et al., 1981
Lithospermum erythrorhizon	Shikonin derivatives	LS + IAA (10^{-6} M), Kinetin (10^{-5} M)	Suspension	Fukui et al., 1990
Lycium chinense	Cerebroside	MS + 2,4-D (1.0 ppm), Kinetin (0.1 ppm)	Suspension	Jang et al., 1998
Mentha arvensis	Terpenoid	MS + BA (5 mg/l), NAA (0.5 mg/l)	Shoot	Phatak and Heble, 2002
Morinda citrifolia	Anthraquinones	B5 + NAA (10^{-5} M), N-Z-amine 0.2%, Sucrose (2%)	Suspension	Zenk et al., 1975
Morinda citrifolia	Anthraquinones	B5 + NAA (10^{-5}M), Kinetin (0.2 mg/l), Sucrose (4%), Pluronic acid F-68 (2% w/v)	Suspension	Bassetti et al., 1995
Mucuna pruriens	L-DOPA	MS + IAA (1 mg/l), BA (1 mg/l), Sucrose (4%)	Suspension	Wichers et al., 1993
Mucuna pruriens	L-DOPA	MS + 2,4-D (2.5 mg/l), Coconut water (10%)	Callus	Brain, 1976
Nandina domestica	Alkaloids	MS + 2,4-D (1.0 mg/l), Kinetin (0.1 mg/l)	Callus	Ikuta and Itokawa, 1988
Nicotiana rustica	Alkaloids	LS + 2,4-D (1 µM), Kinetin (1 µM)	Callus	Tabata and Hiraoka, 1976
Nicotiana tabacum L.	Nicotine	MS + NAA (2.0 mg/l), Kinetin (0.2 mg/l)	Suspension	Mantell et al., 1983
Ophiorrhiza pumila	Camptothecin related alkaloids	LS + 2,4-D (0.22 mg/l), NAA (0,186 mg/l), Sucrose (3%)	Callus	Kitajima et al., 1998
Panax ginseng	Saponins and Sapogenins	MS (without glycine) + 2,4-D (1 mg/l)	Callus	Furuya et al., 1973
Panax notoginseng	Ginsenosides	MS + 2,4-D (2 mg/l), Kinetin (0.7 mg/l), Sucrose (3%)	Suspension	Zhong and Zhu, 1995
Papaver bracteatum	Thebaine	MS + Kinetin (0.47 µM), 2,4-D (4.52 or 0.45 µM), Sucrose (3%)	Callus	Day et al., 1986
Papaver somniferum L.	Alkaloids	MS (without Glycine) + Kinetin (0.1 mg/l)	Callus	Furuya et al., 1972
Papaver somniferum	Morphine, Codeine	MS + 2,4-D (0.1 mg/l), Cystine. HCl (2.5 mg/l), Kinetin (2 mg/l), Sucrose (3%)	Suspension	Siah and Doran, 1991

Table 1. (Continued)

Plant name	Active ingredient	Culture medium	Culture type	Reference
Peganum harmala L.	β-Carboline alkaloids	MS + 2,4-D (2 µM)	Suspension	Sasse et al., 1982
Phytolacca americana	Betacyanin	MS + 2,4-D (5 µM), Sucrose (3%)	Suspension	Sakuta et al., 1987
Picrasma quassioides Bennett	Quassin	B5 medium + 2,4-D (1.0 mg/l), Kinetin (0.5 mg/l), Glucose (2%)	Suspension	Scragg and Allan, 1986
Podophyllum hexandrum royle	Podophyllotoxin	B5 + NAA (4 mg/l), Coconut water (5%), Sucrose (4%)	Suspension	Uden et al., 1989
Polygala amarella	Saponins	MS + 1 mg/l IAA	Callus	Desbene et al., 1999
Polygonum hydropiper	Flavanoids	MS + 2,4-D (10^{-6} M), Kinetin (10^{-6} M), Casamino acid (0.1%), Sucrose (3%)	Suspension	Nakao et al., 1999
Portulaca grandiflora	Betacyanin	MS (without Glycine)+2, 4-D (5 mg/l), Kinetin (0.2 mg/l)	Callus	Schroder and Bohm, 1984
Ptelea trifoliata L.	Dihydrofuro [2,3-b] quinolinium alkaloids	MS + 2,4-D (1 mg/l), Kinetin (0.1 mg/l), Coconut water (5%)	Callus	Petit-Paly et al., 1987
Rauwolfia sellowii	Alkaloids	B5 + 2,4-D (1 mg/l), Kinetin (0.2 mg/l), Sucrose (3%)	Suspension	Rech et al., 1998
Rauwolfia serpentina Benth.	Reserpine	LS + NAA (10 µM), BA (1 µM)	Suspension	Yamamoto and Yamada, 1986
Rauvolfia serpentina x *Rhazya stricta* Hybrid plant	3-Oxo-rhazinilam	LS medium	Callus	Gerasimenko et al., 2001
Rhus javanica	Gallotannins	LS + IAA (10^{-6} M), Kinetin (10^{-5} M)	Root	Taniguchi et al., 2000
Ruta sp.	Acridone and Furoquinoline alkaloids and coumarins	MS + 2,4-D (1 mg/l), Kinetin (1 mg/l)	Callus	Baumert et al., 1992
Salvia miltiorrhiza	Lithospermic acid B and Rosmarinic acid	MS + 2,4-D (0.5 mg/l), BA (0.5 mg/l)	Callus	Morimoto et al., 1994
Salvia miltiorrhiza	Cryptotanshinone	MS + 2,4-D (1 mg/l), Kinetin (0.1 mg/l)	Suspension	Miyasaka et al., 1989
Scopolia parviflora	Alkaloids	LS + 2,4-D (10^{-6} M), IAA (10^{-5} M)	Callus	Tabata et al., 1972
Scutellaria columnae	Phenolics	MS + 2,4-D (0.3 mg/l), Kinetin (1 mg/l)	Callus	Stojakowska and Kisiel, 1999
Solanum chrysotrichum (Schldl.)	Spirostanol saponin	MS + 2,4-D (2 mg/l), Kinetin (0.5 mg/l), Scrose (3-4%)	Suspension	Villarreal et al., 1997
Solanum laciniatum Ait	Solasodine	MS + 2,4-D (1 mg/l), Kinetin (1 mg/l), Sucrose (3%)	Suspension	Chandler and Dodds, 1983a
Silybum marianum	Flavonolignan	Hormone free LS medium	Root	Alikaridis et al., 2000
Solanum paludosum	Solamargine	MS + BA (10^{-6} M), NAA (10^{-6} M) or MS + Kinetin (10^{-6} M) + 2,4-D (10^{-6} M)	Suspension	Badaoui et al., 1996
Tabernaemontana divaricata	Alkaloids	MS + NAA (2 mg/l), BA (0.2 mg/l)	Suspension	Sierra et al., 1992
Taxus spp.	Taxol	B5 medium + 2,4-D (0.2 mg/l), BA (0.5 mg/l), Casein hydrolysate (200 mg/l), Sucrose (3%)	Suspension	Wu et al., 2001
Taxus baccata	Taxol baccatin III	B5 (salts) + 3× B5 vitamins, 2,4-D (2×10^{-2} mM) Kinetin (4×10^{-3} mM) + GA_3 (10^{-3} mM)	Suspension	Cusido et al., 1999
Thalictrum minus	Berberin	LS + NAA (60 µM), 2,4-D (1 µM), BA (10 µM)	Suspension	Kobayashi et al., 1987
Thalictrum minus	Berberin	LS + NAA (60 µM), BA (10 µM)	Suspension	Nakagawa et al., 1986
Torreya nucifera var. *radicans*	Diterpenoids	MS + 2,4-D (10 mg/l), Casamino acid (1 g/l), Coconut mulk (7%), and K^+ instead of $NH4^+$	Suspension	Orihara et al., 2002
Trigonella foenumgraecum	Saponins	MS + 2,4-D (0.25 or 0.5 mg/l), Kinetin (0.5 mg/l)	Suspension	Brain and Williams, 1983
Withaina somnifera	Withaferin A	MS + BA (1 mg/l), Sucrose (3%)	Shoot	Ray and Jha, 2001

Abbreviations: B5 = Gamborg's (1968) medium; BA = 6-Benzyladenine; 2,4-D = 2,4-dichlorophenoxyacetic acid; GA_3 = Gibberellic acid; IAA = Indole-3-acetic acid; IBA = Indole-3-butyric acid; 2iP = N_6-[2-isopentenyl]-adenine; LS = Linsmaier and Skoogs (1965) medium; MS = Murashige and Skoog (1962) medium; NAA = Napthaleneacetic acid.

to produce substances with antitumor, antiviral, hypoglycaemic, anti-inflammatory, antiparasite, antimicrobial, tranquilizer and immunomodulating activities through tissue culture technology.

Exploration of the biosynthetic capabilities of various cell cultures has been carried out by a group of plant scientists and microbiologists in several countries during the last decade. In the last few years promising findings have been reported for a variety of medicinally valuable substances, some of which may be produced on an industrial scale in the near future. The aim of the present review is to focus on the importance of tissue culture technology in production of some of the plant pharmaceuticals reported earlier. We will also describe the successful research on tissue cultures for production of bioactive metabolites performed at our own laboratory.

Tissue Cultures Producing Pharmaceutical Products of Interest

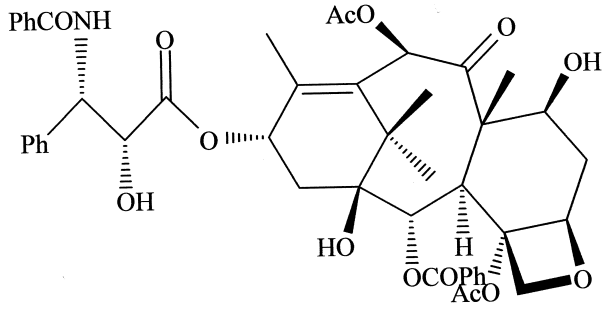
Research in the area of plant tissue culture technology has resulted in the production of many pharmaceutical substances for new therapeutics. Advances in the area of cell cultures for the production of medicinal compounds has made possible the production of a wide variety of pharmaceuticals like alkaloids, 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.

Taxol

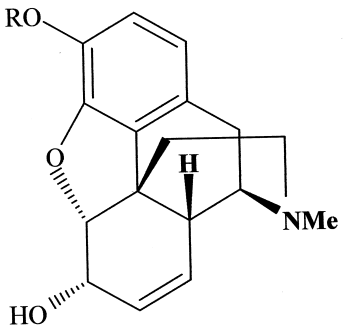
Taxol (plaxitaxol), a complex diterpene alkaloid found in the bark of the *Taxus* tree, is one of the most promising anticancer agents known due to its unique mode of action on the micro tubular cell system (Jordan and Wilson, 1995). At present, production of taxol by various *Taxus* species cells in cultures has been one of the most extensively explored areas of plant cell cultures in recent years owing to the enormous commercial value of taxol, the scarcity of the *Taxus* tree, and the costly synthetic process (Cragg et al., 1993; Suffness, 1995). In 1989, Christen et al. reported for the first time the production of taxol (placlitaxel) by *Taxus* cell cultures. Fett-Neto et al. (1995) have studied the effect of nutrients and other factors on paclitaxel production by *T. cuspidata* cell cultures (0.02% yield on dry weight basis). Srinivasan et al. (1995) have studied the kinetics of biomass accumulation and paclitaxel production by *T. baccata* cell suspension cultures. Paclitaxel was found to accumulate at high yields (1.5 mg/l) exclusively in the second phase of growth. Kim et al. (1995) established a similar level of paclitaxel from *T. brevifolia* cell suspension cultures following 10 days in culture with optimized medium containing 6% fructose. Ketchum and Gibson (1996) reported that addition of carbohydrate during the growth cycle increased the production rate of paclitaxel, which accumulated in the culture medium (14.78 mg/l). In addition to paclitaxel, several other taxoids have been identified in both cell and culture medium of *Taxus* cultures (Ma et al., 1994). Parc et al. (2002) reported production of taxoids by callus cultures from selected Taxus genotypes. In order to increase the taxoid production in these cultures, the addition of different amino acids to the culture medium were studied, and phenylalanine was found to assist in maximum taxol production in *T. cuspidata* cultures (Fett-Neto et al., 1994). The influence of biotic and abiotic elicitors was also studied to improve the production and accumulation of taxol through tissue cultures (Ciddi et al., 1995; Strobel et al., 1992; Yukimune et al., 1996). The production of taxol from nodule cultures containing cohesive multicultural units displaying a high degree of differentiation has been achieved from cultured needles of seven *Taxus* cultivars (Ellis et al., 1996). Factors influencing stability and recovery of paclitaxel from suspension cultures and the media have been studied in detail by Nguyen et al. (2001). The effects of rare earth elements and gas concentrations on taxol production have been reported (Wu et al., 2001 and Linden et al., 2001).

