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Research Article

Production of Adventitious Root and Saponin of *Talinum paniculatum* (Jacq.) Gaertn. in Temporary Immersion Bioreactor

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Abstract: This research is conducted to identify the effects of immersion length and interval on *Talinum paniculatum* (Jacq.) Gaertn adventitious roots' biomass and saponin content in temporary immersion bioreactor. Immersion intervals used in this research are 3, 6, and 12 hours with immersion lengths 1, 3, 5, and 7 minutes. Adventitious roots were induced from leaf explants grown on MS medium added by IBA 2 mg/L. Two grams adventitious root are cultured in temporary immersion bioreactor with each treatment and kept for 28 days. Results of this research indicated different biomass and saponin content caused by each treatment. The best combination was found on immersion length of 5 minutes and 12 hours interval which resulting 3.67 g fresh weight, growth speed 0.027 g/day, spot area of saponin 12.56 mm^2/0.01 g dry-weight, and spot area thickness scale 4+.
Keywords: adventitious roots, saponin, temporary immersion bioreactor, *Talinum paniculatum* (Jacq.) Gaertn

INTRODUCTION

Talinum paniculatum (Jacq.) Gaertn. is a herb with many medicinal properties. In Indonesia *T. paniculatum* known as ginseng Jawa and it roots' contains β-sitosteryl-β-$_{D}$-glicosida known as pharmaceutical products ingredient [1].

T. paniculatum adventitious root induction was successfully done using leaf explants on MS medium added by growth regulator substance IBA 2 mg/L. The disadvantage of using solid medium is that explants only absorb nutrition on the lower side which have direct contact with the medium, so that the result is less optimal compared to using liquid medium [2].

The use of liquid medium on plant tissue culture has some advantages. Liquid medium can provide homogenous culture condition, having faster growth rate, and relatively easy for sterilization [3-4]. However, there are several disadvantages of liquid medium mainly some technical issues such as hyperhydricity, cellular damage caused by propeller rotation when using bioreactor with churning system and oxygen deficiency [5]. Therefore, better methods to regulate aeration on the medium are needed. One of them is using temporary immersion applied to bioreactor known as Temporary Immersion Bioreactor (TIB) [6].

TIB is bioreactor which regulates nutrition and oxygen absorptions of the culture. In this condition, explants are not immersed in the medium all the time. There are several periods when the explants are not immersed [6]. When the explants are not immersed, they are free to absorb oxygen because of low oxygen solubility on immersed condition. Oxygen is needed by culture to unload energy provided by medium in form of sucrose. TIB immersion lengths and intervals can help explants growth [7]. One to fifteen-minute immersion length with 2-12 hours of immersion frequency also affects explants growth of perennial plants [3].

When culture is on immersed condition as a result of one-minute immersion length and 1, 12, 24-hour of immersion intervals on TIB, may cause stress on *Hevea brasiliensis* calluses identified by superoxide dismutase (SOD) [8]. Immersion length and interval in culture could cause abiotic stresses. Abiotic stresses may affect production of secondary metabolite contents [9]. Secondary metabolite synthesis, including saponin is a response to abiotic stress [10]. For example water stress treatment on *T. paniculatum* plant increase its saponin content on water 40% availability [11].

This research is conducted to identify the effects of immersion length and interval on biomass and *saponin* contents of *T. paniculatum* adventitious roots on liquid medium using *temporary immersion system* method on bioreactor.

MATERIAL AND METHODS

Adventitious Roots Induction

Adventitious roots were induced from *T. paniculatum* leaf explants. *T. paniculatum* was obtained from the collection of Indonesian Institute of Sciences, Purwodadi Botanical Garden, Pasuruan, East Java, Indonesia. Leaf explants were cultured on Murashige and Skoog medium (1962) added by IBA 2 mg/L, sucrose 30 gram/L, and agar 12 gram/L. Culture were kept on dark condition at (25±3)°C temperature for two weeks.

Adventitious Root Cultures on TIB

Two-week-old of adventitious roots cultured on solid medium were harvested and weighed as much as 2 grams. The cultures were then immersed in 400 mL liquid MS medium added by IBA 2 mg/L. After four weeks kept on TIB, adventitious roots were harvested and measured its fresh weight, dry weight, and growth speed. The growth speed was measured based on Akalezi [12].

