Optimization of Active Secondary Metabolites in Herbal Drugs

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Abstract

Plants secondary metabolites are a potential renewable source for use in human therapy. Secondary metabolites are widely extracted from the whole plant. Production and extraction of secondary metabolites for therapeutic use from whole plant posses certain problems. These problems include: environmental factors (drought, floods, etc.), disease, political and labour instabilities in the producing countries (often Third World countries), uncontrollable variations in the crop quality, losses in storage and handling. Plant Tissue culture is an attractive and beneficial alternative approach for the production of therapeutically important secondary metabolites in an efficient and time bound manner. Plant cell and tissue cultures hold great promise for controlled production of myriad of useful secondary metabolites on demand. The current review highlights a brief role of plant secondary metabolites in therapy and the history of their production through various techniques involved in plant tissue culture and the best suited media used for the production.

Key Words: Secondary metabolites, Tissue culture, Media, Murashige and Skoog (MS) media, Gibberellin (GA), Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), α-Naphthalene acetic acid (NAA), Gibberellin A3 (GA3), and N6-benzyladenine (BAP), 2,4-Dichlorophenoxyacetic acid (2, 4-D), Medium.

Introduction

Plants have been utilized as medicines for thousands of years. Prior to the nineteenth century, herbal medicines were administered mostly in their crude forms as infusions (herbal teas), tinctures (alcoholic extracts), decoctions (boiled extract of roots or bark), syrups (extracts of herbs made with syrup or honey) or applied externally as ointments (poultices, balms and essential oils) and herbal washes (Hosein *et al.,* 2012). Morphine, produced by the opium poppy, was the first active component isolated from plants in early 19th century. Since then many important drugs, such as artemisinin, atropine, camptothecin, cocaine, codeine, digoxin, papaverine, pilocarpine, podophyllotoxin etc, have been discovered from plants. (Hakkinen and Ritala, 2010). Although modern medicine may be available in developed countries, herbal medicines (phytopharmaceuticals) have often maintained popularity for historical and cultural reasons. Pharmaceuticals and intermediates that are derived from higher plants represent about 25 percent of all prescription drugs. (James C. Linden, Vol VI). Currently, a large number of natural products are produced

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solely from massive quantities of whole plant parts. It has been mentioned that natural habitats for medicinal plants are disappearing fast and together with environmental and geopolitical instabilities; it is increasingly difficult to acquire plant-derived compounds. Moreover, there are several problems associated with the isolation of compounds for production of pharmaceuticals from biomass collected from wild populations of plants. Destruction of plant populations due to over exploitation or natural calamities affects drug supply and the content of bioactive secondary metabolite in the plant. Moreover, wild populations may be represented by various genotypes growing under different environmental conditions which may affect drug profile leading to problems in the purity of the final product (Anrini *et al.,* 2009). This has prompted industries, as well as scientists to consider the possibilities of investigation into tissue culture as an alternative supply for the production of plant pharmaceuticals. (Mulabagal and Tsay, 2004). However, only a few compounds have reached the commercial production scale, including shikonon and paclitaxel. (Hakkinen and Ritala, 2010).

Plant Secondary Metabolites

The plant chemicals used for therapy are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in the growth, development, or reproduction of plants. Secondary metabolites are often colored, fragrant, or flavorful compounds and they typically mediate the interaction of plants with other organisms. Such interactions include those of plant-pollinator, plant-pathogen, and plant-herbivore. Plant secondary metabolites play an indispensable role in the survival of the plant in its environment, often adapting to match the environmental needs.

The secondary metabolites are known to play a major role in the adaptation of plants to their environment, but also represent an important source of pharmaceuticals (Vijaya Sree *et al.*, 2010). Unlike the ubiquitous macromolecules of primary metabolism (e.g. monosaccharides, polysaccharides, amino acids, proteins, nucleic acids, lipids) which are resent in all plants, secondary metabolites with medicinal properties are found only in a few species of plants (Henrich *et al*., 2004)

Secondary herbal metabolites with reported medicinal properties consist of waxes, fatty acids, alkaloids, terpenoids, phenolics (simple phenolics and flavonoids), glycosides and their derivaties. (Cowan, 1995; Eloff, 2001; Satyajit *et al*., 2006).

