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 (antirhythmic), 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 Hoaera ren antisynergrrica 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 Cheng's 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 KNO₃ 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, Y...
Table 1: Details of *in vitro* alkaloids production of some important medicinal plants.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Family</th>
<th>Alkaloids</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ailanthus altissima</em></td>
<td>Simaroubaceae</td>
<td>Alkaloids</td>
<td>SC</td>
<td>Anderson <em>et al.</em> (1987)</td>
</tr>
<tr>
<td><em>Anemone elliptica</em></td>
<td>Apocynaceae</td>
<td>Indole alkaloids</td>
<td>HR</td>
<td>Sauerwein <em>et al.</em> 1991</td>
</tr>
<tr>
<td><em>Anisodus acutangulus</em></td>
<td>Solanaceae</td>
<td>Atropine</td>
<td>HR</td>
<td>Liu <em>et al.</em> 2013</td>
</tr>
<tr>
<td><em>Anisodus luridus</em></td>
<td>Solanaceae</td>
<td>Tropane alkaloids</td>
<td>C</td>
<td>Al-Ashaal <em>et al.</em> 2013</td>
</tr>
<tr>
<td><em>Atropa belladona</em></td>
<td>Solanaceae</td>
<td>Tropane alkaloids</td>
<td>C</td>
<td>Al-Ashaal <em>et al.</em> 2013</td>
</tr>
<tr>
<td><em>Brugmansia candida</em></td>
<td>Solanaceae</td>
<td>Scopolamine / hyosciamine</td>
<td>HR</td>
<td>Pitta-Alvarez <em>et al.</em> 1999</td>
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<tr>
<td></td>
<td></td>
<td>Ajmalicine, Serpentine</td>
<td>C</td>
<td>Morris (1986)</td>
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<tr>
<td></td>
<td></td>
<td>Catharanthine</td>
<td>SC</td>
<td>Zhao <em>et al.</em> (2001), Ramani and Jayabaskaran (2008)</td>
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<td></td>
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<td>Serpentine Roots</td>
<td></td>
<td>Ataei-Azimi <em>et al.</em> 2008</td>
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<td></td>
<td></td>
<td>Indole alkaloid</td>
<td>B</td>
<td>Zhao and Verpoorte (2007)</td>
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<td></td>
<td></td>
<td></td>
<td>SC</td>
<td>Tallevi and Dicosmo (1988), Hanafy <em>et al.</em> 2016</td>
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<td></td>
<td></td>
<td></td>
<td>HR</td>
<td>Hanafy <em>et al.</em> 2016</td>
</tr>
<tr>
<td><em>Catharanthus trichophyllis</em></td>
<td>Apocynaceae</td>
<td>Indole alkaloids</td>
<td>HR</td>
<td>Davioud <em>et al.</em> 1989</td>
</tr>
<tr>
<td><em>Cephaelis ipecacuanha</em></td>
<td>Rubiaceae</td>
<td>Emectic alkaloids</td>
<td>R</td>
<td>Teshima <em>et al.</em> 1988</td>
</tr>
<tr>
<td><em>Choisyra ternata</em></td>
<td>Rutaceae</td>
<td>Furoquinoline alkaloids</td>
<td>SC</td>
<td>Sejoune <em>et al.</em> 1981</td>
</tr>
<tr>
<td><em>Cinchona ledgeriana</em></td>
<td>Rubiaceae</td>
<td>Alkaloid</td>
<td>SC</td>
<td>Koblietz <em>et al.</em> 1983</td>
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<td>HR</td>
<td>Hamill <em>et al.</em> 1989</td>
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<td>Wijnsma <em>et al.</em> 1984</td>
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<tr>
<td><em>Corydalis ophiocarpa</em></td>
<td>Papaveraceae</td>
<td>Isoquinoline alkaloids</td>
<td>C</td>
<td>Iwasa and Takao (1982)</td>
</tr>
<tr>
<td><em>Cinchona officinalis</em></td>
<td>Rubiaceae</td>
<td>Quinine</td>
<td>HR</td>
<td>Geerlings <em>et al.</em> 1999</td>
</tr>
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<td><em>Coscinium fenestratum</em></td>
<td>Menispermaceae</td>
<td>Berberine</td>
<td>SC</td>
<td>Narasimhan <em>et al.</em> 2004</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>Solanaceae</td>
<td>Atropine</td>
<td>HR</td>
<td>Shakeran <em>et al.</em> 2015</td>
</tr>
<tr>
<td><em>Datura innoxia</em></td>
<td>Solanaceae</td>
<td>Tropane alkaloids</td>
<td>C</td>
<td>Kinsara <em>et al.</em> 1994</td>
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<td></td>
<td></td>
<td>hyoscine</td>
<td>C</td>
<td>Siddiqui <em>et al.</em> 2017</td>
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<tr>
<td><em>Duboisia leichhardtii</em></td>
<td>Solanaceae</td>
<td>Tropane alkaloids</td>
<td>C</td>
<td>Yamada and Endo (1982)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scopolamine</td>
<td>HR</td>
<td>Muranaka <em>et al.</em> 1992</td>
</tr>
<tr>
<td><em>Ephedra species</em></td>
<td>Ephedraceae</td>
<td>Ephedrine alkaloids</td>
<td>Callus</td>
<td>O’Dowd <em>et al.</em> 1993</td>
</tr>
<tr>
<td><em>Ephedra intermedia</em></td>
<td>Ephedraceae</td>
<td>Andalkaloids</td>
<td>Callus</td>
<td>Azimi and Hashemloian (2015)</td>
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<td></td>
<td></td>
<td></td>
<td>shoots</td>
<td></td>
</tr>
<tr>
<td><em>Fritillaria unibracteata</em></td>
<td>Liliaceae</td>
<td>Alkaloids</td>
<td>S</td>
<td>Gao <em>et al.</em> 2004</td>
</tr>
<tr>
<td><em>Galanthus transcaucasicus</em></td>
<td>Amaryllidaceae</td>
<td>homolycorine</td>
<td>Bu</td>
<td>Babashpour-Asl <em>et al.</em> 2016</td>
</tr>
<tr>
<td><em>Foeniculum vulgare</em></td>
<td>Apiaceae</td>
<td>Fenchone, flavonoids</td>
<td>SC</td>
<td>Sauerwein <em>et al.</em> 1991</td>
</tr>
<tr>
<td><em>Hyoscyamus muticus</em></td>
<td>Solanaceae</td>
<td>Hyoscyamine</td>
<td>C</td>
<td>Aly <em>et al.</em> 2010</td>
</tr>
</tbody>
</table>
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 raised 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 accommodated in large-scale bioreactor process (Hussain et al. 2012). Large scale production of indole alkaloids from Catharanthus roseus 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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