Efficiency Evaluation of Vanda Tricolor Growth in Temporary Immerse System Bioreactor and Thin Layer Culture System

Rizkita R. Esyanti
Department of Biology, School of Life Science and Technology, Institut Teknologi Bandung, Jln. Ganesha 10 Bandung 40132 Indonesia
Email: rizkita@sith.itb.ac.id

Nikko Adhitama and Robert Manurung
Department of Bioengineering, School of Life Science and Technology, Institut Teknologi Bandung, Jln. Ganesha 10 Bandung 40132 Indonesia
Email: nikko@alumni.itb.ac.id

***Abstract*—*Vanda tricolor* (aliases: *Vanda suavis*) is one of native orchid species found in Indonesia and surrounding countries in SE Asia. It is economically important due to its unique and fragrant smell of its flower. This unique properties increases the demand of this orchid. In order to match the consumer's demand, one of the solutions to mass-produce *V. tricolor* is by using micropropagation method by thin layer liquid medium and temporary immerse system (TIS) bioreactor. In this study, shoot culture of *V. tricolor* is cultivated in full MS media with addition of coconut water using two different systems, thin layer and TIS bioreactor. Two different variations of immersion period of 5 and 10 minutes every 12 hours is given in culture in TIS bioreactor. After 21 days, both systems were analyzed to determine which system and parameter was efficient in accordance to visual and viability analysis, biomass production, and medium utilization (conductivity, and sugar consumption). The result showed that shoots culture in TIS bioreactors are more viable and visually better than in thin layer system. Thin layer system has the highest sugar utilization and conductivity decrease with the value of 4.854 g and 7.52 mS respectively. Largest biomass production occurs in thin layer system with specific growth rate of 0.056/day. Based on visual and viability analysis, biomass production, and medium utilization, this study conclude that TIS bioreactor is more efficient in producing *V. tricolor* shoot.**

***Index Terms*—immersion period, temporary immersion system, thin layer, vanda tricolor**

I. Introduction

Vanda tricolor is one of native orchid species found in Indonesia and some surrounding countries in SE Asia. It is economically important due to its fragrant smell and uniqueness of the flower [1]. The unique properties of the flower has led to massive exploitation, so that the population of this orchid in their natural habitat is significantly decreasing. In order to fulfill demand of consumer, a sustainable method to produce this orchid is required. Conventional method to reproduce the orchid by using nursery is time consuming as well as not economical in term of production and maintenance cost. One of the sustainable method is by micropropagation. This method has proven to be quite efficient for mass production of plants with minimum use of space and resources. However, micropropagation using solid or liquid medium culture face several technical problems, such as uneven nutrient uptake and distribution, hyperhydricity, and the extensive use of man labor [2].

One way to overcome the aforementioned problems is by making modification to the liquid medium culture system by using Temporary Immerse System (TIS). TIS bioreactor is a modified liquid culture system where the immersion of explant happens periodically [3]. In this bioreactor, the medium contained nutrients is periodically immersed the explant thus eliminate the chance of anoxia or hyperhydricity, while the explant still acquire sufficient nutrient supply [4]. Conclusively, this system not only combines the advantages of solid and liquid culture system but also eliminates the disadvantages of both. Previous studies of *Phalaenopsis* micropropagation using TIS bioreactor showed a significant increase of biomass compared to that by using liquid and solid medium [5]. Other studies using *Prunus* and *Malus* showed that the multiplication rate did not differ on solid medium and in TIS bioreactor, but hyperhydricity was present to a certain degree on solid medium but never in temporary immersion system [6].

As previous studies using *Phalaenopsis* orchid showed better result in TIS bioreactor system, studies using other types of orchids is yet to be done. Therefore, in this research, shoot culture of *V. tricolor* orchid was cultivated in full MS media with the addition of coconut water in two different systems, i.e. thin layer and TIS bioreactor. Two different variations of immersion period of 5 and 10 minutes every 12 hours were also applied to the culture in TIS bioreactor. After 21 days, both systems

Manuscript received April 29, 2015; revised July 2, 2015.

doi: 10.18178/joaat.3.1.63-66

were analyzed to determine which system and parameter were more efficient based on the visual and viability analysis, biomass production, and medium utilization (conductivity, and sugar consumption).