Morphine and Codeine

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 production of these compounds. Production of morphine and codeine in morphologically undifferentiated cultures has been re-



Taxol

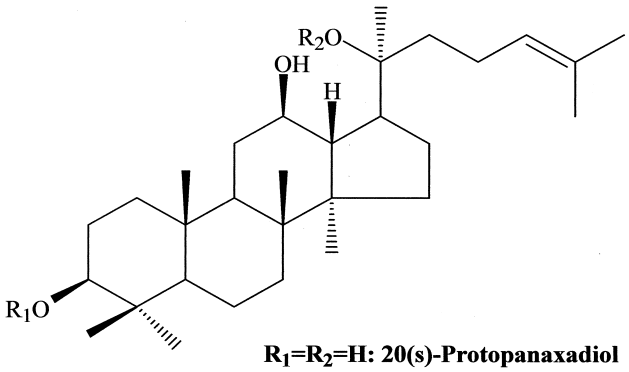


R= H: Morphine
R=Me: Codeine

ported (Tam et al., 1980; Yoshikawa and Furuya, 1985). Removal of exogenous hormones from large-scale culture systems could be implemented using a two-stage process strategy by Siah and Doran (1991). Without exogenous hormones, maximum codeine and morphine concentrations were 3.0 mg/g dry weight and 2.5 mg/g dry weight, respectively, up to three times higher than in cultures supplied with hormones. Biotransformation of codeinone to codeine with immobilized cells of *P. somniferum* has been reported by Furuya et al. (1972). The conversion yield was 70.4%, and about 88% of the codeine converted was excreted into the medium.

Ginsenosides

The root of *Panax ginseng* C.A. Mayer, so-called ginseng, has been widely used as a tonic and highly prized medicine since ancient times (Tang and Eisenbrand, 1992a). Ginseng has been recognized as a miraculous promoter of health and longevity. The primary bioactive constituents of ginseng were identified as ginsenosides, a group of triterpenoid saponins (Huang, 1993a; Proctor, 1996; Sticher, 1998). Among them, ginsenoside Rg_1 is one of the major active molecules from *Panax ginseng* (Lee et al., 1997). Chang and Hsing (1980a) obtained repeatable precocious flowering in the embryos derived from mature gin-



R_1=R_2=H: 20(s)-Protopanaxadiol

Ra: R_1= glucose-6-1-glucose-6-1-glucose
R_2= glucose-3-1-glucose-3-1-glucose

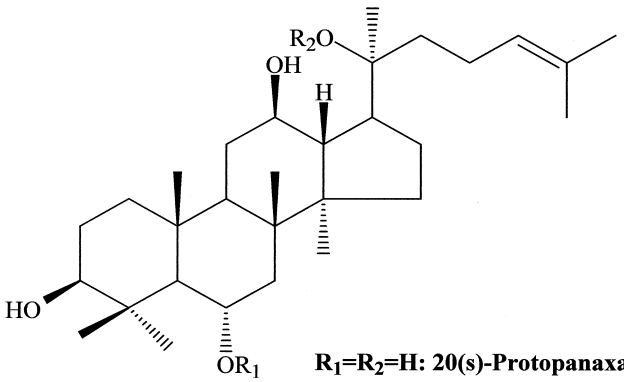
Rb_1: R_1= glucose-2-1-glucose
R_2= glucose-6-1-glucose

Rb_2 : R_1= glucose-2-1-glucose
R_2= glucose-6-1-arabinose(pyr)

Rb_3: R_1= glucose-2-1-glucose
R_2= glucose-6-1-xylose

Rc: R_1= glucose-2-1-glucose
R_2= glucose-6-1-arabinose(fur)

Rd: R_1= glucose-2-1-glucose
R_2= glucose



R_1=R_2=H: 20(s)-Protopanaxatriol

Re : R_1= glucose-2-1-rhamnose
R_2= glucose

Rf: R_1= glucose-2-1-glucose
R_2= H

Rg_1: R_1= glucose
R_2= glucose

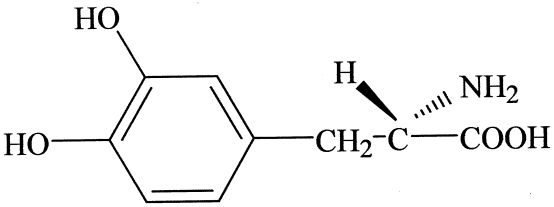
Rg_2: R_1= glucose-2-1-glucose
R_2= H

Structures of Ginsenosides

seng root callus cultured on a chemically defined medium. Also, plant regeneration through somatic embryogenesis in root-derived callus of ginseng has been reported (Chang and Hsing, 1980b). In recent years ginseng cell culture has been explored as a potentially more efficient method of producing ginsenosides. The effect of medium components like carbon (Furuya et al., 1984; Choi et al., 1994), nitrogen (Franklin and Dixon, 1994), and phosphate (Zhang and Zhong, 1997) concentrations and plant growth hormones (Furuya, 1988) were thoroughly studied to increase the production of ginsenosides. Influence of potassium ion was also studied (Liu and Zhong, 1996). Large-scale suspension culture of ginseng cells was first reported by Yasuda et al. (1972). Later on an industrial-scale culture process was initiated by Nitto Denko Corporation (Ibaraki, Osaka, Japan) in the 1980s using 2000 and 20000-1 stirred tank fermentors to achieve productivities of 500-700 mg/l per day (Furuya, 1988; Ushiyama, 1991). This process is considered an important landmark in the commercialization of plant tissue and cell culture on a large scale. In addition to this, *Agrobacterium tumefaciens* infected root cultures were introduced, productivity of which was found to exceed the callus of normal roots threefold (Choi et al., 1989). Other types of tissue cultures, such as embryogenic tissues (Asaka et al., 1993) and hairy roots transformed by *Agrobacteria* (Yoshikawa and Furuya, 1987; Hwang et al., 1991; Ko et al., 1996) have been examined. Yu et al. (2000) reported ginsenoside production using elicitor treatment. These developments indicate that ginseng cell culture process is still an attractive area for commercial development around the world and it possesses great potential for mass industrialization. Concentration of plant growth regulators in the medium influences the cell growth and ginsenoside production in the suspension cultures (Zhong et al., 1996). Recent studies have shown that addition of methyl jasmonate or dihydro-methyl jasmonate to suspension cultures increases the production of ginsenosides (Wang and Zhong, 2002). Also, jasmonic acid improves the accumulation of gensinosides in the root cultures of ginseng (Yu et al., 2002).

L-DOPA

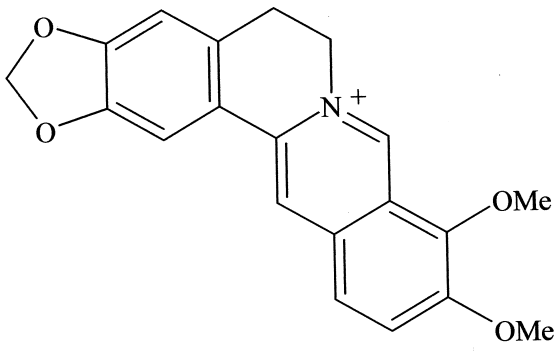
L-3,4-dihydroxyphenylalanine, is an important intermediate of secondary metabolism in higher plants and is known as a precursor of alkaloids, betalain, and melanine, isolated from *Vinca faba* (Guggenheim, 1913), *Mucuna*, *Baptisia* and *Lupinus* (Daxenbichler et al., 1971). It is also a precursor of 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. The widespread application of this therapy created a demand for large quantities of L-DOPA at an economical price level, and this led to the introduction of cell cultures as an alternative means for enriched production. Brain (1976) found that the callus tissue of *Mucuna pruriense* accumulated 25 mg/l DOPA in the medium containing relatively high concentrations of 2,4-D. Teramoto and Komamine (1988) induced callus tissues of *Mucuna hassjoo, M. Pruriense,* and *M. deeringiana* and optimized the culture conditions. The highest concentration of DOPA was obtained when *M. hassjoo* cells were cultivated in MS medium with 0.025 mg/l 2.4 -D and 10 mg/l kinetin. The level of DOPA in the cells was about 80 mmol/g-f.w.



L-DOPA

Berberine

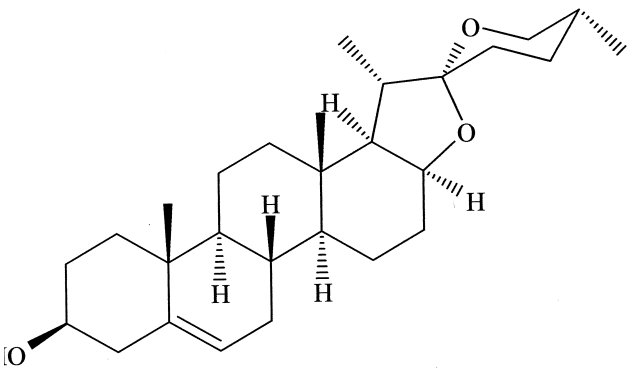
Berberine is an isoquinoline alkaloid found in the roots of *Coptis japonica* and cortex of *Phellondendron amurense*. This antibacterial alkaloid has been identified from a number of cell cultures, notably those of *Coptis japonica* (Sato and Yamada, 1984), *Thalictrum* spp. (Nakagawa et al., 1984; Suzuki et al., 1988), and *Berberis* spp. (Breuling et al., 1985). The productivity of berberine was increased in cell cultures by optimizing the nutrients in the growth medium and the levels of phytohormones (Sato and Yamada, 1984; Nakagawa et al., 1984, 1986; Morimoto et al., 1988). By selecting high yielding cell lines, Mitsui group produced berberine on a large scale with a productivity of 1.4 g/l over 2 weeks. Other methods for increasing yields include elicitation of cultures with a yeast polysaccharide elicitor, which has been successful with a relatively low producing *T. rugosum* culture (Funk et al., 1987). The influence of spermidine on berberine production in *Thalictrum minus* cell cultures has been reported by Hara et al. (1991).



Berberine

Diosgenin

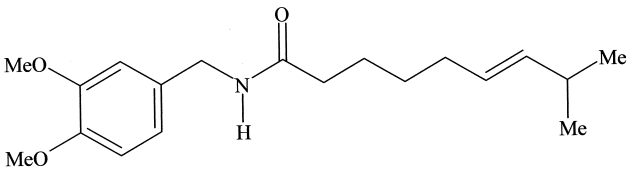
Diosgenin is a precursor for the chemical synthesis of steroidal drugs and is tremendously important to the pharmaceutical industry (Zenk, 1978). In 1983, Tal et al. reported on the use of cell cultures of *Dioscorea deltoidea* for production of diosgenin. They found that carbon and nitrogen levels greatly influenced diosgenin accumulation in one cell line. Ishida (1988) established *Dioscorea* immobilized cell cultures, in which reticulated polyurethane foam was shown to stimulate diosgenin production, increasing the cellular concentration by 40% and total yield by 25%. Tal et al. (1983) have been able to obtain diosgenin levels as high as 8% in batch-grown *D. deltoidea* cell suspensions. However, the daily productivity was only 7.3 mg/l. Several other groups have also attempted cell cultures for diosgenin production (Heble et al., 1967; Brain and Lockwood, 1976; Jain and Sahoo, 1981; Jain et al., 1984; Emke and Eilert, 1986; Huang et al., 1993). Kaul et al. (1969) studied the influence of various factors on diosgenin production by *Dioscorea deltoidea* callus and suspension cultures. The search for high-producing cell lines coupled to recent developments in immobilized cultures and the use of extraction procedures, which convert furostanol saponins to spirostanes such as diosgenin, should prove useful in increasing productivity in the years to come.



Diosgenin

Capsaicin

Capsaicin, an alkaloid, is used mainly as a pungent food additive in formulated foods. It is obtained from fruits of green pepper (*Capsicum* spp.). Capsaicin is also used in pharmaceutical preparations as a digestive stimulant and for rheumatic disorders (Sooch et al., 1977). Suspension cultures of *Capsicum frutescens* produce low levels of capsaicin, but immobilizing the cells in reticulated polyurethane foam can increase production approximately 100-fold (Lindsey and Yeoman, 1984). Further improvements in productivity can be brought about by supplying precursors such as isocapric acid (Lindsey and Yeoman, 1984). Lindsey (1985) reported that treatments which suppress cell growth and primary metabolism seem to improve capsaicin synthesis. A biotechnological process has been developed for the production of capsaicin from *C. frutescens* cells (Lindsey et al., 1983). Holden et al. (1988) have reported elicitation of capsaicin in cell cultures of *C. frutescens* by spores of *Gliccladium deliquescens*. The effects of nutritional stress on capsaicin production in immobilized cell cultures of *Capsicum annum* were studied thoroughly by Ravishankar et al. (1988). Biotransformation of externally fed protocatechuic aldehyde and caffeic acid to capsaicin in freely suspended cells and immobilized cells cultures of *Capsicum frutescens* has also been reported (Ramachandra Rao and Ravishankar, 2000).



