Chapter 1
Production of Plant Secondary Metabolites: Current Status and Future Prospects

P. Silpa, K. Roopa, and T. Dennis Thomas

Abstract Plants are the prime life-supporting system on earth. Despite its use as food, it is also utilized as a source of life-saving drugs for majority of the population in the world. Many plants yield phytochemicals known as secondary metabolites, which are pharmaceutically important and are extracted directly from the plants collected from natural habitat. Regardless of conventional methods, biotechnological approaches especially plant tissue culture techniques play a unique role in producing and extracting secondary metabolites at industrial level. This book chapter discusses the various strategies adopted for secondary metabolite production in plants.

Keywords Secondary metabolites · Tissue culture · Metabolic engineering · Abiotic stress

1.1 Introduction

Plants have been the go-to resource for most of man's needs from aboriginal times. Recently, there is an increased interest in medicinal plants due to the rise in the use of herbal medicine and its therapeutic effects. Plants being a great source of bioactive secondary metabolites play a vital role in the field of drug development (Jose and Thomas 2014). Thus, the alternation of these active components through tissue culture and other biotechnical methods acts as a pillar in drug research. However, the requirement of high quantity of raw material is the main hurdle in using plant material as a key resource in drug development. Modifying the genetic makeup through metabolic engineering and high biomass production through tissue culture of these plants would lead to quality and quantity efficient production of bioactive compounds required for drug research.

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1.1.1 Plant Secondary Metabolites

Plants synthesize a variety of organic compounds, mainly classified as primary and secondary metabolites. Primary metabolites are required for essential biochemical processes such as growth, development, photosynthesis and respiration. Secondary metabolites are mainly involved in defence and protect against environmental stresses and also give specific colour, odour and tastes to the plants. Bioactive compounds are also used in agriculture field to protect crops from pests and also in signal transduction to attract seed dispersal and pollination (Wink 2003). Plant secondary metabolites have no key role in maintenance of the life processes in plants. It forms a significant source for pharmaceuticals, insecticides, flavouring agents, drugs (morphine, codeine, cocaine, quinine, etc.) and many other important biochemicals. In addition to this, certain plant secondary metabolites like phenolics, terpenoids, flavonoids and sulphur and nitrogen derivatives play a critical role to prevent many human diseases (Leicach and Chludil 2014). Biotic and abiotic stresses have a pivotal role in accumulation of secondary metabolites in various plant species (Pavarini et al. 2012). Plant secondary metabolites have been divided into three classes, namely, terpenes, phenolic and nitrogen-containing compounds (Taiz and Zeiger 2004).

1.1.2 Terpenes

Terpenes are one of the largest classes of secondary metabolites, and it is built up from isoprene units. Terpene is derived from the word “turpentine”. Terpenes are further divided into monoterpenes (e.g. carvone, perillyl alcohol, geraniol, limonene), sesquiterpene, diterpene (retinoic acid and retinol), triterpene (lupeol, betulinic acid, olealonic acid) and tetraterpene (lycopene, β-carotene, α-carotene, lutein) based on the number of isoprene units (Thoppil and Bishayee 2011). In higher plants, terpene biosynthesis occurs through two pathways, namely, mevalonic acid pathway and MEP pathway. Minor changes in the enzyme terpene synthases can trigger new catalytic properties easily and thereby induce terpene production (Keeling et al. 2008). Terpenes provide a number of esteemed functions, to attract pollinators, signalling compounds in metabolic pathway, plant-pathogen interactions, environmental stresses and other plant defences (Chen et al. 2011). Certain reports have shown that an endophytic fungus *Hypericum perforatum* helps in the production of terpenes in some plants (Zwenger 2008).

1.1.3 Phenolic Compounds

Tannins, flavonoids, stilbenes, lignans, quinines, phenolic acids, coumarins, etc. are the major phenolic compounds from medicinal herbs. Phenolic compounds form an aromatic ring and contain one or more hydroxyl groups. It possesses various bioactivities like anti-inflammatory, antioxidant, anti-carcinogenic and anti-mutagenic effects (Huang et al. 2010). Phenolics are responsible for the colour of red fruits, wines, and juices and also act as flavouring agents (Cheynier 2012). It can be divided into simple and complex phenolic compounds. These biologically active compounds mainly involved in plant defences during stressed conditions. Flavones and flavanols, isoflavonoids, tannins, anthocyanins, and lignin are included in the complex phenolic compounds. These compounds have significant role in many physiological events like flower and root differentiation, characterization of developmental stages, gene activity determination and growth vigour (Akillioglu 1994).

