

Plant Cell Culture Systems for the Production of Secondary Metabolites - A Review

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Abstract

Plant cell culture systems represent a potential source of valuable secondary metabolites which can be used as food flavourants, colorants, cosmeceuticals, nutraceuticals, and pharmaceuticals. Environmental parameters that effect the cultivation of plants for the production of secondary metabolites include environmental factors, political and labour instabilities in the producing countries, uncontrollable variations in the crop quality, crop adulteration, and losses in post-harvest storage and handling. In most cases, the chemical synthesis of secondary metabolites is either extremely difficult or not economically feasible. The production of useful and valuable secondary metabolites from cell cultures is an attractive alternative to conventional plant cultivation techniques. Cell culture technology was developed as a possible tool to both study and produce plant secondary metabolites. The evolving importance of the secondary metabolites has resulted in a high level of interest in the possibility of altering their production through improving cultivation technology. This review summarizes conventional and alternative plant cell culture techniques for the production of secondary metabolites.

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I. Introduction

Plants have the innate ability of manipulating organic synthesis from the simple molecules such as carbon dioxide, water and inorganic ions, giving a complex array of natural products. This metabolic activity leads to two classes of metabolites: primary metabolites and secondary metabolites (Balandrin and Klocke, 1985; Shimomura et al., 1997). The group of pathways synthesizing simpler but essential molecules for normal physiological growth and energy requirements of plants is called primary metabolism and the products are called primary metabolites. They are widely distributed in nature and are also utilized as food by man. Primary metabolites such as carbohydrates, organic and amino acids, vitamins, hormones, flavonoids, phenolics, and glucosinolates are essential for plant growth, development, stress adaptation, and defence (**Fig. 1**). Besides the importance for the plant itself, such metabolites determine the nutritional quality of food, colour, taste, smell, antioxidative, anticarcinogenic, antihypertension, anti-inflammatory, antimicrobial, immune-stimulating, and cholesterol-lowering properties.

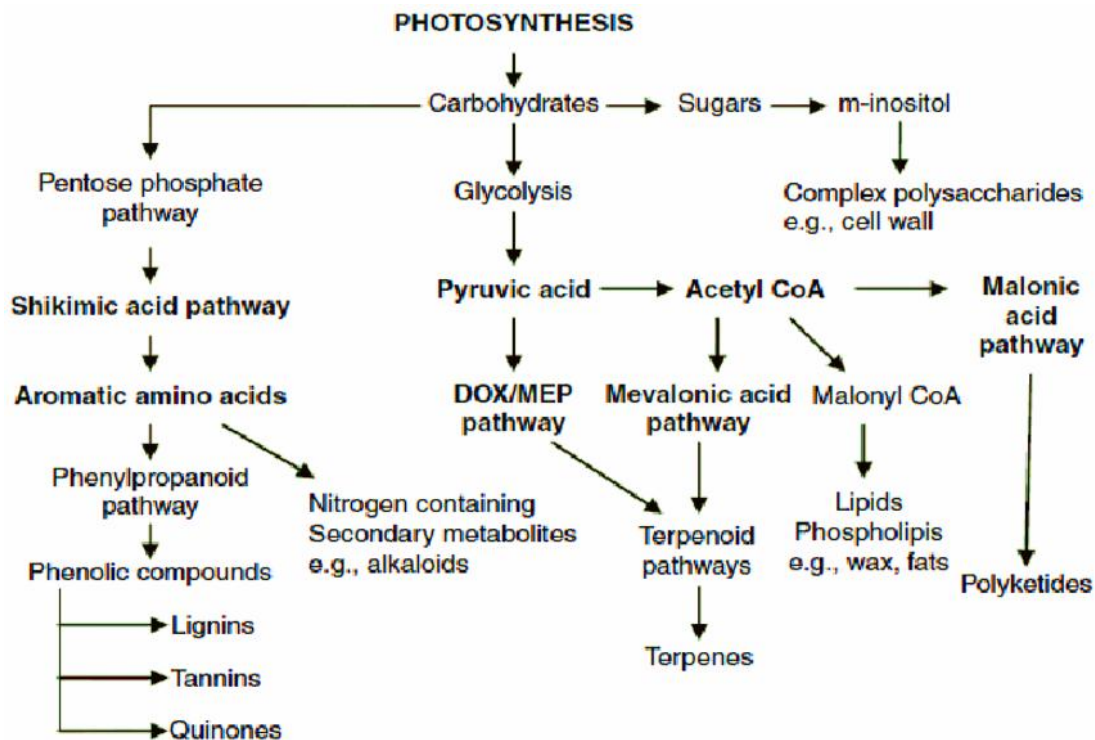


Figure 1.A simplified map of plant metabolism including primary and secondary metabolism end products.

Secondary metabolites are heterogeneous group of natural compounds that may assist in survival and basic functions of the plants, such as symbiosis, metal transport, competition, differentiation and so on (Demain and Fang, 2000). They are also widely used for pharmaceutical, medical, or agricultural purposes (Calvo et. al., 2002) including natural antibiotics which are capable of inhibiting microbial growth (Maplestone^{etal}, 1992; Sekiguchi & Gaucher, 1977; Stone & Williams, 1992). Secondary metabolites have a scientifically proven effect on health but many of these effects are still unknown and their effects are currently being intensively investigated and researched. Secondary metabolites can be classified based on the chemical composition, chemical structure, the biosynthetic pathway or their solubility in various solvents. Secondary metabolites can be divided into three large categories, namely alkaloids, terpenes and phenolics.

