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Production of Medicinal Compounds from Endangered and Commercially Important Medicinal Plants through Cell and Tissue Culture Technology for Herbal Industry

Hemant Sood

Abstract

Plant cell culture technologies have made possible the production of a wide variety of pharmaceuticals such as alkaloids, terpenoids, steroids, saponins, monoterpenes, flavonoids and amino acids. The standardization of technologies for the production of plant metabolites through cell cultures helps in understanding the biology of their biosynthesis and accumulation. Various factors such as physical, chemical, nutritional, and genetic influence the production of metabolites in plant cell cultures. The controlled production of plant metabolites through cell cultures provides a suitable alternative not only in relieving pressure from natural habitats of plant species but also provides conditions suitable for year-round production of metabolites. The production of plant metabolites has been enhanced by exposing the cultured cells to biotic and abiotic elicitors. Off late, the induction of hairy roots has been found suitable in the production of metabolites synthesized in various parts of plants. The lack of proper understanding about the biology of biosynthesis of plant metabolites has been a major stumbling block, in addition to poor amenability of medicinal and aromatic plant species to *in vitro* conditions. Continuous efforts are required to be made in upscaling the production of metabolites on large scale. Least attention has been given towards working out the cost-effectiveness of metabolite production through cell cultures.

Keywords: phytochemicals, cell and tissue cultures

1. Introduction

The plant kingdom has provided a wide variety of natural products with diverse chemical structures and a vast array of biological activities, many of which found applications in health sciences. Over 80% of the approximately 30,000 known natural products are of plant origin. In 1985, 3500 new chemical structures were identified out of which 2600 were derived from higher plants and 121 clinically useful drugs were derived from plants [1]. Plants will continue to provide novel products as well as chemical models for new drugs in the near future [2].

Many of the plant species that produce medicinal herbs have been scientifically evaluated for their possible medical applications. The economic importance of phytopharmaceuticals in plants has led to their inevitable collection from their natural habitats and thus creating environmental and geopolitical instabilities posing a threat to their survival. The reckless collection of plants has put several of them under the categories of endangered or at the verge of extinction. This has prompted industries and scientists to find the alternative technologies for the production of phytopharmaceuticals so that the natural habitat of plants can be preserved.

Plant cell cultures have served as potential renewable resources for the production of valuable medicinal compounds, flavors, fragrances, pigments, dyes, cosmetics and fine chemicals. All these compounds belong to a group collectively known as secondary metabolites. The commercial importance of secondary metabolites and the possibilities of their production by means of cell culture technologies have gained great interest in the recent years. The current review is a survey and analysis of current status of various plant cell culture technologies used for the production of medicinally important metabolites. The future prospects of cell culture technologies in light of successful case studies have been reviewed and possible improvements are suggested.

2. Why plant cell cultures?

The capacity of plant cell, tissue and organ cultures to produce and accumulate many of the valuable chemical compounds has been recognized almost since the inception of in vitro technology. The strong and growing demand in today's market place for natural, renewable products has refocused attention on in vitro cell cultures as potential factories for phytochemical production. The advantage of producing plant metabolites in vitro has been in understanding the biology of their biosynthetic activity which ultimately can be enhanced by regulating physical, chemical, nutritional and genetic parameters. 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 callus/cell cultures.

The advances in plant cell culture technologies has made possible the production of a wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids, and amino acids. The production of plant metabolites through cell cultures offer several advantages such as it makes possible to select genotypes with higher production of secondary metabolites, which can be generated on a continuous year round basis under controlled environment. Plant cell cultures eliminate potential political boundaries or geographic barriers which are otherwise to the production of a crop, such as the restriction of natural rubber production to the tropics or anthocyanin pigment production to climates with high light intensity. Many cost effective parameters have been tried for their economic production at large scale or by possible use of plant cell cultures for biotransformation of natural compounds [3].