II. Materials and Methods

A. Plant Source and Material

Vanda tricolor shoots, in vitro grown on solid media, from the collection of tissue culture in Plant Physiology Laboratory, School of Life Science and Technology, Institut Teknologi Bandung were used. Shoots were cultured for 21 days in 1) TIS bioreactor with immersion period for 5 minutes (TIS A) and 10 minutes (TIS B) per 12 hours; 2), and thin layer system with 5 mL medium incubated in continuous 120 rpm gyratory shaker. For both systems, full MS basal medium supplemented with 150 mL/L-medium coconut water, and 30 g/L sucrose were used. The culture conditions were: temperature 25^{o}C and photoperiod of 16/8 (L/D).

B. Temporary Immersion System (TIS) Bioreactor Apparatus

TIS bioreactor used in this study is custom made according to design of RITA (VI-TROPIC, Saint-Mathieu-de-Treviers, France). This bioreactor was a single glass bottle composed of two compartments; one upper chamber contained the explant, the other one contained the culture medium (Fig. 1). Immersion mechanism of explant was driven by pneumatic transfer of liquid medium and without medium renewal.

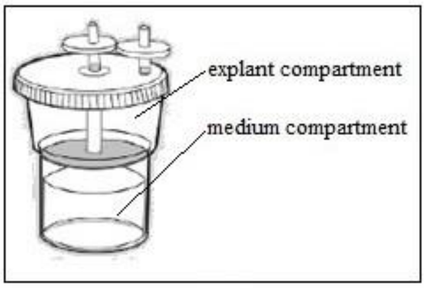

Figure 1. Schematic drawing of RITA bioreactor

C. Experimental Method

For each system, 10 shoots were used and 2 replications were carried out. Medium used in TIS bioreactor was 250 mL while in thin layer system was 5 mL. After 21 days of cultivation, medium and explants were analyzed.

D. Explant Analysis

Explant analysis, include fresh weight (FW) and dry weight (DW) were obtained using a precision balance (Sartorius ED-822) at the beginning and end of the experiment after previously dehydrated in the oven at 80^{o}C for 48 hours.

E. Medium Analysis

Sugar content was measured by BRIX sugar determination using previously calibrated optical refractometer, while conductivity test was measured by using conductivity meter probe (Eutech Instruments, Texas).

III. Result and Discussion

Explant analysis, in term of visual and survivability of the shoots, showed a different result between two systems. Shoots obtained from thin layer system were mostly pale-green colored with fragile appearance which could be an indication of hyperhydricity (Fig. 2). Hyperhydricity on liquid medium culture systems were frequently observed [7], and shoots were not likely to survive acclimatization process [8]. Browning and necrosis were also observed in thin layer system. Browning may be caused by phenol buildup within the shoot as the explants underwent preparation process in the beginning of experiment. However, phenol buildup may also be caused by plant's stress response when surrounding condition incompatible [9]. This buildup may lead to cell death which make the shoots obtained from thin layer system might not viable and survivable. Shoots grown in TIS bioreactor, both in TIS A and B, showed better result where no hyperhydricity or necrosis observed (Fig. 3). In conclusion, TIS bioreactor system produced more viable *V. tricolor* shoots than in thin layer system. This is in agreement with previous studies [6].

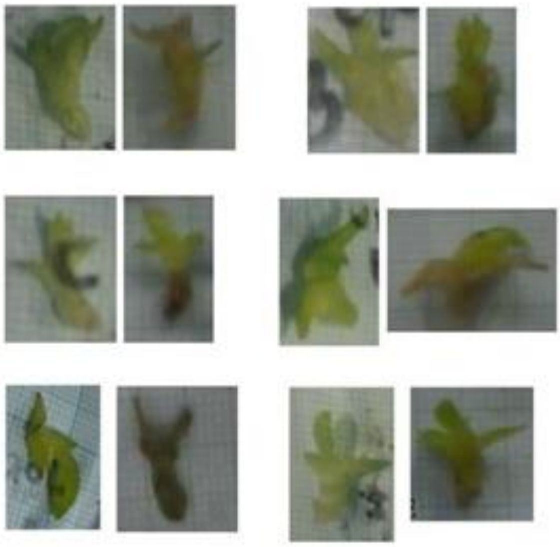

Figure 2. *Vanda tricolor* obtained from thin layer system

Ratio of dry weight/fresh weight showed that both systems were similar, although thin layer system had slightly smaller ratio than TIS bioreactors (Table I). However, FW/DW ratio might also vary and it was related to medium composition, culture system, and plant species used [10]. Growth kinetics from two systems revealed that thin layer system had higher growth rate than TIS bioreactors (Table I), which correspond to higher biomass production. This result was somewhat different when compared with previous studies using *Phalaenopsis* orchid which showed that TIS bioreactor had higher biomass production than in liquid culture system [5].