Analysis of Saponin

Dried of adventitious roots (100 mg) were soaked in 10 mL ethanol, and then warmed in waterbath at 80°C for 30 minutes. Extract were saturated in waterbath at 80°C for 3 haours until the volume was obtained 0.2 mL. Extract and saponin standard were spotted on silica gel GF_{254} and eluted in propanol:water (14:3). The spot was detected by anisaldehide-sulfic acid (Merck) and warmed in the oven at 110°C for 6-10 minutes. The saponin standars (Calbiochem) will give green to black color.

RESULTS AND DISCUSSION

Adventitious Roots Culture in Temporary Immersion Bioreactor

Adventitious roots growth in temporary immersion bioreactor was increased after being cultured for four weeks. It is indicated by increasing fresh biomass. Almost all of adventitious roots biomass increased, except for 3-hour immersion interval with 1 and 3-minute immersion lengths treatment. The highest biomass obtain was on 12-hour immersion interval with 5-minute immersion length. The improvement or reduction of fresh biomass is proportional to dry biomass improvement or reduction and growth speed (Figure 1).

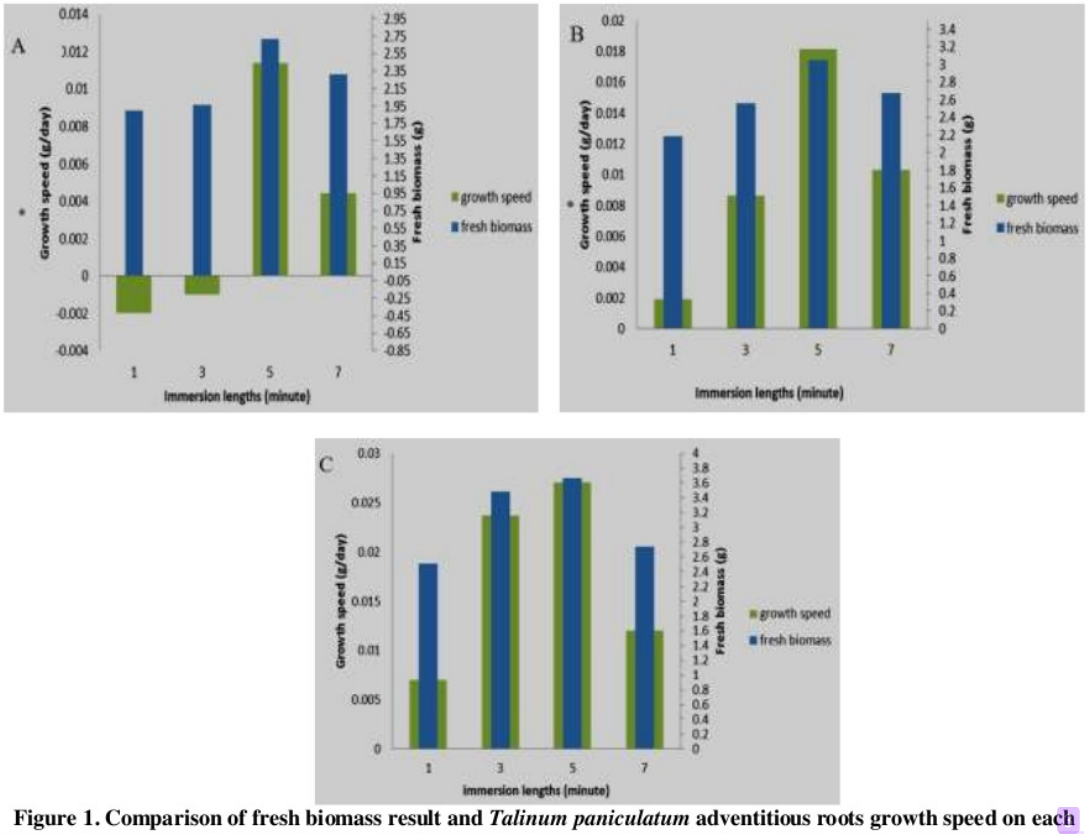


Figure 1. Comparison of fresh biomass result and *Talinum paniculatum* adventitious roots growth speed on each treatment after being kept for 4 weeks in temporary immersion bioreactor using MS medium and IBA 2 mg/L. A: 3-hour interval, B: 6-hour interval, C: 12-hour interval