3. Production of phytochemicals through cell culture technologies

3.1 Callus/cell suspension

Callus/cell suspension cultures have been the prime focus of various studies aimed at the production of phytochemicals of not only medicinal value but also of other industrially important metabolites. Callus is a proliferating mass

of undifferentiated cells, which can be established from different explants of a plant species under in vitro conditions on suitable nutrient media. Once the callus is derived from high metabolite producing explants, their suspension cultures can be established by transferring those calli into liquid media under continuous agitation. Zenk [4] successfully established cell lines of different plants capable of producing high yields of secondary compounds in cell suspension cultures. The production of solasodine from calli of *Solanum elaeagnifolium* and cephaeline and emetine from callus cultures of *Cephaelis ipecacuanha* were successfully achieved [5]. Some of the notable cell culture methods which have been employed for large scale production of metabolites are production of taxol from cell suspension cultures of *Taxus mairei* [6]; production of paclitaxel and its related taxanes from different *Taxus* species; production of berberine through cell suspension culture of *Coptis japonica*; production of vincristine and vinblastine from *Catharanthus roseus* [7, 8], and production of taxoids from cell suspension cultures of *Taxus cuspidate* [9] (Table 1).

3.2 Hairy root cultures

Off late, the cultivation of hairy roots has been seen as a sustainable strategy for the production of medicinally important metabolites of plants not only due to the reason that harvesting roots has been destructive for the plants in nature but also

Plant species	Active ingredient	Reference
<i>Agave amaniensis</i>	Saponins	Andrijany et al. (1999)
<i>Allium sativum</i> L.	Alliin	Malpathak and David (1986)
<i>Coptis japonica</i>	Berberine	Suzuki et al. (1988) and Morimoto et al. (1988)
<i>Duboisia leichhardtii</i>	Tropane alkaloids	Yamada and Endo (1984)
<i>Gentiana</i> sp.	Secoiridoid glucosides	Srrzypezak et al. [10]
<i>Panax ginseng</i>	Saponins and sapogenins	Furuya et al. [11]
<i>Papaver bracteatum</i>	Thebaine	Day et al. (1986)
<i>Rauvolfia serpentine</i> × <i>Rhazya stricta</i> hybrid plant	3-Oxo-rhazinilam	Gerasimenko et al. (2001)
<i>Scutellaria columnnae</i>	Phenolics	Stojakowska and Kisiel (1999)
<i>Tecoma sambucifolium</i>	Phenylpropanoid glycosides	Pletsch et al. (1993)
<i>Taxus mairei</i>	Taxol	Wu et al. [6]
<i>Taxus</i> spp.	Terpenes, sterols, flavonoids	Lish et al. (2002)
<i>Catharanthus roseus</i>	Catharanthine	Zhao et al. [7, 8]
<i>Panax notoginseng</i>	Gensenoside	Zhong and Zhong (1995)
<i>Papaver somniferum</i>	Morphine and codeine	Shia and Doran (1991)
<i>Podophyllum hexandrum royle</i>	Podophyllotoxin	Chattopadhay et al. [12]
<i>Salvia fruticosa</i>	Rosmarinic acid	Karam et al. [13]
<i>Picrorhiza kurroa</i>	Picroside-1	Sood et al. (2010, 2011) and [14]
<i>Taxus cuspidate</i>	Taxoids	Ketchum et al. [9]

Table 1.
Bioactive secondary metabolites produced through shoot/callus cultures/suspension cultures.

due to the ease of growing hairy roots in mass cultures in the absence of external hormones, absence of geotropism and high branching, etc. Furthermore, hairy roots produce secondary metabolites for larger periods of time, unlike natural roots which are not only in limited supply but are available at specific times in a year. For these reasons, switching from culturing natural plant-organs to hairy roots is considered as an attractive alternative for the production of many valuable natural secondary metabolites [15].

For establishing hairy root cultures, the plants are infected by *Agrobacterium rhizogenes* which induces hairy roots by the transfer of T-DNA from Ri plasmid into the plant genome. This ability of *A. rhizogenes* has led to studies on it as a source of root-derived pharmaceuticals [16]. Important metabolites produced through hairy roots are serpentine production from *Catharanthus roseus*, ajmalicine from *Rauvolfia micrantha* [17] and ginkgolides from hairy roots of *Ginkgo biloba* [18]. Large scale production of ginsenoside from *Panax ginseng* hairy roots has been achieved by optimizing organic nutrients in bioreactor for enhancing their production. Recent developments have indicated that hairy root culture technology has moved from small laboratory scale to a large scale industrial production. For example, the German Co. RooTec has been carrying out production of camptothecin and podophyllotoxin through hairy root cultures. In a cross-species co-culture system, hairy roots of *Linum flavum* have been found to increase the production of podophyllotoxin by 240% in the cell suspensions of *Podophyllum hexandrum*. It has been reported that secondary metabolites accumulating in aerial plant have also been accumulated in the hairy roots such as artemisinin which was thought to accumulate only in the aerial parts of *Artemisia annua* also accumulated in the hairy roots. Higher production of forskolin in transformed roots of *Coleus forskolli* was achieved by using various concentrations of auxins and auxin conjugates, cytokinins and GA3 [19]. The enhanced production of picroside-1 has been reported through hairy root cultures of *P. kurroa* [20].

