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BIOREACTOR'S IN PRODUCTION OF PLANTING MATERIAL IN ORNAMENTAL CROPS

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Conventional micropropagation with solid or semi solid media is a typically labor-intensive technique of propagating elite clones of commercial ornamental crops and also limited because of high labor costs. Other disadvantages are costly devices, low proliferation rate, long multiplication cycle before hardening and transplanting, and higher mortality resulting from pathogens. These disadvantages are the major drawbacks of conventional micropropagation for scaling up of potential ornamental crop species. At present application of bioreactors is limited in ornamental micropropagation, however it can be utilized as an efficient tool for the production of elite planting material. Bioreactor not only facilitates optimum growth conditions but also offers many advantages to achieve both maximum yield and high quality of propagules, or to bring down the production costs as low as possible. Now, this technology gaining popularity in developing countries for commercialized micropropagation of ornamental crops including Orchids, Lilium, Hippeastrum, Gladiolus, Spathiphyllum, Anthurium, Chrysanthemum, Gerbera, Anoectochilus and Tulips *etc.*, via thin cell layer, meristem culture, organogenesis and somatic embryogenesis. A range of bioreactors were developed and utilized to verify the effectiveness for mass propagation of ornamental plants. Protocorm-like bodies (PLBs) were induced from flower stalk of *Phalaenopsis* by using suspension culture under bioreactors. Many *Lilium* varieties were successfully multiplied in bioreactors. *Lilium* cv. Marcopolo showed a positive growth of bulblets nearly 10 folds faster than that of the solid medium in balloon type bubble bioreactor (BTBB). Modern bioreactor technology along with liquid culture gives a wider control over conventional micropropagation system. However, monitoring of important process parameters such as temperature, pH, and concentrations of oxygen and carbon dioxide inside the bioreactor vessel needs to be worked out for each plant.

Key words: *Bioreactor; In vitro propagation; Ornamental crops; Somatic embryogenesis.*

Introduction

With the globally advancement of a greenhouse technology, a plant biotechnology and marketing strategies, floriculture has significantly increased over the last two decades and there is a great potential for a continued further growth in both domestic and international markets that offers the great opportunities for developing of new ornamental products. Worldwide, about 156 ornamental plants are propagated through tissue culture. It is reported that geophytes, Tulip, Lilium, Narcissus, Gladiolus, Iris and Hyacinthus build 90% of the market. Among them there are Tulip and Lilium, lead cut flower trade worldwide [4]. Geophytes are of a great economic value as they are used in medicine, food and landscape sectors and propagated with tissue culture techniques [24]. Recent technological advancement of propagation have been developed which could help growers to meet the standard of the global floriculture industry in the next century. Micropropagation has increasingly become a valuable biotechnological tool and widely applied in both research and development of ornamentals assisting breeders to develop new cultivars more rapidly.

The commercial micropropagation of ornamental plants via bioreactor is limited and their scaling up is not as easy as in other sector. So, there is a need to develop a range of protocols to minimize the problems of a conventional micropropagation. A bioreactor works

on liquid nutrient or liquid/air inflow and outflow systems. Liquid cultures have been used for *in vitro* propagation through somatic embryogenesis pathways or direct organogenesis. It is designed for an intensive micropropagation, and gives control over micro-environmental conditions (aeration, temperature and agitation). Paek *et al*. [15] categorized bioreactors system into three groups: 1) bioreactors producing biomass (cells, tissue, organogenic callus, embryogenic propagules, roots and shoots); 2) bioreactors producing metabolites and enzymes; 3) bioreactors used for biotransformation. Bioreactor along with suspension culture gives an alternative ways to minimize the cost of production. Levin and Vasil [12] reported a mass multiplication of horticultural plants by using Bioreactor systems with liquid media. Takayama and Misawa [23] reported first time micropropagation of Begonia by using shake cultures with bioreactor. Cardarelli and Suárez, [3] describe the use of Temporary Immersion Bioreactors (TIB) systems for large-scale micropropagation of horticultural crops. Recent progress has been restricted to only few flower crops. However, application of a bioreactor micropropagation in ornamental crops is still in its infancy. This review will assess the current status of bioreactor in micropropagation of ornamentals like *Rose, Lilium, Tulip, Gladiolus, Chrysanthimumm, Zenthideschia, Cyclamen, Gerbera* species.