On treatments with same immersion interval and different lengths, it indicated different biomass results. It may be caused by immersion lengths which determine contact time between explants and its media, so that the explants are able to absorb oxygen. On 1 and 3-minute of immersion length treatments, nutrition absorption by explants is not optimal, so that it only produces little biomass. Meanwhile, on 7-minute immersion length treatment, explants growth is limited. It is assumed that the most optimum absorption occurs on 5-minute immersion length, resulting high biomass.

The length of contact time between explants and its media on short interval treatments may cause slow growth speed. It may be caused by the frequency of immersion causing oxygen deficiency. This condition results low respiration rate, resulting low ATP obtained and slow growth rate.

Frequent explants immersion may inhibit oxygen absorption causing low biomass production as seen on 3-hour and 6-hour intervals. On 12-hour interval, the explants are not frequently immersed resulting optimal oxygen absorption and high biomass production.

On high intervals, explants indicated positive growth speed and relatively high growth rate. It may be caused by explants absorbing much oxygen when in not being immersed condition resulting high respiration rate and more ATP produced used in cell growth. This finding conform the previous research conducted by Riyadi and Sumaryono [13] on sago somatic embryo growth using temporary immersion system which also finds that 12-hour immersion interval produced the highest fresh biomass.

The best immersion length and interval combination is found on 5-minute length and 12-hour interval immersion indicated by the highest biomass product. It is possibly because of nutrition and oxygen absorptions were optimum at this combination resulting optimal growth of adventitious roots. Different combinations of immersion length and intervals may result optimal growth because of optimal oxygen and nutrition absorptions [7]. Another possibility is because of water deficiency stress on the treatment. In this treatment explants only have contact with water twice a day. Roots growth will be faster in water stress condition compared to growth in water sufficient condition [14-16].

Saponin Content of Adventitious Roots

Saponin content of adventitious roots were indicated by dark green spot with Rf value 0.63 on TLC plate (Figure 2). Semiquantitatively, saponin content is determined by measuring the width and thickness of spot area (Figure 3).