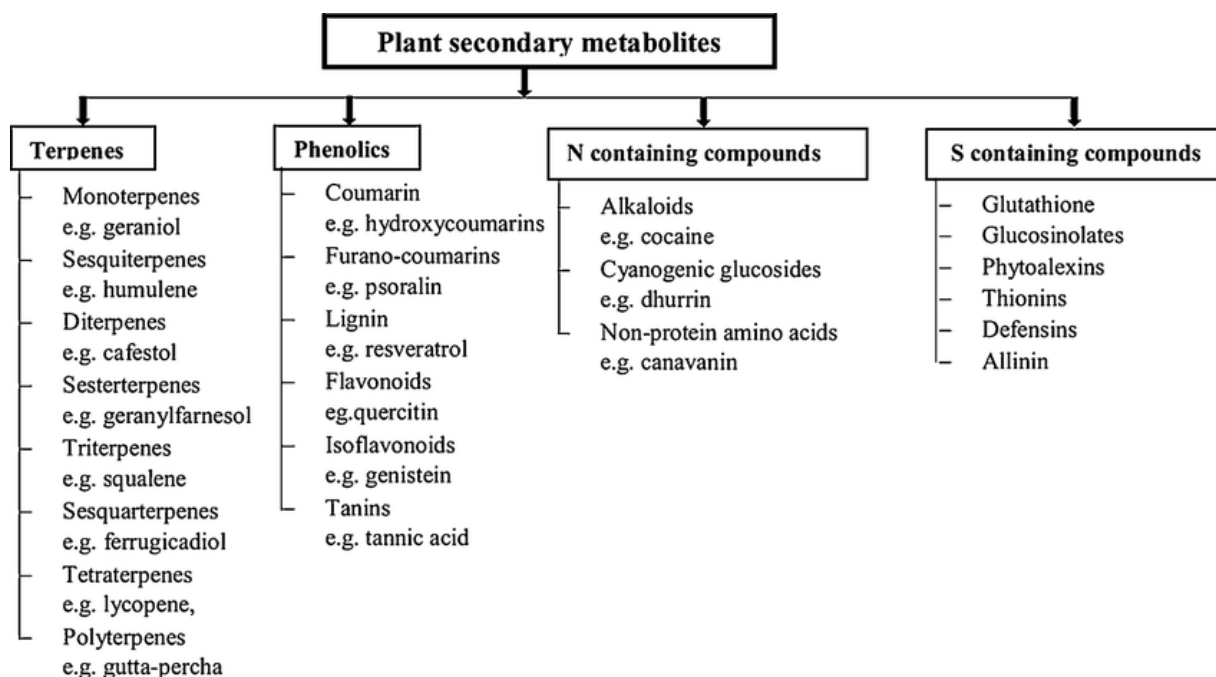


Figure 2. Types of secondary plant metabolites.

Plant cell and tissue cultures can be established routinely under sterile conditions from explants, such as plant leaves, stems, roots, meristems, etc., both for multiplication and extraction of secondary metabolites. Shoot, root, callus, cell suspension, and hairy root culture are used to synthesize metabolites of interest. When the secondary metabolite is restricted to specialized part or organs in host plant, differentiated micro-plant or organ culture is the method of choice. Atropine and Scopolin from *Datura stramonium* are produced in its roots, and hence *in vitro* root culture is preferred for tropane alkaloid synthesis (Donchevaet *et al.*, 2004). Similarly, antidepressant hypericin and hyperforin are localized in foliar glands of *Hypericum perforatum*, which have not been synthesized from undifferentiated cells (Fett–Neto *et al.*, 1995). Recent advancements in the field of secondary metabolite production in cell cultures include the treatment of plant cells with biotic and/or abiotic elicitors. Gibberellic acid, Methyl jasmonate, polyethylene glycol and ultraviolet light are the commonly used elicitors. Gibberellic acid was used as an effective elicitor for the production of Tanshinones from *Salvia miltiorrhiza* hairy roots (Yuan *et al.*, 2008). Methyl jasmonate is an established and effective elicitor used in the production of Taxol from *Taxus chinensis* (Kumari *et al.*, 2009).

The production of metabolites through hairy root system based on inoculation with *Agrobacterium rhizogenes* has garnered much attention of late. The quality and quantity of secondary metabolite by hairy root systems is same or even better than the synthesis by intact host plant root (Liu *et al.*, 2010). In addition, high growth rate, stable genetic makeup, instant growth in plant tissue culture media without plant hormones are some of the major advantages that hairy root cultures have over conventional cell culture techniques. Srivastava and Srivastava (2007) have recently summarized the attempts to adapt bioreactor design to hairy root cultures; stirred tank, airlift, bubble columns, connective flow, turbine blade, rotating drum, as well as different gas phase reactors have all been used successfully. Transgenic hairy roots generated through *Agrobacterium rhizogenes* have not only paved way for plantlet generation but also for synthesis of desired product through transgenic hairy root cultures.

