2011

In vitro micropropagation of medicinal plants by tissue culture

Sidhu, Y.

Sidhu, Y. (2011) 'In vitro micropropagation of medicinal plants by tissue culture', The Plymouth Student Scientist, 4(1), p. 432-449.
http://hdl.handle.net/10026.1/13944

The Plymouth Student Scientist
University of Plymouth

*The Plymouth Student Scientist, 2010, **4**, (1), 432-449*

In vitro micropropagation of medicinal plants by tissue culture

Yaadwinder Sidhu

Project Advisor: Stuart Lane, School of Biomedical & Biological Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA

Abstract

Tissue culturing of medicinal plants is widely used to produce active compounds for herbal and pharmaceutical industries. Conservation of genetic material of many threatened medicinal plants also involves culturing techniques. This work reviews *in vitro* micropropagation techniques and gives examples of various commercially important medicinal plants. The effect of media formulations and culturing techniques on the growth and multiplication of medicinal plants; and on the production of secondary metabolites is also reviewed. Another method of obtaining secondary metabolites is biotransformation; some famous pharmaceuticals obtained by this method are discussed briefly.

Introduction

Since ancient times, mankind has been dependent on plants for food, flavours, medicinal and many other uses. Ancient written records of many civilizations (i.e. Egyptian, Roman, Chinese) give strong evidence regarding use of medicinal plant [1],for example ayurveda documents record the use of medicinal plants to cure many ailments [2-3].

At present there are many well established herbal and plant medicine practices (Ayurvedic medicine in India) which are popular in many parts of the world. The World Health Organisation (WHO) reported that 80% of people in the developing world use medicinal plants for their primary health care [4]. Table 1, shows some important medicinal plants with their key metabolites and uses.

The use of herbal medicines is growing in developed countries, presently 25% of the UK population use herbal medicine [4-5]. About 40% of compounds used in pharmaceutical industry are directly or indirectly derived from plants [6-7] because the chemical synthesis of such compounds is either not possible and/or economically not viable [8]. Therefore a large number plant species (especially medicinal) are under threat of extinction because of their over exploitation [4, 7, 9].

Table 1: Some medicinal plants and their uses.

Medicinal plant	Medicinal uses	Reference
Baliospermum montanum (Willd.)	To cure Jaundice, skin disease, snake bite, asthma, bronchitis and abdominal tumours.	[10]
Rauvolfia serpentina L.	To treat High blood pressure, insomnia anxiety and central epilepsy	[11-12]
Woodfordia fruticosa (L.)	Treating Dysentery, rheumatism, anti -tumour and anti-HIV.	[13]
Artemisia vulgaris L	Treating liver disorders , choleretic and for amenorrhoea and dysmenorrhoea , diabetes, epilepsy , psychoneurosis, depression, irritability, insomnia and anxiety stress.	[14-15]
Asparagus adscendens	Treating spermatorrhoea, chronic leucorrhoea, Diarrhoea, Dysentery, Senile pruritus, Asthma and fatigue Insulin-enhancing activity	[16-17]
Labisia pumila (Bl.) F. Vill.	Treatment of dysentery, flatulence, dysmenorrhoea and gonorrhoea	[18-19]
Ceropegia candelabrum L	Diarrhoea and dysentery	[20]
Melaleuca alternifolia	Antimicrobial , Antifungal and Anti inflammatory	[21-24]
Vitis thunbergii	Arthritis, Eye irritation and Hepatitis	[25-26]
Centella asiatica (L.)	Healing wounds, diarrhoea, eye infections and partial relief from leprosy.	[27]
Curculigo orchioides	Spasmolytic and anti cancer, piles jaundice, asthma, Diarrhoea	[28]

In last two decades there has been a great increase in research on medicinal plant. A number of new medicines have been discovered and advancements in production technology to harvest pharmaceutical important metabolites. During this period there has also been an increase in research publication on medicinal plants. Figure 1 shows publication numbers for medicinal plant and traditional medicines in Africa. Similar trends in medicinal plant research were also seen in the other parts of world [29]. Due to successful research carried out during this period plant tissue culture is an established field in plant cell biotechnology.

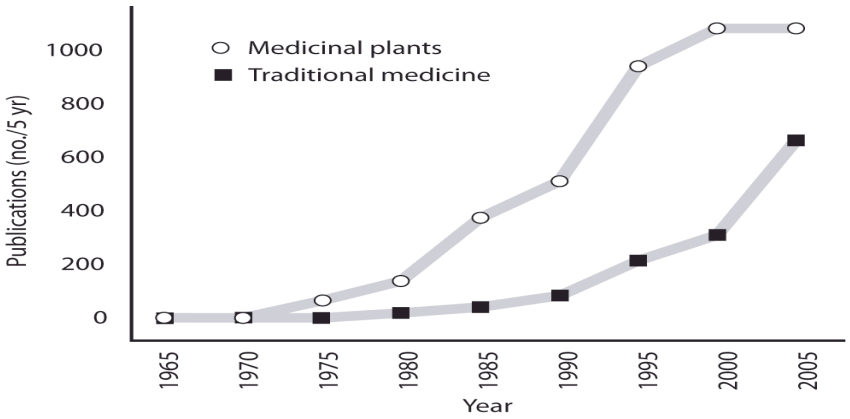

Figure1: Graph showing the publications of medicinal plants and traditional medicine from 1965-2005 in Africa (Adapted from Janick and Whipkey, 2007).

An increase in medicinal plant exports from 375,000 tonnes in 1991 to a staggering 600,000 tonnes in 2002 was reported by Food and Agriculture Organisation (F.A.O) [30]. Global exports valued at 1.12 billion US$ in 1991 rose to 1.51 billion US$ dollars in 1995-1996, but came down to 1.1 billion US$ dollars in 2002. Fall in unit prices in 2002 was due to increase in product production [30]. Medicinal plant imports also showed similar pattern of increase over this period. China, Hong Kong, The United States and Japan are major importers of medicinal plants and plant products. In Europe, Germany is largest importer because many world leading pharmaceutical companies are based there [30]. China and India are the largest markets for medicinal plants in the world. Other major exporters are the Republic of Korea, Chile, Brazil and Thailand [30]. Between 2006 and 2007 a rise in international market prices of many medicinal extracts and essential oils was seen [31-32]. Table 2 shows prices of plants extracts over this period.

