Large scale cultivation of plant cell and tissue culture in bioreactors

Ozlem Yesil-Celiktas, Aynur Gurel and Fazilet Vardar-Sukan

Department of Bioengineering, Faculty of Engineering, Ege University
35100 Bornova-Izmir, Turkey

1. Introduction

Bioactive compounds extracted from plants are widely used. The natural habitats for a large number of plants are rapidly destroyed leading to extinction of many valuable and even endemic species. Studies on the production of plant metabolites by callus and cell suspension cultures have been carried out on an increasing scale since the end of the 1950's. The prospect of using such culturing techniques is for obtaining secondary metabolites, such as active compounds for pharmaceuticals and cosmetics, hormones, enzymes, proteins, antigens, food additives and natural pesticides from the harvest of the cultured cells or tissues.

The large scale cultivation of tobacco and a variety of vegetable cells was examined from the late 1950's to early 1960's initiating more recent studies on the industrial application of plant cell culture techniques in many countries.

The first patent for the cultivation of plant tissue was received in 1956. Shortly afterwards, the National Aeronautics and Space Administration (NASA) started to support research in the field of plant cell cultures for regenerative life support systems (Sajc et al., 2000).

Correspondence/Reprint request: Dr. Ozlem Yesil-Celiktas, Department of Bioengineering, Faculty of Engineering, Ege University, 35100 Bornova-Izmir, Turkey. E-mail: ozlemyesil@gmail.com
In parallel to these achievements, industrial companies in Japan have tried to apply this technology for the commercial production of useful compounds in collaboration with some university groups. For instance, The Japan Tobacco Inc.’s interest involved around mass-production of tobacco cells as raw materials of cigarettes. Meiji Seika in Japan also elucidated the fundamentals of production of *Panax ginseng* cells in large volumes. The work was followed by Nitto Denko Co. which started manufacturing cell mass of ginseng commercially. Other firms such as Ajinomoto and Nippon Shin-yaku also made efforts to increase the level of accumulation of alkaloids, steroids and other secondary products in cultured cells (Misawa, 1994). Consequently, plant cell culture techniques have been used increasingly in basic research to improve cognitive interrelations and finally for exploitation in industry.

The field of plant cell culture covers diverse sub-fields providing alternative approaches which may be attractive under certain circumstances; if the source plant:

- is difficult to cultivate,
- has a long cultivation period,
- produces a commercially significant compound that can not be chemically synthesized in large scale,
- has a low metabolite of the compound of interest.

Furthermore, in some cases mass cultivation of the source plant may not be possible under natural conditions due to environmental, ecological or climatic conditions and/or insufficient agricultural lands may limit the cultivation processes. Secondary metabolites can be produced by plant cell and tissue culture techniques under controlled and reproducible conditions, independent of geographic and climatic conditions.

Biotechnological cultivation of plant cells and tissues involve two major methodologies namely, cell culture studies and clonal propagation techniques. Cell culture studies begin with callus initiation using *in vitro* cultures for the purpose of determining the medium that best adapts for cultivation. When calli are obtained, they can undergo somaclonal variation, usually during several subcultures (Gurel, 1989). When genetic stability is reached, callus lines need to be screened in order to evaluate the productivity of each cell line so that the best performing lines can be taken to cell suspensions. Various approaches can be used to increase the production of secondary metabolites in cell suspensions but elicitation is usually one of the most successful. The final step is the bioreactor studies leading to a possible commercial production of secondary metabolites. This
is a critical step as various problems can arise when scaling-up from shake flasks to bioreactors.

However bioreactors act as a biological factory for the production of high-quality products and provide many advantages listed as follows (Tautorus et al. 1991; Fulzele, 2000; Su, 2006).

- controlled supply independent of plant availability,
- increased working volumes,
- homogeneous culture due to mechanical or pneumatic stirring mechanism,
- better control of cultural and physical environment, therefore easy optimization of growth parameters such as pH, nutrient media, temperature, etc. for achieving metabolite production,
- reproducible yields of end product under controlled growth conditions,
- enhanced nutrient uptake stimulating multiplication rates and yielding a higher concentration of yield of bioactive compounds,
- simpler and faster harvest of cells,
- the opportunity to perform biosynthetic and/or biotransformation experiments related to metabolite production with enzyme availability.
- easier separation of target compounds because of lower complexity of extract,
- better control for scale-up,

Successful operation of these bioreactors often requires the implementation of aeration technologies which constantly need to be improved. Glass and stainless steel bioreactors incorporating impellers are often used for the production of cell and/or tissue culture at industrial scale. However, it is of prime importance to design bioreactor configurations which can provide adequate mixing and mass transfer while minimizing the intensity of shear stress and hydrodynamic pressure. Thus, problems arise when scaling up from pilot plant fermentors to large scale bioreactors.

As for the clonal propagation of a plant in large scale, regeneration from cells belonging to an explant of plants is potentially very useful. Liquid media have been used for plant cells, somatic embryos, and organ cultures in both agitated flasks or various types of bioreactors. Scale up of plant regeneration in a liquid medium is easier than on a solid medium (Okamoto et al. 1996). Although the use of bioreactors has been directed mainly for cell suspension cultures and secondary metabolite production, research directed at improving bioreactors for somatic embryogenesis has been reported for several plant species as well. Utilization of bioreactors for clonal propagation
has been attracting interest recently due to scale-up and automation advantages (Gurel, 2009). Bioreactors have also been used for the cultivation of hairy roots mainly as a system for secondary metabolite production (Ziv, 2000). Furthermore, innovative processes have been proposed for producing secondary metabolites selectively by enzymatic reactions (Takemoto, 2009; Abraham et al., 2005).

In spite of all these advantages, cell culture techniques have not been effectively exploited in industrial scale bioreactors due to the slow growth rates of plant cell cultures and the high capital costs involved and have been limited to a handful of applications such as the production of shikonin from *Lithospermum erythrorhizon*, berberine from *Coptis japonica* and ginsenosides from *Panax ginseng* (Kieran et al., 1997). Phyton Biotech located in Germany which is another good example of industrial exploitation, owns and operates the world's largest cGMP plant cell culture facility with bioreactors up to 75000 L in size and specifically designed to meet the need of plant cells in culture. The total production capacity of the Taxanes, one of its products, runs up to 880.000 Litres/year (Phyton Biotech, 2006). On the other hand, the large scale commercial propagation of plant material based on plant tissue culture was pioneered in the USA. More recently, some companies from Israel, USA and UK have shifted their production facilities to developing countries.

The aim of this book is to critically outline the recent developments in large scale cultivation of plant cell and tissue cultures by presenting scale-up studies and state-of-the-art technologies, while focusing on different cell culture applications and bioreactors. Moreover, a SWOT (Strengths, Weaknesses, Opportunities, Threats) analysis has been carried out to evaluate the methodologies intended for scale-up.
2. Bioreactor considerations

Plant cell suspension, organ cultures and hairy roots are potential sources of secondary metabolites and recombinant proteins. As they are produced under controlled conditions in bioreactors, variations in product yield and quality are minimized and consequently product registration process is simplified.

Different bioreactor designs are available providing the optimum environment for effective cell growth and secondary metabolite production. The bioreactors used for various plant cell cultures are classified as mechanically agitated, pneumatically driven, disposable and non-agitated (Fig. 1).

The most common bioreactor is the stirred tank bioreactor and sufficient know-how exists about the design. In order to minimize the shear forces, numerous modifications have been developed by employing a variety of impeller designs and seals (Fig. 1 A1). Examples of varying capacities from 1–2 L lab scale to 20,000 L industrial scale systems exist in the literature. For instance, biomass with a density of 6.3 g dry weight L⁻¹ was produced from *Acer pseudoplatanus* using a 20 L stirred tank bioreactor with a magnetic stirrer which is regarded as safer than the mechanical stirrers against contamination risks (Asenjo and Merchuk, 1995). Despite their popularity, stirred tank bioreactors have several limitations, such as high power consumption, high shear, and problems with sealing and stability of shafts in tall bioreactors. Horizontal vessels, or rotary drum reactors (Fig. 1 A2) have significantly higher surface area to volume ratio than other reactor types. As a consequence, mass transfer is achieved with comparably less power consumption. Rotary drum reactors used for the cultivation of high-density plant suspensions have shown advantages in terms of suspension homogeneity, low shear environment and reduced wall growth, over either airlift or stirred tank reactors (Saje et al., 2000). However, the disadvantage is their comparatively high energy consumption in industrial scale operations.

A bubble column bioreactor (Fig. 1 B1) is a reactor, in the shape of a column, in which the reaction medium is kept mixed and aerated by the introduction of air at the bottom (IUPAC, 1997). The main advantages of bubble column bioreactors are the low capital costs, uncomplicated mechanical configurations and not too high operational costs due to low energy requirements. On the other hand, they are less suitable for the processes where highly viscous liquids exist. An airlift bioreactor (Fig. 1 B1-5) is defined as a bioreactor in which the reaction medium is agitated and aerated by the introduction of air or another gas mixture and the circulation is
Figure 1. Depictions of bioreactor types for plant cell and tissue cultures. (A) Mechanically agitated bioreactors; (1) stirred tank reactor equipped with various propellers (spin, helix, bladed and paddle), (2) rotary drum tank reactor, (B) Pneumatically driven bioreactors; (1) bubble column, (2) internal loop airlift, (3) external loop airlift, (4) propeller loop, (5) jet loop, (C) Hydrodynamically driven disposable bioreactors; (1) Wave&Undertow bioreactor, (2) Biowave reactor, (D) Non-agitated bioreactors; (1) packed bed, (2) fluidized bed, (3) membrane reactor, (Adapted from Sajc et al., 2000 and Eibl and Eibl, 2008).
enhanced by internal draught tubes or external loops. Thus, the reactor volume is separated into gassed and ungassed regions generating a vertically circulating flow (IUPAC, 1997). Airlift bioreactors can supply the low O₂ demands of plant cell cultures with low shear effects. Breuling and coworkers (1985) reported the production of protoberberine from *Berberis wilsonae* using 20 and 200 L airlift bioreactors. Maximum biomass density was stated as 23 g dry weight L⁻¹ in 200 L reactor. Airlift bioreactors have several advantages such as combining high loading of solid particles, providing good mass transfer, relatively low shear rate, low energy requirements, and simple design. The major drawback is their unsuitability for high density plant cultures. Stirred tank bioreactors are therefore preferable for culturing plant cell suspensions at high densities (Tanaka, 2000).