II. Plant cell and tissue culturesystems

Plant cell and tissue culture technology has many advantages over field cultivation of many plants, as well as drawbacks. Some plants do not withstand field cultivation due to the impact of biological influences (pathogen sensitivity and insects) in nature (Mulabagal and Tsay, 2004), there is risk of extinction for over-harvested plants and geopolitical barriers limit their accessibility. Plant cell and tissue culture has the potential to overcome many of these barriers and they also offer an attractive alternative for the production of high-value secondary metabolites.

- Plant cells are biosynthetically totipotent which means plant cells in culture retain their complete genetic information and therefore have the ability to produce a range of secondary metabolites that are found in the parent plant (Ramachandra and Ravishankar,2002).
- Product profiles of the *in vitro* plant culture and parent plant can differ, thus novel metabolites can be produced (Ramachandra and Ravishankar,2002).
- Plant cell culture biotransformation systems can allow for the conversion of inexpensive precursors to novel or valuable compounds (Ramachandra and Ravishankar,2002).
- Plant cultures can accumulate higher levels of the plant derived product through optimization of cultural conditions (Ramachandra and Ravishankar,1998).
- The plant derived metabolite can be produced all year round as *in vitro* plant cultures are independent of climate or season (Ramachandra and Ravishankar,2002).
- A definite advantage of plant cells for metabolite production is its ability to outweigh whole plant production systems by by-passing the long development times, variations in product yield and quality. It also eliminates contamination with fertilizers and pesticides. Good Manufacturing Practice (GMP) is easily implemented at all stages of the metabolite production, *in vitro* (Hellwig *et al.*,2004).

There are many different types of cell and tissue culture systems which include undifferentiated (callus and suspension cultures) and differentiated cultures (root, shoot and transformed hairy root cultures). The type of culture chosen depends on whether the same metabolites as the parent plant can be produced by the plant cell or tissue culture, the product profile, the genetic stability and the growth profile of the plant cell or tissue culture. The key characteristics of the different plant cell and tissue culture systems are described in Table 1. The major advantage of hairy root cultures is that it produces compounds that are similar to roots of the parent plant, they are genetically stable and they grow faster than untransformed roots and shoots.

Table 1: Characteristics of different types plant cell and tissue cultures

Characteristics	Undifferentiated cell mass (Callus & cell suspension cultures)	Organ cultures (Roots & shoots)	Transformed tissues (eg; hairy roots)
Metabolite production	Produces same metabolites as parent plant (Ramachandra and Ravishankar, 2002)	Produces metabolites that require cell differentiation (Fu, 1998)	Produces root-derived metabolites (Giri and Narasu, 2000a)
Product profiles	Differ from parent plant (Fu, 1998)	Similar to parent plant (Endo and Yamada, 1985)	Similar to parent plant (Spencer <i>et al.</i> , 1990)
Genetic stability	Low (Charlwood and Moustou, 1988; Miura <i>et al.</i> , 1988)	High (Charlwood and Moustou, 1988; Miura <i>et al.</i> , 1988)	High (Airdet <i>et al.</i> , 1988)
Growth profile	Rapid growth cycles (Fu, 1998)	Slow growth cycles	Growth faster than untransformed roots/shoots (Flores <i>et al.</i> , 1987)

2.1 Callusculture

A callus culture is an undifferentiated tissue that develops around an injured or cut part of a plant (leaves, stems or roots) in nature or *in vitro*. It is these undifferentiated cell masses, in friable form, that are integral for the production of homogenous cell suspension cultures. When cultured *in vitro*, all the needs, both chemical (growth medium) and physical of the plant cells have to be met. One of the most commonly used plantgrowth medium, Murashige and Skoog (MS) was developed for tobacco tissue culture (Murashige and Skoog, 1962). The significant feature of MS medium is its very high concentration of nitrate, potassium and ammonia. The culture medium for *in vitro* cultivation of callus cultures consists of four components: the essential elements which can be divided into three categories: micronutrients, macronutrients and iron source; organic supplements to supply vitamins and/or amino acids; a fixed carbon source which is usually sucrose and plant growth hormones (Dixon and Gonzales,1994).

Most plant tissue culture media contain two classes of plant growth hormones, cytokinins and auxins. These are usually used together in the culture medium. The ratio of auxin to cytokine plays an important role in determining the type of culture that will be established. An intermediate ratio favors callus induction and continued growth of the callus tissue without differentiation. An auxin promotes callus induction from explant tissue, cell elongation and maintains the callus tissue in an undifferentiated state while the cytokine allows for stimulation of plant cell division (Dixon and Gonzales, 1994). Therefore, plant growth hormones function synergistically to promote culture growth. Figure 4 illustrates the results obtained from a study which evaluated the effects of different hormones in callus and cell suspension cultures of *Gymnemasylvestre*. This plant originates from India and is used to treat diabetes as it is also known to produce gymnemic acid based bioactives (Gopi and Vatsala, 2006).