Table 2: International prices of plant extracts in 2006 and 2007.

Plant and Extract	*Price in US$/kg 2006*	*Price in US$/kg 2007*
Echinacea herb *(Echinacea purpurea)*	30-33	39
Saw palmetto fruit *(Serenoa repens)*	40-45	45-48
Lycopene from Tomato *(Lycopersicon esculentum)*	--	195

Micropropagation
Micropropagation is the process of vegetative growth and multiplication from plants tissues or seeds. It is carried out in aseptic and favourable conditions on growth media, using various plant tissue culture techniques [5, 33-34]. Tissue culture is based on concept of totipotency; the ability of plant cells and tissues to develop into whole new plant [35]. Gottlieb Haberlandt (1854-1945), a German botanist is considered as the father of plant tissue culture, was the first to separate and culture plant cells on Knop's salt solution in 1898 [36].

In conventional cultivation many plants do not germinate, flower and produce seed under certain climatic conditions or have long periods of growth and multiplication. Micropropagation insures a good regular supply of medicinal plants, using minimum space and time [37]. The advantages of *In vitro* micro propagation of medicinal plant are listed below:

1. Higher rate of multiplication.
2. Environment can be controlled or altered to meet specific needs of the plant.
3. Plant available all year round (independent of regional or seasonal variation)
4. Identification and production of clones with desired characteristics.
5. Production of secondary metabolites.
6. New and improved genetically engineered plant can be produced.
7. Conservation of threatened plant species.
8. Preservation of genetic material by cryopreservation.

Explant source
Explant is material used as initial source of tissue culture. Tissue culture success mainly depends on the age, types and position of explants [38] because not all plant cells have the same ability to express totipotency [10, 39-40]. The most commonly used explants are shoot tips, nodal buds and root tips. Large explants can increase chances of contamination and small explants like meristems can sometimes show less growth [35, 40].

Sterilisation
Microbial contamination of plant tissue culture is a common problem [7, 35, 41]. Common bacterial contaminants are *Bacillus, Pseudomonas, Staphylococcus and Lactobacillus* [33, 41-42]. Microbes multiply and compete with growing explant for nutrients, while releasing chemicals which can alter culture environments e.g. pH and inhibit explants growth or cause death [40-41]. Explants are cleaned by distilled water and sterilized using mercuric chloride, ethyl alcohol, and liquid bleach [7, 37, 43]. Sterilization of laboratory instruments is carried out by autoclaving, alcohol washing, baking, radiations, flaming and fumigation [44]. A considerable decrease in bacterial contamination was seen by using ultrasonic sonicator [45].

Tissue culture Media
Culture media contains vital nutrients and elements for *in vitro* growth of plant tissues [35, 38-39]. Choosing the right media composition is important for successful tissue culturing [34, 38, 46]. Medium contains a carbon source (sucrose), macro and micro nutrients, vitamins, hormones and other organic substances [34-35]. A wide range of media are available for plant tissue culture, but MS [39] medium is commonly used

[34, 38-39]. Other media used are Linsmaier-Skoog (LS) [47], Schenk and Hilderbrandt (SH) [48], WPM (Woody plant medium) [49], and the Nitsch and Nitsch (NN) [50]. Agar is not essential media component but is used as gelling agent [34, 44]. It prevents death of cultured cells due to submerging and lack of oxygen in liquid medium [34]. The *pH* of culture media is normally between 5.0-6.0, and is also very important as it affects uptake of ions [34].

The affects of media composition were demonstrated when tissue culturing *Vitis thunbergii* using WPM, MS and NN medium [25]. WPM medium was found to enhance shoot proliferation the most. Whereas, explants cultured on MS medium showed increased plant growth, leaf formation and root induction. NN medium explants contained higher amounts of chlorophyll but showed lower growth [25].

Culture Browning

Explants in cultures release phenol compounds, which are oxidised by enzymes known as polyphenol oxidase, and cause the media to turn brown [7, 51]. Browning can be minimized by adding antioxidants or phenol absorbents for e.g. ascorbic acid, glutathione, activated charcoal and polyvinylpyrrolidone [43] or by transferring explants into new culture media on regular intervals [7, 13].

Plant growth hormones

Growth hormones regulate various physiological and morphological processes in plants and are also known as plant growth regulators (PGRs) or phytohormones [34, 52]. PGRs are synthesized by plants; therefore many plant species can grow successfully without external medium supplements [53-55]. Hormones can also be added into cultures to improve plant growth and to enhance metabolite synthesis [34, 44]. As observed in *B. Montanum* and many other species, *in vitro* growth and shoot formation was not achieved without adequate concentrations exogenous hormones [10]. However, inadequate or excessive amount of growth hormones can cause morphological and physioloical abnormalities [56]. Table 3 Shows different types of hormones and their functions in plants.

Table 3: Plant growth hormones and their functions.

Categories	Functions	Growth hormones	References
Auxins	Cell division, elongation and root differentiation.	Indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid naphthalene acetic acid (NAA), 2,4,5-trichlorophenoxy acetic acid naphthoxyacetic acid (NOA)	[7, 34, 44].
Cytokinins	Cell division, shoot induction, development and proliferation	benzyl amino purine (BAP), isopentenyl adenine (IPA or 2i-p), kinetin (furfurylamino purine), 4-hydroxy-3-methyl-trans-2-butenylaminopurine (zeatin)	[7, 34, 44].
Gibberellins	Growth, elongation and flowering	GA_3	[34-35]

Tissue culture techniques

There are many types of tissue culture techniques available for micropropagation and plant regeneration. Some commonly used are listed in this section:

Callus Cultures

Callus is an undifferentiated mass of tissue which appears on explants within a few weeks of transfer onto growth medium with suitable hormones [34]. Callus formation occurs from revered process of cell differentiation, known as dedifferentiation or redifferentiation [35]. Different growth hormones are used to promote callus induction and development. In *Cephaelis ipecacuanha* 2, 4-D and NAA along with kinetin promoted callus induction and growth [57]. New plants can be successfully regenerated from callus through organogenesis [58].