It is also worth mentioning the use of disposable bioreactors such as the Wave&Undertow bioreactors (Fig. 1 C1) and the Biowave (Fig. 1 C2) which are modern alternatives to traditional cultivation systems. These bioreactors consist of a sterile plastic chamber that is partially filled with media (10–50%), inoculated with cells and discarded after harvest. Two novel flexible plastic-based disposable bioreactors have been reported lately. The first one, was based on the principle of a wave and undertow mechanism that provided agitation while offering convenient mixing and aeration to the plant cell culture contained within the bioreactor. The second one was a high aspect ratio bubble column bioreactor, where agitation and aeration were achieved through the intermittent generation of large diameter bubbles, "Taylor-like" or "slug bubbles". It was possible to increase the volume from a few liters to larger volumes up to several hundred liters with the use of multiple units. The cultivation of tobacco and soya cells producing isoflavones was described up to 70 and 100 L working volumes for both types. Cleaning, sterilization, and maintenance operations were reduced or eliminated with the disposable bioreactors (Terrier et al., 2007).

Although packed bed (Fig. 1 D1) and membrane reactors (Fig. 1 D3) are advantageous in terms of immobilizing large amounts of cells per unit volume, diffusional limitations of mass transfer and difficulties in handling gaseous components can limit the use of both configurations. (Sajc et al., 2000).

*Table 1* summarizes the major studies involving plant cell and tissue cultures with different types of reactors. As can be seen from literature, plant cell and tissue cultures have been cultivated in reactors of various designs and configurations in scales ranging between 0.25 and 14 L.
2.1. Production of secondary metabolites by plant cell suspension cultures

The main advantages associated with in vitro plant systems include the manipulation of environmental conditions, the rapidity of production, and the use of simpler and cheaper downstream processing schemes for product recovery from the culture medium (Doran, 2000). Plant cell culture is a flexible system that is easily manipulated to increase product yields (Roberts and Shuler, 1997). Cultures of plant cells are not limited by environmental, ecological or climatic conditions (Zhong et al., 1995). Suspended cells with high embryogenic potentials should be selected to maintain a stable production of quality somatic embryos (Ibaraki and Kenji, 2001). Plant cell cultures combine the merits of whole-plant systems with those of microbial and animal cell cultures for the production of valuable therapeutic secondary metabolites (Hellwing et al., 2004).

Recent progress in molecular biology, enzymology, physiology and process technology of plant cell cultures suggest that these systems will become a viable source of important natural products (Dicosmo and Misawa, 1995).

The capability to cultivate plant callus cells and organs in liquid media has also made an important contribution to modern plant biotechnology with respect to the production of commercially valuable compounds (Su and Lee, 2007). Callus cells obtained from the transgenic plants can be grown in simple, chemically defined liquid media to establish transgenic cell suspension cultures for recombinant protein production (Su, 2006).

Furthermore, the use of whole plants for the synthesis of recombinant proteins has received a great deal of attention recently due to advantages in economy, scalability and safety compared with traditional microbial and mammalian production systems. However, plant cells generally have a longer doubling time than bacterial or yeast cells. Genetic instability associated with de-differentiated callus cells due to somaclonal variation is a potential disadvantage in using cultured plant cells for recombinant protein production (Su, 2006).

Oxygen transfer, fluid mixing and the magnitude of the acceptable shear rates are the primary parameters in choosing a suitable bioreactor. For successful cultivation of plant cell suspensions, the bioreactor has to provide good mixing at low shear stress levels with a moderate oxygen supply. Alternative designs to conventional stirred tank bioreactors have led to the use of different impellers for the cultivation of plant cell suspensions. For instance, helical impellers proved to be low shear and provided good mixing with viscous non-Newtonian fluids (Cormier et al., 1996). Indeed, the
majority of plant cell culture studies in laboratory scale which have provided basic information for large scale cultivation have been stirred tank bioreactors equipped with different impellers. Usage of airlift bioreactors were reported for the suspension cultivation of Frangula alnus cells to produce anthraquinones (Sajc et al., 1995) and for plantlet production by Oxalis triangularis (Teng & Ngai, 1999). Cell suspensions of Lilium Oriental Hybrid were cultivated in balloon type bubble bioreactors to produce bulblets (Lian et al., 2003).

The adoption of plant cell cultures as an industrial process depends greatly on the economics of such a process. The multicycle or draw-fill culture technique offered by Lipsky (1992) is one method for improving the productivity and, hence, cost of a process. Mathematical models have been devised for the functional relationships between the nominal costs of biomass and secondary metabolites and the plant cell growth characteristics in a multicycle growth system. The models were used to evaluate the data obtained with cultures of Dioscorea deltoidea (which produces diosgenin) and Panax ginseng, grown in various types of bioreactors. The multicycle system gave an increase of 1.5–2 in biomass productivity compared with batch culture, but was probably only commercially viable if the cost of the process in the bioreactor was at least 30 times that of the medium and if an inoculum of about 30% of the culture of the previous cycle was left in the bioreactor. In the multicycle system incompletely utilized nutrient or metabolite accumulation can only reach 1.43 times or less that of the initial values. With the P. ginseng culture, about 75% of the calculated maximum cell packing density per fresh weight (≈ 530 g l\(^{-1}\)) in this regime was achieved. The possibility of growth in the standard bioreactor of a shear sensitive type culture was shown with a marine impeller speed up to 330 cm s\(^{-1}\).

2.2. Production of secondary metabolites by organ cultures

Regeneration is provided by two morphogenic pathways: organogenesis (the formation of unipolar organs) and somatic embryogenesis (the production of bipolar structures, somatic embryos with a root and a shoot meristem). When plant somatic cells are isolated and cultured in vitro conditions, they are capable of expressing totipotency. The injured cells in the outer layers of the isolated explant evolve ethylene that induces dedifferentiation. Cell division can occur in an unorganized pattern with callus formation, or in an organized pattern with the formation of meristematic centers directly in the explant tissues. Meristematic centers form directly on the explant in some plants and can develop into either
shoots, roots or somatic embryos. Organ cultures are used for secondary metabolite production as well as for plant propagation.

2.2.1. Shoots

The development of spherical meristematic or bud clusters in liquid cultures was shown to provide a highly proliferative and rapid growing system amenable to automated inoculation, control of the medium components, mechanical separation, and efficient delivery to the final stage for plant growth and development (Ziv, 2000).

There are few examples in the literature focusing on secondary metabolite production from shoot cultures. Shoot cultures of *Spathiphyllum cannifolium* (Dewir et al., 2007) and *Stevia rebaudiana* (Sreedhar et al., 2008) were carried out in bubble column bioreactors in order to produce flowers and biomass, respectively. Organ culture of *Lavandula officinalis* was cultivated in 5 L bubble column bioreactor to obtain rosmarinic acid (Wilken et al., 2005). Moreover, shoot culture of *Ananas comosus* was performed in a 10 L airlift bioreactor (Firoozabady & Gutterson, 2003) and organ culture of *Hypericum perforatum* in 2 L stirred tank bioreactor to produce hypericin (Wilken et al., 2005) (Table 1). The necessity to expose shoot cultures to light can be a problem with large stirred tank bioreactors made from steel (Bourgaud et al. 2001).

2.2.2. Hairy roots

Hairy roots grow rapidly, show plagiotropic growth, and are highly branched on phytohormone-free medium (Hu and Du, 2006). *Agrobacterium rhizogenes*-derived hairy roots and plants have application for many different areas. Hairy root cultures have been tested extensively in root nodule research, for production of plant secondary metabolites (Christey, 2001). Transformed roots have been widely studied for *in vitro* production of secondary metabolites in many plant species and for artificial seed production. Hairy root cultures produce secondary metabolites over successive generations without losing genetic or biosynthetic stability (Giri ve Narasu, 2000). Many aspects of plant secondary metabolite biosynthesis have been studied using transformed root cultures (Kuzovkina and Schneider, 2006). The strong corelation between secondary metabolite production and morphological differentiation gives more impetus to the application of organized cell cultures for large scale production of phytochemicals.

Intergeneric co-culture of genetically transformed hairy roots and shooty teratomas is effective for improving tissue specific secondary metabolites.
Table 1. Laboratory scale plant cell and tissue culture studies useful for large scale cultivation in different types of bioreactors.

