



Research review paper

Production of biomass and useful compounds from adventitious roots of high-value added medicinal plants using bioreactor

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ABSTRACT

The increasing global demand for biomass of medicinal plant resources reflects the issues and crisis created by diminishing renewable resources and increasing consumer populations. Moreover, diverse usage of plants and reduced land for cultivation in the world accelerated the deficiency of plant resources. In addition, the preparation of safety of plant based medicine whips up demand for biomass of valuable medicinal plants. As one of alternative approach to upswing the productivity of plant-based pharmaceutical compounds, automation of adventitious root culture system in air-lift bioreactor was adopted to produce cosmic amount of root biomass along with enriched diverse bioactive molecules. In this review, various physiological, engineering parameters, and selection of proper cultivation strategy (fed-batch, two-stage etc.) affecting the biomass production and secondary metabolite accumulation have been discussed. In addition, advances in adventitious root cultures including factors for process scale-up as well as recent research aimed at maximizing automation of the bioreactor production processes are also highlighted. Examples of the scale-up of cultures of adventitious roots of *Morinda citrifolia*, *Echinacea purpurea* and *angustifolia*, *Hypericum perforatum* and *Panax ginseng* by applying 20 L to 10,000 L bioreactors in our lab were demonstrated with a view of commercial application.

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Contents

1. Introduction	1255
2. Adventitious root culture	1256
3. Bioreactor cultivation	1256
4. Optimization strategy to improve secondary metabolite production	1258
5. Scale-up of culture process	1260
6. Case studies	1260
6.1. Adventitious root cultures of <i>Morinda citrifolia</i>	1260
6.2. Adventitious root cultures of <i>Echinacea</i> (<i>purpurea</i> and <i>angustifolia</i>)	1261
6.3. Adventitious root cultures of <i>Hypericum perforatum</i> (L.)	1263
6.4. Adventitious root cultures of <i>Panax ginseng</i>	1264
7. Conclusion	1264
Acknowledgments	1265
References	1265

1. Introduction

Medicinal plants are inexhaustible source of life saving drugs for majority of the world's population. Cultivation of plant cells, tissues or organs for the production of pharmaceutically valuable compounds

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of commercial interest has gained popularity over the last few years (Canter et al., 2005; Murthy et al., 2008). Recently those compounds are isolated by solvent extraction from the naturally grown whole plants, and are used as pharmaceuticals, nutraceuticals, pigments, food stuffs and cosmetics. The evolving demand of secondary metabolites in recent years resulted in a great interest, in secondary metabolism, especially in the possibility to alter the production of bioactive molecules by means of cell culture technology (Vijaya et al., 2010). However, the production of secondary metabolites by cell culture or from the plants grown in nature is not always satisfactory. Because, high water content in cells, foaming and wall growth in bioreactor and unstable production of metabolites are the main obstacles. In contrast, biosynthesis of metabolites in plants grown in nature is often restricted to species or genus, or might be activated only during a particular growth and developmental stage, under specific season, or by nutrient availability and pesticide contamination. Moreover, for medicinal purpose, destruction of plants continuously from their natural stands has caused a major threat to the plant species for their existence (Abdullah et al., 2000; Chattopadhyay et al., 2002). Clearly, the development of alternative and complimentary methods to whole plant cultivation for the stable production of biologically important secondary metabolites is an issue of considerable socioeconomic importance. For these reasons in the past several decades, a lot of efforts have been put into plant cell, tissue and organ culture as an alternative method to whole plant cultivation for the production of pharmacologically important plant secondary metabolites (Rao and Ravishankar, 2002).

Recent advances in plant biotechnological research have shown that bioreactor cultivation of adventitious root is an attractive and alternative method to the whole plant, cell or hairy root culture for biomass and bioactive compound production. Adventitious roots induced under sterile condition in phytohormone supplemented medium have shown high rate of proliferation, tremendous potentialities of accumulation and stable production of valuable secondary metabolites (Hahn et al., 2003; Yu et al., 2005). Therefore, to overcome the aforementioned problems, bioreactor technology is needed for the cosmic-scale cultivation of adventitious roots as a source of valuable biologically important plant-derived secondary metabolites. Bioreactor culture system provides better advantages than the traditional tissue culture system because the culture condition in a bioreactor can be controlled by online monitoring of important process parameters such as temperature, pH, and concentrations of oxygen and carbon dioxide inside the bioreactor vessel. The nutrient concentration can be optimized and nutrient uptake can also be enhanced by continuous medium circulation. Additionally, production cost and time can be reduced by enhancing cell proliferation and regeneration rates, product quality can be controlled, product can be free of pesticide contamination, and product can be harvested all year round to meet the increasing global demand (Paek et al., 2005; Sivakumar et al., 2005).

In spite of potential advantages of secondary metabolite production by plant cell cultures, only paclitaxel, shikonin, ginsenosides and berberine have been produced on a commercial scale, and those process plants are located in USA, Japan, South Korea and China, respectively (Bourgaud et al., 2001; Sivakumar et al., 2005). To produce large quantities of the plant-derived bioactive molecules for application in diverse human therapy and cosmetics, adventitious root culture using automated bioreactor technology of various medicinally important plants have to be developed. Recently in our laboratory, we have established adventitious root culture systems of several valuable medical plants such as *Morinda citrifolia*, *Echinacea purpurea* and *angustifolia*, *Hypericum perforatum* using large scale (500–1000 L) bioreactors. Previously, we have reported adventitious root culture system of *Panax ginseng* using 10-ton scale bioreactors for the production of ginsenosides (Paek et al., 2009; Sivakumar et al., 2005). In this review, a technology for cultivating adventitious roots from those valuable medicinal plants using a high-tech bioreactor system for

the production of anthraquinone, rubiadin, caffeic acid derivatives, hypericin and ginsenoside (Fig. 1), as well as advancements of ginsenoside production from adventitious roots of *P. ginseng* will be highlighted.

2. Adventitious root culture

Plant roots are the potential source of bioactive molecules that include a bewildering diversity of metabolites and bona fide proteins, and therefore, considered as the site of unique secondary metabolism of the whole plant (Bais et al., 2001; Flores et al., 1999). Current advances in plant biotechnology provides opportunity to culture plant cells, tissues and organs for the production of useful secondary metabolites instead of whole plant cultivation. However, large scale production of useful bioactive molecules by plant cell culture for commercial point of view have been known to be very difficult due to poor productivity and instability of plant cell culture, as well as some valuable compounds are not synthesized in the undifferentiated cells (Kim et al., 2002; Rao and Ravishankar, 2002; Verpoorte et al., 2002). Considering these facts, adventitious root culture in large scale bioreactor regarded as a promising approach for the production of secondary metabolites of pharmaceutical and nutraceutical interest.

Adventitious roots induced under aseptic conditions in a suitable phytohormone supplemented medium showed higher growth rate and active secondary metabolism (Hahn et al., 2003). In addition, adventitious roots are the potential biological material for stable commercial production of valuable secondary metabolites without foreign gene under in vitro conditions. Compared to cell cultures, adventitious roots showed higher stability in their growing environment and synthesize cosmic amounts of secondary metabolites into their intercellular spaces, which can be more easily extracted, and can be grown in a phytohormone amended medium with low inoculum but a high growth rate (Sivakumar, 2006). A list of pharmaceutically important medicinal plants, in which adventitious roots have been induced and cultured successfully for the efficient production of high value secondary metabolites, is depicted in Table 1.

Although, hairy root cultures are often considered as an efficient alternative method of some plant species, but hairy root cultures usually produce opine like substrates which are lethal to mammalian cells (Choi et al., 2000). Moreover, this complex structure makes hairy root culture difficult to use as crude extracts, because the purification cost for opine like substrates is too high to compete with field grown plants. Compared with hairy root culture, adventitious root culture is free from opine like toxic compound, as well as the changes in carbon assimilation mode has dramatic effects on the patterns of bioactive molecules produced by adventitious root. Thus, adventitious root culture provides a system to study the coordination between primary and secondary metabolism.