Biotechnological Approaches in The Production of Secondary Metabolites

Tissue culture is a propagation method used to produce plants under sterile conditions. This method uses plant explants (plant parts i.e leaves, shoot, hair etc) or seeds that have been sterilized before being placed in containers with a growing medium (usually a gel) that has some nutrients added. The explants or seeds, the containers and the medium have all been sterilized, and this (if successful) prevents any cut or torn tissue, or the entire explant or seed itself, from becoming infected with a microorganism of some kind and rotting during the time these plant parts require to become rooted or to multiply.

The main difference between tissue culture and propagation done by cuttings, pullings, divisions or seeds is that the plants grow faster (the nutritious gel medium helps) and one can multiply them rapidly by giving them the right hormones and dividing them regularly. Theoretically, an infinite number of plants can be created from just one piece of tissue, and many plants in a relatively brief period of time.

Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon source and some gelling agents in case of solid medium (Murashige and Skoog, 1962). Murashige and Skoog medium (MS medium) is most extensively used for the vegetative propagation of many plant species in vitro. The pH of the media is also important that affects both the growth of plants and activity of plant growth regulators. It is adjusted to the value between 5.4 - 5.8. Both the solid and liquid medium can be used for culturing. The composition of the medium, particularly the plant hormones and the nitrogen source has profound effects on the response of the initial explants (Hussain *et al.,* 2012)

Plant tissue culture involves mainly four stages:

- Stage I. Establishment of an aseptic (sterile) culture.
- Stage II. The multiplication of propagules (a propagule is any part of a plant used to make or become new plants).
- Stage III. Preparation of propagules for successful transfer to soil (rooting and "hardening" (acclimating) outside of sterile conditions in regular growing media).
- Stage IV. Establishment in soil (or other appropriate growing medium).

Modern plant tissue culture is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet. A list of some medicinal plants for *In vitro* production of secondary metabolites is summarized in Table 1.

Plant tissue culture is viewed as a potential mean of producing useful plant products. This is normally achieved under controlled conditions, according to demand and reduced cost and requirements. Also, tissue cultures have produced compounds previously undescribed and cultures of higher plant cells may provide an important source of new economically important compounds (Butcher, 1977; Constabel and Tyler, 1994.)

Different tissue culture types include callus culture, cell suspension culture, protoplast culture, root culture, shoot tip and meristem culture, embryo culture and microspore culture. Different strategies have been used with the aim of increasing the production of bioactive secondary metabolites in plant cell cultures. These include screening and selection of high producing cell lines, optimization of nutrient media for growth and production, organ culture, culture of immobilized cells etc. (Verpoorte *et al., 2002*). Another promising tool to improve product yield in cell culture is the use of biotic and abiotic elicitors (DiCosmo, and Misawa, 1985); feeding of biosynthetic precursors is yet another effective technique. A novel strategy developed is the in vitro cross-species coculture, through which metabolites produced by one species can be taken up by another species for biochemical conversion (Mahagamasekara and Doran, 1998; Pereira *et al.*, 2000; Lin *et al.*, 2003).

A number of plant species have been used for generation and propagation of cell-suspension cultures, ranging from model systems like *Arabidopsis*, *Catharanthus* and *Taxus*, to important monocotyledon or dicotyledonous crop plants like rice, Soya bean, alfalfa and tobacco. Cell suspension cultures systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantages of this method are that it can ultimately provide a continuous reliable source of natural products. In recent years, traditional system of medicine has become a topic of global importance. Discoveries of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last few years (Vijaya Sree *et al*., 2010).

While research to date has succeeded in producing a wide range of valuable secondary metabolites in unorganized callus or suspension cultures, in other cases production requires more differentiated microplant or organ cultures. This situation often occurs when the metabolite of interest is only produced in specialized plant tissues or glands in the parent plant. A prime example

is ginseng (*Panax ginseng*). Since saponin and other valuable metabolites are specifically produced in ginseng roots, root culture is required in vitro. Similarly, herbal plants such as *Hypericum perforatum*, which accumulates the anti-depressant hypericins and hyperforins in foliar glands, have not demonstrated the ability to accumulate phytochemicals in undifferentiated cells (Karuppusamy, 2009).

