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

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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
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°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.

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°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 1. Schematic drawing of RITA bioreactor

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].
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.

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.

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.

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REFERENCES


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