3. Bioreactor cultivation

Advancement of biotechnological research has made plant cell or root suspension cultures capable of producing particular medicinal compounds at a rate similar or superior to that of naturally grown whole plants. However, to fulfill the demand of the increasing population, the major challenge is how to adopt those technologies under laboratory conditions for large scale production of bioactive molecules from plant cells or roots that are reproducible, safe, and economically viable. In this regard, application of bioreactor technology is the key step toward commercial production of bioactive molecules by plant biotechnology. Compared to naturally grown “whole wild plants” or traditionally grown “whole transgenic plants” their production in bioreactors ensures defined control process conditions. Thus, minimizes or even prevents variations in yield and quality of the products, which simplifies process validation and product registration (Eibl and Eibl, 2008; Sivakumar, 2006). Moreover, proper engineering of

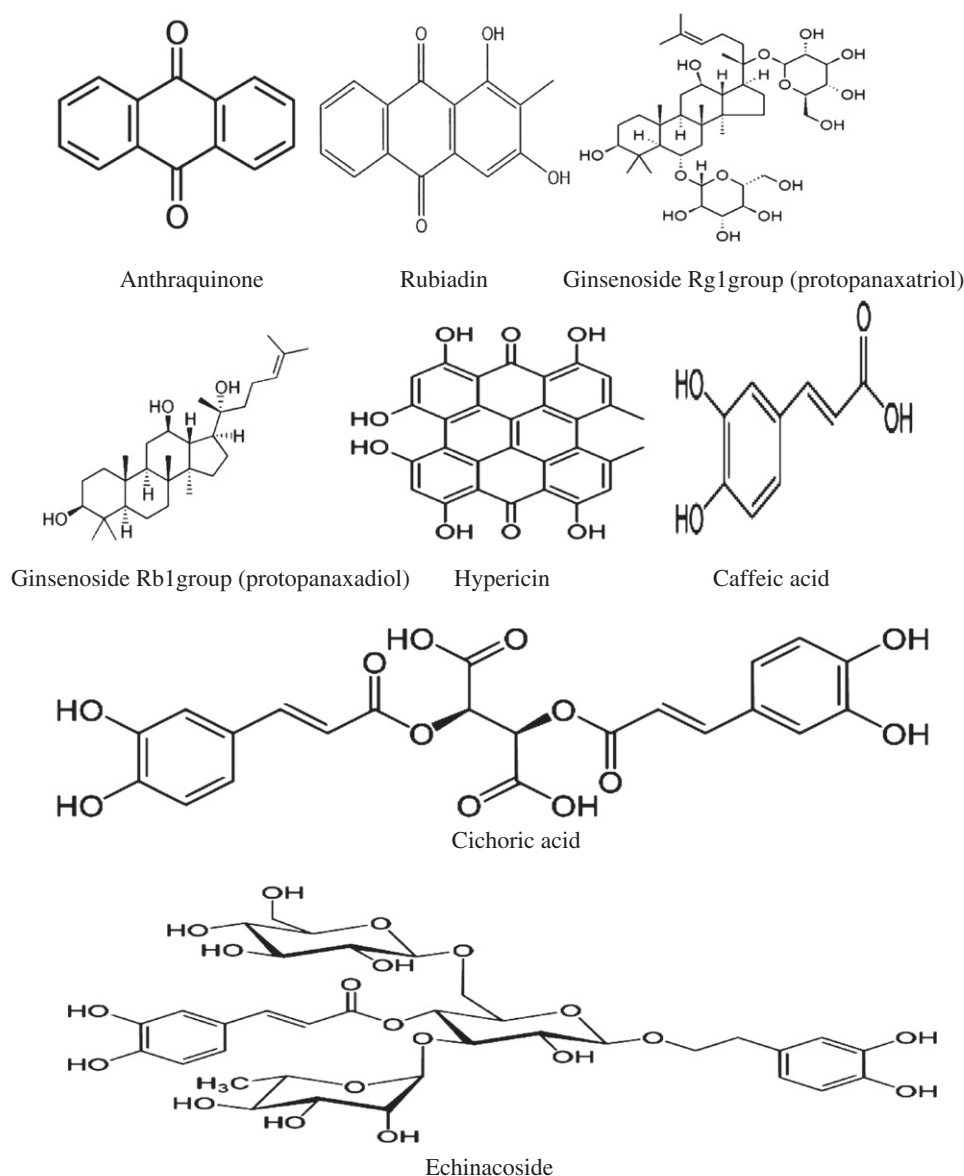


Fig. 1. Chemical structures of major bioactive compounds of *Morinda citrifolia* (anthraquinone, rubiadin), *Panax ginseng* (Rg1 and Rb1 group), *Hypericum perforatum* (hypericin), *Echinacea purpurea* (cichoric acid) and *E. angustifolia* (echinacoside).

bioreactor significantly affects cultivation results by accomplishing and controlling the optimal environment for effective cell or root growth and production of secondary metabolites. Moreover, the important process parameters inside the bioreactor vessel such as pH, temperature, dissolved oxygen and carbon dioxide evolution rate can be monitored and controlled (Sivakumar, 2006). Indeed, to design an appropriate bioreactor system for a particular bioprocess, intensive research efforts on the biological system, such as cell growth, metabolism, genetic manipulation, and protein or other product formation are needed to understand about the physical and chemical environments required for the efficient cultivation of plant cells or roots (Zhong, 2010).

The successful cultivation of plant cell or root suspensions can now be possible at small scale in bioreactors of various configurations. However, many of the unique properties of plant cell or root suspension cultures such as fluid mixing, shear sensitivity, low oxygen requirements and slow growth rates are manifested in complex ways at large scale to commercial scale production. With increase in the scale up process, mixing inside the reactor becomes difficult; resulting in non-uniform concentration of the nutrients and limited

oxygen transfer to respiring roots or cells. The wall growth and clumping of cells inside the reactor vessel caused by modified rheological nature of the fluid, result in sedimentation that significantly affects cell growth and product formation during bioreactor cultivation (Chattopadhyay et al., 2002; Zhong, 2010). These problems underscore a more rigorous analysis of bioreactors to be used for the large scale cultivation of adventitious roots for metabolite production.

Fluid mixing inside the reactor vessel is an important factor because mixing promotes better growth by accelerating the transfer of nutrients from liquid and gaseous phases to roots or cells and the dispersion of air bubbles for effective oxygenation (Zhong et al., 2002). Cells at the core of roots would be exposed to adequate oxygen tensions, high oxygen concentrations near the surface of the roots may become toxic to peripheral cells and caused localized oxidative stress (Shiao and Doran, 2000). Inadequate mixing may lead to clumping of roots or cells, thereby complicating the nature of the reactor system. Moreover, the inner cells of the clumps become nutrient deficient, which may have either an adverse or a positive impact on the cell growth and product formation (Panda et al., 1989). In this aspect, the helical-ribbon impeller has been reported to provide adequate

Table 1

List of plants in which adventitious roots have been induced and cultured successfully for the production of highly-valued secondary metabolites.

