Influence of Explant Position on Growth of *Talinum paniculatum* Gaertn. Adventitious Root in Solid Medium and Enhance Production Biomass in Balloon Type Bubble Bioreactor

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Abstract. *Talinum paniculatum* Gaertn. is one of traditional medicinal plant in Indonesia as an aphrodisiac. This plant has various compounds which is accumulated in roots. In vitro culture of this plant can enhance production of adventitious roots. The aim of this research was to know the influence of explants position on growth of *T. paniculatum* Gaertn. adventitious root in MS solid medium and enhance the production of biomass in balloon type bubble bioreactor. Explants from leaf were cultured at abaxial and adaxial positions in solid MS medium supplemented with IBA 2 mgL^{-1}. Adventitious roots were cultured in bioreactor with various treatments (without IBA, supplemented with IBA 2 mgL^{-1} and supplemented with IBA 2 mgL^{-1} + buffer $NaHCO_3$). Result showed that the main growth of abaxial root was higher than adaxial, however, the total of adaxial root branch was higher than abaxial. The highest biomass production of adventitious root cultured was achieved by MS medium supplemented with IBA 2 mgL^{-1} + buffer $NaHCO_3$. This treatment has produced fresh biomass two fold of initial inoculum.

Keyword: Abaxial, adaxial, biomass, bioreactor, $NaHCO_3$, *Talinum paniculatum*

1. Introduction

Talinum paniculatum Gaertn. has grown in Indonesia and was used as traditional medicinal plant, but growth of this plant is low in natural habitat especially on root organ. It contains various bioactive compounds, including saponin. This compound has been revealed to bring benefit for vitality (aphrodisiac). The accumulation of saponin can be found in root organ, which can be increased with adventitious root culture. Many studies have used the adventitious root culture as one of the techniques to accelerate large-scale multiplication, to increase root biomass and secondary metabolites [1,2,3]. The adventitious root culture can be induced from parts of the plant. In the culture, explant position also can influence the root growth. Some authors reported that explant position can influence the growth of plant tissue culture [4,5]. Technology of root culture in bioreactor is a promising method to produce root biomass and secondary metabolites, because it can be controlled both physically and chemically. One of bioreactors for adventitious root culture is Balloon Type Bubble Bioreactor

(BTBB). Many research used bioreactor to culture various plants such as *Panax ginseng* [6], *Eurycoma longifolia* [7] and *Morinda citrifolia* [8].

One of the cultivation methods in bioreactor is batch cultivation which is characterized by constantly changing environmental conditions. Sajc *et al.* [9] explained it has potential to produce metabolites associated with any kinetic pattern. This cultivation can be used to determine the conditions for maximum biomass productivity. Plant growth hormone such as IBA has potent for induction of root formation and elongation [10,11].

Sodium bicarbonate ($NaHCO_3$) provides high buffer capacity in the cell culture medium thus keeping the pH-value in the physiological conditions during cultivation. The required CO_2 concentration is related to the desired pH-value and the $NaHCO_3$ concentration of the medium [12,13]. The aim of this research was to observe the influence of explants position on growth of *T. paniculatum* Gaertn. adventitious root in MS solid medium and enhance production biomass in BTBB with buffer and batch cultivation.

2. Material and Method

2.1. Plant material and optimization of the culture conditions

The study was carried out in the plant physiology laboratory at Departement of Biology, Faculty of Science and Technology, Airlangga University between December 2015 - July 2016. Adventitious roots were induced from leaves of *T. paniculatum* Gaertn. on MS [14] solid medium supplemented with 7 gL^{-1} agar, 30 gL^{-1} sucrose and 2 ppm indole-3-butyric acid (IBA). Samples of leaf (2 cm length) used were the second leaf from the tip. All of the explants were washed with detergent 10 % (v/v) and thoroughly under tap water for 15 minutes. Then surface of the explants were sterilized 5 % (v/v) $HgCl_2$ for 5 minutes in laminar airflow. After that, explants were washed 3-4 times with sterile distilled water. The cultures were planted in Petri dish containing 25 mL medium and pH was adjusted to 5.8 with 1.0 N HCl or 1.0 N NaOH before adding 7 gL^{-1} agar and autoclaving at 121 ^{0}C for 20 minutes. Different positions of leaf namely abaxial and adaxial were aapplied to verify the suitable culture method for accumulation of main root and root branch of adventitious root. After five weeks, the adventitious root from the first culture were transferred to liquid MS medium supplemented with 2 mgL^{-1} IBA in the balloon type bubble bioreactor (BTBB).