1.1.4 Nitrogen-Containing Compounds

Alkaloids are heterogenous group of secondary metabolites, and in an estimate, about 12,000 alkaloids are isolated from various plants (Ziegler and Facchini 2008). The three major alkaloids, cyanogenic glycosides and glucosinolates and nonprotein amino acids, are the three groups of nitrogen-containing compounds. They mainly act as growth regulators, provide protection from predators and also maintain ionic balance. Cyanogenic glycosides and glucosinolates send out volatile poisons and also play a key role in defence mechanisms (Taiz and Zeiger 2004).

1.2 Production of Plant Secondary Metabolites

Plant secondary metabolites are antibiotic, antifungal or antiviral agents that have the primary function of protecting the plants from disease-causing organisms or pathogens. Plants are mainly exploited for pharmaceuticals, food colours, flavours, fragrances and sweeteners. In this high-tech era, man mainly depends on plant bioactive components for the development of drugs and plant-derived drugs, and intermediates constitute about 25% of the total prescription drugs. Biotechnological approaches such as plant tissue culture techniques have great potential as an alternative for production of useful medicinal compounds like alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids and amino acids. Some commercially available secondary metabolites which are available in the market include shikonin and taxol. In natural conditions, uniform availability of secondary metabolites is not possible due to several reasons. However, cultivation of plant cells or tissues in

aseptic conditions in a bioreactor often leads to consistent production of secondary metabolites with improved quality and yield (Fowler 1985).

For improving the production of secondary metabolites, a number of strategies like screening and selection of high-yielding cell lines; culture of cells from various organs such as shoots, roots, leaves, callus, etc.; suspension culture; induction by elicitors; metabolic engineering; and optimization of media and plant growth regulators were adopted (Anand 2010).

Plant cell and tissue culture holds great potential for controlled production of numerous secondary metabolites which could be useful for various purposes. Secondary metabolite production under controlled condition is devoid of any environmental fluctuations and is cost-effective (Rao and Ravishankar 2002). Plant cell and tissue culture helps to produce important bioactive compounds, and advances in this area may enhance the production of these compounds. The secondary metabolites produced by plant tissue and organ culture are similar to secondary metabolites produced by intact plants. Commercially produced shikonin and taxol are now available in markets. About 20 recombinant proteins like enzymes, antibodies, growth factors and edible vaccines have been produced from tissue culture techniques. Large-scale production of secondary metabolites can be achieved through cell suspension culture by transferring friable callus to suitable medium. The advantage of using cell suspension culture for secondary metabolite production over field-grown plant is that it is devoid of production interfering compounds (Filova 2014).

Production of secondary metabolites in higher plants can be achieved through different explants under sterile condition. Plant cell and tissue culture technique is routinely employed to extract secondary metabolites from various plant species (Table 1.1). Many secondary metabolites are synthesized from primary metabolites in higher plants. About 100,000 secondary metabolites have been isolated from higher plants (Jeong and Park 2006). Its production is usually in lesser quantities, and it is determined by developmental stage and physiology of plants. Medium optimization is required to enhance the production. In many cases, suspension culture, organ culture, embryo culture and callus culture have been successful in increasing the yield of secondary metabolites. The interested metabolites may sometimes synthesize in plant from specialized tissue or organs. For example, saponin is produced from the root of *Panax ginseng*, and hence for large scale, in vitro production of saponin from *P. ginseng* requires root culture. At present several medicinal plants have been utilized to establish various culture systems like callus culture, organ culture, hairy root culture and suspension culture. There are many biotechnological approaches to produce bioactive components and are briefly described below.