Plant species	Metabolites	Importance	References
<i>Panax ginseng</i>	Ginsenosides	Anticancer, antifatigue, immunostimulant, anti-inflammatory, antioxidant	Paek et al. (2009); Choi et al. (2000); Jeong et al. (2006); Kim et al. (2005); Son et al. (1999)
<i>Panax notoginseng</i>	Saponins	Immunostimulant, anticancer	Gao et al. (2005)
<i>Morinda citrifolia</i>	Antraquinone, rubiadin, phenolics, flavonoids	Anticancer, antibacterial, antiviral, hepatoprotective, antioxidant, antiallergic	Baque et al. (2010a, 2010b, 2010c, 2011); Baque (2011)
<i>Echinacea purpurea</i>	Caffeic acid derivatives	Immunostimulant, anti-inflammatory, antioxidant	Wu et al. (2006, 2007a); Jeong et al. (2009b)
<i>E. angustifolia</i>			
<i>Hypericum perforatum</i>	Hypericin, hyperin	Antidepressive, antifungal, anti-inflammatory, antimycobacterial	Cui et al. (2010a, 2010b, 2010c); Cui (2011)
<i>Eleutherococcus koreanum</i>	Eleutheroside B, E	Antifatigue, analgesic	Lee et al. (2011)
	Chlorogenic acid		
<i>Pelargonium sidoides</i>	Coumarin	Antivirus, antibiotic	Jeong (2009)
<i>Scopolia parviflora</i>	Hyacynamine (alkaloid)	Anticholinergic activity	Kang et al. (2004)
<i>Iris germanica</i>	Irisgenin, Iristectorigenin A (flavonoids)	–	Akashi et al. (2005)
<i>Raparus sativus</i> L. cv. Peking koushin	Anthocyanin	Food coloring	Betsui et al. (2004)
<i>Rhu javanica</i>	Galloylglucoses, riccionidin A (polyphenols)	Antioxidant	Taniguchi et al. (2000)
<i>Dubosia myoporoides</i>	Scopolamine, hyoscyamine	Spamolytic, kydriatic agents	Yoshimatsu et al. (2004)
<i>D. leichhardtii</i>			
<i>Cornus capitata</i>	Tannins	Antioxidants	Tanaka et al. (2001)
<i>Anthemis nobilis</i>	Geranyl isovalerte	Anti-inflammatory, fragrance, essential oil	Omato et al. (1998)
<i>Andrographis paniculata</i>	Andrographolide	Antimalarial, antipyretic	Praveen et al. (2009)

mixing at the high density of plant cell suspension cultures due to its proper impeller design (Jolicœur et al., 1992).

Optimum air supply inside the bioreactor culture vessel is another important factor, because high air supply may lead to severe foaming, which has considerable impact on the cell growth and product formation (Zhong et al., 1992). It has been reported that foaming of plant cell suspensions has been correlated with aeration rates and extracellular protein concentrations. To control foaming, a number of antifoams have often been used. However, in some cases this resulted in reduction in cell growth and product formation (Wongasmuth and Doran, 1994). The observed response of plant cells or roots to hydrodynamic stress associated with air supply or agitation of suspension cultures can be attributed to the physical properties of the cultured cells, e.g., thick cellulose containing cell wall, and presence of large vacuoles.

Additionally, the impact of shear stress caused by elevated levels of air supply on plant cells or roots is cell damage, which can be quantitatively measured by using a number of system responses such as reduction in cell viability, and release of intercellular compounds (Meijer et al., 1993), changes in morphology or aggregate formation patterns (Kieran et al., 1995) and changes in metabolism (Zhong et al., 1994). These problems could be solved by directing research effort to the development of shear resistant cell lines, as well as the air-lift bioreactor should be advised for getting optimal oxygen transfer rate and considerable growth. Another suitable alternative approach for transferring gas without affecting cell viability is bubble free aeration of the culture fluid through a moving membrane (Chattopadhyay et al., 2002; Drapeau et al., 1986; Kieran et al., 1997). A stirred tank bioreactor should not be addressed for mass production of adventitious roots because of high shear stress and high electric charge. However, among the different kinds of bioreactors, pneumatically agitated air-lift and bubble column bioreactors are commercially successful for adventitious root and cell suspension culture because of low shear stress, easy scale-up, low operating and maintenance cost (Hu and Zhong, 2001; Paek et al., 2005; Zhong, 2002). Considering these, we have designed a balloon type bubble bioreactor (BTBB) (Fig. 2), which was found effective to overcome foaming and cell growth on the wall of the reactor vessel, and also found to be reliable for enhancing production of biomass and bioactive compounds of different high value medicinal plants (Paek et al., 2001).

4. Optimization strategy to improve secondary metabolite production

Plant biotechnologists have successfully used several approaches to accomplish overproduction of bioactive molecules in suspension cultures of plant cells or roots. Some of these approaches lead to enhancement in secondary metabolite production within plant cells or roots by strain improvement. Statistically high-producing plants give rise to high-producing cell lines (Deus and Zenk, 1982), but the levels of product synthesis in plant cells or roots have also been shown to variable (Dornenburg and Knorr, 1995). Such variability often leads poor product formation with subculturing, which is attributed to genetic changes by mutation in the cell or root culture, or epigenetic changes caused by physiologic conditions. Hence, information concerning the selection of high producing cell or root lines is as important as the optimization of process parameters in increasing the production of metabolites.

A number of physical and chemical factors that may affect secondary metabolism in plant cell or root suspension cultures have been explored. For instance, optimization of the hormone concentration and their combinations are often effective. High auxin levels, although good for adventitious roots or cell growth, are often deleterious to the desired product formation (Baque et al., 2010a; Chan et al., 2005). It was plausible that inoculum density is an important factor affecting the performance of plant cells, tissues and organ cultures. Plant cell suspension cultures are initiated using relatively high inoculum density as there is a minimum inoculation density below which growth does not occur or is preceded by a lag phase. The actual value of this minimum inoculum size depends on the cell line, nutrient composition of the medium and other culture conditions. Medium conditioning is often used to reduce the inoculum density; however, the effect of medium conditioning has not been fully established and is primarily empirical (Jeong et al., 2009a; Lee and Shuler, 2000).

It has been suggested that the development of specific modeling systems can investigate the growth characteristics of in vitro cultures along with the influence of culture conditions, and also can be helpful in relating the predicted and measured variables for optimized growth and productivity. The successful application of artificial neural network (ANN) based prior estimations of culture ambiance can make

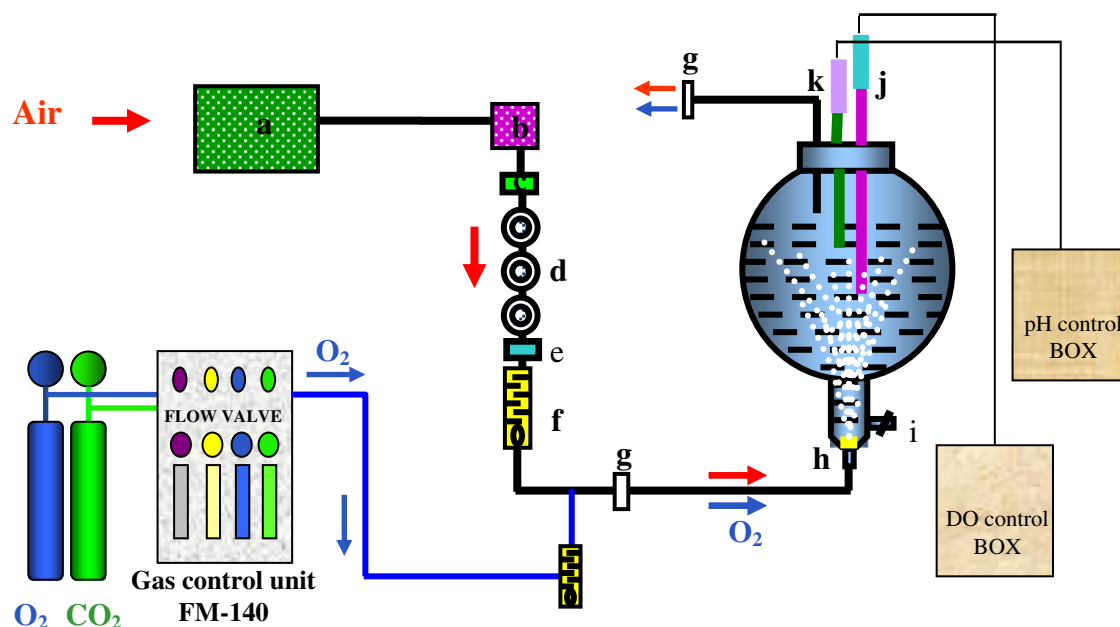


Fig. 2. Schematic diagram of an air-lift bioreactor. a: air compressor, b: air reservoir, c: air after cooler, d: air filter system, e: air dryer, f: air flow meter, g: membrane filter, h: glass sparger, i: medium feeding port, j: DO sensor, k: pH sensor.

