IN VITRO PRODUCTION OF ALKALOIDS: A REVIEW

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To survive in nature, adaptation is a prime necessity for every living thing. Plants produce an array of secondary metabolites which not only play a vital role in adaption but also represent an important source of active pharmaceuticals. Alkaloids are a group of plant secondary metabolites with relatively heavy molecular mass and medicinal properties having nitrogen as a main component . Biological activities of alkaloids include anti cancerous, anti-inflammatory, analgesic and antiproliferative etc. Biotechnology offers a great opportunity to exploit these secondary metabolites via different methods. Plant cell and tissue culture is one of the emerging fields of biotechnology to investigate and enhance the production of secondary metabolites. Undifferentiated cell cultures like callus cultures and suspension cultures have been studied widely for alkaloid production. Along with this, hairy root culture and transformation techniques have also been explored as they are more stable genetically for the production of different alkaloids. Therefore, the present review is mainly focussed on the application of tissue culture technology used for the production of alkaloids in different plant species.

Keywords: Alkaloids, cell suspension culture, hairy root culture, secondary metabolites

Medicinal plants have a unique healing properties. These properties are mainly because of the presence of various secondary metabolites. Secondary metabolites are further widely categorized into alkaloids, flavonoids, terpenoids, phenols and glycosides.

Huge upsurge in scientific investigation has been emphasized in the areas of herbal medicine and traditional remedies (Jain 1991) as modern lifestyle is overshadowed by the synthetic products having side effects. Synthetic drugs which we are using, although are quick in action, and may proves a better way to us but we cannot ignore their side effects into long run. The revival of interest in natural drugs, especially those derived from plants, started in the last two decades mainly because of the widespread belief that green medicines are healthier and safer than synthetic ones (Dixon 2001).

As a result many developed and developing countries are actively engaged in bio-mining medicinal plants for therapeutically precious and biologically active phytochemicals (Sekar *et al.* 2010). This awakening among common people for natural remedies has led to a sudden rise in demand for herbal medicines, followed by belated growth in international awareness about the dwindling supply of the world's medicinal plants (Bodeker 2002).

Demands for wide variety of wild species are increasing with growth in human needs, numbers and commercial trade as a result some wild species are being over-exploited. The capacity for plant cell, tissue, and organ cultures to produce and accumulate many valuable chemical compounds similar to the parent plant in nature has been highlighted since the inception of *in vitro* technology. The strong and growing demand in today's marketplace for natural, renewable products has refocussed attention on *in vitro* plant materials as potential factories for secondary phytochemical products, and has paved the way for new research exploring secondary product expression *in vitro*. The deliberate stimulation of defined chemical products under highly controlled micro-environmental regimes provides an excellent forum for in-depth investigation of biochemical and metabolic pathways (Karuppusamy 2009).

Alkaloids

A large proportion of the drugs used in medicine are either directly isolated from plants or synthetically modified from a lead compound of natural origin. On a global scale,

medicinal plants are mainly used as crude drugs and extracts in our day to day life and are secondary metabolites present in many plants.
In simple words alkaloids are defined as natural substances which react or behaves like base (Bruneton 1999). In other words, alkaloids are heterocyclic biological compounds containing nitrogen as one of their molecules and are pharmacologically active with medicinal and economical value (Aniszewski 1994). Alkaloid widely differentiate into three classes known as true alkaloids, protoalkaloids and pseudoalkaloids (Aniszewski 2007).

a. True alkaloids :-A True alkaloids are mainly derived from amino acid. These type of alkaloids share a heterocyclic ring with nitrogen. They are highly reactive in nature and hence low dose is enough for biological activity. True alkaloids are bitter tasting white solid with an exception of nicotine which has a brown liquid. True alkaloids form water-soluble crystalline salts. The primary precursors of true alkaloids are such amino acids as l-ornithine, l-lysine, l-phenylalanine/l-tyrosine, l-tryptophan and l-histidine (Pelletier 1983, Dewick 2002). Examples of true alkaloids include such biologically active alkaloids as cocaine, quinine, dopamine and morphine.