Table 1.1 Recent studies on secondary metabolites production by using plant cell/tissue cultures

Plant	Compound/s	References
Anoectochilus roxburghii	Kinsenoside	Jin et al. (2017)
Withania somnifera	Withanolides	Ahlawat and Abdin (2017)
Stevia rebaudiana	Antioxidants	Ahmad et al. (2016)
Vitis species	Monoterpene and sesquiterpene	Alonso et al. (2015)
Dysoxylum binectariferum	Rohitukine	Mahajan et al. (2015)
Prunella vulgaris	Antioxidants	Fazal et al. (2014)
Withania somnifera	Withanolide	Sabir et al. (2013)
Vitis species	Nerolidol	Escoriaza et al. (2013)
Solanum tuberosum	Chlorogenic acid (phenolics)	Navarre et al. (2013)
Humulus lupulus	Prenylflavonoid	Matousek et al. (2012)
Ruta graveolens	Umbelliferone	Vialart et al. (2012)
Nicotiana tabacum	Anthocyanins	Huang et al. (2012)
Senecio species	Pyrrolizidine alkaloid	Karam et al. (2011)
Medicago sativa	Saponins	Szakiel et al. (2011)
Brachiaria species	Protodioscin	Barbosa-Ferreria et al. (2011)
Lavandula officinalis	Tetrahydrofurate (THF) acetate derivative	Patel et al. (2011)
Malus species	Anthocyanins	Lin-Wang et al. (2011)
Eugenia uniflora	Tannins and flavonoids	Santos et al. (2011)
Crotalaria retusa	Monocrotaline and pyrrolizidine	Anjos et al. (2010)
Echium plantagineum	Pyrrolizidine (alkaloid)	Lucena et al. (2010)
Vernonia species	Lactones and flavonoids	Keles et al. (2010)
Lychnophora species	Lactones and flavonoids	Gobbo-Neto et al. (2010)
Angelica gigas	Decursin, decursinol angelate	Rhee et al. (2010)
Lavandula officinalis	Deoxyartemisinin	Patel et al. (2010)
Lavandula pedunculata	Essential oils	Zuzarte et al. (2010)
Lavandula vera	Volatiles	Georgiev et al. (2010)
Lithospermum erythrorhizon	Shikonin	Zhang et al. (2010)
Argemone mexicana	Sanguinarine	Trujillo – Villanueva et al. (2010)
Picrorhiza kurroa	Picoside –I	Sood and Chauhan (2010)
Thevetia peruviana	Peruvoside	Zabala et al. (2010)
Abrus precatorius	Glycyrrhizin	Karwasara et al. (2010)
Silybum marianum	Silymarin	Khalili et al. (2010)
Artemisia species	Artemisin	Brown (2010)
Lavandula pedunculata	Camphor and 1,8- cineole	Zuzarte et al. (2010)

1.2.1 Callus Culture

Callus is the mass of undifferentiated cells containing meristematic loci (Bhojwani and Dantu 2013). For induction of calli from explants, 2, 4-D is the most preferred auxin. However, a combination of auxin and cytokinin or high concentration of auxin alone may be used by various workers for callus induction (Filova 2014). For secondary metabolite production, usually non-embryogenic calli were selected which have homogenous mass of dedifferentiated cells. For callus growth and multiplication, auxin is generally preferred, whereas it is omitted for secondary metabolite production. Screening and selection of high-yielding cell lines and standardization of media for optimum secondary metabolite production are some important strategies to improve secondary metabolite production. Suspension culture is an alternative strategy to obtain high level of secondary metabolite production. Small clumps of calli are transferred to liquid medium in flasks, and this will be followed by continuous agitation on an orbital shaker. Agitation often exerts a pressure on the cell clumps resulting in the breaking of larger clumps into smaller aggregates. Agitation also helps in the uniform distribution of cells and better aeration of cells inside medium. High rate of cell division can be achieved in suspension culture than normal callus culture.

1.2.2 Hairy Root Culture

Plant hairy root culture is the most promising technique among root culture for the production of secondary metabolites. Fast hormone-independent growth, genetic stability, lateral branching and lack of geotropism are the major characteristics of hairy roots. Inoculation of *Agrobacterium rhizogenes* helps to synthesize secondary metabolites in hairy roots (Karuppusamy 2009; Palazon et al. 1997).

A. rhizogenes have root-inducing plasmid (Ri plasmid) which contain a T-DNA; during the time of infection, T-DNA is further divided into TL and TR region in some strains (strain A4) of *A. rhizogenes* in induction processes. Two sets of PRi genes, *aux genes* (in TR region) and *rol genes* (in TL region) are involved (Jouanin 1984). Elicitation of hairy root promotes secondary metabolite production and also arrests feedback inhibition, preventing degradation of metabolites in the culture medium (Chandra and Chandra 2011).