Source: Paek et al., 2001.

the whole process of secondary metabolite production through hairy or adventitious root cultures interestingly more feasible, less labor and time consuming (Prakash et al., 2010). In plant tissue culture practices the successful use of ANN has been evidenced by the results of different studies related to biomass estimation of cultured cells (Albiol et al., 1995), growth kinetics of somatic embryos (Uozumia et al., 1993; Zhang et al., 1999) and prediction of in vitro culture parameters for maximum biomass yields in hairy root cultures (Prakash et al., 2010).

Appropriate nutrients, their optimal concentrations and environmental factors are also often beneficial to accelerate the yield and productivity of desired metabolites in plant suspension cultures. However, it is essential to explore the optimum concentration of nutrient, and to investigate the effect of selected medium components on growth as well as product synthesis, and strike a balance between the two for uplifting yield and productivity. This is quintessential particularly for plant secondary metabolism, because conditions suitable for growth may negatively affect the product formation and vice versa (Chattopadhyay et al., 2002). For instance, decrease of medium initial ammonium ion's from 20 mM to 2 mM led to onefold increase of taxol production in *T. chinensis* (Zhou and Zhong, 2010), while high ammonium ions in the medium inhibited biosynthesis of ginsenosides in *P. ginseng* (Paek et al., 2009), phenolics and flavonoids in *H. perforatum* (Cui et al., 2010a) and *E. angustifolia* (Wu et al., 2006). By contrast, the optimal ratio of ammonium to nitrate promoted growth and productivity in adventitious root suspension cultures of these plants. Thus, optimization of different process parameters is the key challenge toward scale-up of suspension cultures in commercial scale.

It has been claimed that one of the major obstacles in the industrial application of plant cell, tissue or organ culture for the efficient production of commercially important bioactive compound is the low product yield (Zhou and Zhong, 2010). Although, optimization of process parameters can lead to an enhancement in secondary metabolite production. However, most often research with plant cell or root cultures fails to produce the target product. In these cases, strategies to enhance the production of desired metabolites must be considered (Jeong et al., 2009a; Kim et al., 2005). With this context, several techniques have been adopted to improve production of plant-derived

secondary metabolites such as elicitation, two-phase and two-stage culture system, precursor feeding, genetic transformation, metabolic and bioreactor engineering, and integrated bioreactor technology (Abdullah et al., 2005; Chong et al., 2005a; Hahn et al., 2003; Zhong, 2002; Zhou and Zhong, 2011a, 2011b). Of the various efforts, elicitation is seen as an effective strategy to enhance production of commercially important bioactive compounds such as anthraquinones (Komaraiah et al., 2005), ginsenosides (Kim et al., 2004; Paek et al., 2009; Wang et al., 2005; Yu et al., 2002), paclitaxel and other taxanes (Zhong, 2002; Wang and Zhong, 2002a, 2002b; Dong and Zhong, 2002), saikosaponin (Chen et al., 2007) and scopolamine (Biondi et al., 2000).

Elicitors are compounds from various biotic or abiotic sources that can modulate a plant defense reaction, which may enhance secondary metabolism in plant cell and root cultures (Zhao et al., 2005). The elicitor-mediated defense responses involve signal perception of elicitor by a cell surface receptor. Thereafter, transduction of some major cellular and molecular events, resulting induction of gene expressions escorting to the synthesis of various proteins relating to plant defense responses and secondary metabolite synthesis and accumulation (Goel et al., 2011; Sudha and Ravishankar, 2002). The best-studied examples of elicitor-mediated induction of secondary metabolite in hairy root culture are that of jasmonic acid (JA)-induced biosynthesis of terpenoid indole alkaloids (Peebles et al., 2009). The JA and allied compounds also act as transducer of elicitor signals for biosynthesis of various groups of secondary metabolites including alkaloids (Gaviraj and Veeresham, 2006), coumarines and furocoumarins (Staniszewska et al., 2003), anthraquinones and saponins (Nakanishi et al., 2005) in hairy roots and cell suspension cultures. It has been proposed that many elicitors stimulate endogenous JA biosynthesis following octadecanoid pathway in plants, which in turn activates defensive genes eventually yielding a variety of JA-induced proteins (Pozo et al., 2005). Therefore, a transcriptome analysis of a JA or methyl jasmonate (MeJa)-challenged tissue can be helpful in discovering and exploring those genes which actively play a prominent role in biosynthetic pathways (Goel et al., 2011).

The effect of jasmonates on secondary metabolism has been studied in detail for alkaloid biosynthesis, in which jasmonate acts by coordinate activation of the expression of multiple biosynthesis

genes. However, how the JA signal is transduced to affect gene expression is putative. It is tempting to speculate that jasmonate-induced secondary metabolic pathways might be regulated by ORCA-like AP2/ERF-domain transcription factors (Memelink et al., 2001). Therefore, it is suggested that this could be mechanism by which MeJa treatment induce biosynthesis of secondary metabolites in plants. This treatment induces abiotic stresses to the plant cell or root cultures that trigger the biosynthesis of secondary metabolites. This leads to obtain nutritional value and anti-carcinogenic properties of cells or roots through natural molecules instead of foreign genes (Sivakumar and Paek, 2005). Nevertheless, jasmonates are also reported to be highly efficient elicitors for taxoid production by *T. chinensis*, and induction of ginsenoside biosynthesis and alteration of ginsenoside heterogeneity in cell suspension cultures of *P. notoginseng* (Qian et al., 2004a, 2004b; Wang and Zhong, 2002c; Wang et al., 2006). In addition, elicitation of MeJa has been shown to be responsible for the de novo biosynthesis of ascorbic acid in cell suspension cultures of *Arabidopsis* and tobacco Bright Yellow-2 (BY-2). In BY-2 cells, the induction of vitamin C biosynthesis by MeJa treatment coincides with enhanced transcription of at-least two late MeJa responsive genes encoding enzymes (Wolucka et al., 2005).

Despite of potential advantages of elicitation to enhance biosynthesis of bioactive molecules, researchers have claimed that adventitious root cultures exposed to MeJa resulted in strong inhibition of root growth but enhanced the accumulation of scopolamine and hyoscyamine in *Scopolia parviflora* (Kang et al., 2004) and ginsenosides in *P. ginseng* (Murthy et al., 2008; Paek et al., 2009). In this regard, two-stage culture system should be addressed; cultivation of adventitious roots without elicitor for biomass accumulation followed by addition of potential elicitor in the second stage for enhancing metabolites without decreasing root biomass. However, the potentiality of the elicitors and their optimal concentration should be considered. Moreover, precursors of biosynthetic pathways could be used in plant suspension cultures to stimulate biosynthesis of target metabolites. In this case, factors such as concentration of precursors and their time of addition should be considered when applying the precursors to the cell or root culture medium. For example, a step feeding strategy, in which 50 g L⁻¹ cholesterol was added in 4 installments during different phases of growth, enhanced the production of alkaloid from 63 mg L⁻¹ to 106 mg L⁻¹ in a 6 L stirred tank bioreactor (Panda et al., 1992) which highlighted the importance of the physiological state of the culture for effective transformation of the precursor to alkaloid.

5. Scale-up of culture process

Scale-up of the culture in a large scale bioreactor is the key challenge toward commercial exploitation of plant-derived secondary metabolites. Because, the environment in which plant cells and roots cultured can be affected when cultures are transferred from shake flask to bioreactors, and further scaling-up from a pilot scale to industrial scale. During this process, reduced productivity has often been observed, which probably due to the changing effect of shear stress, oxygen supply and gas composition in bioreactors (Zhong, 2001, 2002). The large scale cultivation of plant cell was first reported by Tulecke and Nickell (1959). In the last few decades, considerable efforts have been paid in the field of plant cell fermentation and scaling-up. Plant cells can now be cultivated in a volumes of 75,000 L, specific bioreactor systems for culturing plant cells have been established, and productive plant cell culture systems have been developed (Dornenburg and Knorr, 1995). Despite of sporadic reports, only few studies have been commercially successful for the production of adventitious root biomass and bioactive compounds at the industrial scale reactors. The successful scale-up of ginseng adventitious root culture in 500 L balloon type bubble bioreactor (BTBB) was achieved by Choi et al. (2000) and in 10,000 L BTBB by Paek et al.