b.Protoalkaloids: These alkaloids whose nitrogen atom is not a part of heterocyclic ring are known as Protoalkaloids (Jakubke 1994). Protoalkaloids are simple alkaloids in structure and forms minor group among all alkaloids (Aniszewski 2007).

c.Pseudoalkaloids: Pseudoalkaloids are compounds derived from the amino acid pathways from the precursors of amino acids (Jakubke 1994).
Alkaloids are widely used and identified as morphine (pain killer), codeine (antitussive), papaverine (phosphodiesterase inhibitor), ephedrine (stimulant), ajmaline (antirrhythmic), quinine (antimalarial), reserpine (antihypertensive), galanthamine (acetylcholine esterase inhibitor), scopolamine (travel sickness), berberine (psoriasis), caffeine (stimulant), capsaicin (rheumatic pains), colchicine's (gout), yohimbine (aphrodisiac), pilocarpine (glaucoma), and various types of cardiac glycosides (heart insufficiency) (Wink *et al.* 2005).

Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants. Since it was observed, that production of secondary metabolites is generally higher in differentiated plant tissues, there were attempts to cultivate whole plant organs, i.e. shoots or roots under *in vitro* conditions with the aim to produce medicinally important compounds (Biondi *et al.* 2002).

Suspension culture

One of the most successful method of extraction of secondary metabolites from plant cells is suspension culture (Giri and Zaheer 2016). The capacity of plant callus cells and organs cultivation in liquid media has made an important contribution to modern plant biotechnology with respect to the production of commercially valuable compounds (Su and Lee 2007). The homogeneity of an *in vitro* cell population, the large availability of material, the high rate of cell growth and the good reproducibility of conditions make suspension cultured cells suitable for the analysis of complex physiological processes at the cellular and molecular levels. Moreover, plant cell cultures provide a valuable platform for the production of high-value secondary metabolites and other substances of commercial interest (Moscatiello *et al.* 2013). Callus and suspension culture have been carried out in several plants for the production of alkaloids. Panda *et al.* (1992), studied that *in vitro* raised plant *Holaerrena antidysentrrica* produces alkaloid conessine, a therapeutic drug for dysentery and helminthic disorders almost 4.25 times more than that of nature grown plant. Another study done by Cheng *et* *al*. (2006), shows increase alkaloid production in *in vitro* raised plant *Corydalis saxicola*. According to Chengs observation a remarkable improvements of both biomass accumulation and alkaloid production were successfully obtained by manipulating inoculum size and sucrose concentration. Studies done in the plant *Tinospora cordifolia* shows higher accumulation of alkaloids berberine and jatrorrhizine (protoberberine alkaloids) in both callus and cell suspension cultures (Chintalwar *et al.* 2003).