Suspension culture

Suspension cultures are formed *in vitro* when friable calli are grown on liquid media in suitable container and constantly agitated to provide suspension of free cells [34-35]. Conical flasks are used because of their large surface area which helps in maintaining liquid medium and continuous gas exchange [35]. Suspension cultures are of two types batch and continuous. In batch cultures a portion of initial cell suspension is taken and sub-cultured on to fresh media at regular intervals [34]. In continuous cultures fresh medium is added into existing culture and excessive cell suspensions are removed on regular intervals [35]. Suspension cultures are widely used in large scale production of secondary metabolites [6]. Bioreactors like Chemostat are specially designed instruments to carry out continuous cultures on large scale [34].

Somatic embryogenesis

Somatic embryogenesis is process by which a non zygotic embryo is produced from plant tissue or cell, which can develop into a new plant [59-60]. Formation of somatic embryos occurs in two steps, first callus is cultured onto auxin rich medium (2,4-D commonly used) forming embryogenic clumps, these clumps are then transferred into medium without auxins resulting in the formation of mature embryos [34, 59]. Growth and formation of mature embryos depends on auxins and nitrogen levels in the medium [34]. Successful plant regeneration has been achieved in aloe and *Vitis vinifera* by somatic embryogenesis [45, 61].

Protoplast Cultures

Protoplasts are plant cells in which the cell wall has been removed by enzyme digestion or mechanical process [34]. Protoplasts are isolated by dipping plant tissue into hypertonic solution, causing the plasma membrane to shrink away from cell wall [62]. Now cell wall can be removed by enzymatic digestion (pectinase and cellulose) or by mechanical methods [34, 62]. Successful plant regeneration was achieved by protoplast culture in *A. judaica and E. spinosissimus* [63].

Shoot formation

For successful plant regeneration by tissue culture first shoots are formed by culture of explants or callus on media containing growth hormones (mainly cytokinins but sometimes auxins also). Different types and concentrations of growth hormones are used for shoot formation as shown in Table 4. Even in same plant species, shoot

activity with type and level of growth hormones. For example the best shoot induction was observed for the *Aloe barbadensis* on MS medium supplemented with 2 mg L^{-1} BAP and 0.5 mg L^{-1} NAA [55]. Basal medium containing 4 mg L^{-1} BAP and 1 mg L^{-1} of IAA, increased the number and growth rate of multiple shoots [64]. While higher rates of shoot proliferation was also reported in *Aloe barbadensis* on MS medium containing 0.8 mg L^{-1} of IAA and 2.25 mg L^{-1} of BA [65]. In case of *Aloe vera,* MS medium containing 2.0 mg L^{-1} BA, 0.5 mg L^{-1} KIN and 0.2 mg L^{-1} NAA showed rapid shoot multiplication [66] and MS medium containing 1.0 mg L^{-1} BA and 0.2 mg L^{-1} reported the best growth and most number of shoots in *Aloe vera* [67].

Table 4: Affects of various types and concentrations of growth hormones on shoot activity.

Name	Explant	Medi a	Hormone	Result	Observation	Ref.
Baliospermum montanum	Shoot tips and nodal buds	MS	BAP 2.0 mg l^{-1}	Max imum shoot induction, shoots numbers and height	Kn caused fewer and weaker shoots	[10]
Woodfordia fruticosa (L.)	Shoot tips	SH	0.5 mg l^{-1} BAP And NAA 1.0 mg l^{-1} BAP during subculture	Max imum shoot induction, shoots numbers and height	Increase shoot multiplication in subculture	[13]
Vitis thunbergii	Auxiliary shoot buds	WPM MS NN	0.6 mg l^{-1} BA	BA in WPM best for length and shoot proliferation.	2-ip and kinetin were less effective than BA	[25]
Curculig o orchioides	Shoot apices	MS	BA 0.44 µM	Max imum shoot induction, shoots numbers and height	No increase in shoot acitivity at higher conc.	[28]
Withania somnifera	Shoot tips	MS	BA 2.0 mg/L NAA 0.2mg/L	Max imum shoot induction, shoots numbers and height		[68]
Hoslundia opposita	Nodal buds	MS	4.4 µM BA	Most and longest shoots.	Higher levels of BA decreased shoot number.	[37]

Root formation

After producing healthy shoots, plantlets transferred to different media for root formation. Auxins are mainly used in root induction and their effect varies with type and concentration used in different plant species [69] as shown in Table 5 NAA and IBA are most commonly used anxins [34].

For example, in *Aloe barbadensis* MS medium containing 0.5 mg L^{-1} NAA showed 95% rooting with healthy and thick roots, however increasing the NAA concentrations lowered the quality and number of roots [55] and MS medium containing 1 mg L^{-1} IAA was more effective for rooting than medium without any growth hormones [64]. In *Aloe vera*, IAA showed very poor effects at all concentrations whereas MS medium containing 0.2 mg L^{-1} of NAA produced most number of roots as compared to 0.2 mg L^{-1} IBA [66].

In *Woodfordia furticosa,* medium containing 0.1- 1.0 mg L^{-1} of IAA showed fast growing and thick roots whereas very low root development in same concentrations of IBA [13].

Table 5: Effects of growth hormones on root formation in medicinal plants.

Name	Medium	Growth Hormone	Results	Ref.
Aloe vera L.	MS	0.2 mgl $^{-1}$ NAA	Best rooting activity	[66]
Aloe barbadensis	MS	I mgl $^{-1}$ IAA	long, thick and most number of roots	[64]
Aloe barbadensis	MS _ strength	0.5 mgl $^{-1}$ NAA	long, thick and most number of roots	[55]
Baliospermum montanum (Willd.)	MS _ strength	1.0 mg l $^{-1}$ IBA and 0.5 mg l $^{-1}$ IAA	Thick and Long roots produced	[10]
Curculigo orchioides	MS _ strength	0.53 μM NAA	Best number roots produced	[28]
Hoslundia opposita	MS	3.6 μM IAA IBA	Long and thick roots. IBA caused callusing	[37]
Vitis thunbergii	MS	0.5 mg l $^{-1}$ NAA	High root ing, some callus ing	[25]

Secondary Metabolites in Plant cell and tissue cultures

Unlike humans and animals, plants are not mobile which makes them very susceptible to attack from pests and predators. To overcome this problem, during metabolism plants produce enormous number of compounds as part of defence mechanism [8, 70]. These compounds are not essential for primary functions like growth, photosynthesis and reproduction and are called secondary metabolites. Secondary metabolites are used as pharmaceutical, agrochemicals, aromatics and food additives [8, 71]. Plant derived compounds include many terpenes, polyphenols, cardenolides, steroids, alkaloids and glycosides [6, 43]. Figure 2 shows different groups of chemical compounds produced found plants.