(2009). A 150-fold growth was achieved when adventitious roots were grown in 500 L BTBB for 7 weeks compared to the cells and hairy root cultures as reported earlier by Asaka et al. (1993).

Scaling-up of adventitious root culture for the production of biomass and secondary metabolites involves: induction of adventitious roots from the selected explants, optimization of process parameters of adventitious root suspension culture in shake-flask or bioreactors, studies on the growth kinetics and exploring suitable techniques for higher accumulation of metabolites without affecting root growth, and cultivation of adventitious roots in a pilot scale and commercial scale bioreactors. The last step could be downstream processing for the recovery of desired metabolites (Murthy et al., 2008).

It is well-documented that plant cells require less oxygen compared to microbial cells, because of their slow metabolism. It has been plausible that high oxygen concentration is often deleterious to the metabolic activities of the cells, and high sparge rates may remove carbon dioxide and other nutrients from the culture medium. In these cases, the air-lift bioreactor is considered as the most desirable reactor for efficient cultivation of suspension cells or roots (Dornenburg and Knorr, 1995; Smart and Fowler, 1981).

6. Case studies

6.1. Adventitious root cultures of *Morinda citrifolia*

M. citrifolia (L.), known commercially as Noni belongs to the Rubiaceae Coffee family has been used in folk remedies by Polynessians for over 2000 years, owing to its broad range of therapeutic effects such as anticancer, antibacterial, antiviral, antifungal, antitumor, treatment of cold and flu, analgesic, hypotensive, anti-inflammatory, antiallergic and immune enhancing effects (Solomon, 1999; Wang et al., 2002). Isabel Abbott, a former botanical chemist, stated that, “peoples are crazy about Noni” because of its effectiveness against diabetes, high blood pressure, cancer, and many other illness (Abbott, 1985). The major bioactive compounds in Noni are polyphenolics, organic acids and alkaloids, and of the phenolic compounds, the mostly reported ones are anthraquinones (Wang and Su, 2001; Wang et al., 2002).

The demand for Noni roots and extracts has been increased in the past few decades. For medicinal purpose, field cultivation of Noni roots, require 2–5 years, and in regions with high temperature and humidity levels (Ahmed et al., 2008). Moreover, Noni is susceptible to attack by a wide array of pests and diseases, and continuous harvesting from its natural stands has caused the rapid depletion of the mother plants. To overcome these aforementioned problems, adventitious root cultures of this plant in large scale bioreactors could be a promising alternative approach for high density production of root biomass and valuable bioactive molecules such as anthraquinones (AQ), rubiadin, phenolics and flavonoids.

The cell suspension culture of *M. citrifolia* was first reported by Zenk et al. (1975) for AQ production, in which under optimal culture conditions the yield of AQ in leaf-originated suspended cell culture was more than 10 times higher compared to differentiated roots. In addition, enhanced induction of AQ biosynthesis in cell suspension cultures of *M. citrifolia* and other species by attaining exogenous addition of JA, MeJa, salicylic acid (SA), polyunsaturated fatty acids (linoleic acid, linolenic acid and arachidonic acid), chitosan, fungal elicitors, ultrasonication, and controlled feeding of sucrose in the growth medium has previously been reported by several authors (Chong et al., 2005a, 2005b; Komaraiah et al., 2005). Considering these, leaf-originated cell suspension cultures of *M. citrifolia* were established in our laboratory using 5 L BTBB (Ahmed et al., 2008; Shim et al., 2010). However, the large scale cultivation was aggravated due to low content of AQ (17.46 mg g⁻¹ DW), continuous foaming and wall-growth in bioreactors during cell suspension cultures of *M. citrifolia* (Abdullah et al., 2000; Ahmed et al., 2008).

Foaming in bioreactor which may be evolved toward the end of batch culture due to the presence of cells, may cause reduction in working volume of the reactor. Cells entrapped in stable foam inside bioreactor may form a crust, attached to the reactor wall, or around probes and sample ports affecting the operation of the probes and creating problems for sampling of suspension cells (Wongasmuth and Doran, 1994). Moreover, the accumulating necrotic cells may release by-products such as protease, peptides and fatty acids that can arrest cell growth (Piehl et al., 1988; Su, 1995). A lot of efforts have been paid by the researchers over the last decades to overcome foaming, among which the use of antifoam is hindered. Because, the presence of antifoam in the culture medium reduce oxygen diffusion and gas transfer rate to the cells which consequently affects biomass yield (Smart and Fowler, 1981). Moreover, addition of some antifoams may also be prohibited especially manufacturing of food stuffs and pharmaceutical products (Wongasmuth and Doran, 1994). Therefore, as an alternative approach, we have established adventitious root culture system in pilot scale bioreactor (500 L), and was found to be reliable to overcome those aforementioned problems.

The adventitious roots were induced from leaf explants of in vitro grown plantlets of *M. citrifolia*. The selected explants were placed on solid full-strength MS medium supplemented with 1 mg L⁻¹ indole-3-butyric acid (IBA) and 30 g L⁻¹ sucrose, and kept under a fluorescent light (Baque et al., 2010b). A series of experiments were conducted in shake-flask to establish an efficient adventitious root suspension culture system for enhancing root growth and accumulation of bioactive compounds. Overall, increases of root growth and accumulation of AQ, phenolics and flavonoids were achieved at quarter-strength of MS medium supplemented with 5 mg L⁻¹ IBA, 10 g L⁻¹ sucrose and 15 g L⁻¹ (fresh weight, FW) inoculum size under darkness at 23 ± 2 °C for 4 weeks (Baque et al., 2010a, 2010b, 2010c, 2011). Based on the results of the shake-flask optimized culture, a large scale (3 to 20 L), and subsequently a pilot-scale (100–500 L BTBB) adventitious root culture system were also established. In large scale bioreactor culture, the enhancement of root growth and metabolites accumulation were observed in the half-strength MS medium containing 5 mg L⁻¹ IBA, 10 g L⁻¹ sucrose and 15 g L⁻¹ inoculum density when the cultures were agitated with an aeration volume of 0.05 vvm (air volume/culture volume/min) under darkness for 5 weeks. Whereas, in pilot scale bioreactor culture, full-strength MS medium supplemented with 5 mg L⁻¹ IBA and 30 g L⁻¹ sucrose was found to be optimal for upswing productivity of both biomass and metabolites (Baque, 2011). These results clearly demonstrate the augmented ability of optimization of culture process to produce cosmic amount of desired metabolites for commercial point of view.

As an enhancement strategy, various elicitors such as MeJA, SA, lactalbumin hydrolysate (LH) were added in the culture to elucidate their optimal concentration and time of application. The addition of 150 μM MeJA during inoculation was found to be the most effective elicitor on AQ biosynthesis (2-fold over control) but strongly repressed root growth. To overcome detrimental effect of MeJA on root growth, two-stage culture system was adopted: addition of 150 μM MeJA in the culture after 4 weeks and harvested after 1 week of elicitation was shown to be effective for enhancing biosynthesis of AQ, phenolics and flavonoids without decreasing root growth. Later, we were able to increase AQ (up to 22–41%), phenolics (24%) and flavonoids (21–35%) content in adventitious roots harvested from 500 L BTBB compared to 3–20 L BTBB by applying 150 μM MeJA as two-stage culture system (Baque, 2011).