3.3 Elicitation of phytochemicals production in callus/cell/hairy root cultures

The lower yield of phytochemicals in plant cell cultures prompted researchers to look into various other means of enhancing their production. The recognition that certain specific secondary metabolites such as phytoalexins are produced by plants in response to microorganisms has led to the concept of using such stimulators (known as elicitors) for in vitro cultures. The substances used as elicitors can be of biotic or abiotic origin [21]. The plants also elicit the same response when challenged by compounds of pathogenic origin [22]. The elicitation of cell suspension cultures or hairy root cultures with biotic or abiotic elicitors has been found to enhance the rate of production as well as the yields of plant secondary metabolites [23].

The biotic elicitors are substances of biological origin, which include fungal homogenate, chitosan, microorganisms (*Pseudomonas aeruginosa*, *Bacillus cereus*), glycoprotein or intracellular proteins whose function are coupled to receptors and act by activating or inactivating a number of enzymes or ion channels [24]. Abiotic elicitors include physical and chemical stresses such as UV radiations, temperature, antibiotics, salts of heavy metals, etc. [22].

Various fungal elicitors including cell wall fragments, polysaccharides, glycoproteins and oligosaccharides have been used for the production of secondary metabolites in many plant spp. and their cell cultures. The cell extracts and filtrates of four species of fungi were used for the production of taxol from elicited cell cultures of *Taxus* sp. [25]. The cell wall fractions of *Aspergillus niger* have been used as an

elicitor in cell suspension cultures of *Taxus chinensis* thereby resulting in more than two fold increase in taxol yield and about six fold increase in total secretion.

Jasmonic acid (JA) and its methyl esters, methyl jasmonate (MJ) have been reported as key signaling compounds in the process of elicitation leading to the accumulation of various secondary metabolites. Lu et al. (2001) reported 28 fold higher saponin production in the elicited cultures of *Panax ginseng* by using yeast extract and methyl jasmonate as elicitors. Production of many valuable secondary metabolites using various elicitors have been reported successfully in various other plant species [26–29]. Enhanced production of podophyllotoxin in suspension cultures of *Linum album* was reported by using biotic (yeast extract) and abiotic (Ag^+ , Pb^{2+} and Cd^{2+}) elicitors.

Methyl jasmonate, vanadyl sulphate and chitosan were used for enhancing the production of ginsenoside from hairy root cultures of *P. ginseng* [23].

Pitta-Alvarez and Giulietti et al. (2000) used jasmonic acid and aluminium chloride as elicitors for enhancing the production of scopolamine and hyoscyamine in hairy root cultures of *Brugmansia candida*. Bacterial elicitors like *Bacillus cereus*, *Staphylococcus aureus*, etc. have been used for enhancing scopolamine production from the adventitious hairy roots of *Scopolia parviflora* (Table 2).

