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THE EFFICIENCY OF GROWTH MEDIUM BIOCONVERSION INTO BANANA (*MUSA ACUMINATA*, AA) PLANTLET BIOMASS IN TEMPORARY IMMERSION SYSTEM (TIS) RITA® BIOREACTOR WITH DIFFERENT IMMERSION PERIODS

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ABSTRACT

A temporary immersion system (TIS) was developed as an alternative solution to produce large quantity of banana (*Musa acuminata*, AA) plantlet in relatively short period, i.e. by using RITA® **(**Recipient for Automated Temporary Immersion System**)**. In this study, we evaluated the effect of immersion period of medium on growth and development of banana somatic embryo as well as the medium bioconversion into production of shoot biomass. Somatic embryos of *M. acuminata* were grown in full strength MS medium supplemented with 2 ppm BAP (benzylaminopurine). RITA® bioreactors were set up with two different immersion periods, 1 min and 15 min, scheduled every 6 h and incubated for 21 days. The result indicated that immersion period affected embryo's germination and growth rate (μ). Fifteen-minutes immersion showed greater percentage of germination (93.44 ± 0.59%), growth rate (0.042 ± 0.001 g/day), and productivity (0.025 g. L_{medium}^{-1}. $hari^{-1}$) compared to that immersed for 1 min (81.49 ± 0.34%, 0.037 ± 0.001 g/day, and 0.019 g. L_{medium}^{-1}. $hari^{-1}$ respectively). Additionally, the pattern of sucrose, mineral, and inorganic compounds consumption followed the growth of cell biomass for both systems. In conclusion, RITA® system with longer immersion period supports better efficiency of medium bioconversion.

KEYWORDS: Musa Acuminata (AA), TIS RITA®, Immersion Period, Growth Rate, Medium Bioconversion, Somatic Embryo

INTRODUCTION

Bananas (*Musa* spp.) are one of the most important horticultural crops in tropical and sub-tropical regions of the world. Bananas become a substitute staple food for millions of people (such as in Papua, Indonesia), because of its' culture and also it has excellent nutritional value. Based on data released by the Indonesian Center of Statistics (BPS) [1], the average total consumption of bananas in the household in Indonesia from 2002 to 2012 is quite high, amounting to 7.674 kg/capita/year. However, this high demand could not be provided by the national stock, yet Indonesia still require to import.

Generally, bananas are cultivated by conventional methods and in vitro cultivation. Conventional cultivation includes vegetative planting bananas using young shoots or part of the tuber. This method takes a long growth period and prone to various diseases [2]. As an alternatives, in vitro culture offers more effective and better controlled condition for

cultivation of banana.

Various studies on in vitro banana propagation have been done, including in vitro culture method in bioreactor TIS (Temporary Immersion System). This method is a promising since banana seedlings could be propagated in large quantities with in a relatively short time. TIS provide conditions in which explants do not always submerged with medium, thus avoid hyperhydricity. Certain physical conditions in the reactor has to be optimized for the growth of biomass in the reactor itself, one of which is the period of the immersion [3].

Some banana cultivars that have been successfully in vitro cultivated using TIS bioreactor were *Musa* spp. cv. Dwarf Cavendish [4], *Musa* spp. cv. Grande Naine AAA [5, 6], and *Musa* AAB [7, 8]. However, only few studies reported the cultivation of diploid banana (*M. acuminata*, AA). Therefore, this study was conducted to determine the potential of *Musa acuminata* (AA) cultivation in the TIS RITA® bioreactor system, by evaluating the effect of immersion period of medium on growth rate and development of *M. acuminata* (AA), productivity, and the rate of nutrient uptake by *M. acuminata* (AA) biomass during cultivation.

MATERIALS AND METHODS

A. Culture Medium

Medium was consisted of MS salts supplemented with 2 ppm BAP (benzylaminopurine), and 30 $g.L^{-1}$ sucrose. The pH of medium was adjusted to 5.8 and autoclaved at 121 ^{0}C for 15 minutes.

B. Acclimatization of *M. Acuminata* (AA) Somatic Embryos in Liquid Media

Somatic embryos culture in solid medium were subcultured into thin layer liquid medium culture aseptically. 1 g of *M. acuminata* (AA) somatic embryos were cultivated in 5 mL of medium and incubated at a rotary shaker at 90 rpm in room temperature (25 °C). The acclimatization stage lasted for a week before entering into the bioreactor .

C. Shoot Culture in RITA® Bioreactor

Embryos were taken from the thin layer culture, which have been maintained previously, weighed, and counted under a stereo microscope, then cultivated in 250 mL of medium in the bioreactor aseptically. Bioreactors assembled and the air flow was controlled by an automatic timer with two different immersion periods: (1) 1 min; (2) 15 min, scheduled every 6 h.