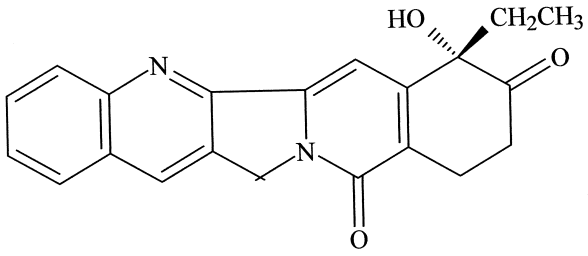
Capsaicin

Camptothecin

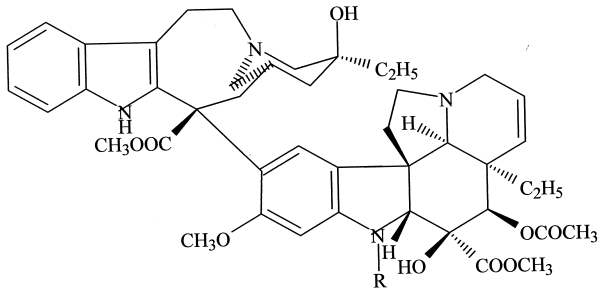
Camptothecin, a potent antitumor alkaloid was isolated from *Camptotheca acuminata.* Sakato and Misawa (1974) induced *C. acuminata* callus on MS medium containing 0.2 mg/l 2,4-D and 1 mg/l kinetin and developed liquid cultures in the presence of gibberellin, L-tryptophan, and conditioned medium, which yielded camptothecin at about 0.0025% on a dry weight basis. When the cultures were grown on MS medium containing 4 mg/l NAA, accumulation of camptothecin reached 0.998 mg/l (Van Hengal et al., 1992). 10-Hydroxycamptothecin, a promising derivative of camptothecin is in clinical trials in the US.

Vinblastine and Vincristine

The dimeric indole alkaloids vincristine and vinblastine have become valuable drugs in cancer chemotherapy due to their potent antitumor activity against various leukemias and solid tumors. These compounds are extracted commercially from large quantities of *Catharanthus roseus*. Since the intact plant contains low concentrations (0.0005%), plant cell cultures have been employed as an alternative to produce large amounts of these alkaloids. Vinblastine is composed of catharanthine and vindoline. Since



Camptothecine

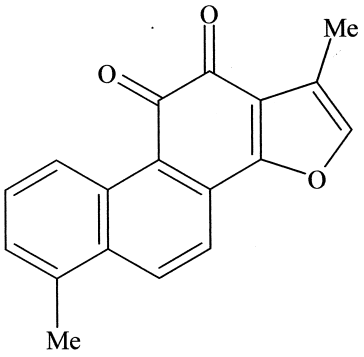


R=CH_3: Vinblastine
R=CHO: Vincristine

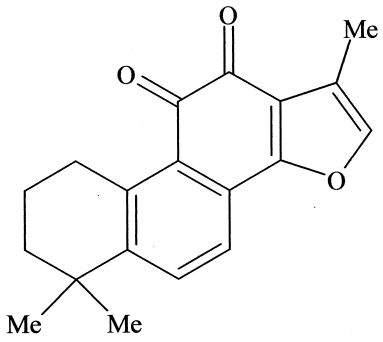
vindoline is more abundant than catharanthin in intact plants, it is less expensive. Misawa et al. (1988) established an economically feasible process consisting of production of catharanthine by plant cell fermentation and a simple chemical or an enzymatic coupling. The significant influence of various compounds, like vanadyl sulphate, abscisic acid, and sodium chloride on catharanthin production have been described by Smith et al. (1987). Endo et al. (1988) attempted synthesis of anhydrovinblastine (AVLB from catharanthine and vindoline through enzymic coupling followed by sodium borohydride reduction). A crude preparation of 70% ammonium sulphate precipitated protein from the cultured cells of *C. roseus* was used as an enzyme source. The reaction mixture contained catharanthine, vindoline, Tris buffer, Ph 7.0, and the crude enzyme; the mixture was incubated at 300°C and for 3 h. The products of the reaction were various dimeric alkaloids including vinamidine, 3(R)-hydroxyvinamidine, and 3, 4-anhydrovinblastine. Dimerization using ferric ion catalyst in the absence of enzyme resulted in anhydrovinblastine and vinblastine in 52.8% and 12.3% yields, respectively. The yield of vinblastine via chemical coupling was improved in the presence of ferric chloride, oxalate, maleate, and sodium borohydride. Influence of various parameters like stress, addition of bioregulators, elicitors and synthetic precursors on indole alkaloids production were studied in detail by Zhao et al. (2001a and b). Also, metabolic rate-limitations through precursor feeding (Morgan and Shanks, 2000) and effect of elicitor dosage on biosynthesis of indole alkaloids (Rijhwani and Shanks, 1998) in *Catharanthus roseus* hairy root cultures have been reported.

Tanshinones

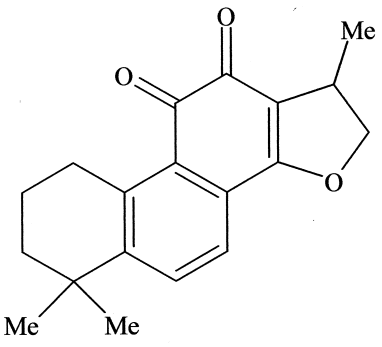
Tanshinones are a group of quinoid diterpenoids believed to be active principles of Danshen (*Salvia miltiorrhiza*), a well known traditional Chinese medicine. Tanshinone I and cryptotanshinone prevent complications of myocardial ischemia; tanshinone II A has undergone



Tanshinone-I



Tanshinone-II A

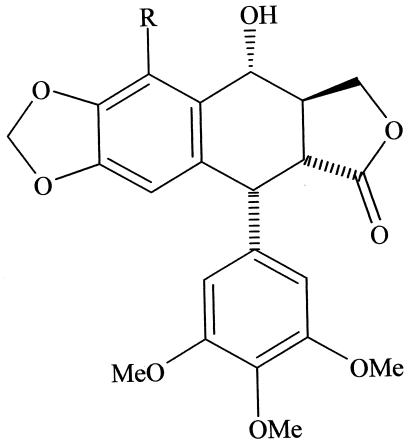


Cryptotanshinone

successful clinical trials for the treatment of angina pectoris in China (Bruneton, 1995). Plant cell and organ culture technology provide an alternative means of producing these active ingredients. Nakanishi et al. (1983) established a cell line containing abundant amounts of cryptotanshinone from *S. miltiorrhiza*. Adventitious root cultures of *S. miltiorrhiza* and the culture conditions for high yield production of tanshinones in the adventitious roots were reported by Shimomura et al. (1991). Diterpenoid production in Ti-transformed root or hairy root cultures of *S. miltiorrhiza* has also been established by Hu and Alfermann (1993). In these cultures, although relatively high tanshinone production was achieved, the morphological characteristics of the hairy roots require special bioreactors for the cultivation, which has hindered the scale-up of such processes.

Podophyllotoxin

Podophyllotoxin is an antitumor aryltetralin lignan found in *Podophyllum peltatum* and *Podophyllum hexandrum*. It also serves as a starting material for the preparation of its semisynthetic derivatives, etoposide and teniposide, widely used in anti-tumor therapy (Issell et al., 1984). These plants, which grow very slowly, are collected from the wild and are thus increasingly rare. This limits the supply of podophyllotoxin and necessitates the search for alternative production methods. Cell cultures of *P. peltatum* for production of podophyllotoxin was first attempted by Kadkade et al. (1981, 1982). To increase the yield of podophyllotoxin, Woerdenberg et al. (1990) used a complex of a precursor, coniferyl alcohol, and b-cyclodextrin to *P. hexandrum* cell suspension cultures. The addition of 3 mM coniferyl alcohol complex yielded 0.013% podophyllotoxin on a dry weight basis, but the cultures without the precursor produced only 0.0035%. Smollny et al. (1992) reported that callus tissues and suspension culture cells of *Lilium album* produced 0.3% podophyllotoxin. Several other tissue culture approaches have been studied to increase the yields (Berlin et al., 1988; Van Uden et al., 1989; Hyenga et al., 1990). Since 5-methoxypodophyllotoxin, an analogue of podophyllotoxin, has strong cytostatic activity (Berlin et al., 1988), many researchers have tried to improve its yield through tissue cultures (Van Uden et al., 1990; Wichers et al., 1990).



R=H : Podophyllotoxin
R=OMe: 5-Methoxypodophyllotoxin

Studies on *In Vitro* Cultures and Production of Important Secondary Metabolites in the Author's Laboratory

Even though several types of cell culture methods are being used to produce important bioactive secondary metabolites, use of cell suspension cultures is preferred for large-scale production due to its rapid growth cycles. Thus cell suspensions are used for generating large amounts of cells for quantitative or qualitative analysis of growth responses and metabolism of novel chemicals. Based on the exciting results in production of medicinal compounds reported above using cell suspension cultures, we have successfully established cell suspension cultures for the production of taxol from *Taxus mairei*, imperatorin from *Angelica dahurica*, and diosgenin from *Dioscorea doryophora* at our research center. We have also succeeded in propagating some of the valuable Chinese medicinal herbs and estimating their active ingredients quantitatively using high performance liquid chromatography (HPLC). The work carried out at our research centers is summarized in the following sections.

Production of Taxol from Taxus mairei by Cell Suspension Cultures

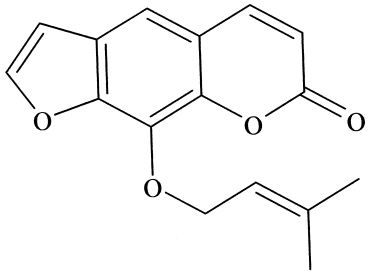
Taxol, a complex diterpene alkaloid, is an anticancer drug found in 1971, by Wani et al. from the Pacific yew tree, *Taxus brevifolia* (Wani et al., 1971). At present the drug is approved for clinical treatment of ovarian and breast cancer by the Food and Drug Administration (FDA, USA). It also has significant activity against malignant melanoma, lung cancer, and other solid tumors (Wickremesinhe and Arteca, 1993, 1994). However, the supply of taxol for clinical use is limited. It depends on extraction from yew trees, and the bark is the only commercial source. The thin bark of the yew tree contains 0.001% taxol on a dry weight basis. A century-old tree yields an average of 3 kg of bark, corresponding to 300 mg of taxol, approximately a single dose in the course of a cancer treatment. Because of the scarcity of the slow growing trees and the relatively low taxol content (Cragg et al., 1993), alternative sources are needed to meet the increasing demand for the drug. The total synthesis of taxol on an industrial-scale seems economically unrealistic due to the complexity of the chemical structure of this molecule (Holton et al., 1994; Nicolaou et al., 1994). The plant cell culture of *Taxus* spp. is considered one possible approach to providing a stable supply of taxol and related taxane compounds (Slichenmyer and Von Horf, 1991).

To exploit the source of taxol, we collected different tissues of *Taxus mairei*, a species found in Taiwan at an alti-

tude of about 2,000 m above sea level. The extracts of bark and leaf tissues were analyzed using HPLC for the content of taxol and taxol related compounds. The HPLC analysis revealed that amounts of taxol and taxol related compounds varies in individual plants, and the principle components such as docetaxel, baccatin III, and 10-deacetylbaccatin in leaf extract were higher than those in bark extracts (Lee et al., 1995). *Taxus mairei* calli were induced from needle and stem explants on Gamborg's B5 medium supplemented with 2 mg/l 2,4-D or NAA. Different cell lines were established using stem and needle derived callus. One of the cell lines, after precursor feeding and 6 weeks of incubation, produced 200 mg taxol per liter of cell suspension culture.

Formation of Imperatorin from Angelica dahurica var. formosana by cell Suspension Cultures

Angelica dahurica var. *formosana* commonly known as "Bai-Zhi" in Chinese is a valuable medicinal herb used in the treatment of headache and psoriasis in China (Zhou, 1980). The constituent imperatorin is believed to be the major active ingredient for curing skin disease (Zhou et al., 1988). *Angelica dahurica* var. *formosana* is a perennial and indigenous plant in Taiwan (Chen et al., 1994). We have studied cell suspension cultures of *Angelica dahurica* var. *formosana* for the production of imperatorin. *Angelica dahurica* var. *formosana* plants were obtained from their natural habitat in the Yang-Ming National Park of Taiwan. The callus was induced from petiole explants on a medium supplemented with 1 mg/l 2, 4-D and 0.5 mg/l kinetin. The resultant callus was used in establishing the cell suspension culture. By increasing the phosphate concentration in the basal medium to 2 mM and using an ammonium to nitrate ratio of 2:1, it was possible to increase the production of imperatorin in cell suspension cultures. Glucose was found to be a better carbon source than sucrose and fructose. The addition of 0.5-1 mg/l of BA to the culture medium increased imperatorin yield, while addition of auxins to the culture medium decreased it. Supplementing the medium with 20 g/l of the adsorbent Amberlite XAD-7 increased imperatorin yield 140-fold (Tsay et al., 1994; Tsay, 1999).