Plant species	Secondary metabolites	Elicitor	Reference
<i>Catharanthus roseus</i>	Ajmalicine, vincristine, vinblastine	a. <i>Pythium</i> sp. b. Yeast elicitor, MeJA c. <i>Trichoderma viride</i> d. <i>Pythium aphanidermatum</i> e. Jasmonic acid	DiCosmo et al. (1987), Menke et al. (1999), Zhao et al. [7, 8] and Namdeo et al. (2004)
<i>Picrorhiza kurroa</i>	Picroside-1	Seaweed extract	[14]
<i>Datura stramonium</i>	Alkaloids (tropane)	<i>Phytophthora megasperma</i>	Kurosaki et al. (2001) and Dorenburg et al. (1994)
<i>Azadirachta indica</i>	Azadirachtin	Jasmonic acid, salicylic acid	Satdive et al. [30] and Funk et al. [31]
<i>Papaver somniferum</i>	Codeine, morphine	Fungal spores	Heinsterin et al. (1985)
<i>Dioscorea deltoidea</i>	Diosgenin	<i>Rhizopus arrhizus</i>	Rokem et al. (1984)
<i>Hyoscyamus niger</i> , <i>H. muticus</i>	Hyoscyamine, scopolamine	Fungal elicitor, MeJA	Singh (1995)
<i>Rauwolfia canescens</i>	Raucaffrincine	Yeast elicitor, MeJA	Gundlach et al. (1992) and Parchmann et al. (1997)
<i>Panax ginseng</i>	Saponin	Oligogalacturonic acid low energy ultra sound	Threfal and Whitehead (1988) and Hu et al. (2003a,b)
<i>Hyoscyamus muticus</i>	Sesquiterpenes	<i>Rhizoctonia solani</i>	Singh (1995)
<i>Lithospermum erythrorhizon</i>	Shikonin	Endogenous source	Fukui et al. [32]
<i>Taxus chinensis</i>	Taxol	Fungal elicitation	Wang et al. (2001)
<i>Taxus brevifolia</i> , <i>T. cuspidate</i>	Taxol, Baccatin III	Fungal elicitor	Yukimuni et al. [33], Hefner et al. (1998) and Luo et al. (2001)

Table 2.
Elicitors used for the production of secondary metabolites by cell cultures of medicinal plants.

3.4 Factors influencing the biosynthesis and accumulation of medicinal phytochemicals

Knowledge about biosynthetic pathways for secondary metabolite production open avenues for the targeted production of medicinal compounds as reported by Varun et al. [34] where he proposed the biosynthetic pathways for the production of picroside-1 and picroside-2 of *Picrorhiza kurroa* an endangered herb of North-Western Himalayas, having hepatoprotective iridoid compounds. Varun et al. [35, 36] optimized preparative RP-HPLC method for the isolation and purification of picrosides in *Picrorhiza kurroa*.

For maximizing the production and accumulation of secondary metabolites through plant cell cultures, specific physical conditions such as type and composition of nutrient media, type and source of explant for initiating cell cultures, incubation temperatures and intensity of light, etc. are of paramount importance.

3.5 Culture medium

The tissue culture media are the basic support system for the growth and development of plant cell cultured in vitro. The activities of basic primary metabolism are largely influenced by the basal media considered to be common to most of the plant species. However, the differentiation or dedifferentiation of plant tissue cultures is influenced by the combinations of growth hormones mainly auxins and cytokinins (Table 3). The manipulation of media components have been reported to influence the biosynthesis and accumulation of secondary metabolites in plant cell cultures. Different strategies have been employed for improving secondary metabolite production in suspension cultures. The influence of media constituents and nutrient stress affect the production of diosgenin from callus cultures of *Dioscorea deltoidea*. The production of gentipicroside and swertiamarin was enhanced on MS medium supplemented with kinetin, NAA and 3% sucrose in suspension cultures of *Gentiana davidii* [44].

The productivity of picroside-1 was increased by optimizing the concentration of nutrients in growth medium and levels of phytohormones in the shoot cultures of *Picrorhiza kurroa* [45, 46]. Elevated sucrose levels from 3 to 6% were favourable in some cultures whereas addition of fructose promoted paclitaxel production in *Taxus* cell cultures [6]. Supplementation of MS medium with seaweed extract also contributed in enhancement of picroside accumulation in shoot cultures of *Picrorhiza* species [14].

Plant species	Metabolites	Reference
<i>Camptotheca acuminata</i>	Camptothecin	Lorence et al. [37]
<i>Ginkgo biloba</i>	Ginkgolides	Ayadi et al. [18]
<i>Gmelina arborea</i>	Verbascoside	Dhakulkar et al. [38]
<i>Linum flavum</i>	Coniferin	Lin et al. [39]
<i>Papaver somniferum</i>	Morphine, sanguinarine, codeine	Le Flem et al. [40]
<i>Panax ginseng</i>	Ginsenoside	Palazon et al. [23]
<i>Pueraria phaseoloides</i>	Puerarin	Shi and Kintizos et al. [41]
<i>Rauwolfia micrantha</i>	Ajmalicine, ajmaline	Sudha et al. [17]
<i>Saussurea medusa</i>	Jaceosidin	Zhao et al. [42]
<i>Solidago altissima</i>	Polyacetylene (cis-dehydromatricaria ester)	Inoguchi et al. [43]

Table 3. Pharmaceutical metabolites produced by hairy root cultures.