The use of bioreactor is advancement for a commercial propagation of floriculture crops. Computer automated bioreactors offer many advantages over agar-based cultures, including a better control of the culture conditions viz; optimal nutrients and PGR supply according to the developmental stage; renewal the medium during the culture period; filtration of the medium for exudates; contamination control. The main problem with Agar based medium is that nutrients dissolved in water is bound to the gel by a matric force. Bioreactor along with liquid medium provides greater water availability than agar based medium [1]. They have described a number of advantages over conventional semi-solid micropropagation, including increase in multiplication rates, reduction in space, energy and labor. These cost-benefit advantages are getting more attention to use liquid systems. Industrial production through embryo cultures using bioreactors is promising for commercial plant micropropagation.

Establishment of cultures in liquid medium is an important step towards automation [2]. Liquid culture systems facilitates uniform conditions for *in vitro* propagation viz, ultra filtration for sterilization is possible, the media can renewed easily without changing the glass vessels. Liquid medium was first used by [6] for culturing isolated cells from bracts of *Lamium purpureum* in Knop's solution supplemented with sucrose. Liquid culture systems have significant effects on the multiplication rates and morphology of shoots, somatic embryos, micro-tubers or bulblets produced *in vitro* [17]. Liquid cultures have been used for plant culture in both agitated and non-agitated vessels and in bioreactors, for somatic embryogenesis and organo-genic pathway [25]. With Bioreactor technology bigger size containers can be used, whereas agar based media necessitate flat culturing. It was abundantly clear that the technology offered new ways to achieve high plantlets yields *in vitro*, at low cost, suitable for research and commercial activities.

The objective of this review is to consolidate the work done so far in the bioreactor system by different workers in ornamental plants and plan for the future improvement of the system after assessing the problems in production of planting material under different bioreactor vessels.

Materials and methods

Reliable micropropagation methods of ornamental plants have been successfully developed by different scientist across the world for use in basic scientific investigations and scaling up of bioreactors.

Table 1

Methods of bioreactors use

Crop	Plant Propagules	Media	Culture type	Stastical analysis	Reference
Lilium	Bulb Scales	MS with BAP and NAA combinations	Airlift Bioreactor	Duncan's multiple range test, at 5% level.	Hee *et al*., 2001 [7]
Lilium	Bulblets	Half strength MS solid medium with 0.3 mg l^{-1} benzyladenine, 0.3 mg l^{-1} naphthalene acetic acid (NAA), and 30 g l^{-1} sucrose	Four types bioreactor: 1. Non stirred jar type, 2. Stirred jar type, 3. Air-lift column type 4. air-lift balloon type	Duncan's multiple range test, at 5% level.	Kim *et al*., 2001 [9]
Lilium	Bulblets from meristem culture	MS with 9% succrose	Baloon type bubble bioreactor	Duncan's multiple range test ($P \leq 0.05$).	Lian *et al*., 2003 [13]
Chrysanthemum	Axillary buds	MS media with 0.5 mg l^{-1} benzyladenine and 30 g l^{-1} sucrose	Column-type bioreactors systems:	Duncan's multiplication range test, at 5% level.	Hahn and Paek, 2005 [15]
Stevia	Shoot tips with a pair of fully opened leaves	MS with various concentrations of IAA, IBA, 2,4-D	Bubble column bioreactor	Duncan's multiple range test at 5% level.	Sreedhar *et al*., 2008 [21]
Zantedeschia (Calla lily)	Shoot tip	MS modified with 100 mg l^{-1} myo-inositol, 1 mg l^{-1} thiamine, 30 g l^{-1} sucrose and 4 mg l^{-1} benzyl amino purine (BAP).	Temporary Immersion System	Dunnentt´s C tests, at 5% level.	Sanchez *et al*., 2009 [19]
Gerbera	Axillary shoots with at least one leaf blade 3–3.5 cm long	Murashige and Skoog (MS) medium with half the level of nitrate	Temporary immersion bioreactor (TIB; BIT®)	Student's *t* test and Tukey's multiple range test at $P \leq 0.05$.	Frometa *et al*., 2017 [5]
Lilium	*In vitro* regenerated bulblets (5-7 mm in size)	MS liquid medium supplemented with 60 g l^{-1} sucrose, 4.4 μM BA and 1.1 μM IAA	4 liter balloon type bubble bioreactor (BTBB)	Duncan's multiple range test, at 5% level.	Cardarelli and Suarez, 2017 [3]

The R.I.T.A.® system: "Reactor of Automatized Temporary Immersion", and B.I.B.® system: "Bioreactor of Immersion by Bubbles".