Imperatorin

Production of Diosgenin from Dioscorea doryophora by Cell Suspension Culture

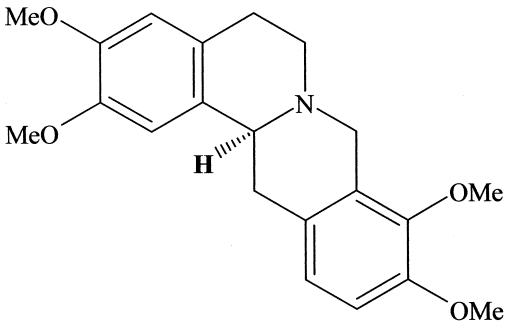
Dioscorea spp. (Dioscoreaceae) are frequently used as a tonic in Chinese traditional medicine. *Dioscorea doryophora* Hance tubers are in high demand as they are used not only as crude drug but also as food. The most active ingredient discovered in the tuber is diosgenin, which can be used as a precursor for many important medicinal steroids, such as prednisolone, dexamethasone, norethisterone, and metenolone (Tsukamoto et al., 1936).

In order to increase diosgenin yield and facilitate the purification process, we have established a cell suspension culture of *Dioscorea doryophora* Hance (Yeh et al., 1994). Cell suspension cultures were obtained from microtuber and stem node-derived callus in liquid culture medium supplemented with 0.1 mg/l 2,4-D, 3% sucrose and incubated on a rotary shaker at 120 rpm. Although 6% sucrose was found to be optimum for the growth of cell suspension culture, cells cultured in a 3% sucrose medium produced more diosgenin. Analysis by HPLC revealed that both stem-node and microtuber derived suspension cells contained diosgenin. The microtuber derived cell suspension culture contains 3.2% diosgenin per gram dry weight while the stem-node derived cultures contain only 0.3%. As the amount of diosgenin obtained from a tuber-derived cell suspension is high and comparable with that found in the intact tuber (Chen, 1985), a cell suspension culture can be used to produce diosgenin.

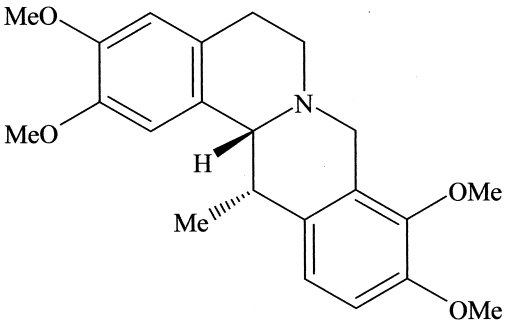
Formation and Analysis of Corydaline and Tetrahydropalmatine from Tubers of Somatic Embryo-Derived Plants of Corydalis yanhusuo

The genus *Corydalis* (Fumariaceae or Papaveraceae) comprises about 320 species, widely distributed in the northern hemisphere, of which around seventy species have been used in traditional herbal remedies in China, Japan, and Korea (Kamigauchi and Iwasa, 1995). The dried and pulverized tubers of *C. yanhusuo*, also called *Rhizoma Corydalis* or yan-hu-suo are a rich source of several pharmacologically important alkaloids (Huang, 1993b). These are used in traditional Chinese medicine to treat gastric and duodenal ulcer, cardiac arrhythmia disease (Kamigauchi and Iwasa, 1995), rheumatism and dysmenorrhea (Tang and Eisenbrand 1992b). *Corydalis yanhusuo* is a slow-growing herb susceptible to fungal diseases which cause serious crop loss and also affect tuber quality. To achieve high productivity, homogeneity, and good quality tubers, pathogen-free planting material must be obtained (Sagare et al., 2000). Plant regeneration via in vitro culture of *C. yanhusuo* would be useful for quick, mass propagation of this important medicinal plant.

A protocol for complete plant regeneration via somatic embryogenesis from tuber derived callus, and production of bioactive compounds such as D, L-tetrahydropalmatine and D-corydaline from the tubers of somatic embryo-derived plants has been standardized in our laboratory (Lee et al., 2001). Primary callus was induced by culturing ma-



Tetrahydropalmatine

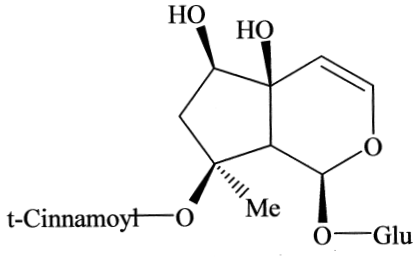


D-Corydaline

ture tuber pieces on a medium supplemented with 2.0 mg/l BA and 0.5 mg/l NAA in darkness. Somatic embryos were induced by subculturing the primary callus on medium supplemented with various concentrations of cytokinins, within 2 weeks of culture in light. The converted somatic embryos of *C. yanhusuo* were cultured for one month on different treatments (growth regulators) in order to promote tuberization and access their effect on accumulation of protoberberine alkaloids. After one and six months of culture in different treatments, the alkaloid contents in the tuber were analyzed by HPLC. The analysis revealed that, somatic embryos cultured on 0.1 mg/l GA_3 for six months showed high amounts of both D, L-tetrahydropalmatine and D-corydalin in the tubers among these treatments. The highest corydalin content was about 3.8 mg/g dry weight after six months of culture on 0.5 mg/l paclobutrazol. The supplementation of an amino acid precursor such as tyrosine (Staba et al., 1982; Kamigauchi and Iwasa, 1995) to the culture medium may further improve the production of these compounds.

In Vitro Synthesis of Harpagoside, an Anti - Inflammatory Irridoid Glycoside from Scrophularia yoshimurae Yamazaki

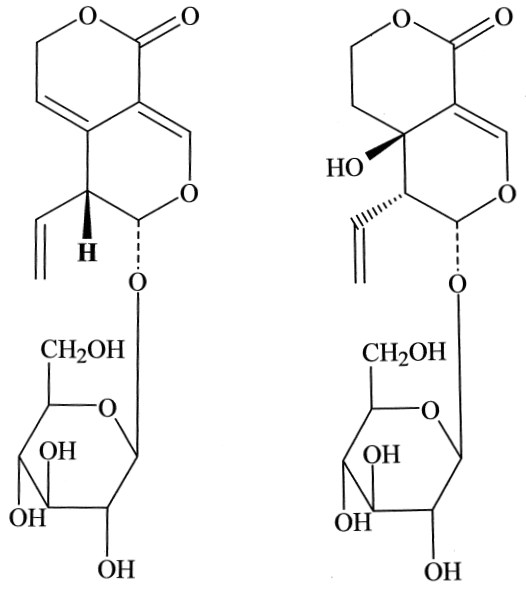
Scrophularia yoshimurae Yamazaki, belonging to the family Scrophulariaceae, is an herbaceous perennial plant 40-60 cm tall that is indigenous to Taiwan. *Scrophularia yoshimurae* is used as "Xuanshen," a substitute for *S. ningpoensis*, in traditional Chinese medicine in Taiwan (Chiu and Chang, 1998). In view of *Scrophularia's* medicinal value, an efficient protocol for micropropagation of *Scrophularia yoshimurae* (Scrophulariaceae) has been developed at our laboratory (Sagare et al., 2001). Multiple shoot development was achieved by culturing the shoot tip, leaf base, stem-node and stem-internode explants on Murashige and Skoog (MS) medium supplemented with 4.44 µM N^6-benzyladenine (BA) and 1.07 µM α-naphthaleneacetic acid (NAA). The shoots were multiplied by subculturing on the same medium used for shoot induction. Shoots were rooted on growth regulator-free MS basal medium, transferred to a soil:peat moss:vermiculite (1:1:1 v/v/v) mixture, and acclimatized in the growth chamber. The content of harpagoside, an anti-inflammatory iridoid glucoside, in different plant materials was determined by HPLC. Harpagoside content in the aerial and underground parts of *S. yoshimurae* was significantly higher than in the marketed crude drug (underground parts of *S. ningpoensis*) and varied with the developmental stage of the plant.



Harpagoside

Gentipicroside and Swertiamarin from In Vitro Propagated Plants of Gentiana davidii var. formosana (Gentianaceae)

The genus *Gentiana* (Gentianaceae) comprises about 400 species distributed throughout the world (Skrzypczak et al., 1993). The bitter principles of Gentianaceae constitute many pharmacologically important compounds, explaining the use of most species of this family in traditional medicine and in the preparation of bitter tonics (Rodriguez et al., 1996). Secoiridoid glucosides are the main compounds with medicinal properties in roots of *Gentiana* species (Skrzypczak et al., 1993). Gentiopicroside and swertiamarin are two important secoiridoid glucosides found in Gentianaceae, the former being quantitatively predominant (Tang and Eisenbrand, 1992c). We have developed a highly reproducible and simple protocol for in vitro propagation of *Gentiana davidii* var. *formosana* (Chueh et al., 2000). Induction of multiple shoots (6.3 shoots per explant) was achieved in the axillary buds of the stem node explants (5 mm long) cultured on Murashige and Skoog (MS) medium supplemented with 4.44 µM N^6-benzyladenine (BA) for a period of two months. A more than twofold increase in the number of shoots per explants



Gentiopicroside **Swertiamarin**

(15 shoots per shoot cultured) was observed when the shoots were subcultured on MS medium supplemented with 1.07 µM α-napthaleneacetic acid (NAA) and 8.88 µM BA. Elongated shoots from the multiple shoots were rooted on MS basal medium supplemented with or without various auxins. The optimum rooting response was obtained on the growth regulator-free medium. Rooted shoots were transferred to a peat moss:vermiculite mixture and acclimatized in the growth chamber under high humidity conditions. The contents of gentiopicroside and swertiamarin, the two important secoiridoid glucosides, in different plant materials were determined by HPLC. The content of gentiopicroside and swertiamarin in the aerial and underground parts of *G. davidii* var. *formosana* was higher than in the marketed crude drug (underground parts of *G. scabra*) and varied with the age of the plant.

Conclusions and Future Perspectives

In vitro propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds. The increased use of plant cell culture systems in recent years is perhaps due to an improved understanding of the secondary metabolite pathway in economically important plants. Advances in plant cell cultures could provide new means for the cost-effective, commercial production of even rare or exotic plants, their cells, and the chemicals that they will produce. Knowledge of the biosynthetic pathways of desired compounds in plants as well as of cultures is often still rudimentary, and strategies are consequently needed to develop information based on a cellular and molecular level. Because of the complex and incompletely understood nature of plant cells in in vitro cultures, case-by-case studies have been used to explain the problems occurring in the production of secondary metabolites from cultured plant cells. A key to the evaluation of strategies to improve productivity is the realization that all the problems must be seen in a holistic context. At any rate, substantial progress in improving secondary metabolite production from plant cell cultures has been made within last few years. These new technologies will serve to extend and enhance the continued usefulness of higher plants as renewable sources of chemicals, especially medicinal compounds. We hope that a continuation and intensification efforts in this field will lead to controllable and successful biotechnological production of specific, valuable, and as yet unknown plant chemicals.

Literature Cited

Alikaridis, F., D. Papadakis, K. Pantelia, and T. Kephalas. 2000. Flavonolignan production from *Silybum marianum* transformed and untransformed root cultures. Fitoterapia **71:** 379-384.

Anderson, L.A., C.A. Hay, M.F. Roberts, and J.D. Phillipson. 1986. Studies on *Ailanthus altissima* cell suspension cultures. Plant Cell Rep. **5:** 387-390.

Anderson, L.A., M.F. Roberts, and J.D. Phillipson. 1987. Studies on *Ailanthus altissima* cell suspension cultures. The effect of basal media on growth and alkaloid production. Plant Cell Rep. **6:** 239-241.

Andrijany, V.S., G. Indrayanto, and L.D. Soehono. 1999. Simultaneous effect of calcium, magnesium, copper and cobalt on sapogenin steroids content in callus cultures of *Agave amaniensis*. Plant Cell Tiss. Org. Cult. **55:** 103-108.

Arntzen, C.J. 1997. High tech herbal medicine: plant based vaccines. Nature Biotechnol. **15(3):** 221-222.

Arrebola, M.L., T. Ringbom, and R. Verpoorte. 1999. Anthraquinones from *Isoplexis isabelliana* cell suspension cultures. Phytochemistry **52:** 1283-1286

Asaka, I., I. Ii, M. Hirotani, Y. Asada, and T. Furuya. 1993. Production of ginsenosides saponins by culturing ginseng (*Panax ginseng*) embryogenic tissues in bioreactors. Biotechnol. Lett. **15:** 1259-1264.

Ayabe, S., K. Iida, and T. Furuya. 1986. Induction of stress metabolites in immobilized *Glycyrrhiza echinata* cultured cells. Plant Cell Rep. **3:** 186-189.

Ayabe, S., H. Takano, T. Fujita, H. Hirota, and T. Takahashi. 1990. Triterpenoid biosynthesis in tissue cultures of *Glycyrrhiza glabra* var. *glandulifera*. Plant Cell Rep. **9:** 181-184.

Badaoui, H. E1., B. Muguet, and M. Henry. 1996. Production of solamargine by *in vitro* cultures of *Solanum paludosum.* Plant Cell Tiss. Org. Cult. **45:** 123-127.

Balandrin, M.J. and J.A. Klocke. 1988. Medicinal, aromatic and industrial materials from plants. *In* Y.P.S. Bajaj (ed.), Biotechnology in Agriculture and Forestry. Medicinal and Aromatic Plant, vol. 4. Springer-Verlag, Berlin, Heidelberg, pp. 1-36.