3.6 Type and source of explant

The type and source of explant has been of major importance in not only establishing successful tissue cultures in any plant species but also of significant importance in producing phytochemicals in vitro. The prime importance of choosing a right explant for the production of phytochemicals lies in the fact that the biosynthesis and accumulation of metabolites is very specific to tissues and organs along with their developmental stages. The tissue and developmental specific accumulation of phytochemicals thus makes it important that appropriate explant be selected for starting plant cell cultures for the production of phytochemicals.

Production of diosgenin has been carried out from cell suspension cultures of different explants of *Dioscorea doryophora* like stem-node, microtuber and intact tuber, etc., along with varying concentrations of sucrose in MS liquid media supplemented with 2 mg/L 2,4-D (0.3–3.5%). Increase in diosgenin production was obtained from tuber derived cell suspensions as compared to intact tuber explant.

Different cell lines were established on B5 medium supplemented with NAA by using stem- and needle-derived callus of *Taxus mairei* and taxol yield of upto 200 mg/L was obtained in precursor fed cell suspensions [47].

3.7 Light and temperature

Plants tissue cultures are largely influenced by the quality and duration of light treatments. There are various case studies in the literature wherein manipulation of light parameters or the temperature regimes has resulted in the alteration in the production of secondary metabolites. Zhang et al. (2005) gave heat shocks of 35–50°C for 30–60 min in the suspension cultures of *Taxus yunnanensis* for enhancing the production of paclitaxel. Production was increased to six fold by pretreatment with abscisic acid. The production of swertiamarin and gentipicroside was enhanced in cell suspension cultures of *Gentiana davidii* by incubating at 25°C and light intensity of 2.33 Lux [44]. Increase in the concentration of glycyrrhizin was found in the root tissue of *Glycyrrhiza uralensis* grown under red light or under low and high intensity of UV-B radiations [48].

3.8 Precursor feeding

Exogenous supply of a biosynthetic precursor to culture medium also increases the yield of the desired products. The concept is based on the idea that any compound which is an intermediate, or is in the beginning of a secondary metabolite biosynthetic route, proves to be a good candidate for increasing the final yield of secondary metabolite. Varun et al. [49] has carried out exogenous feeding of immediate biosynthetic precursor, i.e., cinnamic acid and catalpol in the shoot cultures of *Picrorhiza kurroa* hence stimulated 4.2 fold production of picroside-1. The production of monoterpene alkaloids was increased in cell suspension cultures of *Catharanthus roseus* fed with precursor mevalonic acid, secologanin [50]. Callus cultures of *Dioscorea balcanica* fed with cholesterol, norflurazon as precursors were used for the production of diosgenin, phytosteroids [51]. Hallard et al. [52] used secologanin and tryptamine in cell suspension cultures of *Nicotiana tabacum* for the production of strictosidine. Phenolics compounds were elicited from micropropagated plants of *Calligonum polygonoides* by Owis et al. [53].

Supplementation of media with amino acids has been found to enhance the production of indole alkaloids tropane alkaloid in cell suspension cultures [54, 55]. Addition of phenylalanine to cell suspension culture of *Salvia officinalis* enhanced

the production of rosmarinic acid. The production of taxol from *Taxus* cultures was also increased by using the same precursor [56]. Nicotinic acid was used as a precursor in the hairy root cultures of *Nicotiana rustica* for the production of nicotine. Hakkinen et al. [57] used hyoscyamine as a foreign substrate for enhancing the production of scopolamine in the hairy roots of *N. tabaccum* and found that 85% of the converted scopolamine was released into the medium.

3.9 Genotypic variation

The biosynthesis and accumulation of secondary metabolites or phytochemicals of medicinal value is influenced by the genotype of the target plant species [58]. There are examples wherein genotypic variations have been reported for phytochemical content. However, there has been a technical problem in most of these studies because genotype collections are made from different locations, which vary in altitude, climatic conditions, etc. thus resulting in variation in accumulation of phytochemicals. It would be highly desirable and practically viable if the influence of genotypic variation on phytochemical content is investigated by collecting genotypes of a particular plant species and then growing under uniform environmental conditions. The variation for metabolite content can be done on those genotypic collections.