2.2. Balloon type bubble bioreactor (BTBB) Design

Bioreactor with a capacity of 1 L was filled with 350 mL of liquid MS medium supplemented with 2 ppm IBA and 30 mgL^{-1} sucrose and sterilized by autoclaving at 121°C for 15 min. The adventitious roots were cultured in bioreactor with various treatments (without IBA, supplemented with IBA 2 mgL^{-1} and supplemented with IBA 2 mgL^{-1} + buffer of $NaHCO_3$). Aeration volume was established at 0.2 vvm (volumes of gas per volume of liquid per minute) and the initial inoculum density 2 g/350 mL. Bioreactor was modified with net in middle to support growth of the adventitious root.

2.3. Determination of main root and root branch

After 5 weeks of culture, the main root and root branch were observed and calculated. The main root appeared from explant directly, meanwhile root branch was fixed of main root. The texture color of adventitious root was recorded. All the cultures were maintained for 35 days at 25 ± 2^{0}C, with a 16 hour light (40 μmol m^{-2} s^{-1})/ 8 hour dark photoperiod cycle provided by 40-W white fluorescent tubes.

2.4. Determination of fresh and dry biomass

The cultures were maintained for 35 days and sampling has done every seven days to determine the total sugar, electrical conductivity (EC) and hydrogen ion concentration (pH) of the medium. All the cultures were maintained at 25 ± 2^{0}C, with a 16 hour light (40 μmol m^{-2} s^{-1})/ 8 hour dark photoperiod cycle provided by 40-W white fluorescent tubes. After five weeks, the adventitious was harvested and separated from the medium through a stainless steel sieve. The fresh biomass was recorded after

blotting away the surface water. The dry biomass was measured after drying root and shoot at 60^0C for 48 hour. The growth ratio (GR) was calculated as GR=[harvested DW(grams)–inoculated DW (grams)]/inoculated DW (grams).

2.5. Determination of total sugar, conductivity and hydrogen ion concentration in the medium
Measurement of total sugar, conductivity and hydrogen ion concentration has conducted to liquid MS medium supplemented 2 ppm IBA. The electrical conductivity (EC) was measured using a conductivity meter (Ezdo, Cond5021), total sugar was measured using a refractometer (Atago) and the hydrogen ion concentration (pH) of the culture medium was measured using a pH meter (Boeco, BT-600) at first week until fifth weeks.

2.6. Statistical analysis
For the first culture, the growth of leaves as explant was induced with two treatments (abaxial and adaxial positions) with six replicates. The data was analysed using SPSS software (v.17). Mean separation within column by Independent sample T-test at $P \leq 0.05$. For the second culture, the data of adventitious root was collected in BTBB and was analysed with descriptive analyses using the same software.

3. Result and Discussion

3.1. Determination of main root and root branch of T. paniculatum
In this study, the explant positions in the medium, both abaxial and adaxial of leaves could influence the production of adventitious root especially the main root and root branch. Based on statistic analysis, the main growth of abaxial root was higher than adaxial, but the number of adaxial root branches was higher than abaxial (table 1). The morphology of adventitious based on explant position exhibited similar texture and color (figure 1). Both the culture of abaxial and adaxial positions showed thick texture and white in color.