D. Plantlet and Medium Analysis

After 21 days cultivation, plantlets were harvested, counted (the number and height), weighed, and then dried to obtain the dry weight data. Sucrose remaining content and conductivity of culture medium were also tested. The conductivity was measured by using a conductivity meter (Eutech Instruments Con-110) and the test of sucrose content in the medium was carried out by using a handheld refractometer (Atago U.S.A., Inc Uricon-NE).

E. Data Analysis

Data of dry weight were then transferred into growth curve. Data of sucrose content and conductivity of medium were used to construct a mass distribution model of *M. acuminata* (AA) shoot culture in TIS RITA® bioreactor. The mass

balance modeling was based on the following reaction [9]:

$$0{,}39C_{12}H_{22}O_{11} + 0{,}23NH_4NO_3 + 3{,}43O_2 \rightarrow CH_{1.27}O_{0.43}N_{0.45} + 4{,}07H_2O + 3{,}64CO_2$$

The growth and sucrose consumption curves were developed based on the following equations:

a. Growth Kinetics

Logistic equation [10]:

$$\frac{d}{dt}[X] = \mu X\left(1 - \frac{X}{X_{max}}\right)$$

$$\mu = \mu_{max} * \left(\frac{[C_{12}H_{22}O_{11}]}{k_{C_{12}H_{22}O_{11}} + [C_{12}H_{22}O_{11}]}\right)$$

b. Sucrose Consumption [9]:

$$\frac{d}{dt}[C_{12}H_{22}O_{11}] = -q_{C_{12}H_{22}O_{11}} * X$$

$$q_{C_{12}H_{22}O_{11}} = \frac{\mu}{Y_{x/C_{12}H_{22}O_{11}}}$$

$$Y_{x/C_{12}H_{22}O_{11}} = \Delta X / \Delta[C_{12}H_{22}O_{11}]$$

The productivity of *M. acuminata* (AA) in RITA® system was determined by dividing the addition of biomass dry weight (g) with the volume of medium (L) and cultivation time (days).

F. Statistic Analysis

Data of shoot height were analyzed using student-t test, to compare the average of each treatment. All statistical tests were performed at the 95% confidence interval using SPSS 22.0 (SPSS Inc, USA).

RESULTS AND DISCUSSIONS

Mature stage of *M. acuminata* (AA) somatic embryos (Figure 1 A) were cultured in RITA® bioreactor for 3 weeks. The results showed that the embryos succesfully germinated into plantlets as shown in Figure 2 B and C. The germination frequency of *M. acuminata* (AA) somatic embryos as well as plantlets height generated from the RITA® 1 and 2 system were presented in Table 1. The germination frequency of *M. acuminata* (AA) somatic embryos is higher for the RITA® 2 system (15 min immersion) than the RITA® 1 system (1 min immersion). The average of germination frequency for RITA® 2 is equal to 93.44 ± 0.83% with a height of plantlets produced 2.55 ± 0.72 cm, while for RITA® 1 amounted to 81.49 ± 0.34% with a height of plantlets 1.04 ± 0.42 cm.

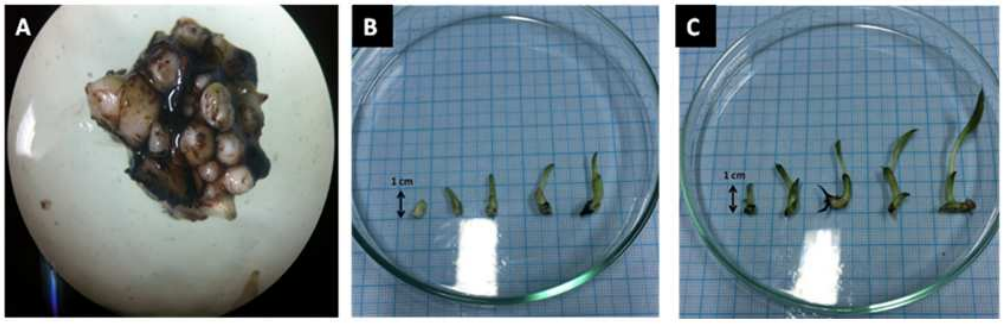

Figure 1: Embryo Somatics and Plantlet of *M. Acuminata* (AA); (A) Examples of *M. Acuminata* (AA) Embryo Somatics; (B) Shoots From RITA® 1; (C) Shoots From RITA® 2

Table 1: Germination Percentage of *M. Acuminata* (AA) Somatic Embryos and Plantlet Height Produced by RITA® 1 Dan 2 Bioreactors

RITA® Bioreactors	AVERAGE NUMBER of EMBRYOS AT BEGINNING ± SD	Average Number of Plantlet Produced ± SD	Germination Percentage (%) ± SD	Average of Plantlet Height (Cm) ± SD
1	92,00 ± 16,97	75,00 ± 14,14	81,49 ± 0,34	1,04 ± 0,42
2	68,50 ± 2,12	64,00 ± 1,41	93,44 ± 0,83	2,55 ± 0,72*

Note: (*)significantly different based on student-t test at 95% confidence level.

