Review

Efficiency of liquid culture systems over conventional micropropagation: A progress towards commercialization

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The most common methods of micropropagation involve the proliferation of shoots via a semi solid system. While such semi solid systems have been moderately to highly successful in terms of multiplication yields, it has become increasingly important to improve productivity and reduce the time taken to multiply commercially important material. Micropropagation by conventional techniques is typically a labor intensive time taking means of clonal propagation. To overcome this, the use of shake cultures utilizing liquid culture medium has been promoted. The liquid medium allows the close contact with the tissue which stimulates and facilitates the uptake of nutrients and phytohormones, leading to better shoot and root growth. Continuous shaking promotes lesser expression of apical dominance which generally leads to induction and proliferation of numerous axillary buds. Further, with in the shake culture conditions, the growth and multiplication rate of shoots is enhanced by forced aeration, since continuous shaking of medium provides ample oxygen supply to the tissue which ultimately leads to their faster growth. Bioreactor provides a rapid and efficient clonal propagation system utilizing liquid medium to avoid intensive manual handling. Automation of micropropagation in bioreactors has been advanced by several authors as a possible way of reducing cost of micropropagation. Micropropagation in bioreactors for optimal plant production depends upon better understanding of physiological and biochemical responses of plant to the signals of culture microenvironment and an optimization of specific physical and chemical culture conditions to control the morphogenesis of plants in liquid culture systems.

Key words: Automation, bioreactor, hyperhydricity and liquid medium.

INTRODUCTION

Micropropagation, popularly known for large-scale clonal propagation, is the first major and widely accepted practical application of plant biotechnology. Now it has gained the status of a multibillion dollar industry through out the world. Initially, the technique of micropropagation for large-scale production of plants was employed basically to ornamental plants only (Ammirato et al., 1989), but recently it has been extended to various vegetable and fruit crops such as potato, strawberry, oil palm, banana, etc. medicinal and aromatic plants and trees (Bajaj, 1986, 1988). Presently, the micropropagation technique is especially being used not only for those plants which are difficult to be propagated through conventional practices, but also for the mass multiplication of existing stocks of germ plasm for biomass energy production and conservation of important, elite and rare plant species that are threatened or on the verge of extinction (Normah et al., 1997; Wawrosch et al., 2001; Dhar et al., 2000; Pania et al., 2000; Michael et al., 2001). The beginning of this industry goes back to about forty years and is based on certain important discoveries. The initial milestone in large-scale production of important plant species through this technique of micropropagation was laid down in the mid of 20th century, when for the first time cultured shoot meristem were used to get virus-free plants of Dahlia and potato (Morel and Martin, 1952, 1955). Skoog and Miller (1957) discovered the role of plant hormones, especially

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of cytokinins in shoot morphogenesis and in the inhibition of apical dominance. The elucidation of the role of cytokinins in apical dominance inhibition, which subsequently results in the release of axillary meristem from dormancy, was the major break-through in this field (Sachs and Thiemann, 1964).

The successful application of such fundamental discoveries to the multiplication of plants by micropropagation has been a key factor in the development of this technology, not only for mass propagation of the existing stocks of germ plasm for biomass production, but also for the conservation of economically important, elite and rare plants. The conventional propagation practices for clonal propagation of such plants are time consuming and labor intensive. During the past couple of decades, there has been an increased interest in problems related to the large-scale plant production as well as in its cost reduction for commercial micropropagation (Donnan, 1986; Chu and Kurtz, 1989; Andrea-Kodym and Zapata-Arias, 2001). At present, being the only commercially exploited tool of plant biotechnology, the micropropagation technique has been applied to about 1000 plant species including crop plants, ornamental plants, medicinal and aromatic plants and trees.