<table>
<thead>
<tr>
<th>Bioreactor</th>
<th>Plant source</th>
<th>Volume (L)</th>
<th>Type of culture</th>
<th>Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stirred tank</td>
<td><em>Elaeis guineensis</em> (oil palm)</td>
<td>0.3</td>
<td>somatic embryogenesis</td>
<td>biomass</td>
<td>Gorret et al., 2004</td>
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<tr>
<td></td>
<td><em>Eschscholtzia californica</em></td>
<td>1</td>
<td>cell suspension</td>
<td>biomass</td>
<td>Tatieck et al., 1990</td>
</tr>
<tr>
<td></td>
<td><em>Cyclamen persicum</em></td>
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<td>somatic embryogenesis</td>
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<td></td>
<td><em>Hypericum perforatum</em></td>
<td>2</td>
<td>organ culture</td>
<td>hypericin</td>
<td>Wilken et al., 2005</td>
</tr>
<tr>
<td></td>
<td><em>Taxus chinensis</em></td>
<td>2.5</td>
<td>cell suspension</td>
<td>taxoid</td>
<td>Zhong et al., 2002</td>
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<td></td>
<td><em>Daucus carota L.</em></td>
<td>3</td>
<td>cell suspension</td>
<td>growth &amp; differentiation</td>
<td>Jay et al., 1992</td>
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<td></td>
<td><em>Azadirachta indica</em></td>
<td>3</td>
<td>cell suspension</td>
<td>biomass &amp; azadirachtin</td>
<td>Prakash &amp; Srivastava, 2007</td>
</tr>
<tr>
<td></td>
<td><em>Azadirachta indica</em></td>
<td>3</td>
<td>cell suspension</td>
<td>azadirachtin (biopest.)</td>
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<td></td>
<td><em>Thalictreum rugosum</em></td>
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<td>high density cell suspension</td>
<td>berberine</td>
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<td><em>Picea stichensis</em></td>
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<td>somatic embryogenesis</td>
<td>germinated embryos</td>
<td>Ingram &amp; Mavituna, 2000</td>
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<td><em>Panax ginseng</em></td>
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<td>adventitious root</td>
<td>ginsenoside</td>
<td>Jeong et al., 2006</td>
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<td></td>
<td><em>Picea mariana</em></td>
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<td>biomass</td>
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<td>cytoplasmic esterase</td>
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<td><em>Anas comosus L.</em></td>
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<td>scopoline, hyoscyamine &amp; tropine alkaloids</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td><em>Picea stichensis</em></td>
<td>5</td>
<td>somatic embryogenesis</td>
<td>germinated embryos</td>
<td>Ingram &amp; Mavittana, 2000</td>
</tr>
<tr>
<td></td>
<td><em>Ananas comosus</em></td>
<td>10</td>
<td>shoot</td>
<td>shoots for propagation</td>
<td>Firoozabady &amp; Gutterson, 2003</td>
</tr>
<tr>
<td>Glass culture vessel</td>
<td><em>Ruta graveolens</em> L.</td>
<td>5</td>
<td>micropropagation</td>
<td>shoots</td>
<td>Diwan &amp; Malpathak, 2008</td>
</tr>
<tr>
<td>Mist</td>
<td><em>Stizolobium hassjo</em></td>
<td>9</td>
<td>hairy root</td>
<td>3,4-dihydroxy phenylalanine</td>
<td>Huang et al., 2004</td>
</tr>
</tbody>
</table>
Figure 2. Hairy root cultures actively growing in hormone-free MS medium in *Rubia tinctorum* L. (Cetin et al. 2005).

This mimics the situation observed in the whole plant where a localized metabolite synthesis is translocated throughout the organs for further bioconversion (Giri and Narasu, 2000). Also, production of two different secondary metabolites is possible simultaneously by adventitious root co-cultures (Wu et al., 2008).

This vast potential of hairy root cultures (Fig. 2) (Cetin et al. 2005) as a stable source of biologically active chemicals has provided the exploitation of *in vitro* system through up scaling in novel bioreactors (Mehrotra et al., 2008).

Hairy root cultures of *Lithospermum erythrorhizon* (Sim & Chang, 1993), *Harpagophytum procumbens* (Ludwig-Muller et al., 2008) and adventitious roots of *Panax ginseng* (Yu et al., 2002; Jeong et al., 2008) and *Scopolia parviflora* (Min et al., 2007) were studied in various volumes of bubble column bioreactors to obtain shikonin, harpagide, ginsenosides and alkoloids respectively. Ginsenoside was also produced in 5 L stirred tank bioreactor using adventitious root culture (Jeong et al., 2006). Hairy root culture of *Stizolobium hassjo* to yield 3,4-dihydroxyphenylalanine was reported using 9L mist bioreactor (Table 1). Present scale-up technology dictates the use of stainless steel tanks for growth of plant cells on an industrial scale. The usage of bioreactors equipped with special hangers inside the vessel is
reported (Rhodes et al., 1989). Hairy root cultures continue to attract interest as a potential resource for large-scale production of commercially valuable compounds.

2.2.3. Somatic embryogenesis

Somatic embryogenesis has been considered to be a distinct developmental pathway, different from either shoot or root organogenesis, in which a single cell gives rise to a structure containing bipolar meristems and with no direct vascular connections to the maternal tissue (Brown et al., 1995). Somatic embryogenesis is also an alternative method for the production of certain metabolites (Tejavathi et al. 2007) and has been regarded as an especially suitable system for the investigation of various morphological and physiological phenomena (Hirai et al. 1997).

In most cases the somatic embryos or the embryogenic cultures can be cryopreserved, which makes it possible to establish gene banks. Embryogenic cultures are also an attractive target for genetic modification (Von Arnold et al. 2002). Plant regeneration from cell suspension cultures offers a suitable in vitro system for the mass propagation of economically important plants. If this could be scaled up to fit bioreactor design, it could become an excellent tool for the large-scale production of somatic embryos that would greatly aid the commercial production of artificial seeds (Patnaik et al. 1997).

The ability to perform all the stages of somatic embryogenesis, from the growth of embryogenic cells to embryo maturation, in liquid media in bioreactors is essential for the successful scale-up of the process (Mavituna and Buyukalaca, 1996).

2.3. In vitro propagation

Propagation of some commercial plants which are difficult to reproduce conventionally by seed or vegetative propagules conventionally is realized by in vitro tissue culture techniques. Plant regeneration can follow the organogenic or embryogenic pathways and is dependent on the manipulation of the inorganic and organic constituents in the medium, the type of explant and the species. In most plants, subcultures in various media are very important for successful regenerations from the callus or directly from the explants (Ziv, 2000).

Four basic methods are used to propagate plants in vitro. Depending on the species and cultural conditions, in vitro propagation can be achieved by:

- enhanced axillary shoot proliferation,
- node culture,
Large scale cultivation of plant cell and tissue culture in bioreactors

- *de novo* formation of adventitious shoots through shoot organogenesis,
- nonzygotic embryogenesis (Trigiano and Gray, 2000).

Micropropagation offers the advantages in quality, quantity and economics over conventional vegetative propagation for many species providing an efficient method for production of a large number of uniform plantlets in a short time (Altman and Colwell, 1997). Furthermore, they enable the rapid propagation of valuable genotypes, production of disease-free plants, non-seasonal production throughout the year and germplasm conservation (Gurel, 2009). The parameters affecting vegetative regeneration such as nutrient and growth regulator levels, light and temperature are adjustable and flexible. Plant propagation and stock plant maintenance require less energy and space (George et al., 2007). Exploring micropropagation to solve special problems connected with selective breeding is expected to prove to be beneficial in the future (Jha and Ghosha, 2005). The other advantages of micropropagation are production of large stocks of true-to-type propagation material as well as a large number of plants from elite or difficult-to-grow and slow-to-grow plants, bringing new plants to the market rapidly, easier transportation of plant material and conservation of plant genetic resources (Trigiano and Gray, 2000; Scragg, 2007).

Clonally propagated plants are suitable materials to improve cultural practices for commercial productions (Jain and Ishii, 2003). Techniques used to propagate commercially important plants *in vitro* are based on axillary branching, adventitious shoot formation and somatic embryogenesis.

Micropropagation by conventional techniques is typically a labour-intensive means of clonal propagation. The work by Paek et al. (2005) describe lower cost and less labour-intensive clonal propagation through the use of modified air-lift, bubble column, bioreactors (a balloon-type bubble bioreactor), together with temporary immersion systems for the propagation of shoots, bud-clusters and somatic embryos. Propagations of *Anoectochilus*, apple, *Chrysanthemum*, garlic, ginseng, grape, *Lilium*, *Phalaenopsis* and potato were described. Studies are being carried out along these lines to improve the productivity and cost efficiency of *in vitro* propagation techniques.

### 2.3.1. Propagation by shoot cultures

Propagation from axillary shoots has proved to be a reliable method for the micropropagation of a large number of species, although not the most efficient procedure. Most commercially micropropagated plants are derived from axillary shoot formations. (Gurel, 2009) and the major application of
shoot cultures is in plant propagation. Compared to other micropropagation methods, shoot cultures:

- provide reliable rates and consistency of multiplication following culture stabilization,
- are less susceptible to genetic variation,
- may provide for clonal propagation of periclinical chimeras (Trigiano and Gray, 2000).

Depending on the species, two methods, shoot and node culture, are used. Both methods rely on the stimulation of axillary shoot growth from lateral buds following disruption of apical dominance of the shoot apex. The axillary shoots produced are either subdivided into shoot tips and nodal segments that serve as secondary explants for further proliferation or are treated as microcuttings for rooting. Shoot culture (shoot tip culture) refers to the in vitro propagation by repeated enhanced formation of axillary shoots from shoot tips or lateral buds cultured on medium supplemented with growth regulators, usually a cytokinin. Node culture, a simplified form of shoot culture, is another method for production from preexisting meristems. Although node culture is the simplest method, it is associated with the least genetic variation (Trigiano and Gray, 2000).

Currently the most frequently used micropropagation method for commercial production utilizes enhanced axillary shoot proliferation from cultured meristems. This method provides genetic stability due to the highly organized structure and is easily attainable for many plant species.