Hairy root culture

Transgenic hairy root cultures have revolutionized the role of plant tissue culture in secondary metabolite production. They are unique in their genetic and biosynthetic stability, faster in growth, and more easily maintained. Using this methodology a wide range of chemical compounds have been synthesized (Shanks and Morgan 1999, Giri and Narasu 2000). Hairy root cultures of many plant species have been widely studied for the production of secondary metabolites useful as pharmaceuticals, cosmetics, and food additives (Christey and Braun 2005, Georgiev *et al.* 2007, Srivastava and Srivastava 2007). Hairy root cultures represent an interesting alternative to dedifferentiated cell cultures for the production of secondary plant products. Because hairy roots originate from a single plant cell infection by *Agrobacterium rhizogenes*, they are usually considered as genetically stable, in contrast with callus lines. Also, in contrast to dedifferentiated cells, the production of secondary metabolites is not repressed during the growth phase of the culture. Therefore, hairy roots usually produce secondary plant compounds without the loss of concentration frequently observed with callus or cell suspension cultures (Bourgaud *et al*. 1997). Indole alkaloids were obtained by hairy root culture from *Catharanthus roseus* L. and their antimicrobial activities were studied by Hanafy *et al.* (2016). The experiment proves maximum accumulation of vinblastine, vincristine and catharanthine in the transgenic hairy roots and also secreted in the liquid culture medium. Another study shows the production of alkaloid benzylisoquinoline from the hairy root culture of *Macleaya cordata* and demonstrated that hairy root system have a huge potential for bioengineering and sustainable production of secondary metabolites like alkaloids and others on commercial scale (Huang *et al.* 2018). *In vitro* production of secondary metabolites can be enhanced by increasing or decreasing the concentration of various compounds used in medium. A study proves that the alkaloid concentrations obtained in the hairy roots were 3-20 times higher in *Atropa belladona* when 35 mM of KNO_3 was used. Increasing the nitrate concentration in the medium of hairy roots also improved the hyoscyamine/ scopolamine ratio (Chashmi *et al.* 2010). Due to its inherent characteristics of hormone autotrophy, uncontrolled growth, biosynthesis, and genetic stability distinctiveness, hairy root cultures have proved to be also a valuable culture system for elicitation experiments. In addition, there are some secondary metabolites that are synthesized only in the roots 14,36,37(Murthy *et al.* 2014, Srivastava and Srivastava 2017, Zaheer *et al.* 2016).

Elicitation

The application of elicitors, which is currently the focus of researches, has been considered as one of the most effective methods to improve the synthesis of secondary metabolites in medicinal plants (Patel and Krishnamurthy 2013). This broader definition of elicitors includes both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitors). Elicitors could be used to enhance plant secondary-metabolite synthesis and could play an important role in biosynthetic pathways to enhanced production of commercially important compounds. Elicitation can be used as one of the important strategies in order to get better productivity of the bioactive secondary products (Chong *et al.* 2005, Smetanska 2008, Sharma *et al.* 2011,

Table 1: Details of *in vitro* alkaloids production of some important medicinal plants.

Plant	Family	Alkaloids	Method	Reference
Aconitum napellus	Ranunculaceae	Aconitine	SC	Hwang *et al.* (2004)
Ailanthus altissima	Simaroubaceae	Alkaloids	SC	Anderson *et al.* (1987)
		Canthinone alkaloids		Anderson *et al*. (1986)
Amsonia elliptica	Apocynaceae	Indole alkaloids	HR	Sauerwein *et al.* 1991
Anisodus acutangulus	Solanaceae	Atropine	HR	Liu *et al.* (2013)
Anisodus luridus	Solanaceae	Tropane alkaloids	HR	Jobanovic *et al*. (1991)
Atropa belladona	Solanaceae	Tropane alkaloids	C	Al-Ashaal et al. (2013)
Brucea javanica	Simaroubaceae	Canthinone alkaloids	SC	Liu *et al.* (1990)
Brugmansia candida	Solanaceae	Scopolamine / hyosciamine	HR	Pitta-Alvarez *et al*. (1999)
Catharanthus roseus	Apocynaceae	Yohimbine, 19-Epivindolinine, Isositsirikine, Vindolinine, Ajmalicine, Horhammerinine, Horhammericine, Lochnericine	SC	Constabel *et al.* (1981)
		Ajmalicine, Serpentine	C	Morris (1986)
		Catharanthine	SC	Zhao *et al*. (2001), Ramani and Jayabaskaran (2008)
		Serpentine	Roots	Ataei-Azimi *et al.* (2008)
		Indole alkaloid	B	Zhao and Verpoorte (2007)
			SC	Tallevi and Dicosmo (1988),
			HR	Hanafy *et al.* (2016)
Catharanthus trichophyllus	Apocynaceae	Indole alkaloids	HR	Davioud *et al.* (1989)
Cephaelis ipecacuanha	Rubiaceae	Emectic alkaloids	R	Teshima *et al*. (1988)
Choisya ternata	Rutaceae	Furoquinoline alkaloids	SC	Sejourne *et al.* (1981)
Cinchona ledgeriana	Rubiaceae	Alkaloid	SC	Koblitz *et al.* (1983)
			HR	Hamill *et al.* (1989)
		Anthraquinones	SC	Wijnsma *et al.* (1984)
Corydalis ophiocarpa	Papaveraceae	Isoquinoline alkaloids	C	Iwasa and Takao (1982)
Cinchona officinalis	Rubiaceae	Quinine	HR	Geerlings *et al.* (1999)
Coscinium fenestratum	Menispermaceae	Berberine	SC	Narasimhan *et al.* (2004)
Datura metel	Solanaceae	Atropine	HR	Shakeran *et al.* (2015)
Datura innoxia	Solanaceae	Tropane alkaloids	C	Kinsara *et al.* (1994)
		hyoscine	C	Siddiqui *et al.* (2017)
Duboisia leichhardtii	Solanaceae	Tropane alkaloids	C	Yamada and Endo (1982)
		Scopalamine	HR	Muranaka *et al*. (1992)
Ephedra species	Ephedraceae	Ephedrine alkaloids	Callus	O'Dowd *et al.* (1993)
Ephedra intermedia	Ephedraceae	Andalkaloids	Callus and shoots	Azimi and Hashemloian (2015)
Fritillaria unibracteata	Liliaceae	Alkaloids	S	Gao et al. (2004)
Galanthus transcaucasicus Fomin	Amaryllidaceae	homolycorine	Bu	Babashpour-Asl *et al.* (2016)
Hyoscyamus muticus	Solanaceae	Hyosciamine	C	Aly *et al.* (2010)