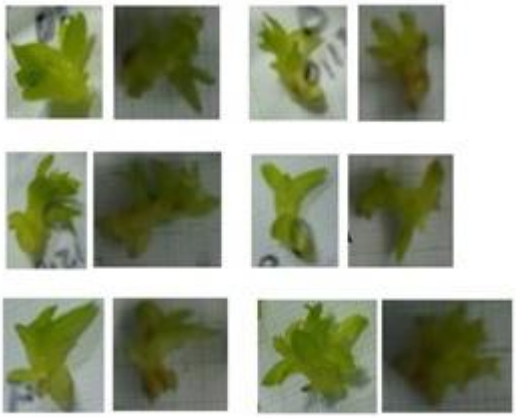

Figure 3. *Vanda tricolor* obtained from TIS bioreactor

TABLE I. EXPLANT ANALYSIS FROM TWO SYSTEMS

System	DW/FW ratio	Growth rate (μ) (/day)	Doubling time (day)
Thin layer	0.0712	0.0564	12.38
TIS A	0.0808	0.0084	27.73
TIS B	0.0782	0.0067	57.76

Medium analysis on sugar utilization and conductivity alteration showed different result in two systems. Thin layer system utilized more sugar (25.28%) compared to TIS bioreactors (6.31%) during 21 days of cultivation (Fig. 4). Higher sugar consumption on thin layer system might correlate with higher biomass production and growth rate in this system as described in Table I. however, at the end of experiment, significant amount of sugar was left unused in the medium of both of culture systems which showed that the systems is not yet efficient.

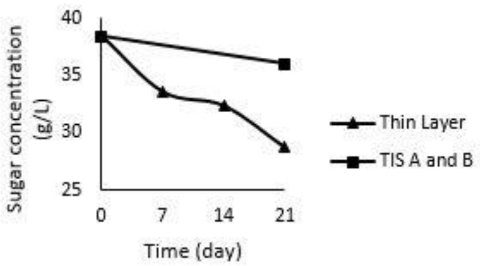

Figure 4. Sugar utilization profile on both systems

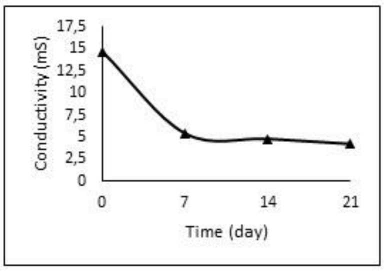

Figure 5. Conductivity decrease in thin layer system

Medium conductivity shows bulk amount of available nutrient inside culture medium. Decrease in conductivity was observed in all systems and this was expected. However, thin layer system had higher conductivity alteration (Fig. 5) compared to TIS bioreactors (Fig. 6). Conductivity alteration occurred due to nutrient uptake from medium by shoots for their growth. Therefore, higher conductivity drop might result in higher growth rate.

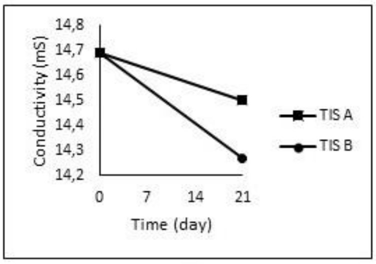

Figure 6. Conductivity decrease in TIS bioreactor

Medium analysis result might explain higher biomass production and growth rate in thin layer system. Although thin layer system produced more biomass and faster growth rate, their ability to sustain shoots' viability and survivability were lower than TIS bioreactor. Thin layer system was designed to maintain medium contact with explant continuously, thus provide better nutrient absorption. However, explants in thin layer system were vulnerable to over-absorption of nutrient and water, due to continuous contact with medium, which might lead to hyperhydricity and cell necrosis TIS bioreactor is designed to prevent over-absorption of water and mineral by periodically immerse the explant in the medium. Slow growth rate and low biomass production observed in this study showed that immersion time and frequency of TIS bioreactors need further optimization.