Results and discussion

Types of propagules produced in the bioreactor

Many Ornamental plant propagules have been cultured in the bioreactor for their mass multiplication. Responses of propagules towards media in bioreactors are quite different

among species or genera and they could be also different from the responses observed under static culture conditions on semi-solid medium. Various types of plant propagules and embryos have been successfully propagated in bioreactors. Storage organs such as bulbs, corms or tubers seem to be the best choice for multiplication in bioreactors. Several examples are as follows:

1. Shoots: Rose, Begonia, Chrysanthemum, Carnation, Gerbera, Anthurium, Petunia;
2. Bulbs: Lilium, Tulip , Zenthedescia;
3. Corms: Gladiolus, Caladium, Crocus;
4. Rhizomes: Orchids.

The propagules produced in the bioreactor easily reestablished in soil, with as little adaptation to *ex vitro* conditions as possible.

Progress in micropropagation automation depends on the use of liquid cultures in bioreactors that can provide rapid proliferation, and separation of plantlets [2]. From the last two decades, bioreactors widely used to optimize the mass regeneration of somatic embryos from embryogenic callus. Takahashi *et al.* [22] achieved virus-free multiplication of Lilium bulblets in a 2000 liter bioreactor. In the case of Lilium cv's Marcopolo, the growth of bulblets in balloon type bubble bioreactor (BTBB) was nearly 10 folds faster than that of the solid medium. In rose, somatic embryogenesis achieved by using zygotic embryo, filament of stamen, leaf, and root [11]. In Phalaenops, Protocorm-like bodies (PLBs) were proliferated from flower stalk by using bioreactors. Charcoal filter attached Bioreactors most suitable for Protocorm like bodies (PLB) culture. About 18,000 PLBs were harvested from 20 g of inoculum (1000 PLB sections) in 2 liter Hyponex medium after 8 weeks of incubation. Hee *et al*. [7] investigated that combination of BAP:NAA (2:0,3) was favorable for Lilium bulblets proliferation inside bioreactors. Kim *et al*., [10] reported that air-lift balloon type favored formation and growth of the lilium bulblets, while stirred jar inhibited bulblet growth. Bulblets in a stirred jar type bioreactor did not survive during the culture period caused by shear stress due to the impeller blades. Schwenkel [20] also reported clonal propagation of *Cyclamen persicum* by using embryogenic cell suspension culture. Lian *et al*. [13] reported that highest number of bulblets (2,0–4,0 g) achived when cultured in immersion type bioreactor for 16 week. Kim *et al*. [9] reported 5000 chrysanthemum plantlets after 12 weeks of culture in 10 liter column type bioreactor system. Pueschel *et al*. [18] highlighted the mass propagation of *Cyclamen persicum* via somatic embryogenesis. Hahn and Paek, 2005 reported that the bioreactor culture favored to the greatest fresh weight, shoot length and leaf area, followed by the ebb and flood culture, while the immersion culture suppressed shoot multiplication of chrysanthemum due to the lack of oxygen and the high water potential. Nhut *et al*. [14] reported that the number of somatic embryos of Lilium derived from embryogenic calli cultured in liquid medium, were more than in solidified medium, 170 rather than 28, and the forms of somatic embryos in different developmental steps (globular-, heart- and cotyledon-shaped) were clearly distinguished under microscope. Ho *et al*. [8] conducted an *in vitro* study with *Lilium formolongi* cvs. Norikula, RaiZen No. 1, RaiZen No. 3, RaiZen Early, and Bailansa. Seedlings of these cultivars were used to induce callus by variously modified MS media, using protocols for flask culture and bioreactor culture. Results showed that the best cell growth and regeneration rate (74±0,14%) of somatic embryos was in a modified 2 liter bioreactor. Sreedhar *et al*. [21] investigated that, the use of modified MS resulted in a high biomass yield 50,68 g dm^{-3} (m/v) of Stevia roots accounting for about 590 microcuttings in three weeks. Best rooting of micro-cuttings occurred in half strength MS medium supplemented with IBA ranging from 4,92 to 7,38 μM, 15 g dm^{-3} sucrose and gelled with 0,8% agar. Sanchez *et al*. [19] studied the effect of immersion frequency and paclobutrazol (PBZ) on Zenthideschia shoot proliferation. This result showed highest biological efficiency