The concept of using shoot meristem culture for *in vitro* propagation originated with the application of this technique to produce virus-free cymbidiums (Morel, 1960) as it was reported that shoot and root apices of virus-infected plants are frequently devoid of pathogens (White, 1934; Kassanis, 1957). Later on, the technique was established as a process of large-scale clonal propagation for virus-free orchids (Morel, 1963).

The technique of shoot meristem culture allows the stem tip to grow on nutrient media, which supports the plant growth. This stem tip is characterized by the presence of apical meristem comprising of meristematic cells capable of division and few leaf primordial cells present at sub-apical region. If a portion of stem beyond the meristematic sub-apical region is included, the procedure is referred to as shoot tip culture rather than meristem culture (Wang and Hu, 1980). The cells of apical meristem possess a high degree of morphogenetic potential for generating plants having the similar genotypic and phenotypic composition as that of the mother source, as these cells tend to be genetically stable. The nutrient medium and phytohormones stimulate and support the development and growth of multiple axillary buds. Three to six fold increases in shoot number can be generally achieved at every 4 - 6 weeks, which results in the production of millions of plants from each cultured shoot meristem in one year. Besides this, there are two other methods, through which the plants can be propagated *in vitro*. These include: (a) de novo formation of shoot meristem in callus tissues and (b) somatic embryogenesis

However, the production of plants through callus tissues is generally avoided because of the frequent occur-

rence of genetically variant plants and even chimeras. These variations are called as 'somaclonal variations' and can also be of two types, either epigenetic variations or heritable variations (Skirvin et al., 1994). The epigenetic or developmental variations are the temporary variations which include phenotypic changes due to the expression of specific genes under a particular set of condition and involve changes in phenotypic and biochemical traits due to karyological variations involving either single or multiple gene changes.

On the other hand, somatic embryogenesis i.e., the formation of embryo like structures from somatic cells and their germination into the complete plants (Bhaskaran and Smith, 1990) has now been proved as potentially most efficient and economic method for the large-scale clonal propagation of plants. The potential of somatic embryogenesis for unlimited multiplication of plants gives tremendous advantage to micropropagation technology. The plants developed from somatic embryos are normally true to type or less variable as compared to the plants developed *via* shoot and root morphogenesis. The developmental pathway of somatic embryos is similar to that of zygotic embryos (Ammirato, 1983; Stange, 1984) but they do differ from their sexual counterparts in that they have bypassed the phase of genetic segregation and recombination during cell division (Swedlund and Vasil, 1985), thus representing an efficient method for clonal multiplication. Development of somatic embryos from a single cell under proper defined nutritional and developmental conditions (Haccius, 1978; Vasil and Vasil, 1982) or from a small group of cells (Browers and Orton, 1982; Ho and Vasil, 1983) may be another possible reason for the lesser or no variation in plants developed from somatic embryo is a bipolar structure in which root and shoot meristem are well defined and established.

There are many advantages of this *in vitro* technique of plant propagation as compared to the conventional procedure (Murashige, 1974, 1978; George and Sherrington, 1984; Pennell, 1984) which made this technique to become a routine procedure for large-scale production of many plant species including crop plants, vegetables, medicinal and aromatic plants, ornamental plants, etc. These advantages are:

1. Micropropagated plants are more or less genetically homogeneous.
2. Shoot tip culture allows the plants to become virus free.
3. Since the culture condition such as light, temperature and humidity are specifically controlled during the procedure, so the plants can be produced irrespective of weather, season and other environment conditions.
4. Propagation through somatic embryogenesis allows higher efficiency of production, therefore a large number of stock plants can be grown by this process in a short time span.

5. Under controlled culture conditions and proper management, a large number of elite stock plants can be maintained in a small area and for a very long time.