Besides propagation, shoot meristems are cultured in vitro for two other purposes: production of pathogen eradicated plants and preservation of pathogen eradicated germplasm. Shoot growth in mature plants is restricted to specialized regions which exhibit little differentiation and in which the cells retain the embryonic capacity for unlimited division. These regions, called apical meristems, are located in the apices of the main and lateral buds of the plant. Cells derived from these apical meristems subsequently undergo differentiation to form the mature tissues of the plant body. Rejeneration of shoots from meristem tips are often used as initial explants for large-scale micropropagation (Pérez-Torner et al. 1999; Trigiano and Gray, 2000; Vila et al. 2002; Goleniowski, et al. 2003; Prehn et al. 2003; Wang et al. 2003; Hosokawa et al. 2004; Verma et al. 2004).

Scaling-up in bioreactors requires the use of liquid instead of agar-gelled media during the proliferation and biomass production stages. This introduces some problems. The culture of plants in liquid medium is known
to cause anomalous morphogenesis, resulting in plant hyperhydricity. The plants that develop in liquid media are fragile, have a glassy appearance, with succulent leaves or shoots and a poor root system. The leaves are the organs that are affected most severely in liquid cultures. The two major processes carried out by the leaves, photosynthesis and transpiration, are not fully functional in hyperhydric leaves and thus cause the poor performance of the transplanted plants *ex vitro* (Ziv, 2000). Another study dealing with the commercial micropropagation was smooth Cayenne pineapple. *In vitro* shoots were used as starting materials, and either longitudinal sections of the shoots or leaf bases were used as the explants to regenerate shoots. When these explants were used, the axillary meristems, which usually remain quiescent during shoot multiplication, were able to form new shoots. Subsequent to the regeneration step, additional multiplication was achieved inside a 10 L periodic immersion bioreactor with shoots immersed in liquid medium for 5-10 min/h (Firoozabady et al., 2003). Currently studies are conducted to overcome these difficulties in shoot culture systems.

Although a number of possible scale up techniques for shoot culture in bioreactors have been described and the mass propagation of shoots of *Stevia rebaudiana* was reported using a bioreactor of 500 L volume (Akita et al. 1994), efficient techniques for the mass propagation of shoots are needed for the production of disease free seed plants.

### 2.3.2. Propagation by hairy root cultures

Hairy root explants can also be propagated clonally and such hairy root lines facilitate studies of plant responses during early stages of mycorrhization. In addition to the speed and ease with which *Agrobacterium rhizogenes* transformed hairy roots can be generated, a valuable characteristic of such roots is that they can be propagated clonally as independent organs, with a vigorous growth that does not require the addition of plant hormones. Furthermore, these root explants have the potential to be colonized by endomycorrhizal fungi *in vitro* (Boisson-Dernier et al. 2001).

Another alternative strategy for evaluating of transformed roots is the production of composite plants. These are generated by inducing transformed roots on non-transformed shoots. These have been widely used in legumes as they represent an ideal system for analyses of gene expression involving infection of rhizobia and nitrogen fixation (Christey, 2001).

*A. rhizogenes* can transfer T-DNA from binary vectors and enable the production of transgenic plants containing foreign genes carried on a second plasmid. This character has been used to produce transgenic plants
(Hu and Du, 2006). *A. rhizogenes*-mediated transformation has also the potential to introduce foreign genes specifically into the root system, such as resistance to root pathogens, pests, resistance to heavy metals. Hairy root cultures represent an ideal system to study root function, the effects of root pathogens and to test genes related with pathogen resistance (Christey, 2001). Transformed hairy roots are very important materials for remediation of soil, groundwater, and biowastes (Kuzovkina and Scheider, 2006).

2.3.3. Propagation by somatic embryogenesis

The embryogenic pathway, as opposed to the organogenic pathway, can be a more efficient and productive system for large-scale clonal propagation (Fulzele and Satdive, 2003). Plant regeneration via somatic embryogenesis is the *in vitro* process used to reduce multiplication time, and potentially offers an efficient system for mass clonal propagation and for regeneration of plants from genetic transformation and somatic hybridisation experiments (Siragusa et al. 2007, Fulzele and Satdive, 2003). The method has the potential to produce a large number of plantlets in a relatively short period of time (Wu et al. 2007).

Somatic embryogenesis may result in less variation through chimerism. However, somaclonal variation will be less likely to take place in direct regeneration, but might be more pronounced in somatic embryos developing

![Figure 3. In vitro propagation of olive (Olea europaea L.) plants (Gurel et al. 2006).](image-url)
Large scale cultivation of plant cell and tissue culture in bioreactors

from continuously cultured callus tissue. Since the embryo contains both a root and an apical shoot meristem, the rooting stage required in conventional \textit{in vitro} bud or shoot propagation technology is obviated. Somatic embryos are small and can be adequately handled in scaled-up procedures. They are amenable to sorting and separation by image analysis, dispensing by automated systems, and can be encapsulated and either stored or planted directly, with the aid of mechanized systems (Ziv, 2000).

Furthermore, manipulation of somatic embryos to function as synthetic seeds would allow clonal germplasm to be stored efficiently in seed repositories if viability can be maintained for adequate lengths of time (Jayasankar et al. 2005).

\textbf{Figure 4.} \textit{In vitro} micropropagation of some aquarium plants (Gurel et al. 2007).
Figure 5. Clonal propagation of *Rubia tinctorum* L. (Cetin et al. 2005).

The technology is currently applied to a large number of agricultural, ornamental, vegetable crop and forestry plant species. *In vitro* propagation of different plants carried out in our laboratories are presented in Fig. 3, 4 and 5.

Conventional *in vitro* plant propagation methods on semi-solid media involve a considerable amount of handling such as transfers to fresh media and plantlet separation (Etienne et al. 1997, Gurel et al. 2006; Gurel et al. 2007; 2007; Cetin et al. 2005). Various liquid medium culture techniques have therefore been developed to reduce labor costs, stimulate growth and improve multiplication rates, and to increase culture uniformity by the elimination of nutritional gradients (Etienne et al. 1997).

Efficient commercial micropropagation depends on rapid and extensive proliferation in conjunction with the use of large-scale cultures for the multiplication phase. Mechanization and automation of the micropropagation process can greatly overcome the limitation imposed by existing conventional labor-intensive methods. Considerable attention has been directed toward buds, shoots, or plantlets during the multiplication and transplanting phases (Ziv, 2000).

However, the establishment of efficient automated micropropagation techniques to overcome some of the present labor input limitations requires, among other technologies, the development of scaled-up liquid cultures in bioreactors. Bioreactors are used in micropropagation for both the embryogenic and organogenic pathways. Successful micropropagation in shake liquid cultures or bioreactors has been reported previously for
ornamental and vegetable crop species, as well as for woody plants. The airlift and immersion bioreactors used in laboratory scale plant cell culture studies provide a basis for large scale cultivation. Micropropagation of potato, Boston fern, banana and gladiolus were carried out at 2 L disposable, presterilized bioreactors to obtain meristem clusters (Ziv et al., 1998). The micropropagation of plantlets from protocorm-like bodies was also realized in 3 L airlift bioreactor (Young et al., 2000). Usage of immersion bioreactors were reported for micropropagation of shoots and buds from Ananas comosus L. (Escalona et al., 1999) and plantlets from Potato Atlantic (Piao et al., 2003) (Table 1).

Nevertheless, two major problems still limit the use of bioreactors for plant culture and micropropagation:

- the phenomenon of hyperhydricity (vitrification), which results from abnormal plant morphogenesis in liquid media
- the high initial capital and operational costs of bioreactors.

While growth retardants added to liquid media were found to reduce shoots into meristematic or bud clusters and thus limit hyperhydricity, simple inexpensive bioreactors are as yet not prevalent. Disposable presterilized plastic film bioreactors may reduce costs and simplify plant processing in scaled-up liquid cultures. The use of clusters can provide an improved system for normal plant development and further growth and hardening after transplanting as the problem of hyperhydricity in liquid cultures is obviated (Ziv et al. 1998).
3. Scale-up

Scale-up generally involves taking a lab-scale bioprocess and replicating it as closely as possible to produce larger amounts of product. A typical scale-up sequence in plant cell and tissue culture studies start with jars, moves to 1 L shake flasks, after that to 1 - 10 L glass bioreactors, then scales up through to stainless steel vessels of varying sizes from 30 – 150 L to 1000 L (Figure 6).

Large scale cultivation of plant cell and tissue culture is an alternative to the traditional methods of plantation. However, plant cell suspensions, shoot and root cultures pose very different problems in bioreactors when compared to microbial cultures during scale-up. Briefly, plant cells grow slowly, the cells are large and generally form clumps which make them more sensitive to shear associated with agitation and exhibit long processing times. Organ cultures are far more sensitive to shear. These characteristics have lead to the necessity to design alternative bioreactor configurations, particularly those that avoid or reduce shear within the large scale bioreactor (Scragg, 2007). Therefore, a lot of effort is currently being put into novel bioreactor designs.

In order to control plant morphogenesis and biomass growth in bioreactors, various culture conditions must be monitored and/or controlled, such as the morphology, oxygen supply and CO₂ exchange, mixing, consequently pH and temperature. Most of these parameters are interrelated, therefore a holistic approach is required for a successful scale-up.

3.1. Culture conditions and requirements

A thorough understanding of substrate requirements as well as culture conditions is essential for the optimization of the scale-up of plant cell and tissue cultures. Carbon and oxygen are two major substrates affecting biomass production therefore the utilization rates of these substrates should be closely monitored. The physical conditions within the reactor are interrelated with the chemical conditions which in turn affect both productivity and cost effectiveness.