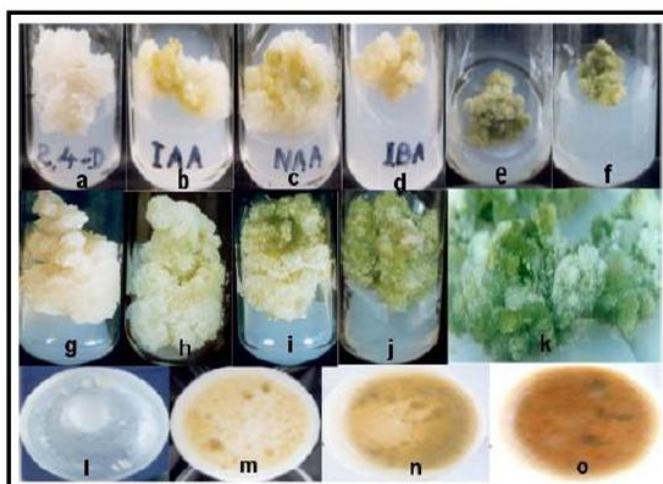


Figure 3: Callus (a-k) and suspension culture (l-o) developments from *G. sylvestre* (Gopi and Vatsala, 2006).

2.2 Plant cell suspension culture

Callus cultures are used to prepare cell suspension cultures by agitating friable callus tissue in liquid medium in shake flasks (Hellwig *et al.*, 2004). Plant cell suspension cultures are complex and heterogenic systems composed of a mixture of single cells and aggregates with different shapes and sizes (Trejo-Tapia *et al.*, 2001). Aggregation occurs as a result of the secretion of extra-cellular polysaccharides which causes the cells to attach to each other after cell division (Taticek *et al.*, 1991; Chattopadhyay *et al.*, 2002). Cell suspension cultures have been researched for the production of secondary metabolites due to their rapid growth cycles as nutrient uptake is enhanced in submerged conditions (Tripathi and Tripathi, 2003). A further advantage of suspension cultures is that secondary metabolites are intracellular based, therefore, enhanced nutrient uptake in liquid cultures allows for the cultivation of plant cell masses at high concentrations before biosynthesis of secondary metabolites is induced. (Tripathi and Tripathi, 2003). Plant cell suspension culture systems can be adapted to fermentation technology and thus bioreactors can be used for large scale production of secondary metabolites (Fu, 1998; Eibl and Eibl, 2008). Furthermore, the environmental factors (temperature, light, pH, gas composition and osmotic pressure) chemical factors (carbon/nitrogen sources), phytohormones, precursors and elicitors (Zhong, 1995; Jaziri *et al.*, 1996; Zhong, 2002) can also be manipulated to influence secondary metabolite production. However, the major disadvantage is that a low concentration of the cell culture derived secondary metabolite limits the industrial exploitation of plant cell suspension cultures (Zhong, 2002). Despite this, plant cell technology has shown to be a promising approach in terms of the production of paclitaxel from *Taxus* species. ESCA genetic (San Carlos, CA, USA) and Phyton (Ithaca, NY, USA) cultivated *Taxus* cell suspension cultures in 2500 litre and 75,000 litre bioreactors respectively (Smith, 1995). Also, Samyang Genex (Taejon, Korea) achieved the commercial production of paclitaxel in plant cell suspension cultures in 2001 (Choi *et al.*, 2002). Other secondary metabolites that have been produced in cell suspension cultures are summarized in Table 2.

Table 2: Group of secondary metabolites produced in cell suspension and tissue cultures of higher plants

Phenylpropanoids	Alkaloids	Terpenoids	Quinones	Steroids
Anthocyanins	Acridines	Carotenes	Anthraquinones	Cardiac
				Glycosides
Coumarins	Betalains	Monoterpenes	Benzoquinones	Pregnenolone
				Derivatives
Flavonoids	Quinolizidines	Sesquiterpenes	Naphthoquinones	
Hydroxycinnamoyl	Furanoquinones	Diterpenes		
Derivatives				
Isoflavonoids	Harringtonines	Triterpenes		
Lignans	Isoquinolines			
Phenolenones	Indoles			
Proanthocyanidins	Purines			
Stilbenes	Pyridines			
Tannins	Tropane alkaloids			

Adapted from Stockigt *et al.*, (1995)

2.3 Organ culture

An organ culture is an isolated organ cultivated *in vitro*. There are two types. Determinate organs which develop to a defined size and shape (example: leaves, flowers and fruits) and indeterminate organs which have the potential to grow without limit (example: shoot and root cultures). The apices (tips) of stems are cultured to cultivate shoot cultures and the apices of lateral roots are cultured to establish root cultures (George *et al.*, 2008). The cultivation of indeterminate organs *in vitro* has been developed for the production of secondary metabolites that require the use of certain biosynthetic enzymes which are only specific to the plant organ of interest (Suzuki *et al.*, 1999; Palazon *et al.*, 2006). Examples of secondary metabolites that have been produced in organ cultures are shown in Table 3.

Table 3: Secondary metabolites produced in organ cultures

Plant	Type of culture	Secondary metabolite	Reference
<i>Silybummarianum</i>	Root	Flavonolignan	Alikaridis <i>et al.</i> , 2000
<i>Withaniasomnifera</i>	Shoot	Withaferin	Ray and Jha, 2001
<i>Mentha arvensis</i>	Shoot	Terpenoid	Phatak and Heble, 2002
<i>Hypericum perforatum</i>	Organ	hypericin	Wilken <i>et al.</i> , 2005
<i>Panax ginseng</i>	Adventitious root	Ginsenoside	Jeong and Park, 2006
<i>Lavandula officinalis</i>	organ	Rosmarinic acid	Wilken <i>et al.</i> , 2005
<i>Scopolia parviflora</i>	Adventitious root	Scopolamine, hyoscyamine and tropane alkaloids	Min <i>et al.</i> , 2007