Barthe, G.A., P.S. Jourdan, C.A. McIntosh, and R.L. Mansell. 1987. Naringin and limonin production in callus cultures and regenerated shoots from *Citrus* sp. J. Plant Physiol. **127:** 55-65.

Bassetti, L., M. Hagendoorn, and T. Johannes. 1995. Surfactant-induced non-lethal release of anthraquinones from suspension cultures of *Morinda citrifolia*. J. Biotechnol. **39:** 149-155.

Baumert, A., D. Groger, I.N. Kuzovkina, and J. Reisch. 1992. Secondary metabolites produced by callus cultures of various *Ruta* species. Plant Cell Tiss. Org. Cult. **28:** 159-162.

Berlin, J., N. Bedorf, C. Mollenschott, V. Wray, F. Sasse, and G. Hofle. 1988. On the podophyllotoxins of root cultures of *Linum flavum*. Planta Med. **54:** 204-206.

Brain, K.R. 1976. Accumulation of L-DOPA in cultures from *Mucuna pruriens*. Plant Sci. Lett. **7:** 157-161.

Brain, K.R. and G.B. Lockwood. 1976. Hormonal control of steroid levels in tissue cultures from *Trigonella foenumgraecum*. Phytochemistry **15:** 1651-1654.

Brain, K.R. and M.H. Williams. 1983. Evidence for an alternative route from sterol to sapogenin in suspension cultures from *Trigonella foenumgraecum*. Plant Cell Rep. **2:** 7-10.

Breuling, M., A.W. Alfermann, and E. Reinhard. 1985. Cultivation of cell cultures of *Berberis wilsonae* in 20 l airlift bioreactors. Plant Cell Rep. **4:** 220-223.

Bruneton, J. 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. Intercept, U.K, pp. 522.

Carrier, D., N. Chauret, M. Mancini, P. Coulombe, R. Neufeld, M. Weber, and J. Archambault. 1991. Detection of ginkgolide A in *Ginkgo biloba* cell cultures. Plant Cell Rep. **10:** 256-259.

Chandler, S. and J.H. Dodds. 1983a. Solasodine production in rapidly proliferating tissue cultures of *Solanum laciniatum* Ait. Plant Cell Rep. **2:** 69-72.

Chandler, S.F. and J.H. Dodds. 1983b. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of *Solanum laciniatum*. Plant Cell Rep. **2:** 205-208.

Chang, W.C. and Y.E. Hsing. 1980a. *In vitro* flowering of embryoids derived from mature root callus of ginseng (*Panax ginseng*). Nature **284:** 341-342.

Chang, W.C. and Y.E. Hsing. 1980b. Plant regeneration through somatic embryogenesis in root derived callus og ginseng (*Panax ginseng* C. A. Mayer). Theor. Appl. Genet. **57:** 133-135, 341-342.

Cheetham, P.S.J. 1995. Biotransformations: new routes to food ingredients. Chem Ind., pp. 265-268.

Chen, A.H. 1985. Study on application of diosgenin-I. Analysis of diosgenin constituent of plants from Taiwan. Science Monthly **43:** 79-85.

Chen, C.C., W.T. Chang, Y.S. Chang, and H.S. Tsay. 1994. Studies on the tissue culture of *Angelica dahurica* var. *formosana* II. Establishment of cell suspension culture and evaluation of cultural conditions. J. Chinese Med. **5:** 123-134. (in Chinese).

Chiu, N.Y. and K.H. Chang. 1998. The Illustrated Medicinal Plants of Taiwan, Vol. 5, SMC Publishing Inc., Taipei, Taiwan, Republic of China, pp. 194. (in Chinese)

Choi, K.T., I.O. Ahn, and J.C. Park. 1994. Production of ginseng saponin in tissue culture of ginseng (*Panax ginseng* C. A. Mayer). Russian J. Plant Physiol. **41:** 784-788.

Choi, K.T., D.C. Yang, and J.C. Park. 1989. Characterization of cell line of ginseng (*Panax ginseng* C.A. Mayer) transformed by *Agrobacterium tumefaciens*. *In* S. Lyams, and G. Takeds (eds.), Proc. Of the 6th Internatl. Congr. of SABRAO, Tokyo, pp. 519-522.

Chueh, F.S., C.C. Chen, A.P. Sagare, and H.S. Tsay. 2000. Quantitative determination of secoiridoid glucoside in *in vitro* propagated plants of *Gentiana davidii* var. formosana by high performance liquid chromatography. Planta Med. **67:** 70-73.

Christen, A.A., J. Bland, and G.M. Gibson. 1989. Cell cultures as a means to produce taxol. Proc. Am. Assoc. Cancer Res. **30:** 566.

Ciddi, V., V. Srinivasan, and V.M.L. Shuler. 1995. Elicitation of *Taxus* cell cultures for production of taxol, Biotechnol. Lett. **17:** 1343-1346.

Cragg, G.M., S. A. Schepartz, M. Suffuess, and M.R. Grever. 1993. The taxol supply crisis. New NCI policies for handling the large-scale production of novel natural product anticancer and anti-HIV agents. J. Nat. Prod. **56:** 1657-1668.

Cusido, R.M., J. Palazon, A. Navia-Osorio, A. Mallol, M. Bonfill, C. Morales, and M.T. Pinol. 1999. Production of taxol and baccatin III by a selected *Taxus baccata* callus line and its derived cell suspension culture. Plant Sci. **146:** 101-107.

Daxenbichler, M.E., C.H. Van Etten, E.A. Hallinan, and F.R. Earle. 1971. Seeds as sources of L-DOPA. J. Med. Chem. **14:** 463-465.

Day, K.B., J. Draper, and H. Smith. 1986. Plant regeneration and thebaine content of plants derived from callus culture of *Papaver bracteatum*. Plant Cell Rep. **5:** 471-474.

De-Eknamkul, D. and B.E. Ellis. 1985. Effects of macronutrients on growth and rosmarinic acid formation in cell suspension cultures of *Anchusa officinalis*. Plant Cell Rep. **4:** 46-49.

Desbene, S., B. Hanquet, Y. Shoyama, H. Wagner, and M. Lacaille-Dubois. 1999. Biologically active triterpene saponins from callus tissue of *Polygala amarella*. J. Nat. Prod. **62:** 923-926.

Dicosmo, F. and M. Misawa. 1995. Plant cell and tissue culture: Alternatives for metabolite production. Biotechnol. Adv. **13 (3):** 425-453.

Doran, P.M. 2000. Foreign protein production in plant tissue cultures. Curr. Opin. Biotechnol. **11:** 199-204.

Dornenburg, H. and D. Knorr. 1999. Semicontinuous processes for anthraquinone production with immobilized *Cruciata glabra* cell cultures in a three-phase system. J. Biotechnol. **50:** 55-62.

Ellis, D.D., E.L. Zeldin, M. Brodhagen, W.A. Russin, and B.H. McCown. 1996. Taxol production in nodule cultures of *Taxus*. J. Nat. Prod. **59:** 246-250.

Emke, A. and U. Eilert. 1986. Steroidal alkaloids in tissue cultures and regenerated plants of *Solanum dulcamara*. Plant Cell Rep. **5:** 31-34.

Endo, T., A. Gooddody, J. Vukovic, and M. Misawa. 1988. Enzymes from *catharanthus roseus* cell suspension cultures that couple vindoline and catharanthine to form α-3, 4, -anhydrovinblastine. Phytochemistry **27:** 2147-2149.

Fett-Neto A, G., J.J. Pennington, and F. DiCosmo. 1995. Effect of white light on taxol and baccatin III. Accumulation in cell cultures of *Taxus cuspidata* Sieb and Zucc. J. Plant. Physiol. **146:** 584-590.

Fett-Neto, A.G., J.M. Stewart, S.A. Nicholson, J.J. Pennington, and F. DiCosmo. 1994. Improved taxol yield by aromatic

carboxylic acid and and amino acid feeding to cell cultures of *T. cuspidata*. Biotechnol. Bioeng. **44:** 967-971.

Franklin, C.I. and R.A. Dixon. 1994. Initiation and maintenance of callus and cell suspension cultures. *In* R.A. Dixon and R.A. Gonzales (eds.), Plant Cell Culture-A Practical Approach, 2nd edn. IRL Press, Oxford, pp. 1-25.

Fujita, Y., Y. Hara, T. Ogino, and C. Suga. 1981. Production of shikonin derivatives by cell suspension cultures of *Lithospermum erythrorhizon*. Plant Cell Rep. **1:** 59-60.

Fukui, H., M. Tani, and M. Tabata. 1990. Induction of shikonin biosynthesis by endogenous polysaccharides in *Lithospermum erythrorhizon* cell suspension cultures. Plant Cell Rep. **9:** 73-76.

Funk, C., K. Gugler, and P. Brodelius. 1987. Increased secondary product formation in plant cell suspension cultures after treatment with a yeast carbohydrate preparation (elicitor). Phytochemistry **26:** 401-405.

Furuya, T. 1988. Saponins (ginseng saponins). *In* I.K. Vasil (ed.), Cell Cultures and Somatic Cell Genetics of Plants, Vol. 5. Academic Press, San Diego, CA, pp. 213-234.

Furuya, T., A. Ikuta, and K. Syono. 1972. Alkaloids from callus cultures of *Papaver somniferum*. Phytochemistry **11:** 3041-3044.

Furuya, T., H. Kojima, K. Syono, T. Ishi, K. Uotani, and M. Nishio. 1973. Isolation of saponin and sapogenins from callus tissue of *Panax ginseng*. Chem. Pharm. Bull. **21:** 98-101.

Furuya, T., T. Yoshikawa, Y. Orihara, and H. Oda. 1984. Studies of the culture conditions for *Panax ginseng* cells in jar fermentors. J. Nat. Prod. **47:** 70-75.

Gamborg, O.L., R.A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soyabean root cells. Exp. Cell Res. **50:** 151-158.

Gantet, P. and J. Memelink. 2002. Transcription factors: tools to engineer the production of pharmacologically active plant metabolites. Trends Pharmacol. Sci. **23:** 563-569.

Gerasimenko, I., Y. Sheludko, and J. Stockigt. 2001. 3-Oxorhazinilam: A new indole alkaloid from *Rauwolfia serpentina* x *Rhazya stricta* hybrid plant cell cultures. J. Nat. Prod. **64:** 114-116.

Giri, A. and M.L. Narasu. 2000. Transgenic hairy roots: recent trends and applications. Biotechnol. Adv. **18:** 1-22.

Goleniowski, M. and V.S. Trippi. 1999. Effect of growth medium composition on psilostachyinolides and altamisine production. Plant Cell Tiss. Org. Cult. **56:** 215-218.

Guggenheim, M. 1913. Dioxyphenylalanin, eine neue Aminosaure aus *Vinca faba*. Z. Physiol. Chem. **88:** 276.

Hagimori, M., T. Matsumoto, and Y. Obi. 1982. Studies on the production of *Digitalis* cardenolides by plant tissue culture. III. Effects of nutrients on digitoxin formation by shoot-forming cultures of *Digitalis purpurea* L. grown in liquid media. Plant Cell Physiol. **23(7):** 1205-1211.

Hahn, J.J., C.A. Eschenlauer, H.M. Narrol, A.D. Somers, and F. Srienc. 1997. Growth kinetics, nutrient uptake, and expression of the *Alcaligenes eutrophus* poly(-hydroxybutarate) synthesis pathway in transgenic maize cell suspension cultures. Biotechnol. Prog. **13(4):** 347-354.

Hansen, G. and M.S. Wright. 1999. Recent advances in the transformation of plants. Trends Plant Sci. **4:** 226-231.

Hara, M., Y. Kobayashi, H. Fukui, and M. Tabata. 1991. Enhancement of berberine production by spermidine in *Thalictrum minus* cell suspension cultures. Plant Cell Rep. **10:** 494-497.

Heble, M. R., S. Narayanaswamy, and M.S. Chadha. 1967. Diosgenin production and beta-sitosterol isolation from *Solanum xanthocarpum* tissue cultures. Science **161:** 1145.

Heble, M.R. and E. Staba. 1980. Steroid metabolism in stationary phase cell suspensions of *Dioscorea deltoidea*. Planta Med. Suppl., pp. 124-128.

Hiatt, A., R. Cafferkey, and K. Boedish. 1989. Production of antibodies in transgenic plants. Nature **342:** 76-78.

Holton, R.A., C. Somoza, H.B. Kim, F. Liang, R J. Biediger, P. D. Boatman, M. Shindo, C.C. Smith, S. Kim, H. Nadizadeh, Y. Suzuki, C. Tao, P. Vu, S. Tang, P. Zhang, K.K. Murthi, L.N. Gentile, and J.H. Liu. 1994. First total synthesis of taxol. 1. Functionalization of the B ring. J. Am. Chem. Soc. **116:** 1597-1600.

Holden, R.R., M.A. Holden, and M.M. Yeoman. 1988. The effects of fungal elicitation on secondary metabolism in cell cultures of *Capsicum frutescens*. *In* R.J. Robins and M.J. C. Rhodes (eds.), Manipulating secondary metabolism in culture, Cambridge University Press, pp. 67-72.