Liquid cultures: efficient cost reduction strategy

In spite of these merits, the micropropagation, technique has certain demerits also which have limited the use and exploitation of this technique at industrial level. The major limitation is the higher cost of plant production. Hence, the most challenging aspect at present is to reduce the production cost, thereby improving the production efficiency (Anderson and Meagher, 1977; Sluis and Walker, 1985; Donnan, 1986; Levin and Vasil, 1989; Aitken-Christie, 1991). Therefore, to overcome this limitation, a number of cost reduction strategies have now been developed. The use of shake cultures utilizing liquid culture medium alone (Weathers and Giles, 1988) or in combination with solid culture medium (Debergh and Maene, 1981; Aitken-Christie and Jones, 1987) have been developed and used by various workers (Earle and Langhans, 1975; Takayama and Misawa, 1981; Takayama, 1991; Paque et al., 1992; Chu et al., 1993). Basically used for plant cells, in somatic embryos and organ cultures, both in agitated flask and bioreactors (Smart and Fowler, 1984; Attree et al., 1994; Tautorus and Dunstan, 1995), the liquid culture medium has been relatively less used for the purpose of micropropagation, However certain merits of this technique are helpful in proving it now as one of the important methods for cost reduction during micropropagation. In liquid medium, the close contact of the tissue with the medium may stimulate and facilitate the uptake of nutrients and phytohromones, leading to better shoot and root growth (Ziv, 1989; Smith and Spomer, 1994; Sandal et al., 2001). The disappearance or lesser expression of activity of apical dominance due to continuous shaking condition of the tissues in the medium is another important feature of liquid cultures, which generally leads to the induction and proliferation of numerous axillary buds. This leads to the development of bud clusters which are amenable to the control of medium components, to mechanical separation and to automated inoculation as an efficient delivery system to the final stage for plant growth (Levin et al., 1997; Ziv et al., 1998). The formation of condensed organized structures in which the shoots are reduced to buds / meristematic tissue in liquid media has been reported for several plant species. These clusters are made up of densely packed meristematic cells, actively dividing and forming new meristemoids on outer surface (McCown et al., 1998, Ziv et al., 1998, 1990; Young et al., 2000). Promotion of larger number of axillary bud Development favors the production of large number of plants which are more or less true-to-type (Takayama and Misawa, 1981; Harris and Mason, 1983; Douglas, 1984; Pierik, 1987; Chu et al., 1993) Furthermore, within the shake culture conditions, the growth and multiplication rate of the shoots is enhanced by forced aeration, since continuous shaking of the medium provides sufficient oxygen supply to the tissue, which ultimately leads to their faster growth. In addition to these advantages, the preparation of liquid medium and handling of shake cultures is easier as compared to the semi-solid one. On the other hand, vitrification in the tissues is the common disadvantage in plant tissues during their *in vitro* propagation using liquid medium (Kevers et al., 1984; Gasper et al., 1987; Debergh et al., 1992). Vitrification also known as Hyperhydricity, designates the hyperhydric malformations frequently affecting herbaceous as well as woody shoots during their *in vitro* vegetative propagation (Kevers et al., 2004). The culture medium is the cause of vitrification of tissues. There are many evidences, which suggest that the reduction of agar concentration or its absence in the culture medium causes vitrification during the growth of the tissue (Hakkaart and Versluijs, 1983; John, 1986; Densco, 1987; Kevers et al., 1987; Bottcher et al., 1988). A number of studies have been undertaken suggestion that not all the plant species show vitrification when cultured on liquid medium. In some culture system where the problem of asphyxiation exists, it can be avoided by taking advent-age of the surface tension and floating explants, so that developing tissue do not get submerged in the liquid medium (Debergh et al., 1981; Leshem, 1983; Skidmore et al., 1988; Dillen and Buysens, 1989).