2.4 *Agrobacterium rhizogenes*-mediated transformation for the induction of hairy rootcultures

Transformed organ cultures are achieved by *Agrobacterium*-mediated transformation which is derived from the gram-negative soil bacteria, *Agrobacterium tumefaciens* and *A. rhizogenes*. These bacteria attack dicotyledonous plant tissues in nature at a wounded site; possibly caused by insect or mechanical damage. The wounded site produces phenolic compounds that attract the bacteria by chemotaxis which subsequently leads to infection of the plant cells by the bacteria (Baladrinet *et al.*, 1985). This activity causes crown gall disease if the plant is infected by *A. tumefaciens*, or hairy root disease if it is infected by *A. rhizogenes* (Escobar and Dandekar, 2003). Hairy root disease is characterized by the formation of a number of small roots with fine hairs that proliferate rapidly at the site of infection (Baladrinet *et al.*, 1985).

Hairy root cultures offer a reliable source of secondary metabolites for large scale feasibility and long-term stability for the following reasons:

- Hairy root cultures have a growth rate as high as or higher than normal roots (untransformed) due to their extensive branching which results in many meristems (Charlwood and Charlwood, 1991; Flores *et al.*, 1999). The average doubling time after inoculation is 24-90 hours (Payne *et al.*, 1991).
- Hairy roots are capable of synthesizing metabolites specific to the parent plant as well as novel metabolites that cannot be detected in the mother plant or other types of *in vitro* plant tissue cultures (Nader *et al.*, 2006).
- Stable integration of the Ri T-DNA (root inducing transfer DNA) into the plant genome allows for the genetic stability of the transformed root cultures. In addition, these root cultures produce secondary metabolites over successive generations without losing their biosynthetic capability (Giri and Narasu, 2000a). Therefore, hairy root lines are a promising source for the constant and standardized production of secondarymetabolites.
- Hairy roots also offer a valuable source of root-derived phytochemicals that can be used as pharmaceuticals, cosmetics and food additives (Giri and Narasu, 2000a).

The infection process and the induction of hairy roots are directed by several regions contained in the Ri (Root inducing) plasmid carried by the bacterium. The *vir* region contains 6 to 8 genes which encode for proteins that are involved in the transformation of the plant cells by transferring the right and left T-DNA (Transfer-DNA) regions (delimited by boarder sequences) of the plasmid into the plant genome (Bensaddek *et al.*, 2008). The right T-DNA region is involved in biosynthesis of auxin and opines (amino acid sugar derivatives) (Gartland, 1995). The left T-DNA-region contains four *rol* genes A, B, C and D which enhance the auxin and cytokine synthesis in the plant cells. These genes are responsible for the hairy root phenotype. The T-DNA regions thus confer the plant cells ability to grow in the absence of exogenous plant hormones (Schmülling *et al.*, 1988; Petersen *et al.*, 1989; Estruchet *et al.*, 1991). The infection and transformation process is illustrated in Figure 4 (Tzfira *et al.*, 2004). The process comprises of ten steps (Tzfira and Citovsky, 2006): **1**) recognition and attachment of the bacterium to the host plant cells; **2**) and the sensing of specific plant signals by *Agrobacterium*'s VirA/VirG two component signal-transduction system; **3**) this leads to the activation of the *vir* gene region and a mobile copy of the T-DNA is then generated by the VirD1/VirD2 protein complex; **4**) the single stranded T-DNA forms a complex with VirD2 protein; this is called the immature T-complex; **5**) this complex then associates with VirE2 to form the mature complex which travels through the pilus and; **6**) host-cell cytoplasm; **7**) and is actively imported into the host-cell nucleus; **8**) once inside the nucleus, the complex is recruited to a point of integration; **9**) stripped of the *vir* proteins and **10**) integrated into the plant host genome. The process described above is used by *A. tumefaciens* to induce crown gall tumors in dicotyledonous plants however Giri and Narasu, (2000a) describes a similar process for *A. rhizogenes*-mediated transformation which involves the Ri plasmid instead of the Ti (Tumor inducing) plasmid.