Advantages of production of secondary metabolites by tissue culture over whole plant extractions:

(1) Reliable, simpler and more predictable production of secondary metabolites; (2) Rapid and efficient isolation of secondary metabolite as compared to extraction from complex whole plants; (3) Interference of the compounds that occur in field grown plants can be avoided in cell cultures; (4) Tissue and cell cultures can yield a source of defined standard metabolites in large volumes. (5) Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions on a continuous year-round basis; (6) Cultured cells would be free of microbes and insects; (7) The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites; (8) Automated control of cell growth and rational regulation of metabolite processes would reduce of labor costs and improve productivity; (9) Organic substances are extractable from callus cultures (Vijaya Sree *et al.*, 2010).

Table 1. List of some medicinal plants for In vitro production of secondary metabolites

S. No.	Name of Medicinal Plant	Secondary Metabolite(s)	Culture Type	Culture Media & Plant growth regulators	Reference
1.	*Aconitum heterophyllum*	Aconites	Hairy root	MS + 2,4-D + Kin	Giri et al., 1997
2.	*Adhatoda vasica*	Vasine	Shoot culture	MS + BAP + IAA	Shalaka and Sandhya, 2009
3.	*Agastache rugosa*	Rosmarinic acid	Hairy root	MS + 2,4-D + Kin + 3% sucrose	Lee et al., 2007
4.	*Agave amaniensis*	Saponins	Callus	MS + Kinetin	Andrijany et al., 1999.
5.	*Ailanthus altissima*	Alkaloids	Suspension	MS + 2,4-D + Kinetin	Anderson et al., 1987.

S. No.	Name of Medicinal Plant	Secondary Metabolite(s)	Culture Type	Culture Media & Plant growth regulators	Reference
6.	*Allium sativum* L.	Allin	Callus	MS + IAA + Kinetin	Malpathak and David , 1986.
7.	*Aloe saponaria*	Tetrahydroan-thracene glucosides	Suspension	MS + 2,4-D + Kinetin	Yagi et al., 1983.
8.	*Ambrosia tenuifolia*	Altamisine	Callus	MS + Kinetin	Goleniowski and Trippi, 1999
9.	*Ammi majus*	Umbelliferone	Shootlet	MS + BAP	Krolicka et al, 2006
10.	*Ammi visnaga*	Furano-coumarin	Suspension	MS + IAA + GA3	Kaul and Staba, 1967
11.	*Anchusa officinalis*	Rosmarinic acid	Suspension	B5 + 2,4-D	De-Eknamkul and Ellis, 1985
12.	*Angelica gigas*	Deoursin	Hairy root	MS (Liq.) + 2,4-D + GA3	Xu et al., 2008
13.	*Anisodus luridus*	Tropane alkaloids	Hairy root	MS + 2,4-D + BA	Jobanovic et al., 1991
14.	*Ammi majus*	Triterpenoid	Suspension	MS + 2,4-D + BA	Staniszewska et al., 2003
15.	*Arachys hypogaea*	Resveratol	Hairy root	G5 + 2,4-D + Kin.	Kim et al., 2008
16.	*Armoracia laphthifolia*	Fisicoccin	Hairy root	MS + IAA	Babakov et al., 1995
17.	*Artemisia absinthum*	Essential oil	Hairy root	MS + NAA + BAP	Nin et al., 1997
18.	*Artemisia annua*	Artemisinin	Hairy root	MS + IAA + Kinetin	Rao et al., 1998
19.	*Artemisia annua*	Artemisinin	Callus	MS + NAA + Kinetin	Baldi and Dixit, 2008
20.	*Aspidosperma ramiflorum*	Ramiflorin	Callus	MS + 2,4-D + BAP	Olivira et al., 2001
21.	*Aspidosperma ramiflorum*	Ramiflorin alkaloid	Callus	MS + 2-4,D + BAP + 30 g/l Sucrose	Olivira et al., 2001
22.	*Astragalus mongholicus*	Cycloartane saponin	Hairy root	MS + 2,4-D + Kin	Ionkova et al., 1997