Hu, Z.B. and A.W. Alfermann. 1993. Diterpenoid production in hairy root cultures of *Salvia miltiorrhiza*. Phytochemistry **32:** 699-703.

Huang, K.C. 1993a. The pharmacology of Chinese herbs. CRC Press, Boca Raton, FL, pp. 21-45.

Huang, K.C. 1993b. Yan Hu Suo- The dried tuber of *Corydalis turtschaninovii* Bess f. *yanhusuo* (Papaveraceae). In: The pharmacology of Chinese herbs, CRC Press, Boca Raton, Florida, U.S.A. pp. 141-142.

Huang, W.W., C.C. Cheng, F.T. Yeh, and H.S. Tsay. 1993. Tissue culture of *Dioscorea doryophora* HANCE 1. Callus organs and the measurement of diosgenin content. Chin. Med. Coll. J. **2(2):** 151-160.

Hwang, B., K.M. Ko, K.H. Hwang, S.J. Hwang, and Y.H. Kong. 1991. Production of saponin by hairy root cultures of ginseng (*Panax ginseng* CA Mayer) transformed with *Agrobacterium rhizogenes*. Korean J. Biotechnol. **34:** 289-296.

Hyenga, A.G., J.A. Lucas, and P.M. Dewick, 1990. Production of tumour-inhibitory lignans in callus cultures of *Podophyllum hexandrum*. Plant Cell Rep. **9:** 382-385.

Ikuta, A. and H. Itokawa. 1988. Alkaloids of tissue cultures of *Nandina domestica*. Phytochemistry **27(7):** 2143-2145.

Ishida, B.K. 1988. Improved diosgenin production in *Dioscorea deltoidea* cell cultures by immobilization in polyurethane foam. Plant Cell Rep. **7:** 270-273.

Issell, B.F., A.R. Rudolph, and A.C. Louie. 1984. Etoposide (VP-16-213): an overview. *In* B.F. Issell, F.M. Muggia, and S.K. Carter, (eds.), Etoposide (VP-16-213)- Current status and new developments. Academic Press Inc, Orlando, pp. 1-13.

Iwasa, K. and N. Takao. 1982. Formation of alkaloids in *Corydalis ophiocarpa* callus cultures. Phytochemistry **21(3):** 611-614.

Jain, M., A.K. Rathore, and P. Khanna. 1984. Influence of kinetin and auxins on the growth and production of diosgenin by *Costus speciosus* (Koen) Sm. callus derived from rhizome. Agri. Biol. Chem. **48:** 529.

Jain, S.C. and S.L. Sahoo. 1981. Growth and production of ste-

roidal sapogenins and glycialkaloids in *Solanum jasminoides* Paxt. suspension cultures. Agri. Biol. Chem. **45:** 2909-2910.

Jang, Y.P., Y.J. Lee, Y.C. Kim, and H. Huh. 1998. Production of a hepatoprotective cerebroside from suspension cultures of *Lycium chinense*. Plant Cell Rep. **18:** 252-254.

Johnson, T., G.A. Ravishankar, and L.V. Venkataraman. 1990. *In vitro* capsaicin production by immobilized cells and placental tissues of *Capsicum annuum* L. grown in liquid medium. Plant Sci. **70:** 223-229.

Jordon, M.A. and L. Wilson. 1995. Microtuble polymerization dynamics, mitotic, and cell death by paclitaxel at low concentration, American Chemical Society Symposium Series, Vol. 583, Chapter X, pp. 138-153.

Kadkade, P.G. 1981. Formation of podophyllotoxin by *Podophyllum peltatum* tissue cultures. Naturwiss **68:** 481-482.

Kadkade, P.G. 1982. Growth and podophyllotoxin production in callus tissues of *Podophyllum peltatum*. Plant Sci. Lett. **25:** 107-115.

Kamigauchi, M. and K. Iwasa. 1995. VI *Corydalis* spp.: *In vitro* culture and the biotransformation of protoberberines. *In* Y. PS. Bajaj (ed.), Biotechnology in agriculture and forestry, Vol. 26. Springer-Verlag, Berlin Heidelberg, pp. 93-105.

Kaul, B., S.J. Stohs, and E.J. Staba. 1969. *Dioscorea* tissue cultures. 3. Influence of various factors on diosgenin production by *Dioscorea deltoidea* callus and suspension cultures. Lloydia **32:** 347-359.

Ketchum, R.E.B. and D.M. Gibson. 1996. Paclitaxel production in suspension cell cultures of *Taxus*. Plant Cell Tiss. Org. Cult. **46:** 9-16.

Khouri, H.E., R.K. Ibrahim, and M. Rideau. 1986. Effects of nutritional factors on growth and production of anthraquinone glucosides in cell suspension cultures of *Cinchona succirubra*. Plant Cell Rep. **5:** 423-426.

Kieran, P.M., P.F. MacLoughlin, and D.M. Malone. 1997. Plant cell suspension cultures: some engineering considerations. J. Biotechnol. **59:** 39-52.

Kim, J.H., J.H. Yun, Y.S. Hwang, S.Y. Byun, and D. I. Kim. 1995. Production of taxol and related taxanes in *Taxus brevifolia* cell cultures: Effect of sugar. Biotechnol. Lett. **17(1):** 101-106.

Kitajima, M., U. Fischer, M. Nakamura, M. Ohsawa, M. Ueno, H. Takayama, M. Unger, J. Stockigt, and N. Aimi. 1998. Anthraquinone from *Ophiorrhiza pumila* tissue and cell cultures. Phytochemistry **48(1):** 107-111.

Kitamura, Y., T. Ikenaga, Y. Ooe, N. Hiraoka, and H. Mizukami. 1998. Induction of furanocoumarin biosynthesis in *Glehnia littoralis* cell suspension cultures by elicitor treatment. Phytochemistry **48(1):** 113-117.

Ko, K.M., D.C. Yang, J.C. Park, K.J. Choi, K.T. Choi, and B. Hwang. 1996. Mass culture and ginsenoside production of ginseng root by two-step culture process. J. Plant Biol. **39:** 63-69. (in Korean).

Kobayashi, Y., H. Fukui, and M. Tabata. 1987. An immobilized cell culture system for berberine production by *Thalictrum minus* cells. Plant Cell Rep. **6:** 185-186.

Koblitz, H., D. Koblitz, H.P. Schmauder, and D. Groger. 1983. Studies on tissue cultures of the genus *Cinchona* L. alkaloid production in cell suspension cultures. Plant Cell Rep. **2:** 122-125.

Krings, U. and R.G. Berger. 1998. Biotechnological production of flavours and fragrances. Appl. Microb. Biotechnol. **49:** 1-8.

Kueh, J.S.H., I.A. MacKenzie, and G. Pattenden. 1985. Production of chrysanthemic acid and pyrethrins by tissue cultures of *Chrysanthemum cinerariaefolium*. Plant Cell Rep. **4:** 118-119.

Kusakari, K., M. Yokoyama, and S. Inomata. 2000. Enhanced production of saikosaponins by root culture of *Bupleurum falcatum* L. using two step control of sugar concentration. Plant Cell Rep. **19:** 1115-1120.

La Count, W., G. An, and J.M. Lee. 1997. The effect of PVP on the heavy chain monoclonal antibody production from plant suspension cultures. Biotechnol. Lett. **19(1):** 93-96.

Lee, Y.J., E. Chung, K.Y. Lee, Y.H. Lee, B. Huh, and S.K. Lee, 1997. Ginsenoside-Rg_1, one of the major active molecules from *Panax ginseng*, is a functional ligand of glucocorticoid receptor. Mol. Cell. Endocrinol. **133:** 135-140.

Lee, C.Y., F.L. Lin, C.T. Yang, L.H. Wang, H.L. Wei, and H.S. Tsay. 1995. Taxol production by cell cultures of *Taxus mairei*. Proc. Symp. on development and utilization of resources of medicinal plants in Taiwan, Taiwan Agricultural Research Institute, Taiwan, April 21, 1995, TARI Special Publication **48:** 137-148.

Lee, Y.L., A.P. Sagare, C.Y. Lee, H.T. Feng, Y.C. Ko, J.F. Shaw, and H.S. Tsay. 2001. Formation of protoberberine-type alkaloids by the tubers of somatic embryo-derived plants of *Corydalis yanhusuo*. Planta Med. **67:** 839-842.

Linden, J.C., J.R. Haigh, N. Mirjalili, and M. Phisaphalong. 2001. Gas concentration effects on secondary metabolite production by plant cell cultures. Adv. Biochem. Eng. Biotechnol. **72:** 27-62.

Lindsey, K. 1985. Manipulation by nutrient limitation of the biosynthetic activity of immobilized cells of *Capsicum frutescens* Mill. ev. *annuum*. Planta **165:** 126-133.

Lindsey, K. and M.M. Yeoman. 1984. The synthetic potential of immobilized cells of *Capsicum frutescens* Mill. Cv. Annuum. Planta. **162:** 495-501.

Lindsey, K., M.M. Yeoman, G.M. Black, and F. Mavituna. 1983. A novel method for the immobilization and culture of plant cells. FEBS Lett. **155:** 143-149.

Linsmaier, E.M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue culture. Physiol. Plant. **18:** 100-127.

Liu, K.C.S., S.H. Yang, M.F. Roberts, and J.D. Phillipson. 1990. Production of canthin-6-one alkaloids by cell suspension cultures of *Brucea javanica* (L.) Merr. Plant Cell Rep. **9:** 261-263.

Liu, S. and J.J. Zhong. 1996. Effects of potassium ion on cell growth and production of ginseng saponin and polysaccharide in suspension cultures of *Panax ginseng*. J. Biotechnol. **52:** 121-126.

Ma, W., G.L. Park, G.A. Gomez, M.H. Nieder, T.L. Adams, J. S. Aynsley, O.P. Sahai, R.J. Smith, R.W. Stahlhut, and P.J. Hylands. 1994. New bioactive taxoids from cell cultures of *Taxus baccata*. J. Nat. Prod. **57:** 116-122.

Malpathak, N.P. and S.B. David. 1986. Flavor formation in tissue cultures of garlic (*Allium sativum* L.). Plant Cell Rep. **5:** 446-447.

Manson, H. S. and C.J. Arntzen. 1995. Transgenic plants as vaccine production system. Trends Biotechnol. **3:** 388-392.

Mantell, S.H., D.W. Pearson, L.P. Hazell, and H. Smith. 1983. The effect of initial phosphate and sucrose levels on nico-

tine accumulation in batch suspension cultures of *Nicotiana tabacum* L. Plant Cell Rep. **2:** 73-33.

Marden, M.C., W. Dieryck, J. Pagnier, C. Poyart, V. Gruber, P. Bournat, S. Baudino, and B. Merot. 1997. Human hemoglobin from transgenic tobacco. Nature **342:** 29-30.

Misawa, M., T. Endo, A. Goodbody, J. Vukovic, C. Chapple, L. Choi, and J.P. Kutney. 1988. Synthesis of dimeric indole alkaloids by cell free extracts from cell suspension cultures of *C. roseus*. Phytochemistry **27:** 1355-1359.

Miyasaka, H., M. Nasu, and K. Yoneda, 1989. *Salvia miltiorrhiza*: *In vitro* production of cryptotanshinone and feruginol. In. Biotechnology in agriculture and forestry, Vol. 7, medicinal and aromatic plants II. *In* Y.P.S. Bajaj (ed.), Springer-Verlag Berlin, Heidelberg, pp. 417-430.

Moreno, P.R.H., R. van der Heijden, and R. Verpoorte. 1993. Effect of terpenoid precursor feeding and elicitation on formation of indole alkaloids in cell suspension cultures of *Catharanthus roseus*. Plant Cell Rep. **12:** 702-705.

Morgan, J.A. and J.V. Shanks. 2000. Determination of metabolic rate-limitations by precursor feeding in *Catharanthus roseus* hairy root cultures. J. Biotechnol. **79:** 137-145.

Morimoto, T., Y. Hara, Y. Kato, J. Hiratsuka, T. Yoshioka, Y. Fujita, and Y. Yamada. 1988. Berberine production by cultured *Coptis japonica* cells in one-stage culture using medium with a high copper concentration. Agri. Biol. Chem. **52:** 1835-1836.

Morimoto, S., Y. Goto, and Y. Shoyama. 1994. Production of lithospermic acid B and rosmarinic acid in callus tissue and regenerated plantlets of *Salvia miltiorrhiza*. J. Nat. Prod. **57(6):** 817-823.

Morimoto, H. and F. Murai. 1989. The effect of gelling agents on plaunotol accumulation in callus cultures of *Croton sublyratus* Kurz. Plant Cell Rep. **8:** 210-213.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15:** 473-497.

Nakagawa, K., H. Fukui, and M. Tabata. 1986. Hormonal regulation of berberine production in cell suspension cultures of *Thalictrum minus*. Plant Cell Rep. **5:** 69-71.