when shoots were immersed in Temporary Immersion System (TIS) every 4 hours (or 6 immersions per day) and when 0,3 mg l^{-1} PBZ was included in the culture medium. Frometa, *et al*. [5] investigated that *in vitro* propagation of Gerbera in a temporary immersion bioreactor for every 8 h immersion with additional ventilation produced more shoots with better morphology than the control. The average multiplication rate was 9 shoots/explants across treatments. The number of shoots/explant increased as culture time increased, more than 87% of plantlets survived in a zeolite and sugarcane filter cake (1:1) substrate. Cardarelli and Suarez [3] found that the application of continuous immersion bioreactors for the micropropagation of lily associated with O_3 treatments was a viable and efficient model for automation and large-scale production of quality shoots.

Current limitations

There are some limitations associated with the use of bioreactors in micropropagation. These include contamination, lack of standard and crop specific protocols and production procedures, hyper-hydricity, and release of phenols or toxic compounds by the cultures. Because of the inherently difficult problems of fungal and bacterial contamination in liquid systems, and the lack of standards information compared to semi-solid growth media, such systems involve higher risk and require greater skill.

Conclusions

Based on above, we suggest that a mass multiplication of a plant cell, tissue and organ by using bioreactor system can be quite a feasible alternative in terms of increase of a multiplication rate and reduction of a production cost when compared with a conventional agar based plant tissue culture method. The simplification of technology could still make bioreactor micropropagation worthwhile for both small and large-scale laboratories in developing countries. Further studies are needed with various ornamental crops to optimize culture conditions for specific genotypes.

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Aswath Ch., Narendera Bh. Биореакторы в прозводстве посадочного материала декоративных растений // Works of State Nikit. Botan. Gard. – 2017. – V. 145 – P. 175-181.

Традиционное микроразмножение с использованием агаризованных или полуагаризованных сред – обычно трудоемкий способ размножения элитных клонов коммерческих декоративных растений, при этом лимитирован высокими лабораторными затратами. Другими ограничениями на этом пути служат дорогостоящие приборы, низкий индекс пролиферации, долгий цикл размножения перед посадкой и пересадкой, и высокая степень гибели от патогенов. Эти ограничения являются главными недостатками традиционного микроразмножения для масштабирования перспективных видов декоративных растений. В настоящее время для микроразмножении декоративных растений применение биореакторов лимитировано, однако, они могут применяться как эффективные средства для производства элитного посадочного материала. Биореактор не только обеспечивает оптимальные условия для роста, но и дает много преимуществ в достижении как максимальной урожайности и высокого качества растениц, но и достаточно снижает затраты на производство. Сегодня эта технология завоевывает популярность в развивающихся странах для коммерческого микроразмножения декоративных растений, таких как орхидеи, лилии, гипеаструм, гладиолусы, спатифилум, антуриум, хризантема, гербера, анектохилус, тюльпаны и т.д., с использованием тонкого слоя клеток, культуры меристем, органогенеза и соматического эмбриогенеза. Часть биорекаторов было разработано и используется для оценки эффективности массового микроразмножения декоратичных растений. Образование протокормо-подобные тел было индуцировано из цветоноса фаленопсиса с использованием суспензионной культуры в биореакторе. Многие сорта лилий успешно размножены в биореакторах. Сорт лилии Марко Поло показал позитивный рост луковичек почти в 10 раз быстрее, чем на агаризованной среде в биореакторе баллонного типа. Современная биореакторная технология с использованием жидких культур обеспечивает более широкое управление традиционными системами микроразмножения. Однако, мониторинг важных параметров процесса, таких как температура, pH, концентрация кислорода и двуокиси углерода внутри биореактора показывает необходимость разработки их для каждого растения.

Ключевые слова: *биореактор; in vitro размножение; декоративные культуры; соматический эмбриогенез.*