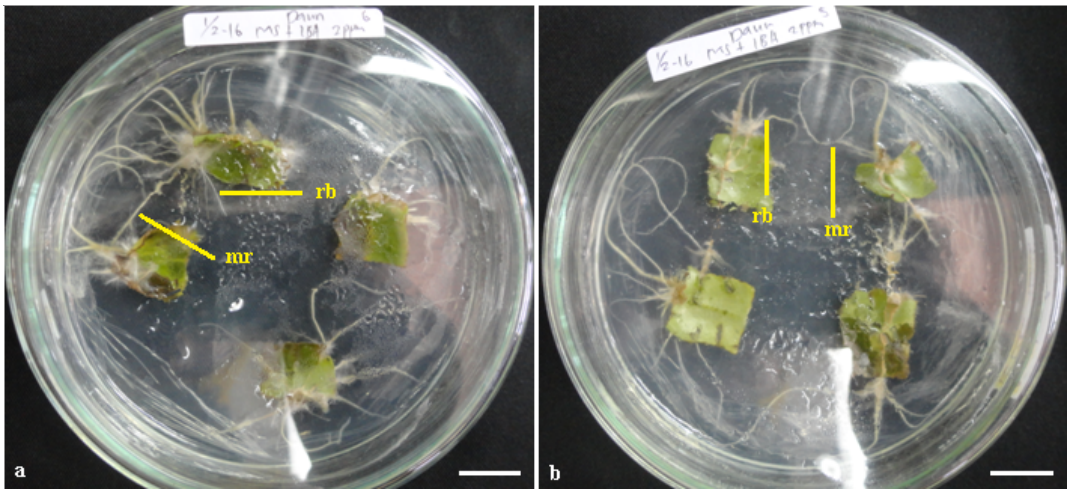

Figure 1. Adventitious root of *Talinum paniculatum* at different explant position treatments in MS medium supplemented 2 mgL^{-1} IBA: a) abaxial, b) adaxial. mr-main root; rb-root branch. (Bar = 1,5 cm).

The culture of adventitious root can be induced from leaf of *T. paniculatum*. In this study, the culture was developed from two different explant positions. It can influence growth of the adventitious root especially at the main root and branch root (table 1). Position of explants in the culture profound

different between abaxial and adaxial (figure 1). Many studies also reported that explant position can improve the plant growth in plan tissue culture [4,5].

Table 1. Number of main roots and root branches from two position of explants (abaxial and adaxial) for five weeks in Petri dish containing MS solid medium with 2 mgL^{-1} IBA.

No.	Treatments	Main Roots (Mean ± SD)	Root Branches (Mean ± SD)
1	Abaxial	34 ± 11.78	18 ± 3.85
2	Adaxial	9.5 ± 2.35	37.2 ± 7.57

Data represents mean values (n=2) with six replicates. Mean separation within column by Independent sample T-test at $P \leq 0.05$

3.2. Biomass of T. paniculatum adventitious root

The highest biomass production of *T. panicualtum* adventitious root was achieved by MS medium supplemented with 2 mgL^{-1} IBA + buffer of $NaHCO_3$. This treatment had produced fresh and dry biomass for 5.2 g and 0.26 g, respectively (figure 2), which was two fold of initial inoculums. Treatment of MS medium with 2 mgL^{-1} IBA without buffer resulted both fresh and dry biomass for 4.33 g and 0.21 g, respectively. The treatment without hormone and buffer produced both fresh and dry biomass lower than the others. Growth ratio in batch with IBA and buffer was 1.53, followed by batch with IBA for 1.13.