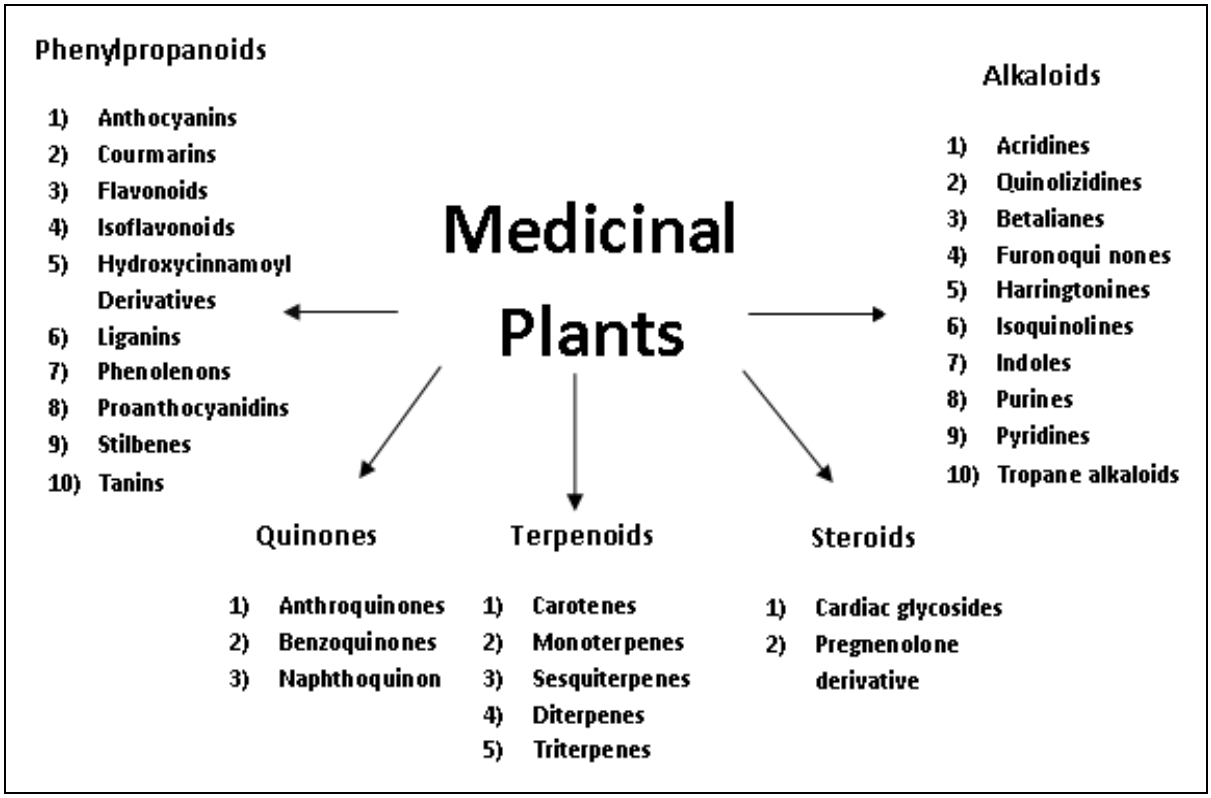

Figure 2: Adapted schematic showing the classification of plant derived compounds [71]

The chemical synthesis of many of these metabolites is only possible in plants [71], resulting in over exploitation and threat of extinction to many medicinal plant species [8]. Totipotency of plant cells ensures that they contain full genetic characteristics of the plant, thus makes it possible to synthesize these compounds *in vitro*. Tissue culture offers an effective and potential alternative of metabolite production because the amount of secondary metabolites produced in tissue cultures can be even higher than in parent plants [71-72]. Advances in plant tissue culture have enabled commercial scale production of plant metabolites. Some pharmaceuticals produced on an industrial scale are shown in Table 6.

Table 6: Adapted table of pharmaceuticals, their uses and source plants [71].

Metabolite	Medicinal Use	Name of Plant
Ajmalicine	Antihypertensive	Catharanthus roseus
Artemisinin	Antimalarial	Artemisia annua
Berberine	Intestinal ailment	Coptis japonica
Camptothecin	Antitumour	Camtotheca acuminata
Capsaicin	Counterirritant	Capcicum frutescens
Castanospermine	Glycoside inhibitor	Castanospermum australe
Codeine, Morphine	Sedative	Papaverus somniferum
Colchicine	Antitumour	Colchium autumnale
Digoxin or Digitoxin	Heart stimulant	*Digitalis lanata or D. purpurea*
Diosgenin	Steroidal precursor	Dioscorea deltoidea
Ellipticine	Antitumour	Orchrosia elliptica
Ginsenosides	Health tonic	Panax ginseng
Forskolin	Bronchial asthma	Coleus forskolii
Podophyllotoxin	Antitumour	Podophyllum petalum
Quinine	Antimalarial	Cinchona ledgeriana
Sanguinarine	Antiplaque	Sanguinaria canadensis
Shikonin	Antibacterial	Lithospermum erythrorhizon
Taxol	Anticancer	Taxus brevifolia
Vincristine Vinblastine	Antileukemic	Catharanthus roseus
Tea tree oil	Antibacterial, Antifungal	Melaleuca alternifolia

In 1983, a Janpanese company (Matusi and Co), pioneered the use of plant cell cultures to produce the secondary metabolite shikonin which have anti HIV properties [6, 73].

Due to lack of knowledge about biosynthetic pathways which produce metabolites in plants, only a small number compounds are derived from tissue cultures on industrial scale [71-72]. Certain problems need to be addressed so that tissue culture can be used to increase the production of secondary metabolite in more plant species. One of the major problems is heterogeneity plant cells and difference in morphological characteristics of each individual cell from the others [73], this has a huge effect in

metabolites yield. To overcome this problem, high yielding cells are indentified and then cloned to produce more cells with similar characteristics. It's reported that careful selection can increase metabolite production by several times [71]. Another problem is plant cells are large and the inflexible cell wall can often be susceptible to damage from industrial equipment [73]. Cells in cultures have long growth and multiplication times thus production processes are very time consuming. In cell cultures, secondary metabolites are stored in vacuoles and releasing them is only possible by permeable membranes. Considerable research has been done to keep cells alive while inducing permeability [71, 73].