IV. CONCLUSION

It could be concluded from this study that TIS bioreactor is more efficient in producing Vanda tricolor shoot, particularly by using system of 10 minute immersion. However, further studies on optimization of immersion time and frequency is required.

ACKNOWLEDGMENT

This research and publication was supported by School of Life Science and Technology, Institut Teknologi Bandung.

REFERENCES

[1] A. W. Purwanto and E, Semiarti, *Pesona Kecantikan Anggrek Vanda*, Yogyakarta: Penerbit Kanisius, 2009, pp. 9-17.

[2] J. Persson, "Evaluation of a new type of temporary immersion system (TIS) bioreactors for plant micropropagation," *Swedish University of Agricultural Sciences*, Jan. 2012, pp. 12.

[3] C. Teisson and D. Alvard, "A new concept of plant in vitro cultivation in liquid medium: temporary immersion," *Current Plant Science and Biotechnology in Agriculture,* vol. 22, pp. 105-110, 1995.

[4] F. Afreen, "Temporary immersion bioreactor: Engineering considerations and applications in plant micropropagation," in *Plant Tissue Culture Engineering*, S. D. Gupta and Y. Ibaraki, Eds., Dordrecht: Springer, 2008, pp. 187-201.

[5] T. Hempfling and W. Preil, “Application of a temporary immersion system in mass propagation of phalaenopsis,” in *Liquid Culture Systems for in vitro Plant Propagation*, A. Kathrine, H. Eide and W. Preil, Eds., Dordrecht: Springer, 2005, pp. 231-241.
[6] C. Damiano, S. L. Starza, S. Monticelli, A. Gentile, E. Caboni, and A. Frattarelli, “Propagation of prunus and malus by temporary immersion,” in *Liquid Culture Systems for in vitro Plant Propagation*, A. Kathrine, H. Eide, and W. Preil, Eds., Dordrecht: Springer, 2005, pp. 231-241.
[7] H. Etienne and M. Berthouly, “Temporary immersion systems in plant micropropagation,” *Plant Cell, Tissue and Organ Culture*, vol. 69, pp. 215-231, 2002.
[8] C. Kevers, T, Franck, R. Strasser, J. Dommes, and T. Gaspar. “Hyperhydricity of micropropagated shoots: A typically stress-induced change of physiological state,” *Plant Cell, Tissue and Organ Culture*, pp. 181-191, 2004.
[9] M. R. Davey and P. Anthony, *Plant Cell Culture*: *Essential Methods*, New York: John Willey&Sons, 2010.
[10] I. S. Park and D. I. Kim, “Significance of fresh weight to dry cell weight ratio in plant cell suspension cultures,” *Biotechnology Techniques*, vol. 7, no. 9, pp. 627-630, 1993.

Rizkita R. Esyanti was born in Indonesia on December 7th 1961. She received her B.S. in Biology from Institut Teknologi Bandung (ITB), Indonesia in 1985 and followed by doctorate degree from University of Wales, Swansea, England in Biology and Biochemistry in 1993. Currently she is Associate Professor in plant science and technology research group in Department of Biology, Institut Teknologi Bandung. Her research interests include biomass and secondary metabolite production from *Catharantus roseus* and *Eurycoma longifolia*.

Nikko Adhitama was born in Samarinda on September 6th 1992. He received his B.Eng. in Bioengineering from Institut Teknologi Bandung in 2015. In mid-2013, he worked as researcher intern in Lembaga Ilmu Pengetahuan Indonesia (LIPI) Biotechnology Research Center. His research interest is mainly on biomass and metabolite production from plant cell using bioreactor application. He is now currentlypursuing his master and doctorate degree in Biotechnology in Osaka University, Japan.

Robert Manurung was born in Tapanuli Utara on December 3rd 1954. He received his B.Eng in chemical engineering from Institut Teknologi Bandung in 1978 and followed by M.Eng. in energy technology from Asian Institute of Technology, Bangkok, Thailand in 1981, and followed by doctorate in chemical engineering from Rijksuniversiteit Groningen, Netherland in 1993 and post-doctorate in The MIT Energy Laboratory, Massachusetts Institute of Technology (MIT), USA in 1994. Currently he is Associate Professor in Department of Bioengineering, School of Life Sciences and Technology, Institut Teknologi Bandung. His research interest is in the field of process development related to biobased products: bioenergy and biochemicals.