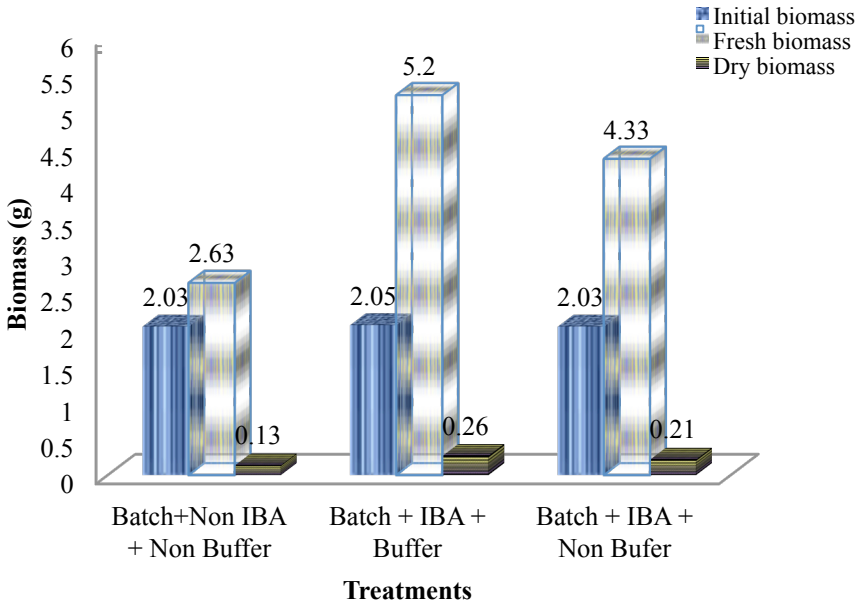

Figure 2. Biomass production of *T. paniculatum* adventitious roots in 1000 mL balloon type bubble bioreactor containing 350 mL MS liquid médium with three treatments for 35 days.

Studies revealed that the production of biomass in liquid culture is influenced by concentration of MS medium, culture method, inoculums, sucrose and plant growth homone [7,8,16,15,17]. Furthermore, Yan *et al*. [18] reported that the plant tissue culture may continuously be provided with better homogenization and hormones which improved the growth of the plantlets. Kuria *et al*. [19] also added that biomass accumulation in liquid medium is higher than that in solid medium. Similarly, Preece [20] reported that employment of liquid media could enhance the growth of root and shoot.

In this study, the tissue cultures were done in batch system. It showed a significant enhancement upon the growth of adventitious root in biorector. Bioreactor method for adventitious root cultures have been developed previously [21]. Sivakumar [22] explained that the bioreactor culture system is more advanced than the traditional plant tissue culture system because the culture conditions in a bioreactor can be optimized. The culture conditions that can be optimized including temperature, pH, concentrations of oxygen, carbon dioxide and nutrients in the medium. Moreover, nutrient uptake of roots can also be enhanced by continuous medium circulation. Furthermore, cell proliferation and regeneration rates can also be increased.