Factors effecting production of Secondary metabolites

Secondary metabolites synthesis is directly related to physical and chemical conditions [6, 73]. Many researchers have reported considerable effects of manipulation of media formulations, growth hormones, temperature and photoperiod on metabolites production. [6].

- **Carbon source**: Sucrose is a commonly used as carbon source and has significant impact on metabolites yield from *In vitro* cultures [71]. Production of rosmarinic acid was enhanced with increasing sucrose levels in the culture media [74]. Instead of sucrose using glucose (in MS medium) as a source of carbon was also found to enhance production of podophyllotoxin in cell cultures of *Podophyllum hexandrum* [75].
- **Media Formulation:** Composition of culture media can affect the yield of active ingredients [6, 74]. In suspension cultures of *Catharanthus roseus,* MS medium was reported to be best for producing serpentine [73]. These effects vary with types of media and plant species.
- **Temperature:** Variation in temperature, photoperiod and pH can cause great difference in product yield [71, 76]. In *Podophyllum hexandrum* cultures, the cultures grown in dark produced better friable calli and higher podophyllotoxin than the ones grown in light [75].
- **Agitation:** Agitating speeds for cultured cells is reported to influence cell viability and product synthesis. *In vitro* grown cells of *Podophyllum hexandrum* showed much more damage at 200 rpm and more viable cells were found between speeds 125- 150 rpm [75].
- **Phosphate:** Production of secondary metabolites has been proved to be effected by phosphate levels in plant cell culture media. In various plant cell cultures, It was noticed that cell growth was promoted but metabolite yield was lowered at higher amounts of phosphate [71].. Production of ajmalicine and phenolics in *Cath. roseus*, caffeoyl putrescines in *Nicotiana tabacum* and harman alkaloids in *Peganum harmala* was reported to be enhanced at lower phosphate levels [71].
- **Nitrogen**: Yield of metabolites containing proteins or amino acid as been observed to be influenced by amounts of nitrogen in culture media. Nitrogen source is an essential component of some culture media for e.g. MS, LS or B5 [38, 71, 73].
- **Plant Growth Hormones**: Level of growth hormones are known to effect production of metabolites [74] For example, the PGR 2,4-D was reported to cease metabolites production [73] whereas an increase in metabolites synthesis was seen with NAA or IAA [71]. In *Catharanthus roseus*, auxins promoted callus induction but auxin removal and cytokinins alone were

found to increase secondary metabolites production [73]. By combining 2.5 mg l^{-1} IBA and 0.1 mg l^{-1} kinetin, the production of ginsenoside saponins in cell cultures of *Panax quinquefolium,* was even higher than in adult plants [77].

- **Gas composition**: Concentration of gases inside culture environment can have significant effects on metabolites yields [6, 71, 73-74]. In cultures of *Panax ginseng* inside a bioreactor*,* it was observed that 40% of oxygen in culture environment gave the highest yields of saponin whereas yield dropped on higher (50%) and lower (20.8% and 30%) concentrations [78].
- **Precursor supplementation**: Supplementing the cell cultures with a compound which is also produced during secondary metabolites production pathway is called as precursor feeding. This process has been reported to enhance the yield of final products in many cell cultures [71].
- **Elicitation**: Process creating artificial pathogen attack scenario by chemical means to start the expression of gene which is associated with secondary metabolites production is called elicitation [79]. Elicitor used to for this purpose can be either biotic or abiotic for e.g.- jasmonic acid, glucan polymers, glycoproteins, fungal cell materials, UV irradiation, salts of heavy metals and many other chemicals [80]. Elicitation is based on the concept that secondary metabolites are produced by plant as part of their defence against pathogen attack and is found very effective in certain compound producing cultures [71]. In *Rauvofja catrescetrs and Eschscholtzia califtnica,* yeast extract (elicitor) increased the production and methyl jasmonate was observed to have similar effects on a number of plant species [79].

Biotransformation

It is the process of chemical conversion or alteration of one compound to another using cultured cells or enzymes action [81]. Plants can synthesize some rare enzymes which can further produce important compounds and chemical synthesis of these compounds is sometimes unfeasible [82]. Different types of cell cultures (suspension and hairy root) are used to transform natural or synthetic aromatic, steroid, alkaloid, coumarin, terpenoid, lignan and many others and flavouring compounds through biotransformation [71, 82]. Process of bio transformation includes reactions such as reduction, oxidation, hydroxylation, acetylation, esterification, glucosylation, isomerization, methylation, demethylation, epoxidation and many more [71]. Table 7 shows biotransformation of some pharmaceutical products.

Table 7: Adapted table showing some famous Pharmacetutical Products of Biotransformation [71].

Plant	Substrate	Product	Reference
Papaver somniferum	Thebiane	Codeine	[83]
Spirulina platensis	Codine	Morphine	[84]
Digitalis lanata L.	Digitoxin	Digoxin	[85]
Capsicum frutescens	Digitoxin	Digoxin, purpureaglycoside	[86]
Eucalyptus Perriniana	Taxol	Taxol derivatives	[87]
Podophyllum hexandrum	Coniferyl alcohol	Podophyllotoxin	[88]
Mucuna Prureins	Tryosine	DOPA(3,4-dihydroxyphenylalanine)	[89]
Catharanthus roseus	Vinblastine Vindoline	Viincrisitn e	[73]
Capsicum frutescens	Isoeugenol	Vinallin, Capsaicin	[90]

Conclusion

Plants are rich sources of pharmaceutically important compounds; but there is a need to synthesis these compounds within laboratory conditions. Micropropagation is an important technology since many secondary plant metabolites can't be synthesised chemically. Many plant species are undiscovered and their medicinal properties unknown; and even the medicinal remedies past down from generations are being lost. Further research and conservation of all plant species including medicinal plants is needed to preserve nature's natural drugs.. Advances in plant tissue culture will enable rapid multiplication and sustainable use of medicinal plants for future generations.