In cell suspension cultures of *M. citrifolia*, Komaraiah et al. (2005) observed a synergistic effect by simultaneously applying 150 μM MeJA and controlled feeding of 2% sucrose, that increased the AQ production to 16.74 mg g⁻¹ DW, which was more than a 4-fold over without MeJA treatment. We achieved 148.35 mg g⁻¹ DW of AQ in adventitious roots (cultured in 500 L BTBB) by attaining exogenous application of 150 μM MeJA after 5 weeks of culture, which was 26-, 2.37-,

12- and 24-fold of AQ content in field grown madder roots (5.70 mg g⁻¹ DW), leaf of green house grown plant (62.39 mg g⁻¹ DW), leaf-originated cells (11.94 mg g⁻¹ DW) and fruit's of green house grown plant (6.15 mg g⁻¹ DW), respectively (Baque, 2011). These results corroborate the feasibility for commercial exploitation of AQ from adventitious root cultures of *M. citrifolia* using large scale bioreactors.

Rubiadin, a major constituent of AQ, is highly valued in pharmaceutical industry due to hepatoprotective (Rao et al., 2006), and anti-tumor promoting activity (Jasril et al., 2003), and also have been found to inhibit lipid peroxidation (Tripathi and Sharma, 1998). Rubiadin was identified and purified from MeJA treated adventitious roots of *M. citrifolia* harvested from pilot scale bioreactor. A reverse-phase HPLC assay method was also developed to quantify rubiadin content in adventitious roots. The HPLC assay of rubiadin was performed by C-18 column using a gradient solvent system of methanol and water with a UV detector at 280 nm (Kim et al., 2010). It is worth noting here that rubiadin was not detected in the various parts (leaf, stem, fruit) of field grown plant of *M. citrifolia* or very few amounts (0.02%) in madder roots; whereas, copious amount of rubiadin (≥0.58%) was detected in adventitious roots compared to ex-vitro roots (Table 2). These results clearly indicate that adventitious root cultures of *M. citrifolia* using large scale bioreactors could be a useful tool for commercial production of AQ, rubiadin, phenolics and flavonoids. Therefore, we have initiated further works, and the commercial application of adventitious root culture of this valuable medicinal plant is now under trail with 1000 L BTBBs in our laboratory.

6.2. Adventitious root cultures of *Echinacea* (*purpurea* and *angustifolia*)

Echinacea, better known as purple coneflower, has gained popularity worldwide because of its increasing medicinal value. Extensive research efforts with this plant can make possible to discover new medicinal compounds, as well as to explore suitable and cost effective technique is needed to facilitate the production of high quality, chemically consistent plant material for drug development and clinical trials (Abbasi et al., 2007a). The potential active compounds in *Echinacea* are caffeic acid derivatives, polysaccharides, alkamids and glycoproteins, that exhibit various clinical effects such as antioxidative, antibacterial, antiviral, antifungal properties and are used for treating common cold, respiratory and urinary diseases (Barrett, 2003). Of the caffeic acid derivatives, cichoric acid (major compound in *E. purpurea*) has been found to have immunostimulatory properties such as the promotion of phagocyte activity in vitro and in vivo. Additionally, cichoric acid has been shown to have antihyaluronidase activity (Bergeron et al., 2002), to exert a protective effect on the free radical-induced degradation of collagen, and has recently been found to inhibit HIV-1 integrase and replication (Lin et al., 1999). Echinacoside, found in *E. angustifolia*, is a broad spectrum antibiotic, inhibiting a broad range of viruses, protozoa, bacteria, and fungi.

Table 2
Quantification of rubiadin content in various plant parts of *M. citrifolia* by HPLC.

Various plant parts	Rubiadin content (%)
Stem	Not detected
Leaf	Not detected
Fruit	Not detected
Ex-vitro roots	0.02
Adventitious roots	0.58

Adventitious roots cultured in 500 L balloon type bubble bioreactor (BTBB) containing full-strength of MS medium supplemented with 5 mg L⁻¹ IBA, 30 g L⁻¹ sucrose and 15 g L⁻¹ of inoculum size with an aeration volume of 0.05 vvm for 5 weeks (150 μM MeJA elicited in the culture after 4 weeks and harvested after 1 week of elicitation).

However, there may not be enough echinacoside in most tissues for the effects to be significant (Cervellati et al., 2002).

Despite for being a major potential source of highly-valued pharmaceutical compounds, as well as has received a global attention because of *Echinacea* is listed as the first among 11 top-selling herbal extracts in North America (Yu and Kaarlas, 2004). However, a range of issues such as contamination of plant materials by microorganisms, pollution from the environment, variability of active components and lack of pure, standardized plant materials for biochemical analysis have restricted the commercial production of this plant (Raman et al., 2004). Considering these, an efficient adventitious root culture system was developed using 20–1000 L BTBB (Jeong et al., 2009b; Wu et al., 2006, 2007a) to produce copious amount of caffeic acid derivatives (Fig. 3A–D). Adventitious roots cultured in bioreactors containing half-strength modified MS medium (Wu et al., 2006) profusely increased (approximately 10-fold) root dry weight and secondary metabolites after 4 weeks of culture. An inoculum density of 7 g L^{-1} FW and an aeration rate of 0.1 vvm were found to be suitable for accumulation of both root biomass and metabolites. Among the caffeic acid derivatives, the accumulation of cichoric acid was the highest (26.64 mg g^{-1} DW), which reflects the feasibility of large scale bioreactor cultivation of *E. purpurea* adventitious roots. After extensive research effort, a pilot scale cultivation of *E. purpurea* adventitious root was established, in which 3.6 kg and 5.1 kg root dry biomass were produced in 500 and 1000 L bioreactors, respectively. The accumulation of 5 mg g^{-1} DW chlorogenic acid, 22 mg g^{-1} DW cichoric acid and 4 mg g^{-1} DW caftaric acid was also quantified in adventitious roots

grown in 1000 L bioreactors (Wu et al., 2007a). Interestingly, these compounds were significantly low in natural roots, as well as natural roots are devoid of chlorogenic acid.

Various enhancement techniques such as elicitation of nitric oxide (NO), medium replenishment, manipulation of incubation temperature and photoperiod have also been imposed to elucidate their involvement in the biosynthesis of secondary metabolites and underlying mechanism involved during adventitious root growth. Adventitious roots elicitor treated with $100 \mu\text{M}$ of sodium nitroprusside (SNP), an exogenous NO producer, enormously enhanced biosynthesis of phenolics, flavonoids and caffeic acid derivatives (especially cichoric acid, which was more than 1.26-fold over control) (Wu et al., 2007b). The application of fed batch cultivation was also proven as an effective strategy. Adventitious roots feeding with 0.5 MS medium at the end of 2nd week increased 1.5-fold root biomass and 1.37-fold cichoric acid compared to without feeding (Wu et al., 2007c).

It is well-known that light is an important factor to regulate almost all plant developmental processes and provides the fundamental building blocks for growth and development (Halliday and Fankhauser, 2003), as well as the biosynthesis of both primary and secondary metabolites (Abbasi et al., 2007b; Hemm et al., 2004; Hoppen et al., 2002). Indeed, an optimum incubation temperature of 20°C was found beneficial for accumulation of root biomass and caffeic acid derivatives in adventitious roots of *E. purpurea*. In addition, maximum biomass accumulation was observed in dark grown cultures, whereas adventitious roots cultured under 3/21 h light and dark photoperiod uplifted biosynthesis of caffeic acid derivatives.



Fig. 3. Cultivation of adventitious roots in liquid-phase air-lift bioreactors (20–10,000 L). Adventitious roots of *Echinacea purpurea*: 20 L, 500 L (A–B) and 1000 L (C–D); *Hypericum perforatum*: 500 L (E–F); Ginseng: 10,000 L (G–I). CBN biotech, a venture company established in Professor Paek's Lab., 2002. Source: Cui, 2011; Murthy et al., 2008; Paek et al., 2009.

Considering this phenomenon, a two-stage culture system was developed, in which adventitious root cultured under dark for the initial 3 weeks stimulated root growth, and irradiate the cultures under 3/21 h light and dark photoperiod in the last 2 weeks enhances biosynthesis of caffeic acid derivatives (Wu et al., 2007d). The definite positive effect of light-mediated enhanced biosynthesis of caffeic acid derivatives has also been reported in hairy root cultures of this species (Abbasi et al., 2007b). The enhanced induction of caffeic acid derivatives and anthocyanins accumulation in *E. purpurea* hairy root culture was found to be well-correlated to an observed light-stimulated activity of phenylalanine ammonia lyase (PAL).