Hyoscyamus niger	Solanaceae	Hyoscyamine and Scopolamine	C	Jasim *et al.* (2012)
Hyoscyamus reticulatus	Solanaceae	Hyoscyamine and Scopolamine	HR	Moharrami *et al.* (2017)
		Tropane alkaloids	HR	Zeynali *et al.* (2016)
Leucojum aestivum	Amaryllidaceae	Galanthamine	P	Ivanov *et al.* (2013)
			S	Georgiev *et al.* (2009)
Macleaya cordata	Papaveraceae	sanguinarine	HR	Huang *et al.* (2018)
Mitragyna speciosa	Rubiaceae	mitragynine	SC	Zuldin *et al.* (2013)
Nandina domestica	Berberidaceae	Alkaloids	C	Ikuta and Itokawa (1988)
Nerium oleander	Apocynaceae	Alkaloids	C	Babcock and Carew (1962)
Nicotiana rustica	Solanaceae	Alkaloids	C	Tabata and Hiraoka (1976)
Ophiorrhiza pumila	Rubiaceae	Camptothecin related alkaloid	C	Kitajima *et al.* (1998)
Papaver bracteatum	Papaveraceae	Thebaine/codeine	C	Kamo *et al*. (1988)
Papaver somniferum	Papaveraceae	Alkaloid	C	Furuya *et al.* (1972)
Peganum harmala	Nitrariaceae	β-Carboline Alkaloid	SC	Sasse *et al.* (1982)
Rauwolfia sellowii	Apocynaceae	Alkaloid	SC	Rech *et al*. (1998)
Rauwolfia serpentina	Apocynaceae	Alkaloid	C	Babcock and Carew (1962)
		Reserpine, Yohimbine, Ajmaline, Ajmalicine, Ajmalidine,	S, L, R	Roja *et al.* (1984)
		Reserpine	SC	Yamamoto and Yamada (1986)
			HR	Mehrotra et al. (2016)
		Ajmaline, Yohimbine, 3-Epi-α-Yohimbine, Ajmalidine	R, L, S	Roja *et al.* (1987)
Rhodophiala bifida	Amaryllidaceae	Amaryllidaceae alkaloids	P	Reis *et al.* (2019)
Scopolia parviflora	Solanaceae	Alkaloid	C	Tabata *et al.* (1972)
Tabernaemontana divaricata	Apocynaceae	Alkaloids	SC	Sierra *et al.* (1992)
Thalictrum rugosum	Ranunculaceae	Berberine	SC	Gugler *et al.* (1988)
Tinospora cordifolia	Menispermaceae	Berberine	P	Priti and Rani 2009
Theobroma cacao	Malvaceae	Purine alkaloids	SC	*Gurney et al.* (1992)