Figure 2 showed the differences in the growth rate of culture in the RITA® 1 and RITA® 2 bioreactors after 21 days of cultivation. Fifteen-minutes immersion showed higher growth rate (0.042 ± 0.001 g/day) and productivity (0,025 g. L_{medium}^{-1}. $hari^{-1}$) compared to that immersed for 1 min (0.037 ± 0.001 g/day and 0,019 g. L_{medium}^{-1}. $hari^{-1}$ respectively).

According to Mordocco et al. [11], configuration of immersion period in RITA® system will greatly determined the productivity of the system, besides genotype and explant size factors.

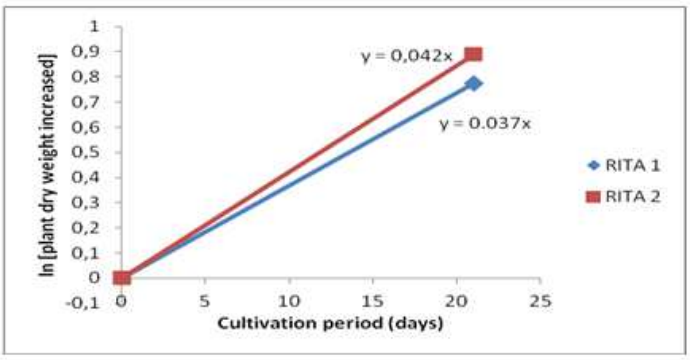

Figure 2: Graph of *M. Acuminata* (AA) Biomass Production in RITA® Bioreactors System after 21 Days of Cultivation

Observation result of *M. acuminata* (AA) shoots in RITA® 1 and 2 system indicated two different growth patterns in shoot culture. The variation of growth pattern was influenced by the different immersion periods of the culture. Immersion period is one of the critical parameters for culture growth, since the period of immersion related to the length of contact between the culture and medium. These conditions determine the absorption of nutrients by explants [3]. The percentage of germination and elongation of shoots on the RITA® 2 system (15 min immersion period) was proportional to the growth rate of the culture when compared with RITA® 1 (1 min immersion period). Similar results were reported by Teisson and Alvard [12] that 15 min immersion every 6 h significantly increased the frequency of embryo germination in

Coffea spp. The frequency of somatic embryos germination obtained in RITA® 1 system was also higher than the study from Escalant et al. [13], in some kind of triploid banana using TIS RITA® system with the same intensity (1 min every 6 h) produced only 60-70% of germination.

Figure 3 showed the pattern of mineral uptake of cultures during 21 days of incubation time. All treated culture consumed minerals contained in the medium, therefore the conductivity of two bioreactors system generally decreased. However, consumption of minerals on RITA® 2 bioreactor culture system (1.568 ± 0.764 mS) was relatively higher compared to RITA® 1 (0.120 ± 0.014 mS). Consumption of mineral and inorganic compounds will be directly proportional to the addition of biomass. The higher consumption of mineral and inorganic compounds, the higher increase of biomass [14].

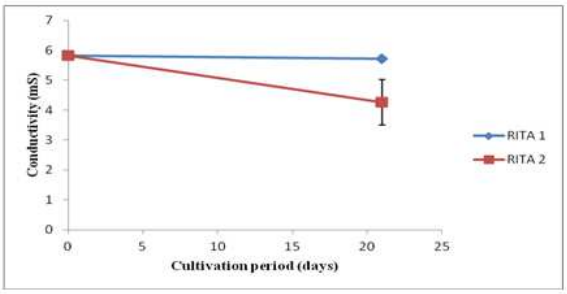

Figure 3: Medium Conductivity of *M. Acuminata* (AA) Culture

Sucrose consumption patterns in bioreactor culture system of *M. Acuminata* (AA) were shown in Figure 4. Sucrose consumption rate of RITA® 2 bioreactor culture system was 0.38 $g.L^{-1}.day^{-1}$ or higher when compared to RITA® 1 (0.05 $g.L^{-1}.day^{-1}$). This sugar consumption rate is positively correlated to the rate of biomass formation. Since sucrose is the only carbon source for cell biomass, it concentration in medium would decrease as the increasing of the weight of biomass [15].