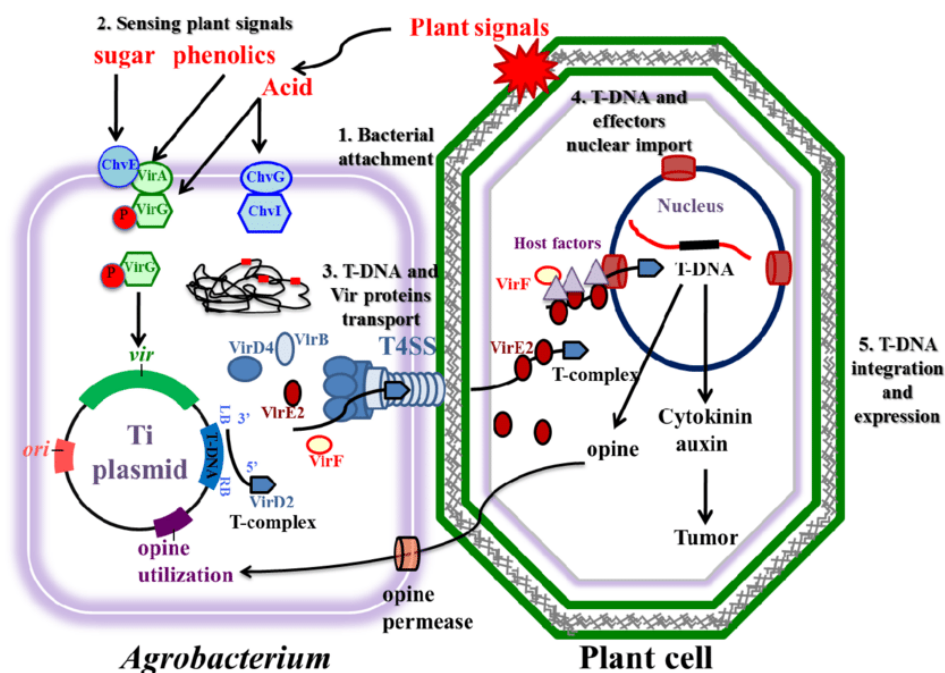


Figure 4: A model of the *Agrobacterium*-mediated genetic transformation process (Tzfira *et al.*, 2004).

The natural transformation process by *A. rhizogenes* can be performed under *in vitro* conditions by using protocols designed for the infection of various plant species. In order for *A. rhizogenes* to mediate the transformation of a plant tissue, contact between bacteria and plant cells must be induced. This can be achieved by direct injection of bacterial suspension into the plant tissue or immersion of the plant tissue into the bacterial suspension. The latter procedure can be enhanced with vacuum filtration (Tomilov *et al.*, 2007). This procedure involves wounding of the explant tissue before inoculation with bacteria. In addition, the use of excised tissues and leaf disks increases the contact surface between the plant tissue and the bacteria (Wang *et al.*, 2002). Alternative procedures such as micro-wounding through electroporation or sonication can be implemented for hard to transform plants (Matsukie *et al.*, 1989; Trick and Finer, 1997; Le Flemet *et al.*, 2004). Transformation of the explant with bacteria occurs during the co-cultivation on solid medium for approximately two to three days. The explant is then transferred to solid medium containing an antibiotic to eliminate the bacteria. Cefotaxime (250-500 mg.l⁻¹) or Timentin (200-300 mg.l⁻¹) is usually used. The explants are then transferred onto hormone-free solid medium and incubated in the dark phase at 20-25°C. Hairy roots appear after 1-4 weeks, these are transferred to Erlenmeyer flasks containing hormone-free liquid medium. The typical phenotype of the transformed root is highly branched and covered with a mass of tiny root hairs. Hairy root cultures have an average doubling time of approximately 2-3 days (Bensaddek *et al.*, 2008). The success of the *Agrobacterium* transformation method can be highlighted by the constantly increasing number of plant species that have been transformed for the establishment of hairy root cultures: 29 species in 1987, 116 species in 1990 and 185 species from 41 families in 2004 (Kuzovkina and Schneider, 2006).

However, the success of the transformation procedure depends on various factors which include: the plant species and age of the plant tissue (younger plant tissues are generally more sensitive to bacterial infection); the explants used for infection (young tissues of sterile plantlets such as hypocotyl segments, cotyledons, petioles and leaves commonly used) and the bacterial concentration used. Sub-optimal concentrations can lower availability of the level of bacteria for transforming the plant cells while high concentrations decrease the availability of the level of bacteria due to competitive inhibition (Kumar *et al.*, 1991; Sevon and Oksman-Caldentey, 2002; Bensaddek *et al.*, 2008). The growth medium also significantly influences hairy root induction. Media containing a high concentration of salt favour hairy root formation of some plants (example, LS medium). Low salt media (example, B5) favour excessive bacterial growth and therefore the explant needs to be treated by transferring it several times onto fresh medium containing antibiotic (Linsmaier and Skoog, 1965; Gamborget *et al.*, 1976).

The choice of the bacterial strain is also an important factor since some plants are very resistant to infection by *Agrobacterium*. Monocotyledonous plants are more difficult to transform than dicotyledonous plants (Bensaddek *et al.*, 2008). There are several types of *A. rhizogenes* strains, examples include: opine,

mannopine, agropine and cucumopine. Opine strains are wild types and have been used to produce hairy roots from medicinal plants (Bensaddek *et al.*, 2008). Mannopine strains transfer only the left T-DNA region (Schmülling *et al.*, 1988; Petersen *et al.*, 1989). Agropine strains transfer both the left T-DNA region and right T-DNA region to the plant host genome (Schmülling *et al.*, 1988; Petersen *et al.*, 1989).