S. No.	Name of Medicinal Plant	Secondary Metabolite(s)	Culture Type	Culture Media & Plant growth regulators	Reference
23.	*Astragalus mongholicus*	Cycloartane	Hairy root	MS + IAA + NAA	Ionkova et al., 1997
24.	*Azadirachta indica*	Azadirachtin	Suspension	MS + 2,4-D	Sujanya et al., 2008
25.	*Azadirachta indica*	Azadirachtin	Suspension	MS + 2,4-D + Cyanobacterial elicitor	Poornasri Devi et al., 2008
26.	*Beeta vulgaris*	Betalain pigments	Hairy root	MS + IAA	Taya et al., 1992
27.	*Brucea javanica*	Canthin	Suspension	MS + IAA + GA3	Wagiah et al., 2008
28.	*Brucea javanica*	Alkaloids	Suspension	MS + 2,4-D + Kinetin	Liu et al., 1990.
29.	*Brugmansia candida*	Tropane	Hairy root	MS + 2,4-D + IAA	Marconi et al., 2008
30.	*Brugmansia candida*	Tropane alkaloid	Hairy root	MS + BA + NAA	Giulietti et al., 1993
31.	*Bupleurum falcatum*	Saikosaponins	Root	B5 + IBA	Kusakari et al., 2000
32.	*Bupleurum falcatum*	Saikosaponins	Callus	LS + 2,4-D	Wang and Huang, 1982.
33.	*Calystegia sepium*	Cuscohygrine	Hairy root	MS + 2,4-D + BA	Jung and Tepfer, 1987
34.	*Camellia chinensis*	Flavones	Callus	MS + 2,4-D + NAA	Nikolaeva et al., 2009
35.	*Camellia Sinensis*	Theamine	Suspension	MS + IBA + Kinetin	Orihara and Furuya, 1990.
36.	*Campanula medium*	Polyacetylenes	Hairy root	MS + IAA + BA	Tada et al., 1996
37.	*Canavalia ensiformis*	L-Canavanine	Callus	LS + NAA + Picloram	Ramirez et al., 1992.
38.	*Capsicum annuum*	Capsaicin	Callus	MS + 2,4-D+ GA3; MS + 2,4-D + Kin.	Varindra et al., 2000; Umamaheswari and Lalitha, 2007
39.	*Capsicum annuum*	Capsaicin	Suspension	MS + 2,4-D + Kinetin	Johnson et al., 1990.

S. No.	Name of Medicinal Plant	Secondary Metabolite(s)	Culture Type	Culture Media & Plant growth regulators	Reference
40.	*Cassia acutifolia*	Anthra-quinones	Suspension	MS + 2,4-D + kinetin	Nazif et al., 2000.
41.	*Cassia obtusifolia*	Anthraquinone	Hairy root	MS + TDZ + IAA	Ko et al., 1995
42.	*Cassia senna*	Sennosides	Callus	MS + NAA + Kin	Shrivastava et al., 2006
43.	*Catharanthus roseus*	Indole alkaloids	Suspension	MS+IAA; MS + NAA + Kin ; MS + 2,4-D + GA3 + Vanadium	Moreno et al., 1993.; Zhao et al., 2001; Tallevi and Dicosmo, 1988
44.	*Catharanthus roseus*	Catharanthine	Suspension	MS + 2,4-D + UV-B radiation	Zhao et al., 2001.; Ramani and Jayabaskaran, 2008
45.	*Catharanthus roseus*	Vincristine	Suspension	MS + 2,4-D + GA3	Lee-Parsone and Rogce, 2006
46.	*Catharanthus Trichophyllus*	Indole alkaloids	Hairy root	MS + IAA + GA3	Davioud et al., 1989
47.	*Cayratia trifoliata*	Stilbenes	Suspension	MS + IAA + GA3	Roat and Ramawat, 2009
48.	*Centella asiatica*	Asiaticoside	Hairy root	Ms + 2,4-D	Kim et al., 2007
49.	*Centella asiatica*	Asiaticoside	Callus	Ms + 2,4-D + Kin	Kiong et al., 2005
50.	*Centella asitica*	Asiaticoside	Shoot	MS + BAP + IAA	Kim et al., 2004
51.	*Centella asitica*	Asiaticoside	Hairy root	MS + 2,4-D	Paek et al., 1996
52.	*Centranthes ruber*	Valepotriates	Hairy root	MS + IAA + Kin	Granicher et al., 1995
53.	*Cephaelis ipecacuanha*	Alkaloids	Root	MS + IAA	Teshima et al., 1988
54.	*Chaenatis douglasei*	Thiarbrins	Hairy root	MS + NAA	Constabel and Towers, 1988
55.	*Chrysanthemum cinerariaefolium*	Pyrethrins	Callus	MS + 2.4-D + Kinetin	Rajasekaran et al., 1991.