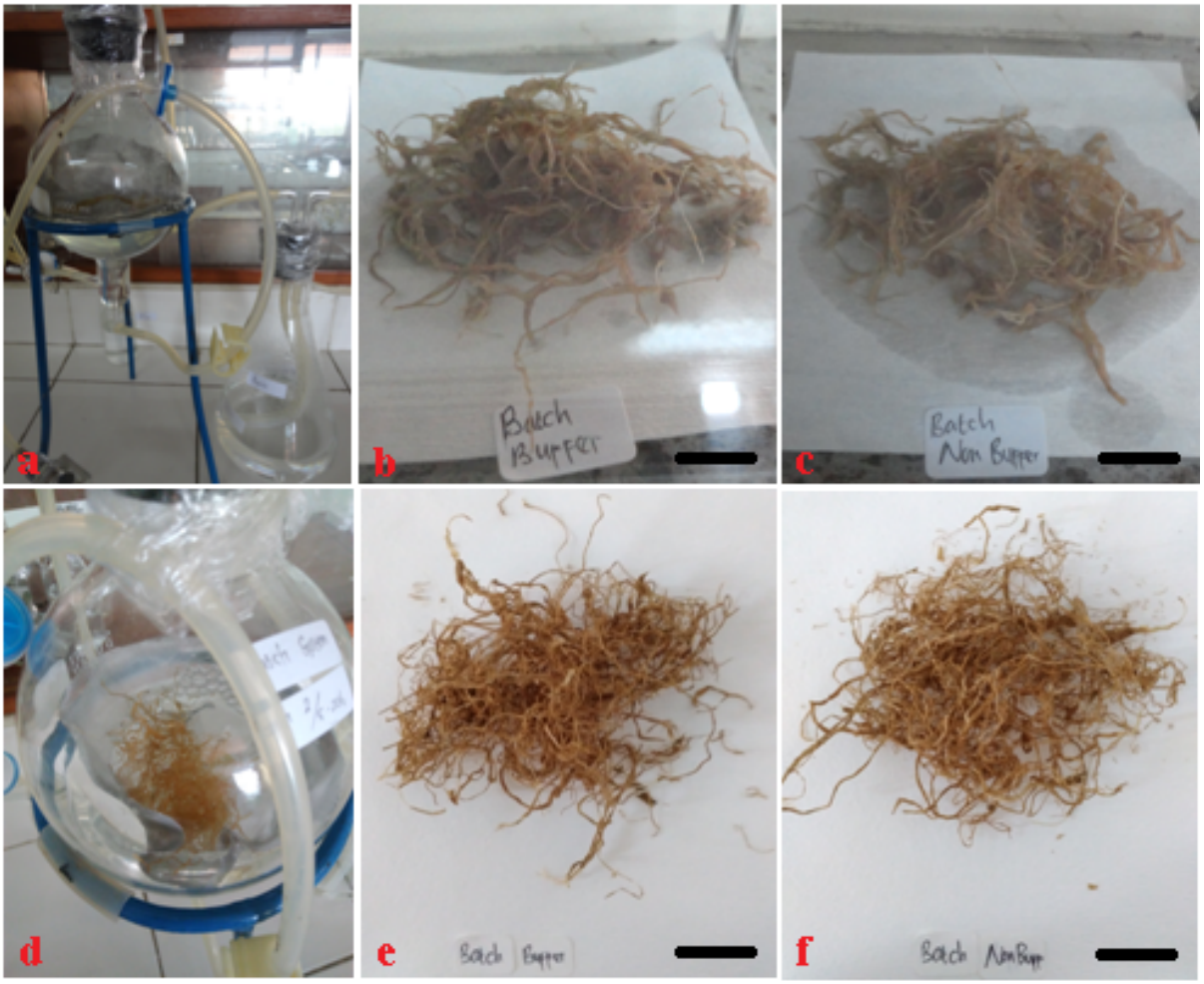

Figure 3. Adventitious root culture of *Talinum paniculatum* Gaertn.: a) balloon type bubble bioreactor ; b-c) fresh biomass in batch buffer and batch non-buffer ; d) culture process ; e-f) dry biomass in batch buffer and batch non-buffer. (Bar = 1,5 cm).

Other than physical parameters, the inoculum density can also influence the growth of adventitious roots. Inoculum density is an important factor affecting growth production in a number of plant cell culture systems. Baque *et al.* [15] reported that inoculum density significantly affect the accumulation of root biomass of *M. citrifolia* during 4 week of culture.

Plant growth hormone such as IBA has potentially induced root formation rather than IAA or synthetic auxins [10]. Yang and Davies [23] showed further evidence of IBA for basipetal transport. The authors stated that IBA can stimulate elongation of subtending nodes, suggesting IBA is transported basipetally in intact pea plants and it has been examined the radiolabeled distribution after application of a rooting solution to the explants base. Zolman *et al*. [11] also reported that auxin

hormone such as IBA and IAA at root elongation and lateral root formation of *Arabidopsis* developmental processes profound sensitive. Moreover, Poupart and Waddell [24] explained that specificity of IBA transport tissue can support the possibility of endogenous auxin in growth and development of some *Arabidopsis* tissues.

Gertlowski and Petersen [25] explained that addition of sucrose can also affect cells metabolisms and the production of metabolites. Lee *et al.* [26] adescribed that sucrose can be hydrolyzed into two monosaccharides, glucose and fructose, by invertase which bounds extracellular and/or cell wall during the initial culture period. In addition, sodium bicarbonate ($NaHCO_3$) provides high buffer capacity in the cell culture medium thus maintaining the pH-value in the physiological conditions during cultivation. The required CO_2 concentration is related to the desired pH-value and the $NaHCO_3$ concentration of the medium. The bicarbonate and carbon dioxide buffering system is the most commonly used to maintain physiological pH. The bicarbonate is a weak buffer with a pKa of 6.1 making a pH range of 7.2-7.6 more difficult to prevent rapid pH changes. This buffer, however, is non-toxic and has nutritional value [12,13].