In many plants, hairy root become green by culturing in continuous exposure of light. It generates photo-oxidative stress in hairy roots (Mukherjee et al. 2014). It produces excess H_2O_2 in the root (Behnke et al. 2010). Phenolics to volatile terpenoids shift occur in green hairy roots of carrot, due to the redirection of primary metabolites towards synthesis of volatile isoprenoid synthesis (Mukherjee et al. 2016).

1.2.3 Organ Culture

Organ culture techniques help the rapid propagation of plants. It also gets a high quantity of bioactive compounds and higher growth compared to plants grown in natural habitats. In *Fritillaria unibracteata*, rapid propagation is achieved through small bulb, and the content of alkaloids is higher in this bulb culture (Gao et al. 1999). Tropane alkaloids hyoscyamine and scopolamine were produced in high quantity in root culture (Fazilatun et al. 2004). Many valuable medicinal compounds were obtained from root culture (Pence 2011; Li et al. 2002). Secondary metabolites produced in plant aerial parts are produced from root culture (Bourgaud et al. 2001; Nogueira and Romano 2002; Smith et al. 2002; Kaimoyo et al. 2008). Organ culture exhibits less sensitivity of shear stress, but in biomass production, they show a high degree of spatial heterogeneity. Ginseng roots are the only example for commercially production of secondary metabolites by organ culture (Hibino and Ushiyama 1999).

1.2.4 Elicitation

Elicitors are substances which produce signals against pathogenic attack resulting in the accumulation of secondary metabolites in plants. Further it can improve the biosynthesis of specific compounds when introduced into a living cell system (Radman et al. 2003). Elicitation is the process in which the living cells will be treated with biotic or abiotic elicitors to obtain enhanced rate of secondary metabolites (Rao and Ravishankar 2002). The most commonly employed biotic elicitors include polysaccharides, glycoproteins, yeast extract and some fungi like *Fusarium oxysporum*, *Aspergillus niger* and *Rhizopus oryzae* (Dornenburg and Knorr 1995). The abiotic elicitors are non-biological origin and are mostly inorganic salts, jasmonic acid, salicylic acid, high pH and environmental stress conditions such as heavy metals, UV radiation, osmotic shock, etc. (Naik and Al-Khayri 2015). High stilbenes accumulation in root cultures of *Cayratia trifolia* was observed by Arora et al. (2009). The addition of alar (N-dimethylamino succinamic acid) along with the elicitor salicylic acid enhanced the stilbenes content up to 12-folds (Arora et al. 2009). In *Centella asiatica*, the presence of triterpenes in callus suspension culture derived from leaves showed an increase after incorporating amino acids (Kim et al. 2004). Adding the amino acid isoleucine at 2 mM in the medium enhanced the production of hyperforin in *Hypericum perforatum* (Karppinen et al. 2007). Hence elicitation is considered as one of the most effective methods for enhancing the secondary metabolite production in cultures (Oksman-Caldentey and Inze 2004). Heavy metals and increasing temperature affect the production of secondary metabolites. In *Robinia pseudoacacia* seedlings exposed to elevated carbon dioxide, high temperature and heavy metals (Pb-Cd) enhanced the production of secondary metabolites (Zhao et al. 2016).

1.2.5 Endophytes

Microbes, such as bacteria or fungus which lives inside the plant without making any indication of disease, are called endophytes. Many useful compounds were obtained from the plants with endosymbionts. For example, *taxol*, an anticancer agent, is obtained from *Taxus brevifolia* when infected with a fungi *Taxomyces andreanae* (Strobel et al. 1993). Accumulation of several valuable metabolites in plants is due to the synergistic effect of both plants and endophytes (Engels et al. 2008). The endophytic fungi of *Pinus sylvestris* and *Rhododendron tomentosum* produce useful secondary metabolites having various antibacterial and antioxidant activities (Kajula et al. 2010). It is reported that some endophytic fungi produce a number of beneficial phytochemicals in Leguminosae family (Wink 2013). In some plants, due to the presence of endophytic fungi, an alkaloid called indolizidine is produced (Ralphs et al. 2008). The endophytic fungus *Phoma medicaginis* produces a compound, hydroxy-6-methylbenzoic acid, which shows a noticeable antimicrobial activity (Yang et al. 1994). Phenylpropanoids, lignins, phenol and phenolic compounds, alkaloids, steroids, etc. are isolated from many mycoendophytes (Herre et al. 2007). Novel metabolites from the endophytes have a vital role in the treatment of many infectious diseases (Rai et al. 2012). It is reported that several genes and protein present in plants were seen in fungi and bacteria. This suggests that horizontal gene transfer may take place from endosymbiotic bacteria and fungi (Wink and Schimmer 2010).