References

1. Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12**, 564-582.
2. Micke, O., J. Hubner, and K. Munstedt 2009. Ayurveda. *Der Onkologe*, **15**, 792-798.
3. Patwardhan, B., D. Warude, P. Pushpangadan, N. Bhatt. 2005. Ayurveda and traditional Chinese medicine: A comparative overview. *Evidence-Based Complementary and Alternative Medicine*, **2**, 465-473.
4. Vines, G. 2004 *Herbal Harvest with future: towards sustainable source for medicinal plants.* Plantlife International, www.plantlife.co.uk
5. Zhou, L.G. and J.Y. Wu, 2006. Development and Application of Medicinal Plant Tissue Cultures for Production of Drugs and Herbal Medicinals in China. *Natural Product Reports*, **23**, 789-810.
6. Stafford, A., P. Morris, and M.W. 1986. *Fowler Plant cell biotechnology: A perspective. Enzyme and Microbial Technology*, **8**, 578-587.

7. Rout, G.R., S. Samantaray, and P. Das, 2000. In vitro manipulation and propagation of medicinal plants*. Biotechnology Advances*, **18**, 91-120.
8. Oksman-Caldentey, K. M. and D. Inzé, 2004. Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. *Trends in Plant Science*, **9**, 433-440.
9. Edwards, R. 2004. No remedy in sight for herbal ransack*. New Scientist*, **181**, 10-11.
10. Sasikumar, S., S. Raveendar, A. Premkumar, S. Ignacimuthu, P. Agastian. 2009. Micropropagation of Baliospermum montanum (Willd.) Muell. Ara.-A threatened medicinal plant. *Indian Journal of Biotechnology*, **8**, 223-226.
11. Singh, P., A. Singh, A.K. Shukla, L. Singh, V. Pande and T.K. Nailwal. 2009. Somatic embryogenesis and in vitro regeneration of an endangered medicinal plant sarpgandha (Rauvolfia serpentina L.). *Life Science Journal*, **6**, 57-62.
12. Ghani. A. 1998 Medicinal plants of Bangladesh - Chemical constituents and uses. Second edition. Asiatic Society of Bangladesh, Dhaka, Pages 337-338.
13. Krishnan, P.N. and S. Seeni. 1994. Rapid Micropropagation of Woodfordia fruticosa (L) Kurz (Lythraceae), A rare medicinal plant. *Plant Cell Reports*, **14**, 55-58.
14. Gilani, A.H., S. Yaeesh, Q. Jamal, M.N. Ghayur. 2005. Hepatoprotective activity of aqueous - Methanol extract of Artemisia vulgaris. *Phytotherapy Research*, **19**, 170-172.
15. Walter, H.L., P.F. Memory, and L. Elvin. 2003. Medical Botany, Second edition. John Wiley and Sons. New Jersey, Pages 345.
16. Mehta, S.R. and R.B. Subramanian. 2005. Direct In vitro Propagation of Asparagus adscendens Roxb. *Plant Tissue Culture*, **15**, 25-32.
17. Mathews, J.N., P.R. Flatt, and Y.H. Abdel-Wahab, 2006. Asparagus adscendens (Shweta musali) stimulates insulin secretion, insulin action and inhibits starch digestion. *British Journal of Nutrition*, **95**, 576-581.
18. Hartinie, M. and G.A. Jualang, 2007. In vitro germination and plantlet establishment of Labisia pumila (Bl.) F. Vill. *Scientia Horticulturae*, **115**, 91-97.
19. Burkill, I.H. 1966. A dictionary of the economic products of the Malay peninsula, 2nd edition, Government of Malaysia and Singapore, Kuala Lumpur, Pages 110.
20. Beena, M.R. and K.P. Martin, 2003. In Vitro Propagation of the rare medicinal plant Ceropegia candelabrum L. through Somatic embryogenesis*. Society for In Vitro Biology*, **39**, 510-513.
21. Mondello, F., F. De Bernardis, A. Girolamo, G. Salvatore and A. Cassone, 2003. In vitro and in vivo activity of tea tree oil against azole-susceptible and -resistant human pathogenic yeasts. *Journal of Antimicrobial Chemotherapy*, **51**, 1223-1229.
22. Terzi, V., C. Morcia, P. Faccioli, G. Vale, G. Tacconi and M. Malnati, 2007. In vitro antifungal activity of the tea tree (Melaleuca alternifolia) essential oil and its major components against plant pathogens. *Letters in Applied Microbiology*, **44**, 613-618.
23. Koh, K.J., A.L. Pearce, G. Marshman, J.J. Finlay-Jones and P.H. Hart, 2002. Tea tree oil reduces histamine-induced skin inflammation. 82nd Annual Meeting of the British Association of Dermatologists. Blackwell Publishing Ltd, Edinburgh, Pages 1080-1095.
24. Hammer, K.A., C.F. Carson, and T.V. Riley, 2004. Antifungal effects of Melaleuca alternifolia (tea tree) oil and its components on Candida albicans,

Candida glabrata and Saccharomyces cerevisiae. *Journal of Antimicrobial Chemotherapy*, **53**, 1081-1085.

