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MULTIPLICATION OF OIL PALM LIQUID CULTURES IN BIOREACTORS

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ABSTRACT

Bioreactor systems can provide quantitative data on oil palm liquid cultures since control of the environmental variables is maintained. Oil palm liquid cultures were multiplied in three bioreactors - B-Braun, Biotron and Sixfors. The cultures showed good proliferation with about 5- to 12-fold weight increment after about 60 days in the B-Braun bioreactor. Increments in fresh weight of four-to five-fold were obtained from the Biotron bioreactor. In the Sixfors system, the increment in cell fresh weight was about three-fold. The production and productivity of cultures multiplied in the bioreactors varied from one clone to another. This study has provided a better understanding of oil palm liquid cultures with regards to their growth in different bioreactors and their potential for commercial applications.

Keywords: oil palm suspension cultures; B-Braun, Biotron, Sixfors bioreactor; proliferation, multiplication, automation.

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INTRODUCTION

In 1991, the first regenerable oil palm embryogenic cell suspension cultures were obtained from oil palm leaf calli (Touchet *et al.*, 1991). Later, mature zygotic embryos excised from fruits were used to generate friable embryogenic suspensions (Texeira *et al*., 1995). Protocols to improve the quality of shoot formation, embryogenic suspension and mass propagation of oil palm liquid suspension culture were also developed by Aberlenc-Bertocci *et al*. (1999), Duval *et al*. (1995) and Wong *et al*. (1999). The subsequent results obtained from suspension cultured clones have been encouraging and field trials are under way to evaluate the performance of the palms (Rival *et al*., 2001; Tan *et al*., 2003). Although cell suspension cultures have been used in oil palm micropropagation, limited data is available on using bioreactors (Tarmizi *et al*., 2004; Gorret *et al*., 2004).

Bioreactors provide many advantages for the growth of plant cell and organ cultures as compared to shake flasks. Most bioreactors are designed with either a mechanical or gas agitation mechanism for mixing to maintain a nearly homogeneous culture. The chemical and physical environment can also be controlled for optimum growth and this can be reproduced on a larger scale according to the most desirable performance observed on the smaller scale.

The use of bioreactors for growth of oil palm suspension cultures has several advantages, including reduced labour/operator handling and amenability to computerized control of the operating conditions. The computerized control and monitoring of conditions in the bioreactor, in turn, make it possible to apply statistical procedures to identify the optimal operating conditions. With such automated systems, the potential to reduce costs and time requirements exists, as well as minimizing the sources of contamination and loss.

The application of bioreactor technology in plant propagation was extensively reviewed by Takayama and Akita (2005). The bioreactor system has proven useful in the multiplication of suspension cultures of *Oryza sativa* L. (Okamoto *et al*., 1996), *Cyclamen persicum* Mill (Hohe *et al*., 1999), *Oxalis triangularis* ssp., *Triangularis* (Teng and Ngai, 1999), *Solanum tuberosum*, L. (Yu *et al*., 2000) and *Rubus chamaemorus* L. (Debnath, 2007). As the potential of the bioreactor system is widely acknowledged, attempts were made to multiply oil palm suspension cultures using various types of bioreactors.

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MATERIALS AND METHODS

Plant Materials

The cultures used were an embryogenic suspension with <2 mm aggregates from clones E34 (mature palm, ortet 0.189/2844), E90 (mature palm, ortet 0.189/1173), E132 (mature palm, ortet 0.373/825), E136 (mature palm, ortet 0.366/73), E139 (mature palm, ortet 0.366/1230), E146 (mature palm, ortet P56/380) and E147 (mature palm P56/360).

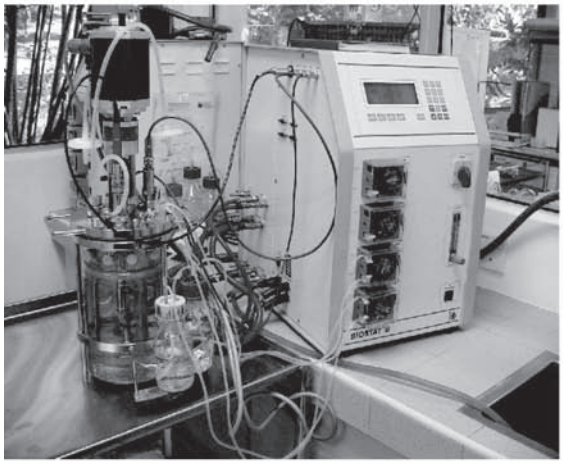
Bioreactor System

The bioreactors used were a B-Braun 'Biostat® B' 2L version 1.0 and a Biotron Version 2.5 system (*Figures 1a* and *1b*). They are basically a bench top and batch culture fermentors with an autoclavable culture vessel - both complete systems with built-in pumps, a vessel with a drive, digital recording and controls.