There are a number of findings related to substrates types and concentrations in order to optimize process parameters. Adding a saccharide mixture to the culture medium as a carbon source to increase the productivity of the secondary metabolites, increasing the productivity of secondary metabolites and shortening the culture time by using a mixture of glucose and fructose or growing plant cells in a medium treated with alkanoic acid or salt are typical examples (Kim et al., 2005; 2006). Choi et al., (1999) suggested increasing the paclitaxel production by inoculating the cells at a high initial concentration and by increasing the saccharide concentration in the medium.
Another innovation focused on modifications of culture conditions such as media composition and operating modes which were found to enhance the yield of various taxanes and taxol-like compounds from cell culture of all *Taxus* species. Particularly preferred enhancement agents included silver ion or complex, jasmonic acid (especially the methyl ester), auxin-related growth regulators, and inhibitors of the phenylpropanoid pathway, such as 3,4-methylenedioxy-6-nitrocinamic acid. These enhancement agents were suggested to be used alone or in combination with one another or other yield-enhancing conditions (Bringi et al., 1997).

These innovations could lead to the economic production of valuable secondary metabolites on an industrial scale using plant cell culture.

### 3.1.1. Cell morphology

Plant cells in suspension cultures display a range of shapes, with the largely spherical and the rod shapes being the most common. Suspension
cultures normally exhibit various degrees of cell aggregation with the aggregate sizes varying dependent on the plant species, growth stage and culture conditions. While some plant cells such as *Nicotiana tabacum* and *Anchusa officinalis*, form fine suspensions with few large aggregates smaller than 1 mm others form aggregates as large as 2 cm in diameter, as in the case of *Panax ginseng* suspension culture (Su, 2006). Unlike cell suspension cultures, clusters that are compact, consist mainly of small meristematic cells and are apparently less shear sensitive (Ziv, 2000).

The morphological character of the plant material, eg.: cell aggregates, calli or hairy roots becomes a more important factor for the design and optimization of the culture conditions as the structure changes from cell aggregates to tissue cultures (Choi, et al., 2006).

### 3.1.2. Aeration and agitation

The requirements for O$_2$ may vary from one species to another. Oxygen must be supplied continuously to provide adequate aeration, since it affects metabolic activity and energy supply. The level of O$_2$ in liquid cultures in bioreactors can be regulated by agitation or stirring methodologies and intensities as well as aeration techniques and gas flow rates, which affect bubble sizes, mixing and circulation times, gas hold-up values and mass transfer coefficients (Vardar-Sukan, 1985; Vardar-Sukan, 1986; Takayama and Akita 1998). Air-flow supply to the reactor determines the degree of aeration and agitation as well as prevents settling of the plant biomass, thus affecting growth and proliferation of the biomass.

The effect of the O$_2$ partial pressure on proliferation and differentiation of embryogenic suspension cultures has been reported for different crops: *Daucus carota, Medicago sativa, Euphorbia pulcherrima, Triticum aestivum* and *Picea abies* (Feria et al., 2003). In these studies high aeration rates appear to reduce the biomass growth (Ziv, 2000). This is attributed is to the stripping of CO$_2$ and essential volatiles from the system (Kato et al., 1975; Smart and Fowler, 1981, Kieran et al., 1997).

In many plants cultivated in bioreactors, intensive aeration, mixing, and circulation cause shearing damage, cell wall breakdown, and thus accumulation of cell debris and release of polysaccharides. Cell death and debris accumulation results in foaming, in turn causing adhesion of cells and aggregates onto the culture vessel walls, and the development of a layer at the upper part of the vessel, thus disrupting the homogeneous culture conditions, causing additional cell debris formation. As the biomass increases and the cultures become viscous, higher rates of aeration are required to allow for oxygen supply and circulation and demand for higher aeration rates that
intensify foaming and the clogging problem (Scragg 1992). Similarly cell aggregates exhibit intercellular mass transfer limitations leading to more vigorous aeration and agitation requirements.

Although plant cells growing in liquid cultures are better exposed to the medium components and the uptake and consumption are faster (Ziv, 2000), individual cells in an organized tissue or cell aggregate can be oxygen deprived within the tissue due to the intra-particle resistances, even if the surface is exposed to oxygen saturated medium. Cultured plants and plant tissue present very large structures which must have considerable oxygen transport rates within the tissue to maintain aerobic respiration. As the oxygen moves into the tissues, it is consumed by respiration. The transport rate through the outermost tissues must be sufficient to supply the oxygen to all tissues that are deeper within (Curtis and Tuerk, 2006). In the case of hairy root cultures, the growth of hairy roots in liquid medium results in the packed root mass playing an inhibitory role in fluid flow and limiting oxygen availability. In addition, the roots hairs play a detrimental role for liquid circulation environment because they inhibit fluid flow, induce the stagnation thus create micro-environmental deficiencies and limit the availability of oxygen.

On the other hand, Shuler (1993) suggested that metabolite productivity might be influenced by the degree of cellular association and be affected by variations in aggregation patterns arising on scale-up. Therefore, the aeration and agitation rates have to be optimized for large-scale production (Ramachandra and Ravishankar, 2002). Oxygen transfer in large scale cultures have to be investigated in-depth since plant cells have a tendency to aggregate. Understanding the oxygen requirements is essential in order to design high performance and more efficient bioreactors for large scale applications.

### 3.2. Mixing

Fluid mixing is an important factor in the process optimization and scale-up of plant cell and tissue culture cultivation and for efficient production of valuable metabolites. However, in most of the studies cited in the literature, mixing time was not controlled at a constant level during cultivation. There is a need to investigate the effect of a constant mixing time on plant cell physiology and metabolism in long-term agitated cultures (Zhong et al., 2002). Nevertheless, environmental factors such as the composition of the gas phase, pH and temperature cannot be controlled precisely while handling large volumes in bioreactors. Thus, the effect of these factors on cell proliferation and differentiation can be investigated in order to improve culture performance (Hohe et al., 1999).
3.2.1. Shear sensitivity

Hydrodynamic shear sensitivity is considered one of the obstacles for large-scale plant cell culture and has attracted considerable research attention. Studies in this field can be expressed in two categories:

- The cells are exposed to shear forces under growth conditions for the entire duration of the cultivation.
- The cells are exposed to well-defined laminar or turbulent flow conditions for short periods of time, generally under non-growth conditions (Kieran et al., 1997; Sajc et al., 2000).

Although, varying levels of shear stresses were identified for different species (i.e., 80-200 Nm\(^{-2}\) for \(M.\) citrifolia, 50 Nm\(^{-2}\) for \(Daucus\) carota), no single mechanism for cell damage has been conclusively identified. Moreover, conducted studies revealed the difficulty of comparing the shear sensitivity of different species and drive conclusions (Sajc et al., 2000). But in general terms, the dependence of the hydrodynamic stress sensitivity of a cell line is predicted to be governed by a combination of effects:

- the species investigated,
- medium components,
- the subcultivation regime,
- age of cells,
- cell morphology,
- types of cells in case of differentiation,
- liquid viscosity,
- degree of aeration.

The study carried out by Meijer and coworkers (1994) supports this general conclusion where plant cell suspensions of different species were subjected to various levels of hydrodynamic stress generated by a Rushton impeller in a fermenter. The cell lines \(Catharanthus\) roseus and \(Nicotiana\) tabacum were capable of growth under conditions of high hydrodynamic stress which were similar to those in a 25 m\(^3\) stirred-tank production fermenter. \(Cinchona\) robusta and \(Tabernaemontana\) divaricata were found to be more sensitive to hydrodynamic stress. The variation in the hydrodynamic stress sensitivity of the cells depended strongly on the cell line used.

In another study by Gong and coworkers (2006), the responses of suspension cultured \(Taxus\) cuspidata in different culture phases were investigated under shear stress using a Couette reactor. It was found that the
pH in medium and peroxide hydrogen ($H_2O_2$) in cells increased more rapidly in the exponential phase than that in lag phase under shear stress. The pH and $H_2O_2$ concentration in the exponential phase were also higher than those in the lag phase. Inhibition studies showed that there existed a 30–45 min delay in the action time of G-protein, $Ca^{2+}$ channel and phospholipase C of *T. cuspidata* cells in the lag phase than that in the exponential phase. Age-related different membrane fluidity and $H^+$-ATPase activity may partially contribute to the observed responses. These early responses might be indicators for selecting shear-resistant cell lines and for cell damage caused by shear stress.

As scaling up based on constant mixing rate is troublesome because of the complex mechanism of shear sensitivity in bioreactors, research efforts can be devoted to identification of shear resistant cell lines in order to overcome shear related obstacles in industrial scale applications.

### 3.2.2. Effects of temperature and pH

The effects of temperature and pH are important parameters in plant cell and tissue cultures and are dependent upon substrate utilization as well as mixing and circulation within the reactor. Insufficient mixing leads to spatial variations within the reactor and the resulting heterogeneity creates gradients of temperature and pH due to variations in physiological performance.

Cultures are maintained at mean temperatures to enhance growth and morphogenesis *in vitro*, which are higher than those for the same plants *in vivo*. The control of the temperature in the liquid medium inside the bioreactor can be easily adjusted by a heating element or by circulating water in a jacket outside the vessel. There is, however, limited information on the effects of temperature in bioreactor cultures, which is usually kept between 17–25°C for induction of callus tissues and growth of cultured cells. But, each plant species may favor a different temperature.

Toivonen and coworkers (1992) found that lowering the cultivation temperature increased the total fatty acid content per cell in dry weight. Akita and Takayama (1994) investigated the temperature effects on potato tuber formation in an airlift bioreactor and reported that a higher number and larger-size tubers developed at 25°C than at 17°C; the higher temperature caused an increase in tuber size. Ziv and Shemesh (1996), working with potato internode explants in liquid cultures, found that tuber formation was best at a 16 h photoperiod and 18/15°C day and night temperature. In another study, the authors investigated the impact of temperature and light quality on biomass accumulation and ginsenoside production by hairy roots cultivated in
5 L bubble bioreactors. Biomass accumulation and ginsenoside production was optimal under 20°C/13°C day (12 h)/night (8 h) cycle. Biomass of hairy roots was highest in the cultures grown under dark or red light while ginsenoside accumulation was optimum in the cultures grown under fluorescent light (Yu et al., 2005). Furthermore, Choi et al. (1999) investigated the effect of the temperature to increase the production level of paclitaxel during the plant cell culture.