Inoculation of arbuscular mycorrhizal fungi (AMF) increases the production of secondary metabolites in economically important plants (Maier et al. 1995). The presence of AMF can considerably enhance the growth and biomass of a plant (Silva et al. 2004). It can also improve the capability to absorb the micronutrients and macronutrients (Chu et al. 2001; Matsubara et al. 2009). Recently, there is an increase in research about the efficiency of AMF in improving the secondary metabolite production (Table 1.2). There is a qualitative and quantitative increase in secondary metabolite production in many plants due to the presence of AMF (Ponce et al. 2004; Ceccarelli et al. 2010). In addition to these, there is an increasing edible vegetable quality triggered by AMF (Baslam et al. 2013).

1.2.6 Nitric Oxide

Nitric oxide plays a crucial role in the production of some important phytochemicals. By the induction of some stresses, accumulation of nitric oxide takes place. Several studies showed that nitric oxide plays a significant role in the development, growth and defence responses of the plant (Flores et al. 2008; Hong et al. 2008). Pharmaceutically important secondary metabolites can be produced by elicitor-induced nitric oxide response (Xu and Dong 2008). Therefore, the significance of nitric oxide can be applied in various biotechnological processes resulting in the production of target secondary metabolites.

Table 1.2 Recent report on influence of inoculation of arbuscular mycorrhizal fungi (AMF) on secondary metabolite production

Host plant	Plant organ	Evaluated phytochemicals	AMF	Effects	References
Moringa oleifera	Leaves	Glucosinolates	*Rhizophagus intraradices*	+ ve	Cosme et al. (2014)
			Funneliformis mosseae	+ ve	
Helianthus annuus	Seeds	Fixed oil	*Funneliformis mosseae*	+ve	Heidari and Karami (2014)
Passiflora alata	Leaves	Total phenols and total flavonoids	*Gigaspora albida*	+ ve	Oliveira et al. (2015)
Stevia rebaudiana	Leaves	Stevioside and rebaudioside A	*Rhizophagus fasciculatus*	+ ve	Mandal et al. (2013)
Anadenanthera colubrina	Leaves	Total phenols, total flavonoids and total tannins	*Acaulospora longula* + *Gigaspora albida*	+ ve	Pedone-Bonfim et al. (2013)
Cucumis sativus	Leaves	Phenols, flavonoids and lignin	*Funneliformis mosseae*	+ ve	Chen et al. (2013)

1.2.7 Abiotic Stress

Abiotic stresses are important for the production of secondary metabolites. Water stress is one of the prominent abiotic stresses which can influence secondary metabolite production. Light has a crucial role in the induction of both primary and secondary metabolites. Light-grown suspension culture displays an increase in phenolic production, in antioxidant activity and also in the total plant metabolite production (Ali and Abbasi 2014). It is reported that there is a link between antioxidant activity and total phenolic content in the suspension culture of *Artemisia absinthium* (Ali and Abbasi 2014). High blue light ratio increases all phenolic acids and flavonoids in some plants (Ouzounis et al. 2014).

1.2.8 Bioreactor

Bioreactors were designed for the commercial production of secondary metabolites. Bubble column bioreactor (Huang and McDonald 2009) and stirred tanks (Su 2006) are the widely used bioreactors for the culture of plant cell. Plants like *Eurycoma longifolia* grow well in bioreactor, and it has a rapid growth compared to that of flask cultures (Lulu et al. 2015). Exposure to UV light brings out secondary metabolite production in bioreactor and also in flask cultures. In *Lavandula vera* cells, rosmarinic acid production showed a 32-fold increase in bioreactors as compared to normal shake flask cultures (Pavlov et al. 2005). Production of secondary metabolites from cells of *Digitalis lantana*, *Catharanthus roseus*, *Hypericum perforatum*,