The restriction map of a Ri plasmid (pRiA4b) of an agropine strain is shown in Figure 5 (Huffman *et al.*, 1984). Hairy roots cell lines obtained by infection with different *A. rhizogenes* strains exhibit different morphologies due to the different Ri plasmids harbored by the strains (Nguyen *et al.*, 1992). The degree of virulence of different strains is also based on the different Ri plasmids harbored by the strains (Bensaddek *et al.*, 2008). For example, LBA 9402 strain is a hypervirulent strain that has been used to transform hard to transform plants such as *Centaurium erythraea*, *Hyoscyamus muticus*, *Saponaria vaccaria* and *Gentiana macrophylla* (Vanhala *et al.*, 1995; Piatczak *et al.*, 2006; Schmidt *et al.*, 2007; Tiwari *et al.*, 2007)

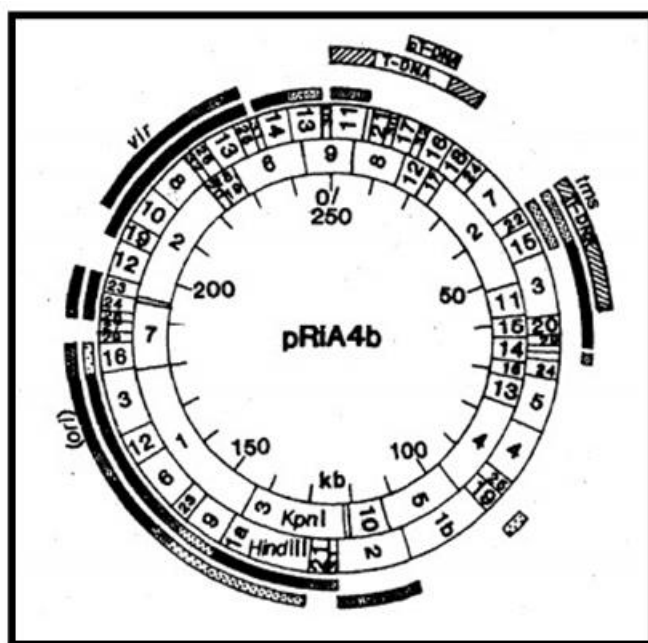


Figure 5: Restriction map of a Ri plasmid (pRiA4b) of an agropine strain. *Vir* represents the virulence region; *ori* is the origin of replication and *tms* represents the auxin genes (Huffman *et al.*, 1984).

2.4.1 Secondary metabolites production in hairy root cultures

Although the biosynthesis of secondary metabolites in hairy roots is genetically controlled, chemical (sucrose concentration, exogenous growth hormone and the nature of the nitrogen source) and environmental factors (light, temperature and pH) can affect growth, total biomass yield and secondary metabolite production (Rhodes *et al.*, 1994; Giriet *et al.*, 1997; Nussbbaumer *et al.*, 1998). In order to maximize biomass and metabolite production, modification of the culture medium is required; this can involve change in the sugar, nitrogen and/or phosphorus source (Bensaddek *et al.*, 2008). Sucrose is the best carbon source; it is hydrolyzed into glucose and fructose during assimilation by the plant cells in culture (Srinivasan *et al.*, 1995). Studies have also been conducted on many plant species whereby the product that leaches into the medium can be recovered by adsorbents. The culture medium is then rejuvenated to maintain the supply of nutrients. Leaching is accomplished by the addition a permeabilization agent (example: Tween 20) into the medium which results in the transient release of secondary metabolites (Boitelet *et al.*, 1996). Pietrosiuk *et al.* (2007) describes the different factors involved in culturing and producing secondary metabolites from *Catharanthus roseus* hairy root cultures. Figure 6 shows *C. roseus* hairy roots cultured from shoot and artificial seed explants. Other examples of secondary metabolites produced in hairy root cultures are shown in Table 4 (Zhou and Wu, 2006).

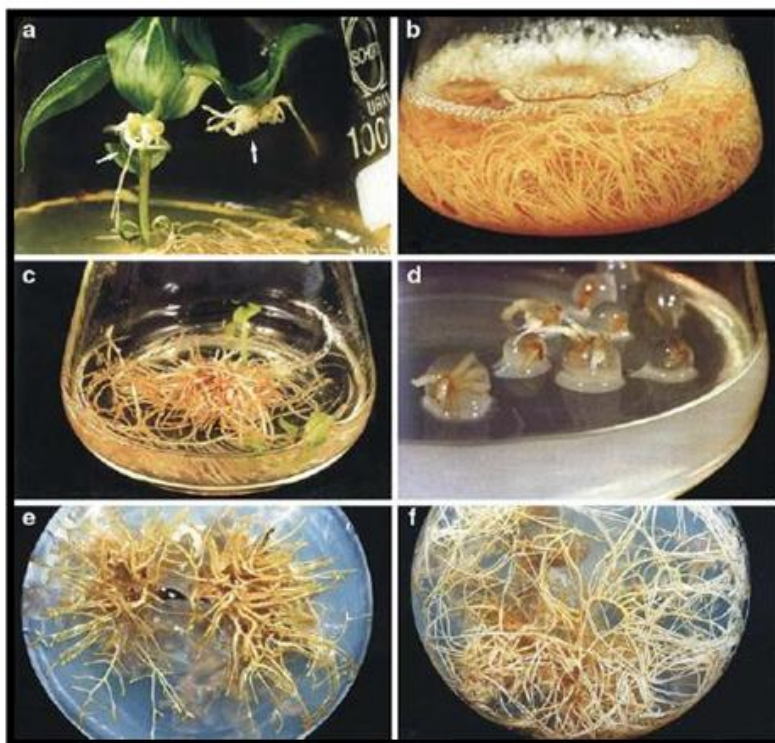


Figure 6: Hairy root cultures of *C. roseus*. Hairy roots emerged at the sites of infection after 7-10 days by using *A. rhizogenes* (ATCC 15843) (a). Hairy roots cultured in B5 medium liquid medium without hormones (b). Transgenic plants regenerated from *C. roseus* hairy roots in half strength B5 medium without hormones (c). Artificial seeds obtained by encapsulating *C.roseus* hairyroots in sodium alginate (d). Hairy roots were cultured from artificial seeds of *C. roseus* on NN medium containing 0.5 mg.l⁻¹IBA and 0.1mg.l⁻¹kinetin(e). Hairy roots were cultured from artificial seeds of *C.roseus* on NN medium containing 0.5 mg.l⁻¹IBA and 0.1mg.l⁻¹BAP (f). The difference in morphology of the hairy roots can be seen when different hormones were used (Pietrosiuket al.,2007).