25. Lu, M.C., 2005. Micropropagation of Vitis thunbergii Sieb. et Zucc., a medicinal herb, through high-frequency shoot tip culture. *Scientia Horticulturae*, **107**, 64-69.
26. Chen, L.G. and C.C. Wang, 2009. Preparative separation of oligostilbenes from Vitis thunbergii var. taiwaniana by centrifugal partition chromatography followed by Sephadex LH-20 column chromatography. *Separation and Purification Technology*, **66**, 65-70.
27. Aziz, Z.A., M.R. Davey, K.C. Lowe and J.B. Power, 2006. Isolation and culture of protoplasts from the medicinal plant Centella asiatica. *Rev. Bras. Pl. Med., Botucatu*, **8**, 105-109.
28. Bhavisha, B.W. and Y.T. Jasrai, 2003. Micropropagation of an Endangered Medicinal Plant :Curculigo orchioides Gaertn. *Plant tissue Culture*, **13**, 13-19.
29. Craker, E.L., Editors- J. Janick and A. Whipkey. 2007. Medicinal and Aromatic Plants—Future Opportunities. Issues in new crops and new uses. ASHS Press, Alexandria, Page 232.
30. Trade in Medicinal Plants. 2005. *F.A.O. Corporate document repository.* Economic and Social Department Food and Agriculture Organization of the United Nations Rome. Accesed 10/11/09 http://www.fao.org/docrep/008/AF285E/AF285e00.htm.
31. Brinckmann, J., 2006. Medicinal Plants & Extracts. Vol.18. International Trade Centre Accessed 10/11/09- http://www.intracen.org/mas/pdfs/print/english/medicinal-plants.pdf.
32. Brinckmann, J., 2007. *Medicinal Plants and Extracts. Vol. 24.* International Trade Centre Accessed10/11/09 http://www.intracen.org/mns/Documents/Medicinal_Plants_Sample.pdf.
33. Leifert, C., W.M. Waites, and J.R. Nicholas, 1989. Bacterial contaminants of micropropagated plant cultures. *Journal of Applied Microbiology*, **67**, 353-361.
34. Bhojwani, S.S. and M.K. Razdan, 1996. Plant tissue culture: Theory and Practice: Developments in crop science Vol. 5. Elsevier, Amsterdam.
35. Fowler, M.R., F.W. Rayns and C.F. Hunter(Editor) 1993. The language and aims of plant cell and tissue culture. In Vitro Cultivation of Plant Cells, Butterworth-Heinemann Ltd, Oxford, Page 1-18.
36. Krikorian, A.D. and D.L. Berquam. 1969. Plant cell and tissue cultures: The role of Haberlandt. *The Botanical Review,* **35**, 59-67.
37. Prakash, S. and J. Van Staden, 2007. Micropropagation of Hoslundia opposita Vahl--a valuable medicinal plant. *South African Journal of Botany*, **73**, 60-63.
38. Gamborg, O.L., T. Murashige, T.A. Thorpe and I.K. Vasil. 1976. Plant-tissue culture media. *Journal of the Tissue Culture Association*, **12**, 473-478.
39. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant*, **15**, 473-497.
40. Staba, E.J. and J.E.A. Seabrook. 1980. Laboratory Culture. Plant Tissue Culture As a Source of Biochemicals, CRC Press, Boca Raton, Pages 1-20.
41. Leifert, C. and W.M. Waites, 1992. Bacterial growth in Plant tissue culture media. *Journal of Applied Bacteriology*, **72**, 460-466.
42. Leifert, C., J.Y. Ritchie, and W.M. Waites, 1991. Contaminants of Plant tissue and Cell cultures. *World Journal of Microbiology & Biotechnology*, **7**, 452-469.

43. Matkowski, A. 2000. Plant in vitro culture for the production of antioxidants -- A review. *Biotechnology Advances*, **26**, 548-560.
44. Rayns, F.W., M.R. Fowler amd C.F. Hunter. 1993. Media design and use. In Vitro Cultivation of Plant Cells, Butterworth-Heinemann Ltd. Oxford, Pages 43-64.
45. Garro-Monge, G., A.M. Gatica-Arias, and Valdez-Melara, 2008. Somatic embryogenesis, Plant regeneration and Acemannan detection in Aloe (Aloe barbadensis MILL.). *Agronomia Costarricense*, **32**, 41-52.
46. Huang, L. and T. Murashige. 1977. Plant tissue culture media:major constituents;their preperation and some applications. *Tissue Culture Assoc*, **3**, 539-548.
47. Linsmaier, E.M. and F. Skoog, 1965. Organic growth factor requirement of tobacco tissue cultures. *Physiol. Plant*, **18**, 100-127.
48. Schenk, R.V. and A.C. Hilderbrandt, 1972. Medium and techniques for induction and growth of monocotyledonous dicotyledonous plant cell cultures*. Canadian Journal of Botany.* **50**, 199-204.
49. Lloyd, G.B. and B.H. McCown, 1980. Commercially feasible micropropagation of mountain laurel (Kalamia latifolia) by use of shoot tip culture*. Proc. Int. Plant Propagators Soc.* **30**, 421-437.
50. Nitsch, J.P. and C. Nitsch, 1969. Haploid plants from pollen grains. *Science*, **163**, 85-87.
51. Bhat, S.R. and K.P.S. Chandel, 1991. A novel technique to overcome browning in tissue culture. *Plant Cell Reports*, **10**, 358-361.
52. Srivastava, L.M., 2002. Plant Growth and Development: Hormones and Environment Academic Press, New York, Pages 140-143.
53. Bhavisha, B.W. and Y.T. Jasrai, 2003. Micropropagation of an Endangered Medicinal Plant :Curculigo orchioides Gaertn. *Plant tissue Culture*, **13**, 13-19.
54. Hussey, G., 1982. In vitro propagation of monocotyledonous bulbs and corms. *Plant Cell, Tissue and Organ Culture*, **19**, 677-680.
55. Baksha, R., M.A.A. Jahan, R. Khatum and J.L. Munshi. 2005. Micropropagation of Aloe barbadensis Mill. through In vitro Culture of Shoot tip Explants*. Plant tissue Culture and Biotechnology*. **15**, 121-126.
56. Bouza, L., M. Jacques, and E. Miginiac, 1994. In vitro propagation of Paeonia suffruticosa Andr. cv. `Mme de Vatry': developmental effects of exogenous hormones during the multiplication phase. *Scientia Horticulturae*, **57**, 241-251.
57. Rout, G.R., S. Samantaray, and P. Das, 2000. In vitro somatic embryogenesis from callus cultures of Cephaelis ipecacuanha A. Richard. *Scientia Horticulturae*, **86**, 71-79.
58. Sears, R.G. and E.L. Deckard, 1982. Tissue cultire variability in wheat- callus induction and plant regeneration*. Crop Science*, **22**, 546-550.
59. Razdan, M.K. 2003. Introduction to Plant Tissue Culture*.* 2nd Edition, Science Publishers, Inc., New Hampshire, Pages132.
60. Zimmerman, J.L. 1993. Somatic embryogenesis- A Model for early development in higher plants. Plant Cell, **5**, 1411-1423.
61. Morgana, C., R. Di Lorenzo, and F. Carimi, 2004. Somatic embryogenesis of Vitis vinifera L. (cv. Sugraone) from stigma and style culture. *Vitis*, **43**, 169-173.
62. Hunter, C.F., 1993. Protoplast and haploid cultures. In Vitro Cultivation of Plant Cells, Butterworth-Heinemann Ltd. Oxford, Pages 82-112.