3.10 Metabolic engineering for the production of phytopharmaceuticals

True metabolic engineering of plant secondary metabolite pathways has been hampered due to the lack of thorough knowledge of biosynthetic pathways and their regulatory mechanisms leading to the formation of desired compounds. Methods like labeled precursor feeding, induced expression of regulatory genes and block competitive pathways and metabolism by antisense genes have been used for enhancing the production of desired metabolites. Yun et al. [59] cloned the hyoscyamine 6-beta hydroxylase gene (*h6h*) of *Hyoscyamus niger* and introduced into *Atropa belladonna* and collected scopolamine from engineered plant. In a later study, Hashimoto et al. (1993) reported fivefold higher concentration of scopolamine from *A. belladonna* hairy roots expressing the same gene than the wild-type hairy roots. Increased alkaloid production by overexpression of genes encoding key enzymes of tropane alkaloid biosynthesis pathway was reported by Palazon et al. [23] and Moyano et al. [60] in *Duboisia* hybrid, *Datura metel* and *Hyoscyamus muticis* hairy roots, respectively. Similarly, Zang et al. (2004) produced 411 mg/L scopolamine in cultivated hairy roots from the simultaneous over expression of *pmt* and *h6h* genes in *H. niger*. Elevated nicotine alkaloid production was achieved in *Nicotiana tabaccum* hairy roots carrying *h6h* gene [57]. Neha et al. [61] reported 2.6 fold increase in picroside-1 production by modulating four integrated secondary metabolic pathways, i.e., methyl erythritol phosphate, mevalonate, iridoid and phenylpropanoid pathway using seaweed extract. Moreover Sharma et al. [62] defined many strategies through metabolic engineering for stimulating the production of bio-active compounds from medicinal plants.

3.11 Upscaling the production of phytochemicals

The production of phytopharmaceuticals in cell cultures coupled with their low yield from natural sources and supply concerns of plant species has renewed interest in up scaling cell culture technology for large scale production. Bioreactors are the key step towards their commercial exploitation because it provides defined

parameters for up scaling the production of phytochemicals or secondary metabolites from plant cell cultures.

Bioreactor is a large culture vessel fitted with microprocessor control unit for the control of pH, temperature, light, dissolved oxygen, gas flow rate, agitation speed, nutrient factors, cell density for optimal growth or production, handling of cultures, nutrient uptake and product harvestation, etc. The success of Mitsui Petrochemical Industry Co. Ltd. in Japan in producing shikonin on a commercial scale from *Lithospermum erythrorhizon* and that of Nitto Denko Corp. Japan in mass production of *Panax ginseng* or ginseng cells have demonstrated the practical feasibility of using cell cultures in the large scale production of secondary metabolites of pharmaceutical importance. Commercial companies like Phytion and Samyang Genex are successfully producing paclitaxel and its related taxanes on large scale [63].

Heble and Chadha (1985) reported the successful cultivation of *Catharanthus roseus* cells in 7–20 L capacity of airlift bioreactor for the production of ajmalicine and serpentine by judicious use of air lift and low agitation. Significant amounts of sanguinarine were produced in cell suspension cultures of *Papaver somniferum* using bioreactors [64]. Ginseng root tissue cultures in 20 ton bioreactor produced 500 mg/L of saponin per day [65]. Hahn et al. [66] have produced ginsenoside from adventitious root culture of *Panax ginseng* through large scale bioreactor system. Chattopadhyay et al. [12] produced podophyllotoxin through cell cultures of *Podophyllum hexandrum* in a bioreactor.

Different types of culture systems have been successfully used such as airlift bioreactors were used for scaling up hairy root production of *Astragalus membranaceus* [67] and *Solanum chrysotricum* [68] and mist bioreactor for hairy root of *Tagetes patula* [69]. Flow diagram of a process for the production of picrosides from *Picrorhiza kurroa* is given below wherein callus cultures/suspension cultures have been established from different explants and accumulation of picrosides is being investigated by HPLC [46, 70] (Figure 1).