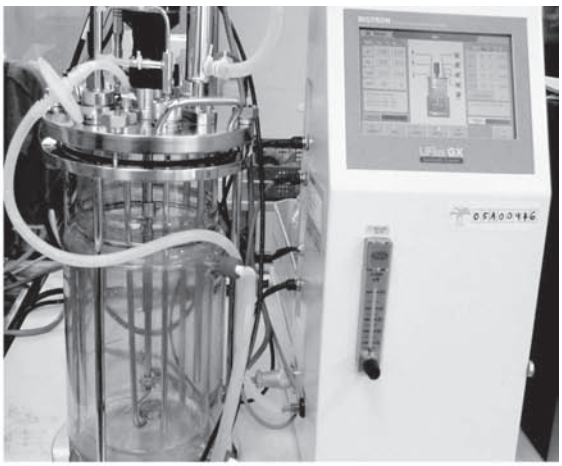
A Sixfors bioreactor with six round-bottom 500 ml glass vessels (Infors, AG CH-4103 Bottmingen/ Switzerland) (*Figure 1c*) was also used.

Media

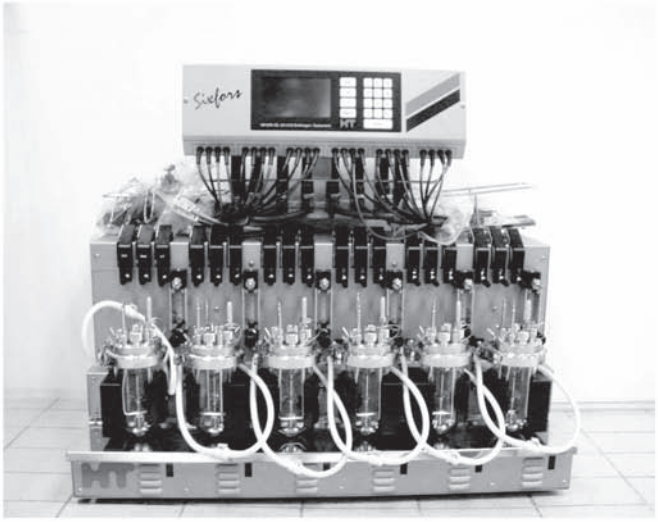
Liquid MS (Murashige and Skoog, 1962) medium with 1 mg litre^{-1} nicotinic acid, 0.1 g litre^{-1} myo-inositol, 0.1 g litre^{-1} L-glutamine and 3% sucrose (castor sugar brand MSM) was used (Rohani *et al.*, 2003). The medium was supplemented with 1 mg litre^{-1} dichlorophenoxyacetic acid (2, 4-D) + 0.1 mg litre^{-1} 1-naphthaleneacetic acid (NAA). This hormonal combination was found effective for culture proliferation in oil palm liquid culture systems (Tarmizi, 2002). The pH of the medium was adjusted to 5.7 before autoclaving at 121°C for 25 min.



a. Bioreactor 'Biostat® B' version 1.0.



b. Bioreactor Biotron Version 2.5.



c. Sixfors bioreactor with six vessels (Infors, AG CH-4103 Bottmingen/ Switzerland).

Figure 1.

Inoculation of Culture

Approximately 4 g to 5 g embryogenic suspension cultures were used to inoculate 1 litre MS medium. The parameter settings for the bioreactor were a temperature of 28ºC and agitation of 60 to 100 rpm. The pH and pO_2 were 5.7 and 20%-40%, respectively.

For the Sixfors system, 4 g culture aggregates were inoculated into 400 ml MS medium. The temperature, aeration and agitation were regulated at 26ºC, 20% dissolved oxygen and 150 rpm.

For comparison, suspensions from the same culture were transferred to 100 ml flasks with inoculation of 0.4 g per 20 ml and incubated in darkness at 100 rpm on an orbital shaker. This shake flask system is used routinely for the proliferation of liquid cultures (Tarmizi, 2002).