Currently our research efforts are focusing on developing large scale bioreactor methodologies for the efficient production of caffeic acid derivatives from adventitious root cultures of *E. angustifolia* (a potential source of echinacosides). Optimization of culturing conditions using shake-flask (Wu et al., 2006), and antioxidative responses of adventitious roots to different levels of CO₂ in bioreactors (Ali et al., 2006a) have previously been reported for this species. As a part of scale-up of culture process, a series of experiments were conducted in our laboratory using 5 L BTBB for efficient production of caffeic acid derivatives. Subsequently, elicitation of MeJa and fed-batch cultivation has also been employed to improve the production of these metabolites. The maximum root dry weight (7.15 g L⁻¹), and accumulation of echinacoside (5.38 mg g⁻¹ DW), cynarin (3.81 mg g⁻¹ DW) and cichoric acid (1.82 mg g⁻¹ DW) were achieved at quarter-strength of MS medium supplemented with 1 mg L⁻¹ IBA and 50 g L⁻¹ sucrose for 4 weeks of culture period. An inoculum size of 13 g L⁻¹ (FW) along with 0.1 vvm aeration rate was found to be optimal for both adventitious root growth and accumulation of caffeic acid derivatives (Cui, 2009).

Medium replenished by quarter-strength of fresh MS medium along with 50 g L⁻¹ sucrose after 2 weeks, enhanced accumulation of root dry mass of 5.99 g L⁻¹ to 7.71 g L⁻¹ DW, but repressed accumulation of phenolics and flavonoids. However, the total production of 71.90 mg L⁻¹ DW caffeic acid derivatives was quantified, which was 1.22-fold and 2.90-fold over control (without feeding) or medium replenished by quarter-strength of fresh MS medium along with 50 g L⁻¹ sucrose after 3 weeks of culture. Furthermore, addition of 100 and 200 µM of MeJa following two-stage culture system (adventitious roots allowed to grow up to 3 weeks and harvested after 1 week of elicitation) enhanced biosynthesis of caffeic acid derivatives (Table 3). The accumulation of 17.83 mg g⁻¹ DW echinacoside and 7.37 mg g⁻¹ DW cichoric acid was achieved by adding 100 and 200 µM of MeJa, respectively; showing 2.87-fold and 7.15-fold over their respective control. These results are the clear indication of augmented ability of MeJa for commercial exploitation of caffeic acid derivatives through large scale bioreactor cultures of *E. angustifolia*. The pilot scale (500–1000 L) bioreactor cultures of *E. angustifolia* adventitious roots are now being under trail to evaluate the feasibility for commercial application.

Table 3
Content of caffeic acid derivatives in adventitious roots of *E. angustifolia* as affected by various concentrations of MeJa after 5 weeks.

MeJa (µmol)	Caffeic acid derivatives (mg g ⁻¹ DW)			
	Echinacoside	caffeic acid	cynarin	cichoric acid
Control	6.20c	–	3.96c	1.03d
100	17.83a	3.49b	6.24a	5.95b
200	16.25b	4.68a	5.87b	7.37a
400	5.73d	1.61c	3.06d	2.87c
800	0.47e	–	0.78e	0.11e

Mean separation by Duncan's multiple range test at *P* 0.05.

Adventitious roots cultured in 5 L BTBB containing quarter-strength of MS medium supplemented with 1 mg L⁻¹ IBA, 50 g L⁻¹ sucrose and 13 g L⁻¹ inoculum size with an aeration volume of 0.1 vvm (cultures were elicited with MeJa after 4 weeks and harvested after 1 week of elicitation).

6.3. Adventitious root cultures of *Hypericum perforatum* (L.)

Hypericum perforatum (L.), commonly known as St. John's wort, has received global attention, owing to its variety of structurally diverse phytochemicals such as flavonols, naphthodianthrones and phloroglucinols, which have been reported to have antidepressant activity in different antidepressant model systems (Barnes et al., 2001; Cirak et al., 2007; Walker et al., 2002). Of the various aforementioned metabolites, research on St. John's wort has focused primarily on hypericin and pseudohypericin as the major constituents responsible for the antidepressant activity (Walker et al., 2002), and recently clinical studies on St. John's wort underscored the possible role of flavonoids in preventing cardiovascular diseases and several kinds of cancer (Chu et al., 2000; Gastpar and Zeller, 2005; Thiede and Walper, 1994).

In the last few years, extracts isolated from *H. perforatum* have received great attention among commodities of the US and Germany. Several herbal life saving drugs and dietary supplements have been marketed with an annual total sales figures were approximately US \$6 billion and 200 million in Europe and US, respectively (Harrison, 1998). However, for medicinal purpose, plants require approximately 2–3 years to mature, the accumulation of secondary metabolite is very low (0.3%), as well as biosynthesis of metabolites also varied up to 50-fold in summer and winter grown plants (Zobayed et al., 2004). Additionally, indiscriminate uses of whole plants for herbal remedy have caused rapid depletion of the mother plants from its natural stands. Although cell, organ and hairy root cultures of *H. perforatum* for mass production of biomass and regulation of metabolite biosynthesis by precursors and elicitors have been reported by several authors (Gadzowska et al., 2007; Liu et al., 2007a, 2007b; Pavlik et al., 2007; Walker et al., 2002; Zobayed et al., 2004). However, the industrial application of this plant is still in its infancy probably due to the slow growth of the cells and restriction of genetically-modified organisms for medicinal purpose and food additives (Hahn et al., 2003; Wu and Zhong, 1999; Yoshikawa and Furuya, 1987). Recently, Cui et al. (2010a, 2010b, 2010c) have established adventitious root culture system of *H. perforatum* for high density production of root biomass and hypericin using BTBB. Adventitious roots (6 g L⁻¹ FW) were inoculated into a 3 L BTBB filled with half-strength MS medium supplemented with 0.1 mg L⁻¹ kinetin with 1 mg L⁻¹ IBA and 30 g L⁻¹ sucrose. After 5 weeks of culture 104.2 g L⁻¹ (FW) adventitious root was harvested, in which 56.47 mg g⁻¹ DW of total phenolics, 35.01 mg g⁻¹ DW flavonoids, 1.39 mg g⁻¹ DW hypericin and 0.97 mg g⁻¹ DW chlorogenic acid were measured. The authors have concluded that the accumulation of hypericin in adventitious root grown in bioreactor was coincided to those in green-house grown plants as previously reported by Zobayed et al. (2004) and Cirak et al. (2007).

Additionally, optimization of various process parameters and effective elicitation methods were also established. The optimal culture condition for effective adventitious root growth and bioactive compound production was achieved by inoculating 3 g L⁻¹ (FW) adventitious roots in BTBB supplemented with half-strength MS medium containing 1 mg L⁻¹ IBA along with 0.1 mg L⁻¹ kinetin, 5:25 (mM) NH₄⁺/NO₃⁻ ratio, 30 g L⁻¹ sucrose and an aeration rate of 0.1 vvm for 6 weeks of culture period. Of the various elicitors (MeJa, SA and LH) and their concentration tested, addition of 100 µM MeJa in the culture after 5 weeks and harvested after 1 week of elicitation was proven effective strategy for uplifting the pace of metabolite biosynthesis (Cui, 2011). Subsequently, a pilot scale bioreactor culture system was also established in which 1.1 kg, 4.7 kg and 6.3 kg root dry mass were measured from 100 L BTBB, 500 L drum and BTBB, respectively (Fig. 3E–F). These cultures were observed less prone to erratic metabolite production compared to the cultures of undifferentiated cells, as well as exhibit lower sensitivity to shear stress than cell suspension cultures. Additionally, identification of hypericin, hyperin, quercetin and chlorogenic acid in adventitious roots harvested from pilot scale bioreactors was also confirmed by LC–MS/MS. The quantification results

of phenolics and flavonoids in adventitious roots were found to be much higher than the field-grown plants (Cui, 2011). These results corroborate the effectiveness of large scale cultivation of *H. perforatum* adventitious roots for rapid and enormous production of valuable secondary metabolites.