63. Pan, Z.G., C.Z. Liu, S.J. Murch, M. El-Demerdash, and P.K. Saxena, 2003. Plant regeneration from mesophyll protoplasts of the Egyptian medicinal plants Artemisia judaica L. and Echinops spinosissimus Turra. *Plant Science*, **165**, 681-687.
64. Ujjwala, J.S., 2007. In vitro Regeneration of Aloe barbadensis*. Biotechnology*, **6**, 601-603.
65. Meyer, H.J. and J. Vanstaden, 1991. Rapid in vitro propagation of Aloe barbadensis Mill. *Plant Cell Tissue and Organ Culture*, **26**, 167-171.
66. Ahmed, S., A.H. Kabir, M.B. Ahmed, M.A. Razvy and S. Ganesan, 2007. Development of Rapid micropropagation methood of Aloe vera L. *Seed Science Journal*, **24**, 121-128.
67. Aggarwal, D. and K.S. Barna, 2004. Tissue culture propagation of elite plant of Aloe vera Linn. *Journal of Plant Biochemistry and Biotechnology*, **13**, 77-80.
68. Saritha, K.V. and C.V. Naidu, 2007. In vitro flowering of Withania somnifera Dunal. - An important antitumor medicinal plant*. Plant Science*, **172**, 847-851.
69. Swamy, S.L., S. Puri and A.K. Singh, 2002. Effects of auxins (IBA and NAA) and seasons on rooting of juvenile and mature hardwood cuttings of Robinia pseudoacaia and Grewia optiva. *New Forest*, **23**, 143-157.
70. Bennett, R.N. and R.M. Wallsgrove, 1994. Secondary metabolites in Plant Defence mechanisms*. New Phytologist*, **127**, 617-633.
71. Ramachandra Rao, S. and G.A. Ravishankar, 2002. Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances*, **20**, 101-153.
72. Parr, A.J., 1989. The Production of Secondary Metabolites by Plant cell cultures. *Journal of Biotechnology*, **10**, 1-26.
73. DiCosmo, F. and M. Misawa, 1995. *Plant cell and tissue culture: Alternatives for metabolite production.* Biotechnology Advances, **13**: p. 425-453.
74. Misawa, M., 1985. Production of useful plant metabolites. (Editor. A. Fiechter). Adv Biochem Eng Biotechnol. Springer-Verlag, Berlin, Pages 59-88).
75. Chattopadhyay, S., A.K. Srivastava, S.S. Bhojwani and V.S. Bisaria, 2002. Production of podophyllotoxin by plant cell cultures of Podophyllum hexandrum in bioreactor. *Journal of Bioscience and Bioengineering*, **93**, 215-220.
76. McChesney, J.D., 1999. Quality of botanical preparations: environmental issues and methodology for detecting environmental contaminants. Botanical Medicine: Efficacy, quality assurance and regulation, Mary Ann Liebert, Inc publishers, New Rochelle, New York, Pages 127-131.
77. Zhong, J.J., Y. Bai, and S.J. Wang, 1996. Effects of plant growth regulators on cell growth and ginsenoside saponin production by suspension cultures of Panax quinquefolium. *Journal of Biotechnology*, **45**, 227-234.
78. Trung Thanh, N., H. Niranjana Murthy, Kee-Won Yu, C. Seung Jeong, Eun-Joo Hahn and Kee-Youp Paek. 2006. Effect of oxygen supply on cell growth and saponin production in bioreactor cultures of Panax ginseng*. Journal of Plant Physiology,* **163**, 1337-1341.
79. Roberts, S.C. and M.L. Shuler, 1997. Large-scale plant cell culture*. Current Opinion in Biotechnology*, **8**, 154-159.
80. Zhong, J.J. 2002. Plant cell culture for production of paclitaxel and other taxanes*. Journal of Bioscience and Bioengineering*, **94**, 591-599.
81. Chasseaud, L.F. and D.R. Hawkins, 2006. Biotransformation of Drugs*.* Encyclopedia of Pharmaceutical Technology: Third Edition. Pages 310 - 324.

82. Giri, A., V. Dhingra, C.C. Giri, A. Singh, Owen P. Ward and M. Lakshami Nasaru. 2001. Biotransformations using plant cells, organ cultures and enzyme systems: current trends and future prospects. *Biotechnology Advances*, **19**, 175-199.
83. Wilhelm, R. and M.H. Zenk, 1997. Biotransformation of thebaine by cell cultures of Papaver somniferum and Mahonia nervosa. *Phytochemistry*, **46**, 701-708.
84. Rao, S.R., U. Tripathi, and G.A. Ravishankar, 1999. Biotransformation of codeine to morphine in freely suspended cells and immobilized cultures of Spirulina platensis*. World Journal of Microbiology & Biotechnology*, **15**, 465-469.
85. Alfermann, A.W., I. Schuller, and E. Reinhard, 1980. Biotransformation of Cardiac Glycosides by Immobilized Cells of Digitalis lanata1. *Planta Med*, **40**, 218-223.
86. Ramachandra Rao, S., T. Usha, and G.A. Ravishankar, 2002. Biotransformation of digitoxin to digoxin in cell cultures of Capsicum frutescens in the presence of β-cyclodextrin. *Biocatal Biotransform*, **20**, 137-143
87. Hamada, H., K. Sanada, T. Furuya, S. Kawabe and M. Jaziri. 1996. Biotransformation of Paclitaxel by Cell Suspension Cultures of Eucalyptus Perriniana*. Natural Product Research*, **9**, 47 - 52.
88. Uden, W.V., A.S. Bouma, J.F. Baracht Waker, O. Middel, H.J. Winchers, P.D. Waard, H.J. Woerdenbag, R.M. Kellogg and N. Pras. 1995. The production of podophyllotoxin and its 5-methoxy derivative through bioconversion of cyclodextrin-complexed desoxypodophyllotoxin by plant cell cultures. *Plant Cell, Tissue and Organ Culture*, **42**, 73-79.
89. Pras, N., H.J. Woerdenbag, and W. VanUden, 1995. Bioconversion potential of plant enzymes for the production of pharmaceuticals. *Plant Cell Tissue and Organ Culture*, **43**, 117-121.
90. Rao, S.R. and G.A. Ravishankar, 1999. Biotransformation of isoeugenol to vanilla flavour metabolites and capsaicin in suspended and immobilized cell cultures of Capsicum frutescens: study of the influence of beta-cyclodextrin and fungal elicitor*. Process Biochemistry*, **35**, 341-348.