Large-scale culture of plants in bioreactors

To reduce the intensive labor requirement along with the production cost during plant propagation by tissue culture technique, there is an immense need of developing scale-up systems and automation (Aitkin-Christie, 1991). Progress in tissue culture automation will depend upon the use of liquid cultures in bioreactors (Sakamoto et al., 1995). Bioreactors are usually described in a biochemical context as self contained, sterile environments which capitalize on liquid nutrient or liquid/air inflow and out flow systems, designed for intensive culture and affording maximal opportunity for monitoring and control over micro environmental conditions (agitation, aeration, temperature, dissolved oxygen pH etc). Since bioreactors provide a rapid and efficient plant propagation system for many important plant species utilizing liquid media to avoid intensive manual handling, this method for large scale production of plants is promising at industrial level. Employing bioreactors with liquid medium for micropropagation is advantageous due to the ease of scaling-up (Preil, 1991), the ability to prevent the physiological disorders of shoot and leaf hyperhydricity (Ziv, 1999) and low production cost as a result. Large-scale plant propagation using bioreactor can also be beneficial in terms of year round production of the propagules of useful plants resulting in comparatively less labor cost and time

(Levin et al., 1988; Preil et al., 1988). The basic function of a bioreactor is to provide optimum physical and chemical conditions, which influence the optimum growth of tissues in a high yielding culture system. The liquid medium in such systems is used to facilitate handling (Leathers et al., 1995). The large scale production of plants using bioreactors employing liquid medium can be achieved through induction of somatic embryogenesis (Stuart et al., 1987; Greidziak et al., 1990; Terashima and Nishimura, 1991; Denchev, 1992) or by using shoot meristem multiplication technique (Hagimori et al., 1984; Takayama, 1986; Katagi et al., 1986; Akita et al., 1994). In spite of great potential of bioreactors for large-scale culture of somatic embryos, there have been only a limited number of successful reports (Chen et al., 1987; Denchev et al., 1992; Hvoslef-Eide et al., 1998). The major limitation in this area of research is that in most of the cases the nutrient requirement for the induction of somatic embryos is quite different from the nutritional requirements for the normal development and conversion of these somatic embryos into complete plantlets. Hence, majority of the studies involving the use of bioreactor systems have followed the concept of batch cultures. Only certain investigations mentioned the results about bioreactor application for micropropagation of plants *via* organogenic tissues like meristem tips, bulblets, corms, microtubers and shoot propagules, etc. (Ziv, 1990; Takahashi et al., 1992; Ziv and Shemesh, 1996; Ziv and Lilien-Kipnis, 1997; Lim et al., 1998; Lee, 1999; Seon et al., 2000). The use of bioreac-tors as a system for plant propagation through organogenic or embryogenic pathway, although limited to a small number of plant species, is presently being applied to several ornamental, vegetable and fruit crop plants (Table 1). The various propagation aspects of several plant species in bioreactor and some major problems associated with the operation of the bioreactors were recently reviewed by various workers (Takayama and Akita, 1998; Ibaraki and Kurata, 2001.

A schematic outline of mass propagation of certain economically important plants using shake flask and bioreactor technique has been proposed earlier (Kukreja and Ahuja, 1994) which involves the following steps:

1. Establishment of aseptic cultures.
2. Rapid growth and multiplication of apical and axillary buds in shake flasks
3. Rapid growth and multiplication of shoot buds in bioreactor culture.
4. Root information and hardening of the *in vitro* raised plants.
5. Climatization of the *in vitro* raised plants under field conditions.

Various physical and chemical factor affecting the growth and proliferation of tissues in a bioreactor culture vessel containing liquid medium have been studied in de-