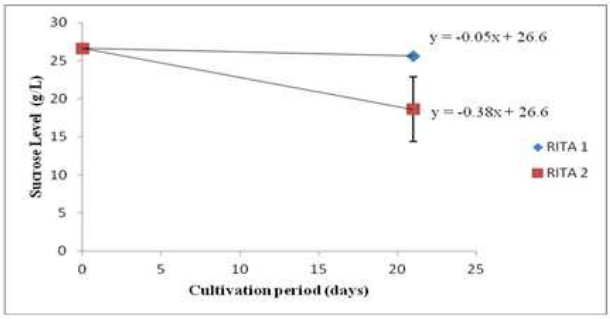

Figure 4: Sucrose Level in *M. Acuminata* (AA) Culture Medium

Table 2 and 3 showed the mass balance of RITA® 1 and 2 culture systems. Our results showed that the hypothetical amount of biomass and the entire value was different from the actual amount of biomass obtained from the measurement of plantlets weight. The hypothetical amount of biomass in RITA® 1 was 0.282 g while the actual was 0.182 ± 0.014 g, and the hypothetical amount of biomass in RITA® 2 was 1.678 g while the actual was 0.225 ± 0.007 g. This could be explained by a mismatch between the root biomass formula of *Atropa belladona* with the shoot biomass formula

of *M. acuminata* (AA). Each cell had a different of substrate consumption pattern and biomass formation pattern. Another factor that might influence the construction of a mass balance model was the limiting substrate in the reaction, as this was not examined in this study. On the other hand, knowledge of the limiting substrate will be very helpful in validating the model that has been built.

Figure 5 showed that every culture system had a curve intersection of sucrose consumption and biomass growth curve at different time. The curve was obtained from equation modeling of the growth kinetics and the consumption rate of sucrose with the help of Berkeley Madonna software. The results of the modeling showed that RITA® 2 culture system had a curve intersection earlier than RITA® 1 system. The maximum biomass amount of RITA® 2 system (0.203 unit of biomass) was higher than the RITA® 1 system (0.18 unit biomass). Based on the analysis of the curve intersection and the maximum amount of biomass, banana culture in TIS RITA® 2 (15 min immersion) was more efficient for bioconversion of substrate into biomass than the same system with shorter immersion period (1 min).

CONCLUSIONS

In vitro propagation of *M. acuminata* (AA) cultured in TIS RITA® bioreactors was potential to be developed as an efficient method of it shoot production. Furthermore, the period of culture immersion was a critical parameter that affected the growth of banana in TIS. Fifteen-minutes immersion in medium was an optimal immersion period for growth of the *M. acuminata* (AA) shoots compared with 1 min immersion. Comparison between the percentage of germination, growth rate, biomass acquisition, as well as productivity of *M. acuminata* (AA) with the consumption of sucrose and nutrition showed that the RITA® culture system with 15 min immersion period gave better results than 1 min immersion period every 6 hours.

Table 2: Culture Mass Balace in Tis Rita® 1 System

Reaction Equation	$0,39c_{12}h_{22}o_{11}$	$0,23nh_4no_3$	$3,43o_2$	$Ch_{1,27}o_{0,43}n_{0,45}$	$4,07h_2o$	$3,64co_2$
begining (mol)	0,078			0,003	0,000	0,000
reaction (mol)	0,003	0,002	0,026	0,007	0,030	0,027
final (mol)	0,075			0,011	0,030	0,027
final (g)	25,581			**0,282**	0,549	1,200

Table 3: Culture Mass Balace in TIS RITA® 2 System

Reaction Equation	$0,39c_{12}h_{22}o_{11}$	$0,23nh_4no_3$	$3,43o_2$	$Ch_{1,27}o_{0,43}n_{0,45}$	$4,07h_2o$	$3,64co_2$
begining (mol)	0,078			0,004	0,000	0,000
reaction (mol)	0,023	0,014	0,206	0,060	0,244	0,218
final (mol)	0,054			0,063	0,244	0,218
final (g)	18,588			**1,678**	4,390	9,597

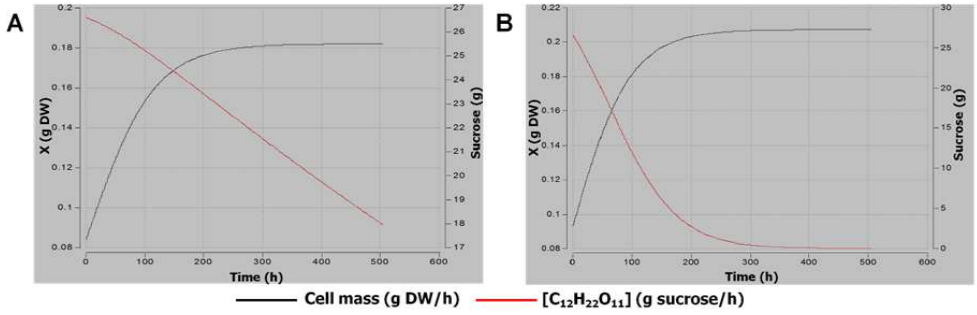

Figure 5: Sucrose Concumption and Biomass Growth Curves; (A) RITA® 1 System; (B) RITA® 2 System

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