The medium pH is usually adjusted to between 5 and 5.9 before autoclaving and extremes of pH are avoided. The optimum pH is determined and controlled using a small scale bioreactor or a jar fermentor with pH control equipment. Each plant species has different optimized conditions both for growth of the cells and for production of useful products, so it is necessary to optimize the conditions in each case. Moreover, temperature and pH gradients due to inhomogeneous mixing are still issues to be tackled. Knowledge produced from immobilized culture studies can be exploited to elucidate the complex interactions between chemical and physical parameters of a plant bioreactor.
4. SWOT analysis for scale-up

SWOT analysis is a general tool intended to be used in the preliminary stages of decision-making and as a precursor to strategic planning in various kinds of applications. It is an examination of internal strengths and weaknesses of a specific topic as well as the opportunities and threats it is facing due to outside factors. In this section, a structured set of information compiled in the form of a SWOT analysis (Table 2) regarding the scale up of plant cell culture, organ culture and propagation has been presented to demonstrate the state-of-the-art and point out areas for further research.

4.1. Plant cell suspension culture

Cell suspension culture has more immediate potential for industrial application than plant tissue or organ cultures, due to the extensive body of expertise acquired from submerged microbial culture studies (Kieran et al., 1997). Production can be more reliable, simpler, and more predictable and isolation of the phytochemical can be rapid and efficient, as compared to extraction from complex whole plants. Cell cultures cannot only yield defined standard phytochemicals in large volumes but also eliminate the presence of interfering compounds that occur in the field-grown plants (Lila, 2005). Therefore the metabolites can be produced under controlled and reproducible conditions, independent of geographical and climatic factors. Moreover, selection of high-growth cell lines to produce the desired metabolites is possible.

Plant cell culture is still lacking long-term commercial success as discussed in many reviews concerning the technology and strategies for its optimization (Kieran et al., 1997). Problems associated to slow growth rates and low products yields (Taticek et al., 1991) affecting intracellular production of valuable metabolites have made plant cell culture-based processes, with a few exceptions, economically unrealistic. Moreover, contamination which is a result of slow growth is very risky and has to be tackled. Plant cell suspensions are shear-sensitive. The large size and rigid cell wall indicate that the cells and in particular the larger organized structures could be sensitive to shear (Altman, 1997).

Oxygen transfer in large scale cultures have to be investigated in-depth and the aeration and agitation rates have to be optimized for large-scale production since plant cells have a tendency to aggregate. Information obtained through immobilized cell cultures can be a starting point shedding light on mass transfer limitations within aggregates.
Table 2. SWOT analysis of scale-up in plant cell and tissue cultures.

<table>
<thead>
<tr>
<th></th>
<th>Strengths</th>
<th>Weaknesses</th>
<th>Opportunities</th>
<th>Threats</th>
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| Plant cell suspension culture | - Has more immediate potential for industrial application than plant tissue or organ cultures  
- More reliable, simpler and predictable production  
- Yield a source of defined standard phytochemicals in large volumes  
- Interfering compounds that occur in the field-grown plant can be avoided  
- Selection of high growth cell lines | - Unattained long term commercial success  
- Instability of cell lines  
- Low yields  
- Slow growth  
- Contamination  
- Lower levels of secondary metabolites compared to differentiated tissues  
- Susceptible to chemical and physical culture conditions such medium composition, mixing, aeration and shear  
- Cell aggregation  
- High production cost | - Developments in alternative bioreactor design and control, Elicitor technology, Metabolic engineering and biotransformation chemistry  
- Consumer trends towards natural products and additives  
- Increased pharmaceutical applications as phytotherapy | - Cheaper conventional production as a result of more efficient agricultural techniques  
- Highly productive plants yielding secondary metabolites of interest using genetically modified organisms (GMOs) |
### Table 2. Continued

<table>
<thead>
<tr>
<th>Organ cultures</th>
<th>Relatively stable shoot and root cultures</th>
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<tbody>
<tr>
<td></td>
<td>Possibility of repeated and regular harvests from the same biomass</td>
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<tr>
<td></td>
<td>Direct shoot regeneration</td>
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<tr>
<td></td>
<td>Lower sensitivity to shear and contamination</td>
</tr>
<tr>
<td></td>
<td>Less time and labor required to generate large number of plants from meristematic shoot tips</td>
</tr>
<tr>
<td></td>
<td>Can be an alternative for the maintenance of material in the gene banks.</td>
</tr>
<tr>
<td></td>
<td>Strong correlation between secondary metabolite production and morphological differentiation</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Inhomogeneous biomass</th>
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<tr>
<td>High degree of spatial heterogeneity</td>
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<tr>
<td>The need for different bioreactor designs</td>
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<tr>
<td>The necessity to expose shoot cultures to light.</td>
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<tr>
<td>Slow growth</td>
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<tr>
<td>Inhibitory role of biomass in the fluid flow</td>
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<table>
<thead>
<tr>
<th>Production of value-added compounds by biotransformations</th>
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<tbody>
<tr>
<td>The ability to engineer the genomic DNA</td>
</tr>
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<td>Similar metabolite pattern in hairy roots to that of plant roots</td>
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<table>
<thead>
<tr>
<th>Cheaper conventional production as a result of more efficient agricultural techniques</th>
</tr>
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<tbody>
<tr>
<td>Highly productive plants yielding secondary metabolites of interest using genetically modified organisms (GMOs)</td>
</tr>
</tbody>
</table>
Table 2. Continued

<table>
<thead>
<tr>
<th>Propagation</th>
<th>Fast proliferation</th>
<th>More expensive than the conventional methods</th>
<th>Market demands for newly bred and ornamental plants</th>
<th>National/Global regulatory and legal restrictions</th>
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<tbody>
<tr>
<td></td>
<td>Production of disease free plant material</td>
<td>Time and labour consuming as a consequence of intensive hand manipulation of various culture phases</td>
<td>Industrial demands for standardized raw materials with high contents of valuable compounds</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The ability to airship large quantities efficiently</td>
<td>Abnormal plant development</td>
<td>Automation through use of liquid cultures and bioreactors</td>
<td></td>
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<tr>
<td></td>
<td>Multiplication of the plants irrespective of the season and weather on a year round basis</td>
<td>Vitrification</td>
<td>Robotics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>True-to-type propagation of plants</td>
<td>Not used commercially for all plant species.</td>
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<td></td>
<td>Production of transgenic clonal plants</td>
<td>Microbial contamination as affected by both the plant material and operation procedures of large scale bioreactors</td>
<td></td>
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<td></td>
<td>Materials for artificial seeds</td>
<td>Recalcitrant plant species</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alternative way to store germplasm</td>
<td>Genetic and epigenetic variations during vegetative propagation</td>
<td></td>
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</table>
Finally, lack of genetic stability (Kieran et al., 1997) is also regarded as a weakness. In terms of economical issues, the production cost of metabolites is still high in spite of remarkable advances in plant cell culture technology. Zenk (1978) stated that industrial plant cell culture techniques would be introduced only if the plant product under consideration is produced at a price equal to or preferably lower than the field-produced product. On the other hand, Drapeau and coworkers (1987) suggested that a quick way to assess the attractiveness of a cell culture method vs. conventional agriculture is to calculate a specific biosynthesis rate "based on the final dry weight and the total time of bioreactor process or land occupation". Shikonin is a good example, in that the long growing period of 3-5 years and strict climatic requirements mean that the cost of the plant raw material is high. With shikonin there is an 830-fold increase in plant cell culture, making it feasible for industrial application. But the technology is still too expensive to produce food stuffs, food additives and pharmaceuticals since these can be more easily produced by alternative ways such as chemical synthesis, extraction from plants or by microbial fermentation (Misawa, 1994). A major aspect which has to be addressed is the problem of contamination in large-scale liquid cultures, which can cause severe losses (Leifert and Waites 1992). Attempts to control contamination in liquid cultures of foliage plants were reported by Levin et al. (1997a), who used continuous filtration systems to control bacterial growth in the medium (Ziv, 2000).

Recent developments in bioreactor design and control, elicitor technology, metabolic engineering and biotransformation chemistry are some of the opportunities identified. Production of podophyllotoxin which is the precursor of a semi-synthetic anticancer drug via biotransformation induced by introduction of coniferin in the suspension culture of Podophyllum hexandrum is a good example. Plant cell culture as a production strategy is gaining even more importance when combined with the consumer demand towards natural products and additives (Taticek et al., 1991). Moreover, increased pharmaceutical applications such as phytotherapy can boost the industrial application of plant cell culture. Since genetic manipulation of cell cultures has a great potential for altering the metabolic profile of plants, profitability on industrial scale is expected to increase in the future (Razdan, 2003).

Reductions in the cost of conventional production due to the development of more efficient agricultural techniques and cultivation of plants with high secondary metabolite content, using genetically modified organisms (GMOs) are regarded as threats for the large scale application of plant cell cultures.
4.2. Organ cultures

Organ culture enables a close control of secondary compound production and repeated and regular harvests from the same biomass (Bourgaud et al., 2001). Shoot and root cultures are relatively more stable. Compared to cell suspension cultures, organ cultures generally display a lower sensitivity to shear stress (Bourgaud et al., 2001) and are fairly well stable in metabolite yields due to their genetic stability (Ramachandra and Ravishankar, 2002). The strong correlation between secondary metabolite production and morphological differentiation gives more impetus to the application of organized cell cultures for large scale production of phytochemicals. The vast potential of hairy root cultures as a stable source of biologically active chemicals has focused the attention of researchers towards the exploitation of this excellent *in vitro* system through up scaling in novel bioreactors which would provide the best conditions for maximum growth. The main constraints for the commercial exploitation and large scale cultures of hairy roots in bioreactor relate to the operational phases in which several parameters are required to be optimized (Mehrotra et al., 2008).