Nakagawa, K., A. Konagai, H. Fukui, and M. Tabata. 1984. Release and crystalization of berberine in the liquid medium of *Thalictrum minus* cell suspension cultures. Plant Cell Rep. **3:** 254-257.

Nakanishi, T., H. Miyasaka, M. Nasu, H. Hashimoto, and K. Yoneda. 1983. Production of cryptotanshinone and feruginol in cultured cells of *Salvia miltiorrhiza*. Phytochemistry **22 (3):** 721-722.

Nakao, K., K. Ono, and S. Takio. 1999. The effect of calcium on flavanol production in cell suspension cultures of *Polygonum hydropiper.* Plant Cell Rep. **18:** 759-763.

Nicolaou, K.C., Z. Yang, J.J. Liu, H. Ueno, P. G. Nantermet, R. K. Guy, C.F. Claiborne, J. Renaud, E.A. Couladouros, K. Paulvannan, and E. J. Sorensen. 1994. Total synthesis of taxol. Nature **367:** 630-634.

Nazif, N.M., M.R. Rady, and M.M. Seif E1-Nasr. 2000. Stimulation of anthraquinone production in suspension cultures of *Cassia acutifolia* by salt stress. Fitoterapia **71:** 34-40.

Nguyen, T., J. Eshraghi, G. Gonyea, R. Ream, and R. Smith. 2001. Studies on factors influencing stability and recovery of paclitaxel from suspension media and cultures of *Taxus cuspidata* cv Densiformis by high-performance liquid chromatography. J. Chromatogr. A. **911:** 55-61.

O'Dowd, N., P.G. McCauley, D.H.S. Richardson, and G. Wilson. 1993. Callus production, suspension culture and in vitro alkaloid yields of *Ephedra*. Plant Cell Tiss. Org. Cult. **34:** 149-155.

Orihara, Y. and T. Furuya. 1990. Production of theanine and other γ-glutamyl derivatives by *Camellia sinensis* cultured cells. Plant Cell Rep. **9:** 65-68.

Orihara, Y., J.W. Yang, N. Komiya, K. Koge, and T. Yoshikawa. 2002. Abietane diterpenoid from suspension cultured cells of *Torreya nucifera* var. *radicans*. Phytochemistry **59:** 385-389.

Parc, G., A. Canaguier, P. Landre, R. Hocquemiller, D. Chriqui, and M. Meyer. 2002. Production of taxoids with biological activity by plants and callus cultures from selected Taxus genotypes. Phytochemistry **59:** 725-730.

Petit-Paly, G., M. Montagu, C. Viel, M. Rideau, and J. Chenieux. 1987. Dihydrofuro [2,3-b] quinolinium alkaloids in cultured cells of *Ptelea trifolia* L. Plant Cell Rep. **6:** 309-312.

Phatak, S.V. and M.R. Heble. 2002. Organogenesis and terpenoid synthesis in *Mentha arvensis*. Fitoterapia **73:** 32-39.

Phillipson, J.D. 1990. Plants as source of valuable products. *In* B.V. Charlwood, and M.J.C. Rhodes (eds.), Secondary Products from Plant Tissue Culture. Oxford: Clarendon Press, pp. 1-21.

Proctor, J.T.A. 1996. Ginseng: Old crop, new directions. *In* J. Janick (ed.), Progress in New Crops. ASHS Press, Arlington, VA, pp. 565-577.

Rajasekaran, T., L. Rajendran, G.A. Ravishankar, and L.V. Venkataraman. 1991. Influence of nutrient stress on pyrethrin production by cultured cells of pyrethrum (*Chrysanthemum cinerariaefolium*). Curr. Sci. **60 (12):** 705-707.

Ramachandra Rao, S. and G.A. Ravishankar. 2002. Plant cell cultures: Chemical factories of secondary metabolites. Biotechnol. Adv. **20:** 101-153.

Ramirez, M., L. Alpizar, J. Quiroz, and C. Oropeza. 1992. Formation of L-canavanine in *in vitro* cultures of *Canavalia ensiformis* (L) DC. Plant Cell Tiss. Org. Cult. **30:** 231-235.

Ravishankar, G.A. and S. Ramachandra Rao. 2000. Biotechnological production of phyto-pharmaceuticals. J. Biochem. Mol. Biol. Biophys. **4:** 73-102.

Ramachandra Rao, S. and G. A. Ravishankar. 2000. Biotransformation of protocatechuic aldehyde and caffeic acid to vanillin and capsaicin in freely suspended and immobilized cell cultures of *Capsicum frutescens*. J. Biotechnol. **76:** 137-146.

Ravishankar, G.A., K.S. Sarma, L.V. Venkataraman, and A.K. Kadyan. 1988. Effect of nutritional stress on capsaicin production in immobilized cell cultures of *Capsicum annuum*. Curr. Sci. **57:** 381-383.

Ray, S. and S. Jha. 2001. Production of withaferin A in shoot cultures of *Withania somnifera*. Planta Med. **67:** 432-436.

Rech, S.B., C.V.F. Batista, J. Schripsema, R. Verpoorte, and A. T. Henriques. 1998. Cell cultures of *Rauwolfia sellowii*: growth and alkaloid production. Plant Cell Tiss. Org. Cult. **54:** 61-63.

Rijhwani, S.K. and J.V. Shanks. 1998. Effects of elicitor dosage and exposure time on biosynthesis of indole alkaloids by *Catharanthus roseus* hairy root cultures. Biotechnol. Prog. **14:** 442-449.

Rodriguez, S., J.L. Wolfender, K. Hostettmann, G. Odontuya, and O. Purev. 1996. Corniculoside, a new biosidic ester secoiridoid from *Halemia corniculata*. Helv. Chim. Acta. **79:** 363-370.

Sagare, A.P., C.H. Kuo, F.S. Chueh, and H.S. Tsay. 2001. *De novo* regeneration of *Scrophularia yoshimurae* YAMAZAKI (Scrophulariaceae) and quantitative analysis of harpagoside, an iridoid glycoside, formed in aerial and underground parts of *in vitro* propagated and wild plants by HPLC. Biol. Pharm. Bull. **24:** 1311-1315.

Sagare, A.P., Y.L. Lee, T.C. Lin, C.C. Chen, and H.S. Tsay. 2000. Cytokinin-induced somatic embryogenesis and plant regeneration in *Corydalis yanhusuo* (Fumaraceae)-a medicinal plant. Plant Sci. **160:** 139-147.

Sajc, L., D. Grubisic, and G. Vunjak-Novakovic. 2000. Bioreactors for plant engineering: an outlook for further research. Biochem. Eng. J. **4:** 89-99.

Sakato, K. and M. Misawa. 1974. Effects of chemical and physical conditions on growth of *Camptotheca acuminata* cell cultures. Agri. Biol. Chem. **38:** 491-497.

Sakuta, M., T. Takagi, and A. Komamine. 1987. Effects of nitrogen source on betacyanin accumulation and growth in suspension cultures of *Phytolaca americana*. Physiol. Plant. **71:** 459-463.

Sasse, F., U. Heckenberg, and J. Berlin. 1982. Accumulation of β-carboline alkaloids and serotonin by cell cultures of *Peganum harmala* L. Plant Physiol. **69:** 400-404.

Sato, F. and Y. Yamada. 1984. High berberine producing cultures of *Coptis japonica* cells. Phytochemistry **23:** 281-285.

Schripsema, J., A. Ramos-Valdivia, and R. Verpoorte. 1999. Robustaquinones, novel anthraquinones from an elicited *Cinchona robusta* suspension culture. Phytochemistry **51:** 55-60.

Schroder, W. and H. Bohm. 1984. Betacyanin concentrations in young cell cultures from *Portulaca grandiflora* an analysis of variation. Plant Cell Rep. **3:** 14-17.

Scragg, A.H. 1997. The production of aromas by plant cell cultures. *In* T. Schepier (ed.), Adv Biochem. Eng. Biotechnol. Vol. 55. Berlin: Springer-Verlag, pp. 239-263.

Scragg, A.H. and E.J. Allan. 1986. Production of triterpenoid quassin and cell suspension cultures of *Picrasana quassioides* Bennett. Plant Cell Rep. **5:** 356-359.

Shanks, J.V. and J. Morgan. 1999. Plant hairy root culture. Curr. Opin. Biotechnol. **10:** 151-155.

Shimomura, K., T. Kitazawa, N. Okamura, and A. Yagi. 1991. Tanshinone production in adventitious roots and regenerates of *Salvia miltiorrhiza*. J. Nat. Prod. **54:** 1583.

Siah, C.L. and P.M. Doran. 1991. Enhanced codeine and morphine production in suspended *Papaver somniferum* cultures after removal of exogenous hormones. Plant Cell Rep. **10:** 349-353.

Sierra, M.I., R. Heijden, T. Leer, and R. Verpoorte. 1992. Stability of alkaloid production in cell suspension cultures of *Tabernaemontana divaricata* during long-term subculture. Plant Cell Tiss. Org. Cult. **28:** 59-68.

Skrzypczak, L., M. Wesolowska, and E. Skrzypczak. 1993. *Gentiana* species XII: *In vitro* culture, regeneration, and production of secoirridoid glucosides. *In* Y.P.S. Bajaj (ed.), Biotechnology in agriculture and forestry, Vol. 21, Medicinal and aromatic plants IV. Berlin, Heidelberg: Springer-Verlag, pp. 172-186.

Slichenmyer, W.J. and D.D. Von Horf. 1991. Taxol: a new and effective anticancer drug. Anti-Cancer Drugs **2:** 519-530.

Smith, J.I., N.J. Smart, M. Misawa, W.G.W. Kurz, S.G. Tallevi, and F. DiCosmo. 1987. Increased accumulation of indole alkaloids by some cell lines of *Catharanthus roseus* in response to addition of vanadyl sulphate. Plant Cell Rep. **6:** 142-145.

Smollny, T., H. Wichers, T. De Rijk, A. Van Zwam, A. Shasavari, and A.W. Alfermann. 1992. Formation of lignans in suspension cultures of *Linum album*. Planta Med. Suppl. **58:** A622.

Sooch, B.S., M.R. Thakur, and G. Kaur. 1977. Evaluation of some chili (*Capsicum annuum* L.) genotypes for capsaicin and ascorbic acid contents. Indian Food Packer. **31:** 9-11.

Srinivasan, V., L. Pestchanker, S. Moser, T. Hirasuma, R.A. Taticek, and M.L. Shuler. 1995. Taxol production in bioreactors; kinetics of biomass accumulation, nutrient uptake, and taxol production by cell suspensions of *Taxus baccata*. Biotechnol. Bioeng. **47:** 666-676.

Staba, E.J., S. Zito, and M. Amin. 1982. Alkaloid production from *Papaver* tissue cultures. J. Nat. Prod. **45:** 256-262.

Sticher, O. 1998. Getting to the root of ginseng. Chem. Technol. **28:** 26-32.

Stojakowska, A. and W. Kisiel. 1999. Secondary metabolites from a callus culture of *Scutellaria columnae*. Fitoterapia **70:** 324-325.

Strobel, G.A., A. Stierle, and J.G.M. Van Kuijk. 1992. Factors influencing the in vitro production of radiolabelled taxol by Pacific yew, *Taxus brevifolia*. Plant Sci. **84:** 65-74.

Suffness, M. 1995. Taxol: Science and Applications. CRC Press, Boca Raton, FL.

Suzuki, M., K. Nakagawa, H. Fukui, and M. Tabata. 1988. Alkaloid production in cell suspension cultures of *Thalictrum flavum* and *T. dipterocarpum*. Plant Cell Rep. **7:** 26-29.

Tabata, M. and N. Hiraoka. 1976. Variation of alkaloid production in *Nicotiana rustica* callus cultures. Physiol. Plant. **38:** 19-23.

Tabata, M., H. Yamamoto, N. Hiraoka, and M. Konoshima. 1972. Organization and alkaloid production in tissue cultures of *Scopolia parviflora*. Phytochemistry **11:** 949-955.

Tal, B., J.S. Rokem, and I. Goldberg. 1983. Factors affecting growth and product formation in plant cells grown in continuous culture. Plant Cell Rep. **2:** 219-222.

Tam, W.H.J., F. Constabel, and W.G.W. Kurz. 1980. Codeine from cell suspension cultures of *Papaver somniferum*. Phytochemistry **19:** 486-487.

Tanahashi, T. and M.H. Zenk. 1985. Isoquinoline alkaloids from cell suspension cultures of *Fumaria capreolata*. Plant Cell Rep. **4:** 96-99.

Tang, W. and G. Eisenbrand. 1992a. *Panax ginseng* C.A. Mayer. Chinese drugs of plant origin. Springer, Berlin, pp. 710-73.

Tang, W. and G. Eisenbrand. 1992b. *Corydalis turtschaninovii* Bess. F. *yanhusuo* Y.H. Chou et C.C. Hsu. *In* W. Tang and G. Eisenbrand (eds.), Chinese Drugs of Plant Origin, Chemistry, Pharmacology, and Use in Traditional and Modern Medicine. Springer-Verlag, Berlin, Heidelberg, pp. 377-393.