tail (Lee, 1997). To obtain normal and healthy plants, accurate monitoring of such factors is very necessary during the growth phase of culture. These physical and chemical factors include dissolved oxygen (DO) of the liquid medium, pH of the medium, inoculum density, culture period, light and temperature conditions and the configuration and type of culture vessel. Lee (1997) studied the precise controlling and monitoring of pH changes during the growth phase of culture and showed that is helped to control the changes of internal physical and chemical factors which subsequently helped in improving plant growth. The dissolved oxygen content (DO) indicates the amount of oxygen available to the tissue in the liquid medium. This available oxygen for plant cell is determined by oxygen transfer coefficient ($K_L a$ values). As the tissue grows, the need of oxygen increases which can be controlled by airflow and conti-nuous agitation which helps in enhancing the availability of dissolved oxygen for the tissue (Jay et al., 1992; Takahashi et al., 1992; Drew, 1997; Lim et al., 1998; Seon et al., 2000). In such cases, oxygen enriched en-vironment in bioreactor culture vessel leads to the better plantlet growth. Current interest towards photoautotrophic micropropagatoin revealed that carbon dioxide and light enriched environment during the *in vitro* culture facilitates and supports the shoot growth (Mousseau, 1986; Kozai et al., 1987; Kozai and Sekimoto, 1988; Infante et al., 1989; Kozai, 1990). It has been observed that CO_2 en-richment in a bioreactor culture vessel during the growth phase of shoots of sweet potato, potato and Chrysan-themum and Chinese fox glove enhanced the growth and production of healthy plantlets (Paek et al., 2001). The cultures growing in a bioreactor vessel containing liquid medium exhibit the following conditions (Takayama, 1991):

1. Floating just beneath the medium surface (Strawberry plants) (Takayama et al., 1987).
2. Freely moving in the medium as in case of Begonia and Gloxinia (Takayama and Misawa, 1981).
3. Sinking or submerged to the bottom of the vessel (Lilies, Gladiolus potatoes)

As the bioreactor is the most suitable vessel for large-scale tissue culture resulting in the production of large number of plantlets in one single batch, once the culture conditions have been established at the shake flask level or in a small scale bioreactor of 250 ml to 1 L capacities, cultures can be easily multiplied in a large-scale bioreac-tor. The size and various designs or configurations, according to the test culture systems have been reported by different workers (Takayama and Misawa, 1981; Katagi et al., 1986; Takayama, 1991; Teng et al., 1993; Akita and Takayama, 1994). The basic construction of bioreactors used for plant propagation is similar to that of used for microbial, animal or plant cell cultures (Akita et al., 1994). Standard configuration of a bioreactor includes bioreactor culture vessel, which provides optimum space

Table 1. Some important plants propagated in bioreactors.

Plant	Propagable units Produced	Reference(s)
Amaryllis hippeastrum	Buds, bulblets	Takayama and Akita, 1998
Ananas comossus	Shoot clusters	-
Apium graveolens	Somatic embryos	Nade et al., 1990
Araceae species	Plants	Takayama and Akita, 1998
Acanthopanax koreanum	Somatic embryos	Son et al., 1999b
Artemisa annua	Plants	Park et al., 1989
Asparagus officinalis	-	Takayama, 1991
Atropa belladonna	-	Takayama, 1991
Begonia	Multiple shoots	Takayama and Misawa, 1981
Brodiaea species	Bud clusters, corms	Ilan et al., 1995
Caladium species	-	Takayama, 1991
Colocasia species	-	Takayama, 1991
Coffea arabica	Shoot clusters, plants	Teisson and Alard, 1995
Cyclamen persicum	Somatic embryos	Hvoslef-Eide and Munstar, 1998
Daucus carota	Somatic embryos	Jay et al., 1992
Dianthus caryophyllus	Shoots, plants	Chatterjee et al., 1997
Dioscorea species	-	Takayama, 1991
Dieffenbachia species	-	Takayama, 1991
Digitalis lanata	Somatic embryos	Greidziak et al., 1990
Eschcholltzia californica	Somatic embryos	Archambault et al., 1994
Euphorbia puleherrima	Somatic embryos	Preil, 1991
Fragaria ananasa	Shoots, plants	Takayama and Akita, 1998
Gentiana species	Plants	Hosokawa et al., 1998
Gladiolus grandiflorum	Bud clusters, corms	Ziv et al., 1994; Teisson and alvard, 1995
Gloximia	Shoots	Takayama, 1991
Hevea brasiliensis	Buds, plants	Alvard et al., 1993; Teisson and Alvard, 1995
Hyacinthus orientalis	Bulblets, plants	Takayama and Akita, 1998
Lilium species	Plants, bulblets	Takayama 1991
Medicago sativa	Somatic embryos	Stuart et al., 1987
Musa species	Buds, plants	Alvard et al., 1993
Nephrolepts exaltata	Buds, plants	Levin et al., 1997; Ziv et al., 1998
Nerine sarniensis	Bulblets, somatic embryos	Lilien-Kipnis et al., 1994; Ziv et al., 1994
Ornithogalum dubium	Shoots, bulblets, plans	Ziv and Lilien Kipnis, 1997
Populus tremula	Bud cluster, shoots	McCown et al., 1988
Picea species	Somatic embryos	Tautorus et al., 1994; Attree et al., 1994
Pinellia ternate	Shoots, corm	Takayama et al., 1991
Pelargonium graveolense	-	Katagi et al., 1986
Phalaenopsis	Protocorm	Young et al., 2000
Solanum tuberosum	Clusters, shoots	Takayama and Akita, 1998; Ziv et al., 1998
Stevia rebaudiana	Shoots	Akita et al., 1994