Panax ginseng, *Sophora flavescens*, etc. has been cultured in various bioreactors (Filova 2014). Sharma et al. (2011) studied the puerarin accumulation in *Pueraria tuberosa* during shoot cultures in static and liquid medium with or without aeration. Shoots were grown in growtek bioreactor with different aeration, and the maximum puerarin content was 1484 μg/g dry weight, which was about 2.3-fold higher than puerarin content recorded in control cultures (Sharma et al. 2011). The genes involving in withanolides production in *Withania somnifera* were upregulated in bioreactor. About 1.5-folds increase in the production of withanolides was found in bioreactor as compared to shake flask (Ahlawat and Abdin 2017). Different bioreactors like continuous immersion bioreactor with net (CIB-N), continuous immersion bioreactor (CIB), temporary immersion bioreactor (TIB) and temporary immersion bioreactor with net (TIB-N) were used for the production of bioactive compounds (Jang et al. 2016). Of these, CIB system was found to be the most efficient bioreactor for the large-scale production of metabolites (Jang et al. 2016). Bioreactor culture of *Anoectochilus roxburghii* accumulated highest level of kinsenoside and other polysaccharides (Jin et al. 2017).

1.2.9 Metabolic Engineering

Metabolic engineering has proven to be a valuable tool for large-scale production of several biomedically as well as industrially relevant secondary metabolites from plants. Current studies employing transgenic and/or recombinant technologies have opened up opportunities for metabolic engineering/metabolomics, leading to the manufacturing of high-value secondary metabolites, even at the commercial level. Metabolic engineering mainly aims at the increase in the production of desired products, brings down the production of unwanted compounds and scales up the yield of novel compounds (Ludwing-Muller et al. 2014). Many of the medicinally and economically important terpenoids are produced through metabolic engineering (Elgar 2017). Patel et al. (2016) reported that the incorporation of squalene synthase gene in isoprenoid pathway of *Withania somnifera* increased the production of bioactive compounds.

1.2.10 Immobilization

Immobilization enhances the secondary metabolite production by entrapping plant cells in suitable matrix, which can protect cells from liquid shear forces and allow better cell-to-cell contacts. The viability of immobilized cells extended over a prolonged period of time (Brodelius 1985). The benefit of immobilized plant cell is that it can extend its production time, making the cells catalyse the same reaction almost indefinitely. The immobilized cells can overcome the task of isolating the compounds from the biomass; rather the products will be delivered in the medium itself.

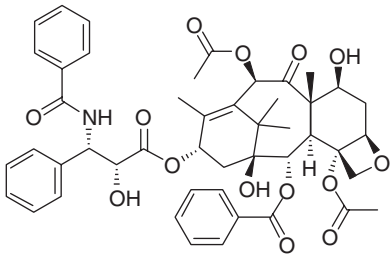

Fig. 1.1 Chemical structure of taxol

It can also perform multienzyme operations, and by using high-yielding cell lines, the productivity can be enhanced significantly (Smetanska 2008).

1.3 Production of Valuable Pharmaceutical Compounds Through In Vitro Culture Techniques

Most of the new therapeutics was evolved from secondary metabolites from plants. Advancement in the plant cell and tissue culture enhanced the production of several pharmaceutically active compounds, and some of such key compounds are described below.

1.3.1 Taxol

Taxol (Fig. 1.1) is a compound extracted from the bark of the Pacific yew tree (*Taxus brevifolia*) which possesses anticancer properties (Oksman-Caldentey and Inze 2004). In polymerized form of microtubules, taxol stabilizes it and thereby causes the death of cells. Due to the huge commercial use of taxol, *Taxus* species have been massively explored. In some studies, it was found that addition of certain amino acids like phenylalanine in the medium yielded maximum taxol in *T. cuspidata* (Long and Croteau 2005). The effect of both biotic and abiotic elicitors positively influenced the yield and accumulation of taxol in some species (Pavarini et al. 2012).

1.3.2 Morphine and Codeine

Morphine and codeine (Fig. 1.2a, b) are the pain-relieving drugs obtained from the members of the family Papaveraceae. Both these compounds occur naturally in Poppy plant (*Papaver somniferum*). Both morphine and codeine were commercially produced in cultures using callus and suspension culture (Yoshikawa and Furuya 1985). The optimum quantity of codeine and morphine was observed in cultures devoid of exogenous hormone (Furuya et al. 1972).