Table4: Hairy root cultures of medicinal plants and their secondary metabolites.

Plant	Product/s
<i>Anisodustanguticus</i>	Scopolamine
<i>Artemisia annua</i>	Artemisinin
<i>Cassia obtusifolia</i>	Steroids
<i>Cassia obtusifolia</i>	Betulinic acid, chrysophanol, physcion, 8-O-methylchrysophanol, 1-hydroxy-7-methoxy-3-methylanthraquinone, 1-O-methylchrysophanol, aloe-emodin
<i>Astragalus membranaceus</i>	Astragaloside IV, polysaccharides
<i>Cyanotis arachnoidea</i>	20-Hydroxyecdysone
<i>Dioscorea ziziberensis</i>	Diosgenin
<i>Glycyrrhiza uralensis</i>	Flavonoids
<i>Gynostemma pentaphyllum</i>	Triterpenoid saponins
<i>Panax ginseng</i>	Saponins
<i>Panax japonicus var. major</i>	Saponins
<i>Panax quinquefolium</i>	Saponins
<i>Phytolacca esculenta</i>	Saponins
<i>Polygonum multiflorum</i>	Rhein, emodin
<i>Pueraria lobata</i>	Isoflavonoids
<i>Rheum palmatum</i>	Anthraquinones
<i>Rheum wittrochii</i>	Aloe-emodin, rhein, chrysophanol, emodin, physcion, 8-O-

	Methylchrysohanol
<i>Salvia miltiorrhiza</i>	Tanshinone I, tanshinone IIA, cryptotanshinone
<i>Saussureainvolucrata</i>	Sytingin, rutin, hispidulin
<i>Solanum photeinocarpum</i>	Glycoalkaloids, saponins
<i>Stephania delavayi</i>	Isoquinoline alkaloids
<i>Taxus brevifolia</i>	Taxol

(Zhou and Wu, 2006)

2.4.1.1 The production of anticancer compounds in hairy rootcultures

Cancer is a leading cause of death worldwide, accounting for 9.6 million deaths in 2018 (Globocan, 2018). Several chemo-preventive agents have been developed and are used for the treatment of cancer but the toxicity of these agents limits their use (Kathiresan *et al.*, 2006). Thus, there is a great interest to research new and better treatments. The plant kingdom is one of the most attractive sources of novel anticancer compounds. Various potent anticancer compounds from higher plants have been identified by the National Cancer Institute in the United States of America through conducting an intensive screening program (Suffness and Douros, 1982). However, there are certain limitations towards using plants; the concentration of the active compounds present in the plants is generally low, the growth rate of plants is slow and geographical or environmental conditions affects the accumulation of the active compounds (Yesil-Celiktaset *et al.*, 2010). Thus, the economical production of the active compounds by extraction of the intact plant is a difficult task. Furthermore, many anticancer compounds isolated from higher plants are secondary metabolites and have complex structures which are difficult to chemically synthesize (Oksman-Caldentey and Inze, 2004). Although plant tissue culture technology is not a very cost-effective option it is undoubtedly one of the most appropriate approaches to solve the above problems if the active compounds could not be manufactured by extraction or chemical processes. Therefore, over the last decade research studies have focused on applying plant tissue culture technology for the possible commercial production of anticancer drugs such as taxol, vinblastine, vincristine, camptothecin derivatives and podophyllotoxin (Table 5).

Table5: Anticancer compounds produced by hairy rootcultures

Plant	Compound/s	Treatment	Reference/s
<i>Taxus cuspidata</i>	Taxol	Ovarian, breast, non-small cell lung	Rowinsky and Donehower, 1995; Kim <i>et al.</i> , 2009
<i>Catharanthus roseus</i> L. (G.) Don	Vinblastine [Velban®], vincristine [Oncovin®]	Acute leukaemia, small-cell lung cancer, cervical, breast, head and neck Cancer	Ataei-Azimiet <i>et al.</i> , 2008; Schmelzer and Gurib-Fakim, 2008
<i>Ophiorrhizapumila</i>	Camptothecin derivatives (irinotecan [Campto®], topotecan [Hycamtin®])	Colorectal and ovarian cancers	Bookman, 1999; Mathijssen <i>et al.</i> , 2001; Vanhoeferet <i>et al.</i> , 2001; Mathijssen <i>et al.</i> , 2002; Sudoet <i>et al.</i> , 2002
<i>Podophyllum hexandrum</i>	Podophyllotoxin (used as a precursor for production of etoposide [VP-16-213] and teniposide [VM-26])	Lung cancer, testicular cancer, a variety of leukemias and othersolid tumours	Holthius, 1988; Stahelin and von Wartburg, 1991; Imbert, 1998; Giri and Narasu, 2000b

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