RESULTS AND DISCUSSION

Multiplication of Cultures in B-Braun System

A good proliferation rate was obtained, demonstrated by an increment of the fresh weight of the cultures from all clones tested between five- to 12-fold after 60 days in the medium treated with 1 mg litre^{-1} 2,4-D + 0.1 mg litre^{-1} NAA (*Figures 2* and *3*). This indicated that growth development in the bioreactor system varied amongst clones and each may require different parameters for optimum proliferation. The control cultures in the shake flasks only increased 2.5-fold in average. Subsequently, all the cultures from the bioreactor were able to regenerate when transferred to solid media (*Figure 4*). Ramets were also established from clone E90 and have been transferred to the pre-nursery and field nursery (*Figure 5*).

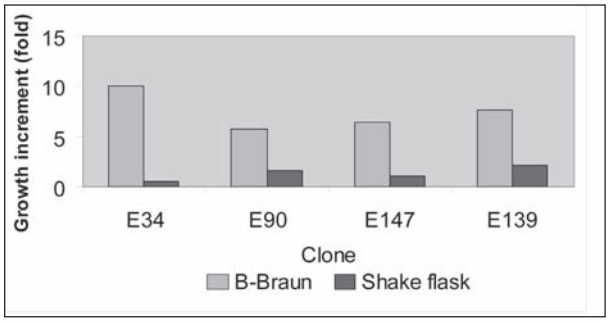


Figure 2. Comparison of growth by oil palm clones in the B. Braun bioreactor and shake flask systems after 60 days in culture.

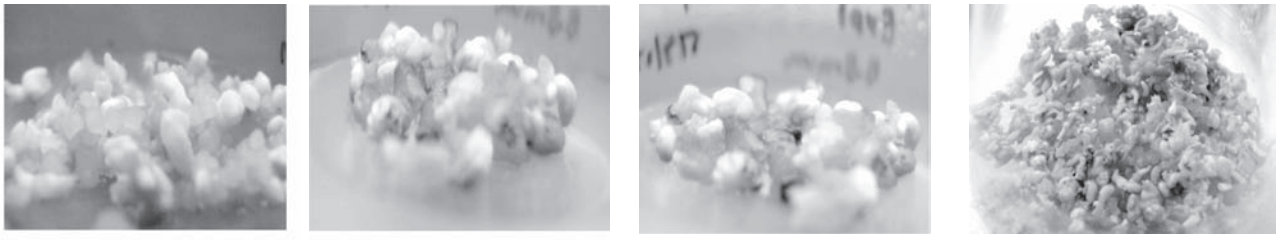
E34 (0.55X)

E90 (0.8X)

E139 (0.75X)

E147 (0.8X)

Figure 3. Culture aggregates of clones E34, E90, E147 and E139 collected from the B-Braun bioreactor after 60 days in culture.



E34 *E90* *E139* *E147*

Figure 4. Cell aggregates of the different clones from the B-Braun bioreactor-cultures starting to regenerate.

a. Pre-nursery (4 months) *b. Field nursery (1 year)*

Figure 5. Ramets derived from clone E90 established in (a) pre-nursery and (b) field nursery.

Multiplication of Cultures in the Biotron System

An increment in fresh weight was also observed from clones multiplied in the Biotron bioreactor. The clones, E90, E139 and E147 increased about three- to five-fold in their fresh weight (*Figure 6*). Even though the increases were not as high as in the B-Braun bioreactor, this Biotron system can also be used for multiplication of oil palm cultures. Further improvement and optimization are currently being conducted on the system.

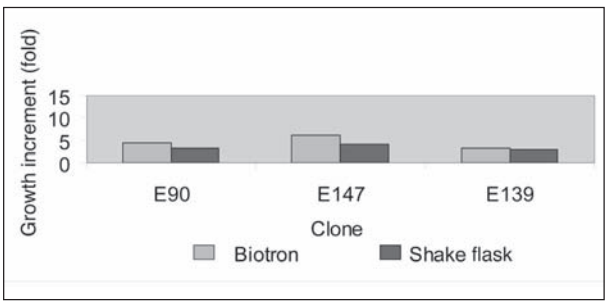


Figure 6. Growth comparison of oil palm clones in the Biotron and Shake flask systems after 45 days in culture.

Multiplication of Cultures in the Sixfors System

After two months in the Sixfors bioreactor, the fresh weight of cell aggregates from clones E132, E136, E146 and E147 were up 1.6-, 2.3- 2.1- and 3.3-fold, respectively (*Figure 7*). The advantage of this system is that running samples in six vessels with regular sampling for certain time periods can be done concurrently. Thus, more clones or samples can be tested simultaneously. Further optimization is being conducted to further improve the system.