Adelberg *et al*. [27] had shown that with gelling agents the nutrients dissolved in the liquid phase are not completely available as water is bound to the gel by a matrix force. That study compared the spent gelled medium and spent the liquid medium and revealed that nutrients remained in agar, and therefore nutrients were somehow unavailable to the plant. It also has been shown that in presence of agar, the resistance of sucrose transfer from medium to the plant is 300× greater than that of sucrose transfer from a shaken liquid to the plant per unit of surface area [28]. Thus, the sucrose availability in liquid medium is higher than in gelled medium [27].

The liquid culture has also beeb reported to be more susceptible with shear stress. Sajc *et al* [9], in their review stated that shear levels which were below levels of causing cell damage while still high enough for efficient mixing in the liquid phase and dispersion of air are considered as important criteria of bioreactor design. Vogelmann *et al.* [29] reported that the critical shear stress is between 80 and 200 N/m2. During the process scale-up, the main goal is to reproduce on a larger scale the conditions found to be optimal on a small scale. The perceived sensitivity of plant cells to hydrodynamic stress associated with agitation and aeration. In addition to damaging and breaking the cells through the hydrodynamic stress generated by aeration, agitation, shaking, pumping, and other operations. The immediate consequence of the shear effect on plant cells is cell damage, which has been quantitatively measured by using a number of system responses such as reduction in cell viability [30], changes in morphology and/or aggregate patterns [31], release of intracellular compounds [32] and changes in metabolism. Liquid culture is often associated with hyperhydricity [33]. In contrast, our results showed that the adventitious roots in the liquid medium did not show any hyperhydricity symptoms. This phenomenon was likely caused by the application of the net in middle of bioreactor. Furthermore, the roots were floated in filter sieve and were not sunk in liquid medium. Therefore, the shear levels in the bioreactor was suitable for a good bioreactor design.

3.3. Total sugar, conductivity and hydrogen ion concentration in the medium

The determination of hydrogen ion concentration (pH), total sugar and electrical conductivity (EC) of the médium was conducted every seven days. Total sugar, pH and EC were decreased, but pH and EC were increased by using buffer for every two weeks (figure 4). Several studies described that liquid medium was examined by electrical conductivity, pH and total sugar. The high conductivity of medium showed that the adventitious root was unavailable to absorb inorganic compounds. Meanwhile, Thanh *et al*. [34] explained that electrical conductivity was used as indirect method to estimate biomass in the cell culture, because it represents the uptake of inorganic compounds by the cell during cultivation. Decreasing amount of organic compounds indicated that cells work and increase biomass of cell, instead. Furthermore, this conditions were supported by the decreasing pH of medium at the end of cultivation. Decreasing pH can decrease the ability of the cells to absorb inorganic compound from the medium [35]. It was likely that the decreasing pH during adventitious root culture was caused by ammonium in MS medium. Ammonium in MS medium is derived from

ammonium nitrate as source of nitrogen whichi is also important as a buffer. When cells need nitrogen, cells will release H^+ to uptake nitrogen source, amonium. Thus, continuous release of H^+ into medium will result acid conditions.