Tang, W. and G. Eisenbrand. 1992c. *Gentiana* spp. 72. *In* W. Tang and G. Eisenbrand (eds.), Chinese Drugs of Plant Origin, Chemistry, Pharmacology, and Use in Traditional and Modern Medicine: Springer-Verlag, Berlin, Heidelberg, pp. 549-

553.

Taniguchi, S., Y. Imayoshi, E. Kobayashi, Y. Takamatsu, H. Ito, T. Hatano, H. Sakagami, H. Tokuda, H. Nishino, D. Sugita, S. Shimura, and T. Yoshida. 2002. Production of bioactive triterpenes by *Eriobotrya japonica* calli. Phytochemistry **59:** 315-323.

Taniguchi, S., K. Yazaki, R. Yabu-uchi, K. Kawakami, H. Ito, T. Hatano, and T. Yoshida. 2000. Galloylglucucoses and riccionidin A in *Rhus javanica* adventitious root cultures. Phytochemistry **53:** 357-363.

Teramoto, S. and A. Komamine. 1988. Biotechnology in agriculture and forestry, Medicinal and aromatic plants IV. Springer-Verlag, *In* Y.P.S. Bajaj (ed.), Berlin, Heidelberg, pp. 209-224.

Teshima, D., K. Ikeda, M. Satake, T. Aoyama, and K. Shimomura. 1988. Production of emetic alkaloids by *in vitro* culture of *Cephaelis ipecacuanha* A. Richard. Plant Cell Rep. **7:** 278-280.

Tsay, H.S. 1999. Tissue culture technology of medicinal herbs and its application of medicinal herbs and its application in Taiwan. *In* C.H. Chou, G.R. Waller, and C. Reinhardt (eds.), Biodiversity and Allelopathy: from Organisms to Ecosystems in The Pacific. Academia Sinica, Taipei, Taiwan, pp. 137-144.

Tsay, H.S., W.D. Chang, C.C. Chen, and Y.S. Chang. 1994. The production of imperatorin from *Angelica dahurica* var. *formosana* by cell suspesion culture. J. Agric. Assoc. China. **168:** 32-48.

Tsukamoto, T., Y. Yeno, and Z. Ohta. 1936. Glycosides of *Dioscorea tokoro* I. Diocin, dioscoreasapooxin and diosgenin. J. Pharm. Soc. Jpn. **56:** 135.

Uden, W., N.N. Pras, J.F. Visser, and T.M. Malingre. 1989. Detection and identification of Podophyllotoxin produced by cell cultures derived from *Podophyllum hexandrum* royle. Plant Cell Rep. **8:** 165-168.

Uden, W., N. Pras, E.M. Vossebeld, J.N.M. Mol, and T.M. Malingre. 1990. Production of 5-methoxypodophyllotoxin in cell suspension cultures of *Linum flavum* L. Plant Cell Tiss. Org. Cult. **20:** 81-87.

Ushiyama, K. 1991. Large-scale cultures of ginseng. *In* A. Komamine, M. Misawa, and F. DiCosmo (eds.), Plant Cell Culture in Japan: Progress in Production of Useful Plant Metabolites by Japanese Enterprises Using Plant Cell Culture Technology. CMC Co. Ltd., pp. 92-98.

Van Hengal, A.J., M.P. Harkes, H.J. Witchers, P.G.M. Hesselinic, and R.M. Buitglaar. 1992. Characterization of callus formation and camptothecin production by cell lines of *Camptotheca acuminata*. Plant Cell Tiss. Org. Cult. **28:** 11-18.

Van Uden, W., N. Pras, J.F. Visser, and ThM. Malingre. 1989. Detection and identification of podophyllotoxin produced by cell cultures derived from *Podophyllum hexandrum* Royle. Plant Cell Rep. **8:** 165-168.

Van Uden, W., N. Pras, E.M. Vossebeld, J.N.M. Mol, and ThM. Malingre. 1990. The production of 5-methoxypodophyllotoxin in suspension cultures derived from *Linum flavum* L. Plant Cell Tiss. Org. Cult. **20:** 81-87.

Venkateswara, R., S. Sankara Rao, and C.S. Vaidyanathan. 1986. Phytochemical constituents of cultured cells of *Eucalyptus tereticornis* SM. Plant Cell Rep. **3:** 231-233.

Venkateswara, R., K. Sankara Rao, and C.S. Vaidyanathan. 1987. Cryptosin-a new cardenolide in tissue culture and intact plants of *Cryptolepis buchanani* Roem. & Schult. Plant Cell Rep. **6:** 291-293.

Villarreal, M.L., C. Arias, A. Feria-Velasco, O.T. Ramirez, and R. Quintero. 1997. Cell suspension culture of *Solanum chrysotrichum* (Schldl.)- A plant producing an antifungal spirostanol saponin. Plant Cell Tiss. Org. Cult. **50:** 39-44.

Wahl, M.F., G. An, and J.M. Lee. 1995. Effects of DMSO on heavy chain monoclonal antibody production from plant cell culture. Biotechnol. Lett. **17(5):** 463-468.

Waller, G.R., C.D. MacVean, and T. Suzuki. 1983. High production of caffeine and related enzyme activities in callus cultures of *Coffea arabica* L. Plant Cell Rep. **2:** 109-112.

Wang, P.J. and C.I. Huang. 1982. Production of saikosaponins by callus and redifferentiated organs of *Bupleurum falatum* L. *In* A. Fujiwara (ed.), Plant Tissue Culture. Maruzen, Tokyo. pp. 71-72.

Wang, W. and J.J. Zhong. 2002. Manipulation of ginsenoside heterogeneity in cell cultures of *Panax notoginseng* by addition of jasmonates. J. Biosci. Bioeng. **93:** 48-53.

Wani, M.C., H.L. Taylor, M.E. Wall, P. Coggon, and A.T. McPhail. 1971. Plant antitumor agents VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc. **93:** 2325-2327.

Wichers, H.J., M.P. Harkes, and R.J. Arroo. 1990. Occurrence of 5-methoxypodophyllotoxin in plants, cell cultures and regenerated plants of *Linum flavum*. Plant Cell Tiss. Org. Cult. **23:** 93-100.

Wichers, H.J., J.F. Visser, H.J. Huizing, and N. Pras. 1993. Occurrence of L-DOPA and dopamine in plants and cell cultures of *Mucuna pruriens* and effects of 2,4-D and NaCl on these compounds. Plant Cell Tiss. Org. Cult. **33:** 259-264.

Wickremesinhe, E.R.M. and R.N. Arteca. 1993. *Taxus* callus cultures: Initiation, growth optimization, characterization and taxol production. Plant Cell Tiss. Org. Cult. **35:** 181-193.

Wickremesinhe, E.R.M. and R.N. Arteca. 1994. *Taxus* cell suspension cultures: optimizing growth and production of taxol. J. Plant Physiol. **144:** 183-188.

Wijnsma, R., J.T.K.A. Go, van I.N. Weerden, P.A.A. Harkes, R. Verpoorte, and A.B. Svendsen. 1985. Anthraquinones as phytolexins in cell and tissue cultures of *Cinchona* sp. Plant Cell Rep. **4:** 241-244.

Woerdenbag, H.J., W. Van Uden, H.W. Frijlink, C.F. Lerk, N. Pras, and ThM. Malingre. 1990. Increased podophyllotoxin production in *Podophyllum hexandrum* cell suspension cultures after feeding coniferyl alcohol as a β-cyclodextrin complex. Plant Cell Rep. **9:** 97-100.

Wongsamuth, R. and M.P. Doran. 1997. Production of monoclonal antibodies by tobacco hairy roots. Biotechnol. Bioeng. **54(5):** 401-415.

Wu, J., C. Wang, and X. Mei. 2001. Stimulation of taxol production and excretion in *Taxus* spp cell cultures by rare earth chemical lanthanum. J. Biotechnol. **85:** 67-73.

Yagi, A., Y. Shoyama, and I. Nishioka. 1983. Formation of tetrahydroanthracene glucosides by callus tissue of *Aloe saponaria*. Phytochemistry **22(6):** 1483-1484.

Yamada, Y. and T. Endo. 1984. Tropane alkaloids in cultured cells of *Duboisia leichhardtii*. Plant Cell Rep. **3:** 186-188.

Yamada, Y. and T. Hashimoto. 1982. Production of tropane al-

kaloids in cultured cells of *Hyoscyamus niger*. Plant Cell Rep. **1:** 101-103.

Yamamoto, O. and Y. Yamada. 1986. Production of reserpine and its optimization in cultured *Rauwolfia serpentina* Benth. cells. Plant Cell Rep. **5:** 50-53.

Yasuda, S., K. Satosh, T. Ishi, and T. Furuya. 1972. Studies on the cultural conditions of plant cell suspension culture. *In* G. Terui (ed.), Fermentation technology today. Society for fermentation Technology, Osaka, Japan, pp. 697-703.

Yeh, F.T., W.W. Huang, C.C. Cheng, C. Na, and H.S. Tsay. 1994. Tissue culture of *Dioscorea doryophora* Hance. II. Establishment of suspension culture and the measurement of diosgenin content. Chinese Agron. J. **4:** 257-268.

Yoshikawa, T. and T. Furuya. 1985. Morphinan alkaloid production by tissues differentiated from cultured cells of *Papaver somniferum* (1). Planta Med. **2:** 110-113.

Yoshikawa, T. and T. Furuya. 1987. Saponin production by cultures of *Panax ginseng* transformed with *Agrobacterium rhizogenes*. Plant Cell Rep. **6:** 449-453.

Yu, K.W., W.Y. Gao, E.J. Hahn, and K.Y. Paek. 2002. Jasmonic acid improves ginsenoside accumulation in adventitious root culture of *Panax ginseng* C.A. Mayer. Biochem. Eng. J. **11:** 211-215.

Yu, K.W., W.Y. Gao, S.H. Son, and K.Y. Paek. 2000. Improvement of ginsenoside production by jasmonic acid and some other elicitors in hairy root culture of ginseng (*Panax ginseng* C.A. Mayer). In vitro Cell. Dev. Biol. **36:** 424-428.

Yukimune, Y., H. Tabata, Y. Higashi, and Y. Hara. 1996. Methyljasmonate-induced overproduction of paclitaxel and baccatin III in *Taxus* cell suspension cultures. Nature Biotech. **14:** 1129-1132.

Zenk, M.H. 1978. The impact of plant cell culture on industry. *In* T.A. Thorpe (ed.), Frontiers of Plant Tissue Culture 1978, University of Calgary; International Association for Plant Tissue Culture, pp. 1-13.

Zenk, M.H., H. El-Shagi, and U. Schulte. 1975. Anthraquinone production by cell suspension cultures of *Morinda citrifolia*. Planta Med. Suppl., pp. 79-101.

Zhang, Y.H. and J.J. Zhong. 1997. Hyperproduction of ginseng saponin and polysaccharide by high density cultivation of *Panax notoginseng*. Enzyme Microb. Technol. **21:** 59-63.

Zhao, J., Q. Hu, Q. Guo, and W.H. Zhu. 2001a. Effects of stress factors, bioregulators, and synthetic precursor on indole alkaloid production in compact callus clusters cultures of *Catharanthus roseus*. Appl. Microbial. Biotechnol. **55:** 693-698.

Zhao, J., W. Zhu, and Q. Hu. 2001b. Enhanced catharanthine production in *Catharanthus roseus* cell cultures by combined elicitor treatment in shake flasks and bioreactors. Enzyme. Microb. Technol. **28:** 673-681.

Zhong, J.J., Y. Bai, and S.J. Wang. 1996. Effect of plant growth regulators on cell growth and ginsenoside saponin production by suspension cultures of *Panax quinquefolium*. J. Biotechnol. **45:** 227-234.

Zhong, J.J. and Q.X. Zhu. 1995. Effect of initial phosphate concentration on cell growth and ginsenoside saponin production by suspended cultures of *Panax notoginseng*. Appl. Biochem. Biotechnol. **55:** 241-246.

Zhou, G.M. 1980. Studies on useful compounds of Bai-Zhi for healing Yin- Hsieh Ping. Chung-Chen-Yau Res. **4:** 33. (in Chinese).

Zhou, G.M., C.G. Yu, Y.C. Han, and C.T. Mun. 1988. Studies on Bai-Zhi. IV. The toxicity test of useful compounds. Med. J. China Hospital **8:** 220-221. (in Chinese).

利用組織培養生產藥用植物重要二次代謝物之技術

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藥用植物是新藥開發最重要的資源，目前已有許多對人類健康有益的藥物，是直接由植物的二次代謝物或將此二次代謝物略加修飾之後所獲得，因此，利用組織培養生產植物有用的二次代謝物或提高二次代謝物產能的技術，最近幾十年頗受世界各國學者的重視。始自 1960年代末期，三角瓶培養系統以及運用許多不同培養策略的植物細胞培養技術，即被應用於生產二次代謝物之研究。本文綜合報導過去十幾年來組織培養技術應用於藥學領域的研究概況，同時也摘要寫出作者十幾年來從事植物組織培養生產二次代謝物之心得，提供讀者參考。

關鍵詞：生物轉換；細胞懸浮培養；毛髮狀根培養；藥學；二次代謝物。