for the tissue to interact with the medium and to grow under aseptic conditions. Impeller or agitator is attached on to a rotating shaft, which is mechanically driven. The main function of impeller is agitation and proper mixing of the medium in order to supply ample oxygen and mineral substrates to the cultured tissue for growth. Shoot and embryo cultures are more sensitive to damages caused by shear stress generated by mechanical agitation. According to their growth habits, the multiple shoots generally do not form dense clumps during growth where mass transfer is severely limited and therefore the mechanical agitation is not always necessary for mass propa-

Table 2. Types of bioreactor used for plant propagation.

Types of bioreactors	Example		Reference(s)
Mechanically agitated bioreactors	a)	Aeration agitationbioreactors	Kessel and Carr, 1972; Preil et al., 1988;
	b)	Rotating drum bioreactors	Stuart et al., 1987
	c)	Spin filter bioreactor	Tanaka et al., 1983
	d)	Stirred tank bioreactors	Styer, 1985; Wheat et al., 1986
			Hooker and Lee, 1990; Lee, 1997
Pneumatically agitated bioreactors	a)	Simple aeration bioreactor	Takayama, 1981, 1986, 1991
	b)	Bubble column bioreactor	-
	c)	Airlift bioreactor	Lee, 1999
	d)	Ebb and flow bioreactor	
Non-agitated bioreactors	a)	Gaseous phase bioreactor	-
	b)	Overlay aerationbioreactor	-

gation of shoots in bioreactors (Akita et al., 1994). Many other modifications in impeller design and their use during shoot growth in the bioreactor culture vessel have been studied, such as non-mechanical agitation (Chen et al., 1987; Stuart et al., 1987), or mechanical agitation (Ammirato and Styer, 1985; Styer, 1985). Aerator or sparger is a stainless steel pipe (bar or ring) with pin holes provided at the bottom of the vessel, through which compressed and filtered sterile air is sparged in the medium. The type of sparger is important because the efficiency of shoot and embryo growth and development depends upon the level of dissolved oxygen and carbon dioxide. Even the size of bubbles produced by sparger holes are important as their larger size along with the velocity of movement can cause damage to the growing cultured tissue due to shear stress (Terahima and Nishimura, 1991; Jay et al., 1992). Air line filter is an autoclavable disposable filter which allows sparging sterile air into the vessel. In addition to this, a bioreactor unit has on line measurement of different culture condi-tions i.e. pH, dissolved oxygen, temperature, gas flow rate, sugar level, foaming, etc.