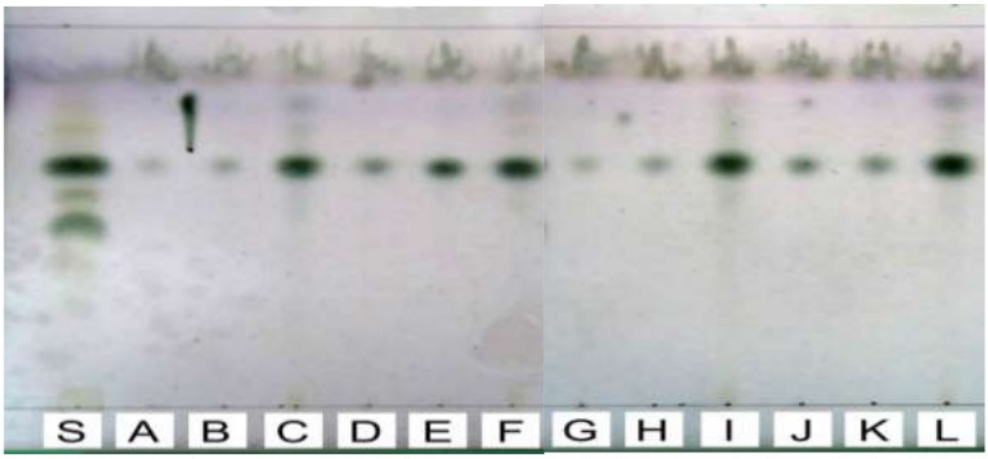


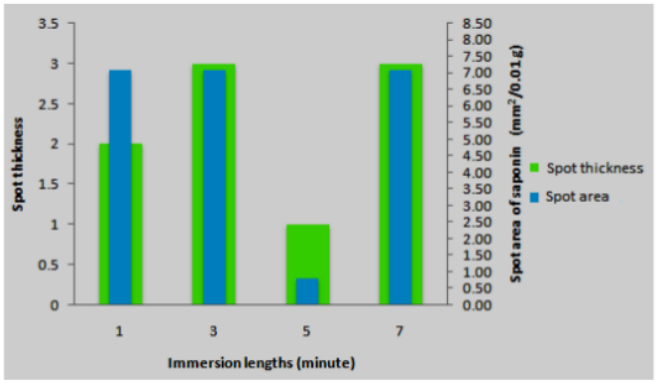
Figure 2. Cromatogram of saponin on TLC plate. S: saponin standard, A: 1-minute immersion length in 3-hour interval, B: 1-minute immersion length in 6-hour interval, C: 1-minute immersion length in 12-hour interval, D: 3-minute immersion length in 3-hour interval, E: 3-minute immersion length in 6-hour interval, F: 3-hour immersion length in 12-hour interval, G: 5-minute immersion length in 3-hour interval, H: 5-minute immersion length in 6-hour interval, I: 5-minute immersion length in 12-hour interval, J: 7-minute immersion length in 3-hour interval, K: 7-minute immersion length in 6-hour interval, L: 7-minute immersion length in 12-hour interval.

The longer immersion interval, the higher saponin content resulted. It may be caused by less contact between root and the medium resulting water and nutrition deficiencies on explants. However, referring to high biomass product on that condition it is possible that the available water and nutrition is used in primary metabolism resulting intermediate compound named squalene. Squalene is precursor of saponin [17].

Synthesis of saponin is initiated by glycolysis process which results pyruvic acid. Pyruvic acid is oxidized into acetyl CoA. Acetyl CoA is the source of Carbon on the synthesis of saponin (Manitto, 1992). Acetyl CoA is synthesized into mevalonic acid which release its CO_2 resulting isoprenoid. Six isoprenoid compounds are condensed resulting squalene. Squalene undergoes cyclisation process into terpenoid compounds. The terpenoid compounds will bind with glucose to form saponin [17].

Saponin content of *T. paniculatum* adventitious root culture is in proportion with its biomass. This phenomenon was coincide with production of biomass and saponin content of *Panax ginseng* in TIB [18].

A



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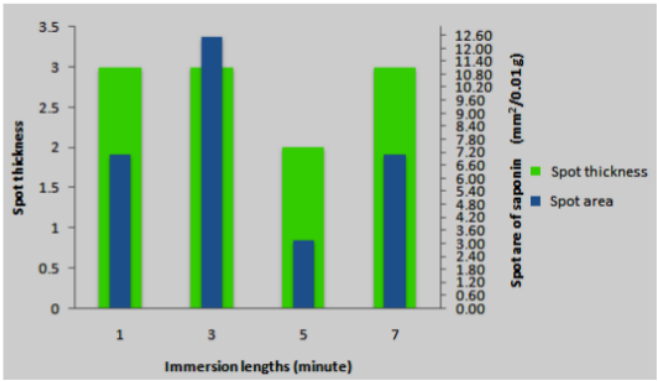

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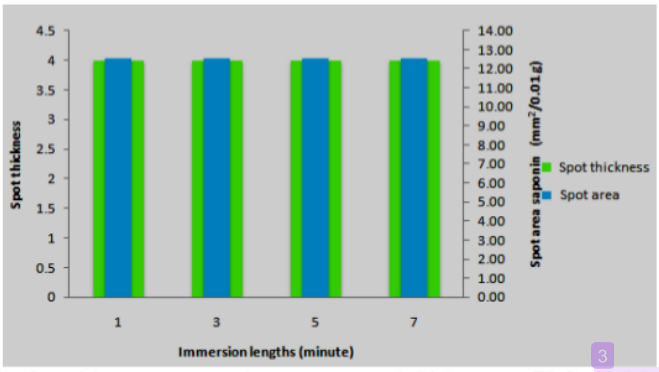

Figure 3. Comparison of adventitious roots saponin spot area and thickness on TLC. A: 3-hour interval, B:6-hour interval, C: 12-hour interval

CONCLUSION

The highest biomass and saponin content of *Talinum paniculatum* adventitious roots on liquid culture using temporary immersion bioreactor method is obtained in combination of 5-minute immersion length and 12-hour immersion interval.

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