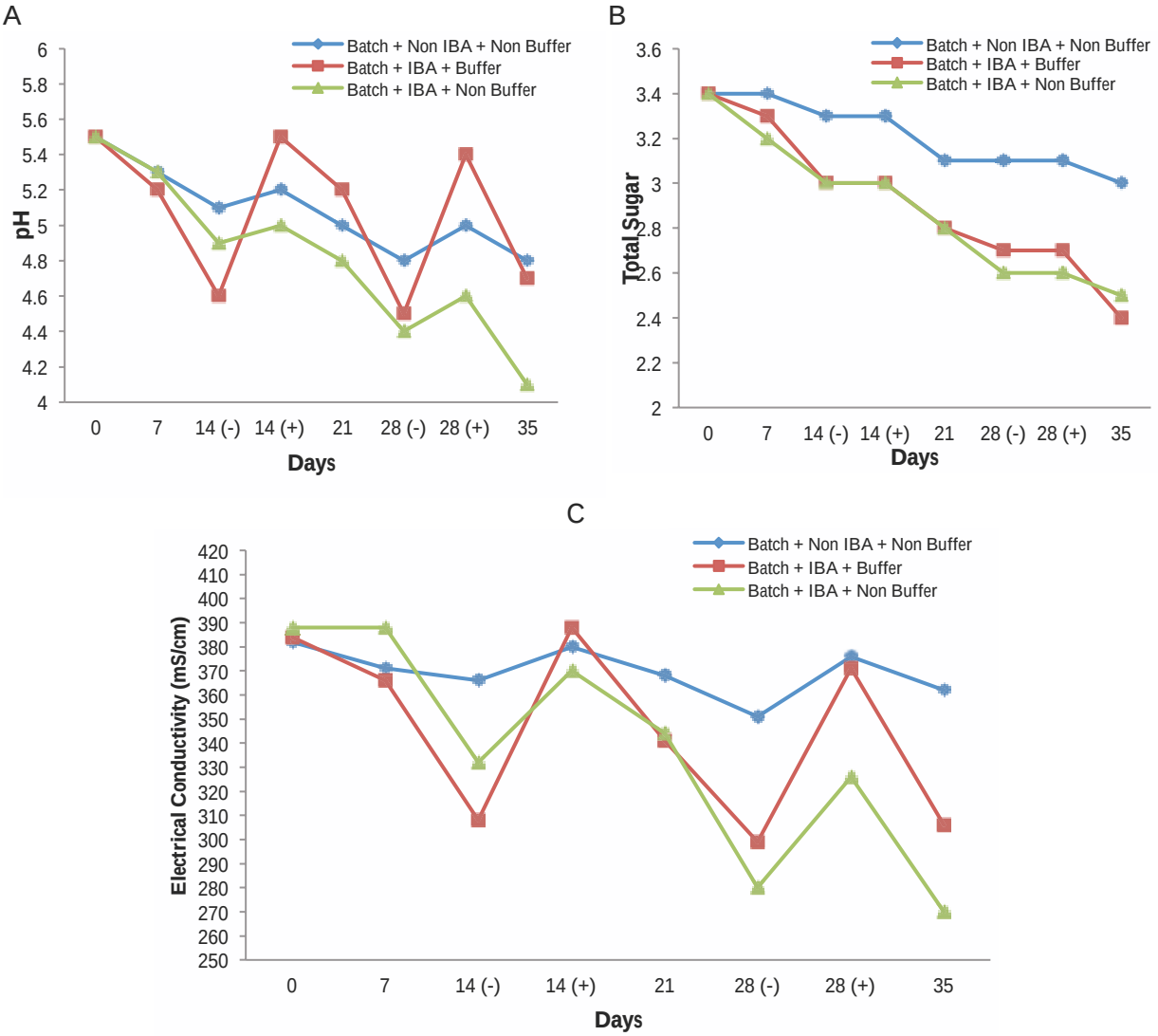

Figure 4. Adventitious root culture condition for 35 days. Culture condition change in (A) hydrogen ion (pH); (B) Total sugar; (C) Electrical conductivity (EC).

The source of carbon in liquid MS medium is sucrose. Sucrose will be hydrolyzed to glucose and fructose in the beginning of tissue culture. During cultivation, cell will consume monosacharides, therefore the measurement of total sugar can be indicated by cells growth [36]. Furthermore, oxygen for aeration in bioreactor should be supplied throughout the cultivation. Indeed, productivity of high-density and high-viscosity cell suspensions is proportional to cell concentration generally, as well as aeration is a major concern in bioreactor design and scale up for plant cell culture [9].

4. Conclusion

In this study, the growth of abaxial main root was higher than adaxial, contrarily to that total number of root branch. Meanwhile, the highest biomass production of adventitious root cultured was achieved

by MS medium supplemented with IBA 2 mgL^{-1} + buffer $NaHCO_3$. This treatment has produced fresh biomass in two fold of the initial inoculum.

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