Types of bioreactors used for micropropagation

Various types of bioreactors have been developed and used in different laboratories for plant shoot cultures. Generally, they are modified in terms of devices providing agitation and vessel configuration (Ziv, 2005; Hvoslef-Eide et al., 2005; Paek et al., 2005; Asenjo and O' Carroll, 1988). Some of the major differences between the bioreactors designed and used for secondary metabolite production and for plant propagation deal with the media manipulations, the relatively large size of tissue structures in the vessel, and in later case in con-trast the relatively small anticipated scale of production system (Vasil, 1991). The different types of bioreactors used for the large-scale plant propagation are presented in Table 2.

Some other bioreactor types have also been reported for plant propagation in which light conditions were developed in culture vessels (Ikeda, 1985). Inoue (1984) described the use of transparent pipes fitted into the bioreactor through which light was emitted. Ikeda (1985) reported an airlift bioreactor equipped with a photo inducing draft tube, which consists of optical fibers introducing light into the reactor vessel.

Advantages and disadvantages of the use of bioreactor culture system for micropropagation

The major advantage of using bioreactor culture system for micropropagation of economically important plants is the potential for scaling-up in lesser time limit, reduction in the production cost as well as an automated control of physical and chemical environment during growth phase of the plant cultures. However, a lack of systematical and factorial experimental knowledge about the interaction between plant physiology and physical parameters of bioreactor designs affects the frequent use of this tech-nology (Paek et al., 2001). Different designs and modify-cations in the agitation systems have made the technique more advantageous. In the case of airlift and column type bioreactors, where simplicity of design and construction, low shear stress, low contamination rate and less power consumption are the positive points towards plant propagation, yet there are certain demerits also include foaming induce by large volumes of air leading to impair-ed gas transfer within the medium (Leathers et al., 1995; Lee, 1997). Another problem in airlift type bioreactors is the evaporation of culture medium. To overcome this problem, addition of a sterile water column or condenser could be helpful. This can also extend the cultivation period (Lee, 1997). To overcome the problems encoun-tered during the use of air lift and bubble column bioreactors, the bottom-type bubble bioreactor (BTBB) was designed, in which foaming was drastically reduced by the use of cell lifting devices or tube(s) at vessel bottom. In addition, there was the provision of gas recycl-

ing system which allowed the examination of different gases in the medium (Seon et al., 1998; Son et al., 1999).

Stirred tank bioreactors (STR) were later designed to ensure the proper mixing of medium and large sized bubbles. The use of mechanically agitated stirrers result-ed in an even flow of the medium in different directions of the vessel, which enhanced the proper oxygenation of the cultured tissue (Hooker and Lee, 1990). The stirred tank reactors (STR) have some demerits also like high shear force, complicated configuration, high contamina-tion rates, difficulty in optimizing culture conditions, etc. Recently, the use of ebb and flow type of bioreactor for plant propagation has overcome a number of problems faced during the culture in other types of bioreactors. This type of bioreactor consists of a fixed or floating support system inside the vessel, which initially helps to hold the explant apart from the agitator (if provided), and later supports the growing shoots. In these reactors, medium is pumped into the vessel from storage tanks through a series of channels evenly to the plant material resulting in fast and better growth. The medium remains in the vessel for some time and then drains back to the storage tank. This has eliminated the problems of those culture sys-tems where different growth phases need different compositions of nutrient concentration (as in case of so-matic embryo development). Under these circumstances the ebb and flow type systems would be helpful because each step can be separated from the other and can be easily monitored and controlled independently. In such systems each step of plantlet production can be carried out in the same culture vessel simply by changing the culture medium. The nutrient medium outflow and the level regulated by specific pumps and controller, facilitate the tissue growth. Besides, the system has the advent-age of short term exposure of growing cultures to plant growth hormones for the induction of different and desirable growth responses i.e., embryogenesis, organ-ogenesis or multiple shoot induction, etc. This system completely eliminates the step of regularly performed sub-culturing, which is the most labor intensive and adds to the production cost of micropropagated plants.

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