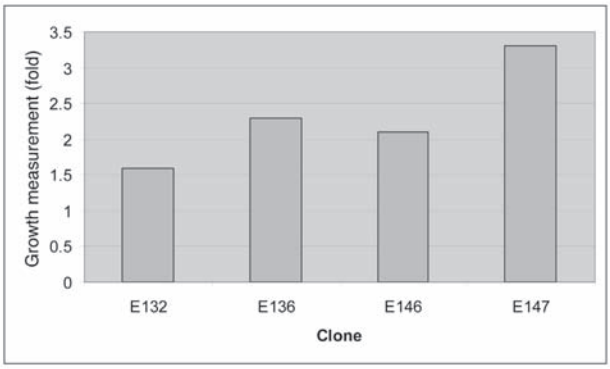


Figure 7. Growth comparison of oil palm clones in the Sixfors bioreactor after 60 days in culture.

Although stirred tank bioreactors have been reported unsuitable for plant cell suspension culture because of the shear stress produced by the impeller, somatic embryos have been successfully grown in various mechanically-stirred tank bioreactor for alfalfa (Stuart *et al.*, 1987), carrot (Kessel and Carr, 1972) and sandalwood (Tautorous *et al*., 1994). It was demonstrated that stirred tank bioreactors effected higher biomass production of *Picea sitchensis* cultures as compared to shake flask, airlift, bubble and hanging stirrer bioreactors (Ingram and Mavituna, 2000). Increase in biomass composing of larger aggregates was found more suitable for propagation in stirred tank as compared to using fine suspension cultures.

In this study, all the three bioreactors are suitable for multiplication of oil palm liquid cultures. For large scale production of embryogenic cultures, the B-Braun and Biotron bioreactors have greater potential in producing more rooted plantlets / ramets than the shake flask system. Based on observations on liquid cultures, 1 g mature aggregates from selected clones can produce about 1000 rooted plantlets after about nine months on regeneration media. If 50 g culture can be collected from the bioreactor, the expected number of rooted plantlets can reach up to about 50 000. For mass propagation of oil palm cultures, bioreactors with a working volume of 1-3 litres are generally sufficient. However, in the oil palm liquid culture system, the risk of clonal abnormality still exists and thus need to be addressed. There is therefore an urgency to develop molecular markers for the screening of good clones derived from bioreactors.

The Sixfors™ bioreactor (Infors AG, Bottmingen, Switzerland) was also useful in the multiplication of oil palm cultures. It permits simultaneous online monitoring and control of the growth conditions, including temperature, agitation, and dissolved oxygen parameters, in six separate reactor vessels. The system can also be supported by analytical equipment, including chromatographic systems for analysis of the culture medium, secondary metabolites, biomass, *etc.* Currently, other culture conditions including sugars, ammonia, nitrate, amino acids and organic acids, are monitored off-line that is samples are withdrawn from the bioreactor, processed and the compounds analysed. This system can be used to gain a better understanding of the behaviour of oil palm cultures with respect to the physical and chemical conditions during propagation in bioreactors.

CONCLUSION

Although cell suspension cultures have been used in oil palm micropropagation (Tarmizi 2002), very limited data is available with bioreactors. Nevertheless, there are several reports on the production of somatic embryos using a bioreactor. The culturing parameters such as pH and DO can be optimized by monitoring and controlling the culture systems. The effect of DO, CO_2, shear stress and bioreactor design on the viability of plant cells and embryoid regeneration have been investigated for different plants and reported to be species dependent (Ibaraki and Kurata, 2001). Furthermore, it is also important to evaluate the effect of different ortets / cell lines of oil palm on their growth in standardized parameters and media using a bench scale bioreactor with several vessels to speed up the experiments.

The application of bioreactors for plant micropropagation is one of the ways to reduce production cost by scaling up and automation. Recent experiments have been restricted to only a few species that, nonetheless, have demonstrated the feasibility of this technology. Innovative technologies to further improve the liquid culture system are being developed at MPOB, *e.g.*, MPOB Fast Transfer Technique (MoFaTT) (Tarmizi and Zaiton, 2005), Simple Impeller with Fast Transfer Technique (SLIM-FaTT) (Tarmizi and Zaiton, 2006a), Two-in-One MPOB Simple Impeller (2-in-1 MoSLIM) (Tarmizi and Zaiton, 2006b) and the MPOB Modified Vessel (MoVess) (Tarmizi *et al.*, 2007). These new technologies which are even more economical and practical and will complement the existing shake flask and bioreactor systems.

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