S. No.	Name of Medicinal Plant	Secondary Metabolite(s)	Culture Type	Culture Media & Plant growth regulators	Reference
56.	*Cinchona ledgeriana*	Quinine	Suspension	MS + Kinetin; B5 + 2,4-D; B5 + 2,4-D + Kinetin	Kuch et al., 1985; Schripsema et al., 1999; Wijnsma et al., 1985
57.	*Cinchona ledgeriana*	Quinine	Hairy Root	MS + 2,4-D	Hamill et al., 1989
58.	*Cinchona succirubra*	Anthraquinone	Suspension	MS + IAA + GA3	Khouri et al., 1986
59.	*Citrus sp.*	Limonin	Callus	MS + 2,4-D + Kinetin	Barthe et al., 1987.
60.	*Coffea arabica L.*	Caffeine	Callus	MS + 2,4-D + Kinetin	Waller et al., 1983.
61.	*Corydalis ophiocarpa*	Isoquinoline alkaloids	Callus	MS + 2,4-D + Kinetin	Iwasa and Takao, 1982.
62.	*Croton sublyratus Kurz*	Plaunotol	Callus	MS + NAA + BA	Morimoto and Murai, 1989.
63.	*Cryptolepis buchanani*	Cryptosin	Callus	B5 + 2,4-D + Kinetin	Venkateswara et al., 1987.
64.	*Cymbopogon citratus*	Essential oil	Shoot	MS + IAA + GA3	Quiala et al., 2006
65.	*Digitalis purpurea L.*	Cardenolides	Suspension	MS+BA	Hagimori et al., 1982
66.	*Vaccinium myrtillus*	Flavonoids	Callus culture	MS + BAP + NAA	Hohtola et al., 2005
67.	*Vinca major*	Vincamine	Hairy root	MS + BAP	Tanaka et al., 2004
68.	*Vitis vinifera*	Anthocyanin	Suspension	MS + BAP + NAA	Qu et al., 2006
69.	*Vitis vinifera*	Resveratrol	Callus	MS + IAA + GA3 + UV	Kin and Kunter, 2009
70.	*Withania somnifera*	Withaferin A	Soot	MS + BA	Ray and Jha, 2001
71.	*Withania somnifera*	Withaferin	Hairy root	MS + IAA + Kintin	Banerjee et al., 1994
72.	*Withania somnifera*	Withanoloid A	Hairy root	MS + IAA + Kin	Murthy et al., 2008

S. No.	Name of Medicinal Plant	Secondary Metabolite(s)	Culture Type	Culture Media & Plant growth regulators	Reference
73.	*Withania somnifera*	Steroidal lactone	Callus	MS + 2,4-D + BA	Mirjalili et al., 2009
74.	*Zataria multiflora*	Rosmarininc acid	Callus	MS + IAA + Kin	Francoise et al., 2007

Discussion and Conclusion

This review reports the current status of medicinal plants and their secondary metabolites production by *in vitro* propagation. The use of in vitro plant cell culture for the production of chemicals and pharmaceuticals has made great strides building on advances in plant science. The increased use of genetic tools and an emerging picture of the structure and regulation of pathways for secondary metabolism will provide the basis for the production of commercially acceptable levels of product. Knowledge of biosynthetic pathways of desired phytochemicals in plants and in cultures is often still in its infancy, and consequently strategies needed to develop an information based on a cellular and molecular level. *In vitro* plant cell cultures have potential for commercial production of secondary metabolites. *In vitro* propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds. The increased use of plant cell culture systems in recent years is perhaps due to an improved understanding of the secondary metabolite pathway in economically important plants. Advances in plant cell cultures could provide new means for the cost-effective, commercial production of even rare or exotic plants, their cells, and the chemicals that they will produce (Baumert *et al.,* 1992).

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