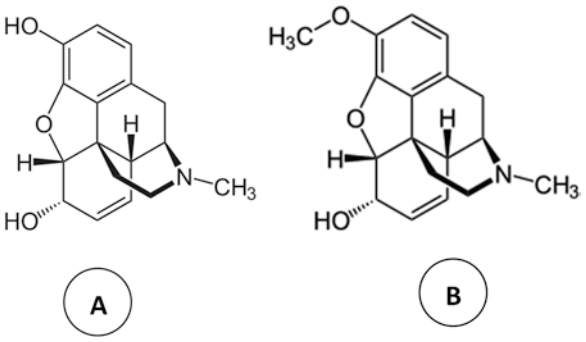

Fig. 1.2 Chemical structure of morphine (**a**) and codeine (**b**)

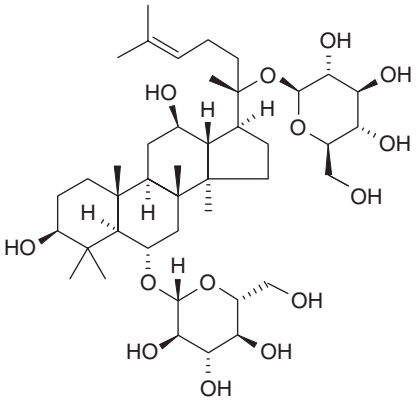

Fig. 1.3 Chemical structure of ginsenoside

1.3.3 Ginsenosides

The active component of the plant *Panax ginseng* is referred to as ginsenosides which are essential for various physiological activities. Chemically ginsenosides are a group of triterpene saponins (Fig. 1.3). The addition of spermidine in the medium enhanced the production of ginsenosides in cultures (Marsik et al. 2014). Further, an elicitor, casein hydrolyzate enhanced ginsenosides production without suppressing the biomass (Marsik et al. 2014). In root culture of *Panax ginseng*, jasmonic acid improves the ginsenosides production (Lambert et al. 2011).

1.3.4 L-DOPA

A nonprotein amino acid, L-DOPA (L-3, 4-dihydroxy phenylalanine; Fig. 1.4), is a potent drug obtained from the plant (*Mucuna hassjoo*) and is mainly used to cure Parkinson's disease (Brain and Lockwood 1976). It is the precursor of many secondary metabolites like alkaloids, melanin and betalain (Daxenbichler et al. 1971). The requirement of large quantities of L- DOPA led to the development of cell

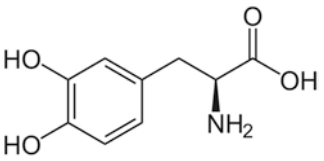

Fig. 1.4 Chemical structure of L-DOPA

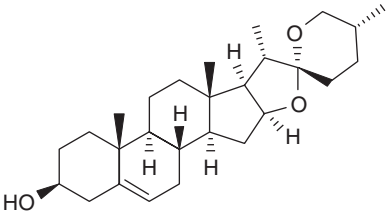

Fig. 1.5 Chemical structure of diosgenin

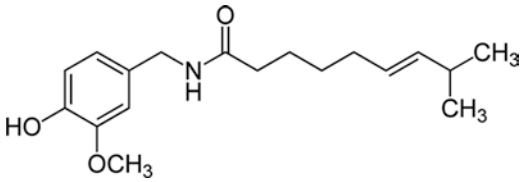

Fig. 1.6 Chemical structure of capsaicin

culture techniques to obtain optimum production of this compound. *Mucuna hassjoo* cells were cultured in MS medium supplemented with kinetin for obtaining optimum quantity of L-DOPA (Vanisree et al. 2004).

1.3.5 Diosgenin

Diosgenin (Fig. 1.5) is a biologically active metabolite, intermediate to various steroid drugs (Tal et al. 1984). Due to its high demand in the market, the production of diosgenin was enhanced by the application of in vitro techniques, and thereby it is beneficial to modern system of medicine. The optimum accumulation of diosgenin in culture was greatly influenced by the carbon and nitrogen level in the medium (Tal et al. 1984).

1.3.6 Capsaicin

The alkaloid, capsaicin (Fig. 1.6), is obtained from *Capsicum* species and mainly serves as a food additive (Ravishankar et al. 2003). The quantity of capsaicin differs significantly in suspension culture and immobilization technique. The amount of capsaicin was comparatively low in suspension culture, whereas it was increased substantially to about 100-fold in immobilization technique (Lindsey 1995). Further, the addition of isocaproic acid in the medium enhanced the production of capsaicin (Lindsey 1995).