6.4. Adventitious root cultures of *Panax ginseng*

Ginsenosides are triterpene saponins that are mostly found in ginseng. So far, more than 150 naturally occurring ginsenosides have been isolated from different types of ginsengs (Christensen, 2008), in which major types are Korean ginseng (*P. ginseng* C. A. Meyer), American ginseng (*P. quinquefolius* L.) and Sanchi (*P. notoginseng*). Of these, *P. ginseng* has high dammarane-type ginsenoside content, which corroborates its higher biological activity than that of other ginseng (Sivakumar et al., 2011). For isolation and purification of these ginsenosides, several efficient methodologies have been established (Engelberth et al., 2010; Qi et al., 2010). Preclinical and clinical studies on ginseng documented that ginsenosides have anticancer (Wang et al., 2009), antihyperglycemic and thermogenic activity (Yang et al., 2010), activation of insulin-independent signaling by Rc type ginsenoside (Lee et al., 2010) and inhibition of pancreatic lipase activity (Liu et al., 2008). Moreover, Rg₃ type ginsenoside is involved in improving insulin signaling and glucose uptake by modulating the expression of IRS-1 and GLUT4 (Kim et al., 2009), and Re type ginsenoside has significant antioxidant efficacy against diabetic microvasculopathy (Cho et al., 2006).

Due to vast potentiality of ginsenosides for biopharmaceutical application, remarkable efforts have been paid in the research and plant factories for the development of efficient methodologies with the view of commercial scale production of ginseng cells and hairy roots. For instance, an industrial scale ginseng cell culture process was initiated in the 1980s at the Nito Denko corporation (Ibaraki, Osaka, Japan) using 2000 and 20,000 L stirred tank reactors (STRs) to produce ginseng biomass. Studies on ginseng cells in STRs claimed that the agitator design and the agitation rate are major factors affecting cell growth and saponin production (Furuya et al., 1984). Alternatively, adventitious root cultures of *P. ginseng* in BTBB regarded as a promising alternative approach due to its fast growing ability, lack of toxicity, easy to harvest and simple to purify compared to cells, hairy roots or in vivo plant products. Understanding from our long-term research efforts and by employing a series of experiments iteratively, we have succeeded in establishing adventitious root (callus-derived) culture system of *P. ginseng* using pilot scale (500–1000 L BTBB and drum type BB) and commercial scale (10,000 L) BTBB (Choi et al., 2000; Paek et al., 2005, 2009; Yu et al., 2000, 2001).

The optimization of growth regulators, organic nutrients, sucrose, nitrogen source, growth kinetics and medium replenishment strategy for enhancing ginsenoside production in different capacities air-lift bioreactors have been reported by several authors (Ali et al., 2005; Jeong et al., 2009a; Kim et al., 2003, 2004; Sivakumar and Paek, 2005; Sivakumar et al., 2005; Yu et al., 2002). In addition, gaseous composition especially O₂, CO₂ and C₂H₄ was also showed profound effect on root growth and metabolite accumulation. The results of gaseous composition have reflected that a 40% O₂ supply was found beneficial for enhancing accumulation of root biomass and biosynthesis of ginsenosides, whereas elevated levels of CO₂ and C₂H₄ were unfavorable to the adventitious root cultures due to their deleterious effect on ginsenosides biosynthesis (Jeong et al., 2006, 2008).

Various attempts such as elicitation of MeJa (Ali et al., 2006a; Kim et al., 2007; Yu et al., 2002), SA and Cu (Ali et al., 2006b, 2007, 2008), linoleic acid (LLA) and α -linolenic acid (α -LNA) (Dewir et al., 2010; Wu et al., 2009) and adaptation of two-stage culture system have been employed to increase ginsenoside yield. The exogenous addition of 100 μ M MeJa was found effective for enhancing ginsenoside biosynthesis but strongly inhibited root growth (Kim et al., 2004; Yu et

al., 2002). However, combination of 25 μ M IBA with 100 μ M MeJa synergistically stimulated both root growth and ginsenoside accumulation compared with 100 μ M MeJa alone. These results suggest that the addition of IBA with MeJa is beneficial to alleviate the inhibitory effect of MeJa on adventitious root growth of ginseng. Moreover, two-stage culture system was also adopted, in which adventitious roots exposed to MeJa significantly stimulated (7-fold) yield of total ginsenosides with differential accumulation of Rb and Rg group compared to no elicitation (Kim et al., 2007). Interestingly, when adventitious root cultures were elicitor treated with α -LNA, production of both root biomass and total ginsenosides (diol and triol groups) increased. This treatment also enhanced nutritive properties of ginseng roots by inducing biosynthesis of essential polyunsaturated fatty acids such as LLA and α -LNA (Dewir et al., 2010; Wu et al., 2009).

Co-culture of interspecies is often effective and interesting phenomenon, and therefore getting renewed interest for the production of wide array of plant secondary metabolites. Because co-culture offers advantages for metabolite production by one organ, which in turn to be excreted in the medium and absorbed by another organ for further biochemical conversion. For instance, co-cultivation of *Cephaelis* and *Mikania* cells stimulated coumarin content, but inhibited the cell growth of *Mikania*. Whereas, interaction between *Mikania* and *Maytenus* cells resulted in increased biomass production of *Maytenus* cells but the friedelin content was declined, suggesting a possible occurrence of allelopathy in this system (Pereira et al., 2000). With respect to this view point, Wu et al. (2008) have established adventitious root co-culture system of *P. ginseng* and *E. purpurea* for the production of ginsenosides and caffeic acid derivatives. Co-cultivation of *Echinacea* root showed negative impact on ginseng adventitious root growth and ginsenoside accumulation, when the inoculum proportions of *Echinacea* roots were enhanced with co-cultures. However, iterative experimental efforts have made the co-culture system successful to produce copious amount of ginsenosides and caffeic acid derivatives by establishing co-culture with higher inoculum proportion of ginseng to *Echinacea* (4:1 and 3:2) followed by elicitor treatment of 200 μ M MeJa after 30 days of culture initiation.

Based on those extensive research efforts, an automated commercial scale bioreactor technology was developed by CBN Biotech Company, South Korea (<http://www.cbnbiotech.com>) for high density cultivation of root biomass and ginsenosides. Since 2002, CBN biotech has been manufacturing ginseng adventitious roots using several 10,000 L bioreactors (Fig. 3G–I) with an average production of 45 tons (FW) per year as per good manufacturing practice guidelines.

7. Conclusion

Advancement of biotechnological research has made adventitious root culture as an attractive alternative to whole plant cultivation for the production of pharmacologically important compounds and has made great strides building on advances in plant sciences. The results of our studies have demonstrated that the use of bioreactor has led to the development of a technology suitable for large scale cultivation of adventitious roots of various plant species. For commercialization of ginseng and *Echinacea* adventitious roots, as well as to elucidate the feasibility for commercial application of other valuable medicinal plants, large scale cultures have been achieved using air-lift bioreactors by determining various factors affecting the production of root biomass and bioactive molecules. However, due attention must be given to the biological and engineering parameters related to the growth and secondary metabolite production by adventitious roots in bioreactor culture. Optimization of medium ingredients by statistical techniques, application of appropriate mathematical models for optimized adventitious root cultivation, feeding strategy of metabolic precursors, exploitation of potential elicitors, and extraction of intercellular metabolites by organic solvents can lead to significant enhancement in productivity of secondary metabolites. Furthermore,

knowledge of biosynthetic pathways of desired compounds in adventitious roots is still in its infancy, and consequently, the understanding of the regulation on secondary metabolic pathways involved on the levels of products, enzymes and genes, including aspects as transport and compartmentation is required.

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