B: Bioreactors; SC: Cell suspension culture; C: Callus; P: Plantlet; R: root; L: lea; S: Shoot; HR: Hairy root culture; Bu: Bulbs

Hussain *et al.* 2012) and lowering production costs (Miao *et al.* 2000, Zhang and Jian-Yong 2003). Elicitors are compounds stimulating any type of plant defence (Radman *et al.* 2003). The secondary metabolites are released due to defence responses which are triggered and activated by elicitors, the signal compound of plant defence responses (Krishnamurthy 2013).

Successful elicitation for alkaloids like ajmaline and ajmalicine from the hairy roots of plants *Rauwolfia serpentina* and *Solanum khasianum* were done using cellulase as biotic elicitor and salt as abiotic elicitor (Srivastava *et al.* 2016).

Another study shows that yeast extract elicitation helps in increasing the vinblastine and vincristine production from in vitro plantlets of *Catharanthus roseus* (Maqsood *et al.* 2017) thus proves that signalling component of yeast extracts in the biosynthesis could be a very effective approach for large scale augmentation of alkaloid yield of pharmaceutical importance.

Other examples of yeast extract induced elicitation of alkaloids *in intro ra*ised plants are

Scutellaria baicalensis (Yoon *et al.* 2000), *Panax ginseng* (Lu *et al. 2001)*, *Centella asiatica* (Kim *et al.* 2007), *Angelica gigas* (Rhee *et al.* 2010) and *Pueraria candollei* (Korsangruang *et al.* 2010).

Genetic transformation

The stable introduction of foreign genetic information into the plants represents one of the significant developments in recent advances of plant biotechnology including high volume production of pharmaceuticals (Hansen and Wright 1999) and opens new avenues for the production of several biologically active natural compounds.

Induction of rol C genes in *Atropa belladona* hairy root lines helps in the stimulation of biosynthesis of tropane alkaloids (Bonhomme *et al.* 2000). Another experiment done on plants *Datura metel* and *Hyoscyamus muticus* shows increased tropane alkaloid synthesis when the hairy root cultures overexpress the pmt gene. It results in more alkaloid synthesis compared to that of control hairy roots. Both hyoscyamine and scopolamine production were improved in hairy root cultures (Moyano *et al.* 2003).

Another study shows the importance of rol B and rol C gene expression as an effective inducer for plant secondary metabolites in *Artemisia carvifolia* for the production of artemisinin (Dilshad *et al.* 2015).

Bioreactors

Application of bioreactor system for large-scale cultivation of plant cells for the production of valuable bioactive compounds in an active field. Plant cells in liquid suspension offer a unique combination of physical and chemical environments that must be accomodated in large-scale bioreactor process (Hussain *et al.* 2012). Large scale production of indole alkaloids from *Catharanthus roses* has been highlighted by Zhao and Verpoorte (2007) emphasizing the strategies and new technologies to improve alkaloid production and bioreactor performance.A surface-immobilized bioreactor for *C. roseus* cell cultures had also been tested (Archambault *et al.* 1990, Archambault 1991). Recently Ramakrishnan and Curtis (2004) developed a trickle-bed bioreactor for root cultures. Several modes such as batch, semi-batch, fed-batch, immobilized culture, and continuous cultures have been used.

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