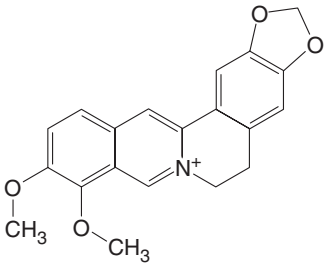

Fig. 1.7 Chemical structure of berberine

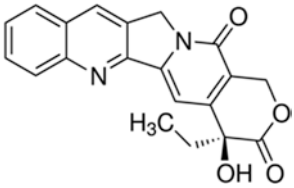

Fig. 1.8 Chemical structure of camptothecin

1.3.7 Berberine

The isoquinoline alkaloid berberine (Fig. 1.7) is obtained from the cell culture of *Coptis japonica* (Vanisree et al. 2004), *Thalictrum* species (Nakagawa et al. 1986) and *Berberis* species (Morimoto et al. 1988). In order to increase the berberine yield in cultures, several elicitors were employed by Funk et al. (1987). Further, Nakagawa et al. (1984) and Morimoto et al. (1988) standardized the nutrient medium for optimum berberine production.

1.3.8 Camptothecin

Camptothecin (Fig. 1.8), a potent antitumor alkaloid, is isolated from the plant *Camptotheca acuminate* (Padmanabha et al. 2006). The production of camptothecin from *C. acuminata* cells in cultures was optimum on MS medium supplemented with 4.0 mg/l NAA (Thengane et al. 2003).

1.3.9 Vincristine and Vinblastine

The plant *Catharanthus roseus* (also known as *Vinca rosea*) contains the vinblastine and vincristine (Fig. 1.9a, b) which are used in chemotherapy (Noble 1990). Due to its irreplaceable medicinal properties, application of biotechnological tools

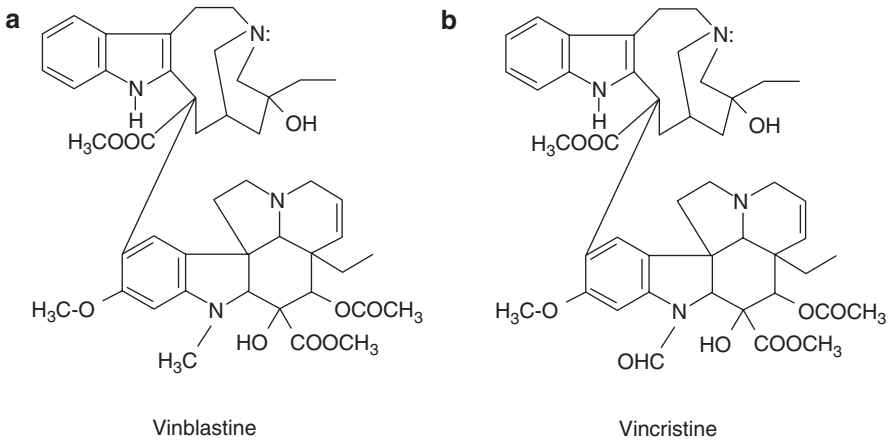

Fig. 1.9 Chemical structure of vinblastine (**a**) and vincristine (**b**)

especially plant tissue culture techniques was employed to produce large quantity of these compounds (Oksman-Caldentey and Inze 2004). Several chemicals such as oxalate, maleate, ferric chloride and sodium borohydride were added in the medium to increase the production of vinblastine (Verma et al. 2007). The other factors which influenced the production of these alkaloids include various stresses such as salinity, drought, heavy metals (Pandey 2017), UV stress (Binder et al. 2009) and presence of elicitors and addition of bioregulators (Zhao et al. 2001).

1.4 Conclusions

Plants are potent source of various useful phytochemicals. The secondary metabolites can be extracted from various parts of the plant. In recent years, there is an increased use of biotechnological tools to obtain continuous and reliable source of secondary metabolites. Among the various techniques, plant cell and tissue culture technology plays a crucial role in secondary metabolite production. The major advantage of using this technology is that it can provide bioactive secondary metabolites in controlled conditions irrespective of season and soil conditions. However, many challenges like difficulties in scaling-up, sustainability of culture and phytochemical recovery, etc. still remain to be challenging factors in this area. Continuous refinements of these techniques are necessary to overcome these limitations.

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