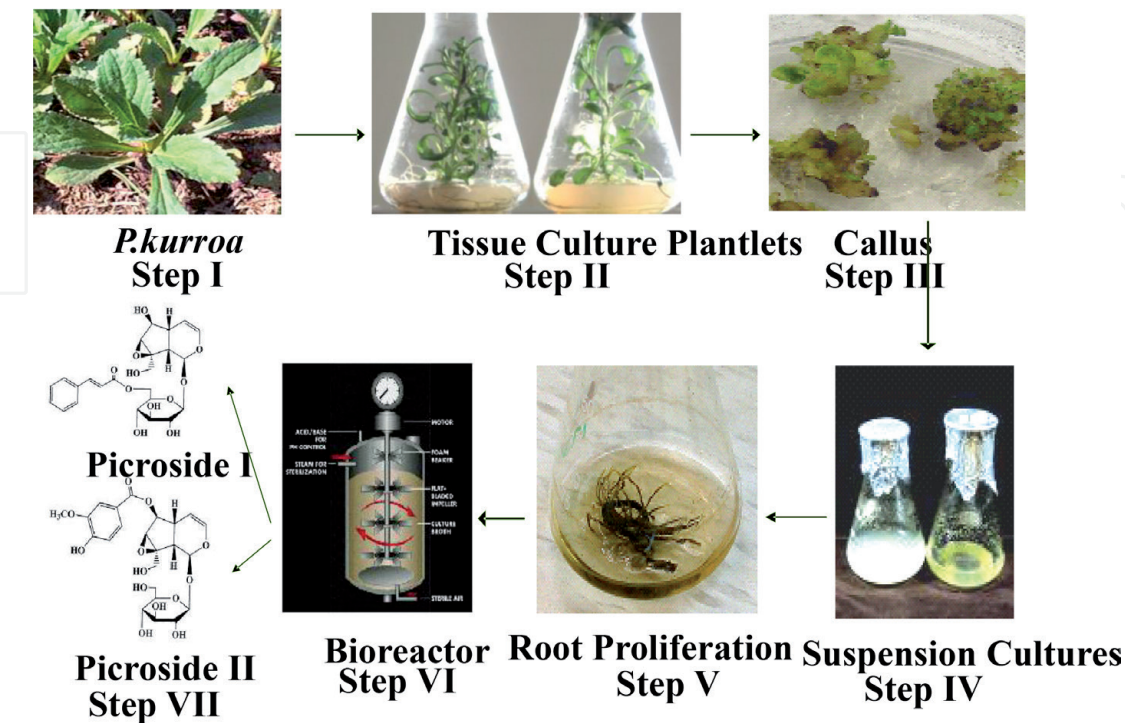


Figure 1.
Pictorial representation for picroside-1 production through plant tissue culture.

3.12 Limitations in cell culture technologies for the production of phytochemicals

The research on in vitro production of phytochemicals has been carried for the past 20 years, however, there are very few case studies where technologies have been upscaled successfully. There has been several shortcomings some of which are mentioned below:

3.12.1 General limitations

- Lack of understanding about the physical environmental and genetic factors controlling the production of pharmaceuticals
- Low yields of pharmaceuticals in tissue cultures
- Lack of information on cost effectiveness in the production of pharmaceuticals through cell cultures
- Poor amenability of most of the plant species producing pharmaceuticals to in vitro conditions
- Use of high sugar concentration (3–8%) or addition of elicitors or precursors increases the production cost considerably
- Infections due to contamination limit the progress of cell cultures
- Lack of knowledge of various molecular events that occur in secondary metabolite biosynthesis

3.12.2 Limitations pertaining to bioreactor conditions

- Cell sedimentation and death due to mass transfer of cells in large vessels limits the supply of oxygen and nutrients
- Plant cells are extremely sensitive to shear forces
- Plant cells have very low doubling time (16–24 h) therefore produce less biomass and relatively produce small amount of secondary metabolites
- For aeration of cells stirring is needed which sometimes cause damage to cells and lower the yield of products

4. Conclusions and future prospects

In spite of bottlenecks in the large scale production of phytopharmaceuticals many technological advancements and refinements have been made in the recent years right from the selection of high yielding cell lines to manipulation of basic chemical, physical and biological parameters. The identification of right explant of proper developmental stage, standardization of optimum nutrient medium resulting in maximum accumulation of pharmaceuticals, optimization of low-cost production technology are some of the areas which warrant immediate attention. Knowledge of the biosynthetic pathways of desired compounds in plants as well as

in cell cultures is still rudimentary, therefore emphasis need to be made generate information based on a cellular and molecular level. Major breakthrough in the metabolomics and its integration with genomics and transcriptomics technologies will help in discovering potential genes of biosynthetic pathways so that closer understanding of the links between different levels in biological systems can lead to better understanding of the molecular biology of secondary metabolite production in plants.

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