Shoot regeneration from shoot apex is direct, relatively simple and is not prone to somaclonal variation and chromosomal abnormalities. Shoot and root development from meristematic shoot tips is also quite simple and needs less time and labor to regenerate large numbers of plants (Saeed et al., 1997). Hence the storage of shoot tip cultures may be an alternative method for the maintenance of material in gene banks for medium and long term storage (Grout and Henshaw, 1978).

One of the major weaknesses encountered with organ cultures in bioreactors is due to the inherent heterogeneous nature of the biomass compared to cell suspensions and thus the high degree of spatial heterogeneity in the reactor. This heterogeneity can be enhanced by consequent mass transfer limitations of oxygen and nutrients.

Root systems of higher plants generally exhibit slower growth and are difficult to harvest. In comparison to a cell suspension culture, the growth of organs in liquid medium results in the packed root mass playing an inhibitory role in fluid flow and limiting oxygen availability. Experience and expertise from packed and expanded bed cultures could be used or adapted to develop more efficient techniques for organ culture cultivation.

The major opportunity identified for this technique is that a wide variety of chemical compounds including aromatics, steroids, alkaloids, coumarins and terpenoids can undergo biotransformations using plant cells, organ cultures and enzymes (Giri et al., 2001). The metabolite pattern found in hairy roots is similar, if not always identical to that of plant roots. Moreover,
the ability to engineer the genomic DNA through tailor-made Ri plasmid is predicted to find a tremendous use in producing novel compounds (Ramachandra and Ravishankar, 2002). The fact that the medium is less amenable to contamination in open conditions (Bourgaud et al., 2001) makes it even more interesting.

Cheaper conventional production and cultivation of genetically engineered plants yielding higher amounts of secondary metabolites are also regarded as threats for large scale application of organ cultures.

4.3. Propagation

Clonal propagation has created new challenges in global trading for producers, farmers, nursery owners, and for rural employment in terms of producing high quality and healthy planting material of ornamentals, forest and fruit trees, propagated from vegetative parts (Anonymous, 2002). The possibility of propagating the plants under a controlled environment, anywhere, irrespective of the season and weather, on a year-round basis (Anonymous, 2002) and cultivation of true-to-type plants have made this technique appealing. Production of very large number of clonal propagules within a short time span and obtaining disease-free plant material by eliminating viral, bacterial and fungal contamination are the major strengths. Moreover, the possibility of air-shipping large numbers of plantlets efficiently and bringing newly bred plants and selections to market quickly and in large quantities (Altman, 1997) are regarded as strengths of propagation. Somatic embryogenesis as a propagation technique provides an ideal system for the investigation of the whole process of differentiation of plants and presents a potential of delivering high-quality products with predictable and reproducible characteristics. Useful systems can be developed to produce transgenic clonal plants as well as to obtain materials for artificial seeds (Zimmerman, 1993) by using this technique. Synthetic seed application is a promising field. Genetically heterozygous plants or plants with unique gene combinations which can not be maintained by conventional seed production techniques due to increases in genetic recombinations at each generation, can be manufactured as synthetic seeds. This technology might provide an alternative to fields on green house nurseries for storing germplasm. Implementation of recycling procedures involving immature somatic embryos can be a challenge for large scale applications. The major emphasis during the production scale-up period should be to overlay the established fundamental principles of manufacturing planning and control (Grossnickle et al., 1996).
The organization of the specialized organs and the nature of the various tissues of cultured material, determine the survival potential of plants ex vitro. Vitrification which is the phenomenon of hyperhydricity in organs and plants in vitro, and in plants after they have been transplanted ex vitro is identified as a weakness. Vitrified organs appear to be water soaked and are brittle with an abnormal structure. Hyperhydric shoots are easily damaged by desiccation and survive very poorly when subcultured or transferred to an external environment (Ziv and Chen, 2007). In economical terms, clonal propagation is again more expensive than the conventional methods of planting and requires several types of skills and a constant monitoring of the input (Anonymous, 2002). The technique is costly due to intensive hand manipulation of the various culture phases. The need for multiple subcultures on different media makes shoot and node culture extremely labor intensive. Presently, total labor costs, typically ranging from 50 to 70% of production costs, limit the expansion of micropropagation industry. Thus the current application of the technology is restricted to high value horticultural crops such as ornamental plants. Expansion of the industry to include production of vegetable, plantation and forest crops depends on the development of more efficient micropropagation systems. Cost-reduction strategies including elimination of production steps and development of reliable automated micropropagation systems will facilitate this expansion (Trigiano et al., 1999). Abnormal plant development is sometimes observed and thus the method can not be used commercially for all plant species (Ziv, 2000). Genetic and epigenetic variations may occur during vegetative propagation (Podwyszynska, 2005) and can seriously impair the quality of the product. Microbial contamination as affected by both the introduced plant material and operation procedures (Ziv, 2000) can be a drawback in large scale bioreactors. Additionally, the existence of recalcitrant species for bioreactor application is regarded as a weakness as such species are difficult to be cultured in liquid medium even if they could be propagated on agar medium (Gray et al., 1995). Moreover, synchronization of development remains one of the major difficulties for moving somatic embryogenesis technique on to an industrial scale (Molle and Freyssinet, 1992). Such synchronization needs to be controlled at all stages of the process. Studies are now under way to understand and control factors leading to morphological heterogeneity of somatic embryos in the bioreactor, in order to achieve more uniform plantlet development in the nursery. Overcoming this obstacle is essential before industrial production can even be envisaged (Barry-Etienne et al., 2002). Despite the difficulties, somatic embryogenesis continues to be a highly attractive biological strategy for large scale production research. The financing and funding of various projects and companies have been erratic,
often resulting in a lack of continuity and instability, as evidenced by ventures such as Plant Genetics, of the United States based example of the scale-up of somatic embryogenesis (Sluis, 2008).

Market demands for newly bred and ornamental plants and industrial demands for standardized raw materials with high valuable compounds contents are considered as opportunities. Hvosleif-Eide and coworkers (2003) proposed three different approaches to scale-up micropropagation which then are referred to as opportunities:

- Standardization of plant tissue
- Automation through use of liquid cultures and bioreactors and
- Robotics

National and global regulatory and legal restrictions are identified as threats. For instance, research on genetically engineered plants is developing at a rapid pace and these GMO’s will be commercially produced by clonal propagation (Sedjo, 2006), but this is very likely to face a barrier because of the regulations.
5. State of the art for large scale cultivation

Shikonin, ginsenosides, taxol and berberine are presently produced on large scale. But, how do we define large scale? The rule of thumb is that any production equal or above 20 L can be considered as large scale. The production of secondary metabolites from *C. roseus* such as alkaloids, ajmalicine and serpentine have been studied intensively and form ideal model systems for testing new methods to stimulate secondary metabolite formation (Asada and Shuler, 1989). Scale-up applications in plant cell and tissue cultures is presented in Table 3. Air driven bioreactors are generally preferred over the agitated tanks, and the stirred tank bioreactors equipped with specially designed impellers are preferred when mechanical agitation is indispensable. The volumes are in the range of 20 to 1000 L yielding secondary metabolites such as alkaloids, ajmalicine, taxol and ginsenosides from undifferentiated biomass and/or roots, shoots.

Only few studies have been carried out for the production of adventitious roots in large scale at the industrial level. The first successful attempt for scale-up was by Choi et al (2000) who have successfully achieved 150-fold growth increases when ginseng adventitious roots were grown in 500 L balloon type bubble bioreactors (air-lift bioreactors) for 7 weeks. The adventitious roots grown in bioreactors contained 1% of dry root weight, which corresponds half of the content for the field grown plants. Another successful example of scale-up process was by Wu et al (2007) who have cultivated adventitious roots of *Echinacea purpurea* in 1000 L air lift bioreactors. They were able to achieve 5.1 kg dry biomass of adventitious roots and these roots were possessing higher amounts of cichoric acid (22 mg/g dry mass), chlorogenic acid (5 mg/g dry mass) and caftaric acid (4 mg/g dry mass).

The profitability of a product is determined by the effectiveness of the scale-up and the resulting cost of the transition into manufacturing. Capital and operating costs associated with most glass and stainless steel bioreactors are high, due to low yields coupled with high sterility criteria subsequent to each culturing cycle. Consequently, the products extracted from cells or tissues grown in such bioreactors are expensive, and cannot at present compete commercially with comparable products produced by alternative techniques.

Industrial scale bioreactor devices are traditionally permanent or semi-permanent structures. Lately a patent has been filed proposing a concept of a disposable bioreactor device for solving the aforementioned problems regarding large scale cell/tissue culture production. The recent literature reveals the predominance of disposable or in other words single use bioreactors.
Table 3. Large scale applications of plant cell and tissue culture.

<table>
<thead>
<tr>
<th>Type of culture</th>
<th>Plant source</th>
<th>Bioreactor</th>
<th>Volume (L)</th>
<th>Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>High density cell culture</td>
<td>Panax notoginseng</td>
<td>centrifugal impeller</td>
<td>30</td>
<td>ginsenosides</td>
<td>Zhong &amp; Zhang, 2005</td>
</tr>
<tr>
<td>Cell suspension</td>
<td>Catharanthus roseus</td>
<td>stirred tank</td>
<td>100</td>
<td>biomass</td>
<td>Fulzele, 2003</td>
</tr>
<tr>
<td>Cell suspension</td>
<td>Lithospermum erythrorhizone</td>
<td>agitated</td>
<td>750</td>
<td>shikonin</td>
<td>Tabata and Fujita, 1985</td>
</tr>
<tr>
<td>Cell suspension</td>
<td>Nicotiana tabacum</td>
<td>bubble</td>
<td>1500</td>
<td>biomass</td>
<td>Kato et al., 1976</td>
</tr>
<tr>
<td>Cell suspension</td>
<td>Taxus cuspidata</td>
<td>balloon type bubble</td>
<td>100 - 500</td>
<td>taxol</td>
<td>Son et al., 2000</td>
</tr>
<tr>
<td>Cell suspension</td>
<td>Coleus blumei</td>
<td>airlift</td>
<td>300</td>
<td>rosmarinic acid</td>
<td>Rosevear, 1984</td>
</tr>
<tr>
<td>Cell suspension</td>
<td>Catharanthus roseus</td>
<td>airlift</td>
<td>20</td>
<td>ajmalicine</td>
<td>Fulzele &amp; Heble, 1994</td>
</tr>
<tr>
<td>Photoautotrophic cell suspension</td>
<td>Chenopodium rubrum</td>
<td>airlift</td>
<td>20</td>
<td>biomass</td>
<td>Fisher &amp; Alfermann, 1999</td>
</tr>
<tr>
<td>Elicited cell suspension</td>
<td>Catharanthus roseus</td>
<td>airlift</td>
<td>20</td>
<td>alkaloid</td>
<td>Zhao et al., 2001</td>
</tr>
<tr>
<td>Propagation of shoots</td>
<td>Stevia rebaudiana</td>
<td>non-agitated</td>
<td>500</td>
<td>shoots</td>
<td>Akita et al., 1994</td>
</tr>
<tr>
<td>Adventitious root</td>
<td>Panax ginseng</td>
<td>balloon type bubble</td>
<td>500</td>
<td>root biomass</td>
<td>Choi et al., 2000</td>
</tr>
<tr>
<td>Adventitious root</td>
<td>Echinacea purpurea</td>
<td>airlift</td>
<td>1000</td>
<td>root biomass</td>
<td>Wu et al., 2007</td>
</tr>
</tbody>
</table>
The disposable device for axenically culturing and harvesting cells and/or tissue in at least one cycle, comprises of a sterilisable disposable container having a top and a bottom end, which can be filled partially with a suitable sterile biological cell and/or tissue culture medium (Shaaltiel, 2005).

In many cases, maintaining sterile conditions outside the disposable bioreactor devices are not possible. Once opened to extract the harvestable yield, these devices have no further use after one culturing cycle. But in spite of this, disposable bioreactors are relatively inexpensive for the quantities and production volumes which are typically required by non-industrial-scale users, and are relatively easy to use by non-professional personnel. In fact this aspect of simplicity of use and low economic cost is a major attraction of disposable bioreactor devices. But on the other hand, the disposable bioreactor devices have very little in common with industrial scale bioreactors structurally, operationally or in the economies of scale (Shaaltiel, 2005) and the performance of electrochemical sensors for pH and oxygen is often compromised.

Moreover, the control systems lack both performance and sophistication when compared with their stainless steel counterparts at large scale applications. Therefore, the usage of disposable bioreactors at industrial scale is still in its infancy.

Shiau (2009) developed a bioreactor for growing fungus, plant cell, tissue, organ, hairy roots and plantlet. The bioreactor comprised a nutrient reservoir, a rotatable culture bed, culture medium, a mist generator and a mist delivery pipe. The nutrient reservoir is used to store a nutrient solution required for the explants and the culture medium is supported on the rotatable culture bed which is rotated at a specific speed. Mists of the nutrient solution is produced by the mist generator and delivered by the mist delivery pipe which has a mist-exit port adjusted to a desired level above the culture medium so as to discharge and spray the mists over the explants.

Although, the commercial production of anticancer drug paclitaxel using plant cell culture was achieved by the Samyang Genex (Taejon, Korea) and Phyton Catalytic (NY, USA) (Zhong et al., 2002), many research studies have been concentrated on increasing the production of taxol-like compounds from various Taxus species.

Apart from these, the majority of the innovations are related to developing methods and optimization of process parameters such as the innovation related to conducting the plant cell culture by adding a saccharide mixture to the culture medium as a carbon source, to increase the productivity of the secondary metabolites, increasing the productivity of secondary metabolites and shortening the culture time by the use of the mixture of glucose and fructose. This could contribute in producing useful secondary
metabolites on an industrial scale using plant cell culture (Kim et al., 2006). Kim and coworkers (2005) developed a method for production of secondary metabolites comprising the step of culturing plant cells in a medium treated with alkanoic acid or salt which may contribute to the commercial production of some secondary metabolites. In another patent, a method of reproducing plants by somatic embryogenesis is described where the embryogenic tissue is placed onto a semi-permeable membrane located in a culture medium and incubating it to produce a normal somatic embryo (Bausher et al., 2004). A method for clonal propagation of scented Pandanus amaryllifolius was patented involving the establishment of aseptic shoot cultures of P. amaryllifolius using both terminal and lateral shoot buds as explants (Neelwarne et al., 2005).

In addition to the well-known applications of plant cell and tissue cultures, another novel approach has been introduced in recent literature, where the enzymatic content of the plant material is used to carry out enzymatic modifications to produce value-added products. Takemoto (2009) proposed an innovative process for producing theaflavin selectively, in large quantity, in high yield, simply, and at low cost. The process was characterized by mixing a processed plant extract containing epicatechin, epigallocatechin, epicatechin-3-O-gallate and epigallocatechin-3-O-gallate with a plant cell culture material having a peroxidase activity to thereby produce theaflavin selectively.

Another innovation focused on a process development for the production of peroxidase which comprised of a plant cell culture producing cells from neem (Azadiracta indica) and nirgundi (Vitex negundo) wherein the peroxidase obtained had higher enzymatic activity not reported earlier. Compact callus aggregates showing tremendous enzyme activity were induced so that it could be recovered and reused. In most of the commercial preparations, the general plant body is used for the extraction of the enzyme thereby leading to the destruction of the plants as well as accumulation of waste materials. Apart from that, the enzyme purification from the general plant body is very difficult and usually nearly 70% of the purified enzyme price may be akin to purification cost. A method was developed by changing the concentrations of hormone combinations and the supplement (coconut water) in order to optimize the biomass growth and enzyme production (Abraham et al., 2008).
6. Concluding remarks

In this study, the large-scale cultivation of plant cell and tissue cultures for the purpose of commercially valuable secondary metabolite production as well as in vitro plant propagation are discussed. With respect to secondary metabolite production by plant cell and tissue cultures, the characteristics and the configuration of the industrial plant, consisting of reactors and separation equipment, determines the economic feasibility of the process. In general, the industrial plant must be capable of for the production of more than one product so that an economic utilization is ensured. Even when the plant is used for a single product, a flexible design allowing for different flows of material, new strains of higher productivity, different heat evolution and aeration requirements should be used.

Technological, economical and scale-up aspects show that air driven bioreactors such as airlift and bubble column reactors are more commonly adapted to large scale applications. The successful and commercial exploitation of plant cell and tissue cultures will depend on multidisciplinary studies of plant morphogenesis in liquid media and on the understanding of the control mechanisms of organ and embryo development from meristematic or bud clusters. The chemical and physical environment, in relation to biomass growth and controlled regeneration, should be further investigated. The chemical composition of the growth media such as the level of carbohydrates and specifically the levels and ratios of growth-promoting and -retarding regulators will need to be further studied in more detail as well as the accompanying physical conditions. The wide spread exploitation of plant cell and tissue cultures for secondary metabolite production will be dependent on the development of low cost, high productivity processes specifically tailor-made for low volume high cost products.

The high efficiency of plant propagation using bioreactors has been revealed by the work of many researchers. Bioreactor technology seems to be applicable to commercial propagation for the several plant species discussed herein. However, many problems still exist in scale-up and in the adaption of this know-how to other plant species. The main cause for the problems comes from the difficulty in the preparation and handling of the bioreactors, in the preparation of ‘seed cultures’ and in determining the optimum culture conditions, which depends mainly on the types of culture, different genera or species of plants, etc. The advantages of bioreactor technology exist in the high efficiency and ease of operation. However, the very special requirements of mass propagation provide significant challenges to process engineers. For
example, the mass propagation of shoots in large scale bioreactors is realized and the propagated shoots can be used as seed plants after acclimatization. In the future, it will be necessary to develop a system of illumination to uniformly propagate shoots. The problems discussed as the weaknesses of the technique have to be overcome before the wide-spread use of the technique for commercial propagation.

In parallel to the future developments in process automation and robotics, bioreactors will be indispensible for industrial scale in vitro plant propagation. Implementations of scale-up and mechanization techniques are mandatory for the expansion of commercial micropropagation. New technologies in robotics and machine vision systems for cutting, sorting, and dispensing have been reported. However, these technologies have actually increased the cost of plant micropropagation.

Automation is potentially effective for the development of a practical embryo production system. It could offer labor cost reductions for the established culture protocol, as well as contribute to the study somatic embryogenesis and to improvement of embryo production efficiency. Automation techniques are expected to contribute to the acquisition of basic knowledge of the biological processes. Methods for monitoring and control of culture parameters will improve the reliability and/or reproducibility of culture processes, replacing expert judgements. Many improvements are still needed for economical automated somatic embryo production systems and this can only be achieved through progress in mechanization techniques coupled with studies in the response and mathematical modelling of biological processes.

Further studies should also be carried out on developing techniques to efficiently remove plants from the bioreactor for transplanting because the cultures often form large clumps.

In conclusion it can be stated that the future success of large scale cultivation of plant cell and tissue culture will depend on the collaborative efforts of researchers from different disciplines as well as the evolving consumer demands and innovative spirit of entrepreneurs.
7. References


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Large scale cultivation of plant cell and tissue culture in bioreactors

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