Preface

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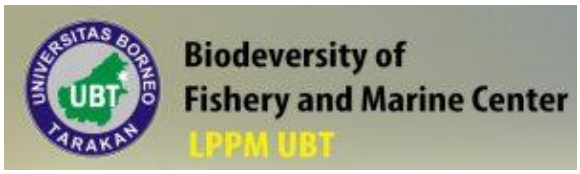
CABE 2021

International Conference on Climate Change, Agriculture, Biodiversity, and Environtment Study (CABE 2021)

Tarakan, North Kalimantan – Indonesia, 23-24 December 2021

Organized By:



Preface

International Conference on Climate Change, Agriculture, Biodiversity, and Environtment Study (CABE 2021) was held in Tarakan, Indonesia. CABE 2021 was hosted by Biodiversity of Fishery and Marine Center LPPM UBT.

The conference is organized by the Research and Community Services Center of Borneo Tarakan University, Indonesia. The primary objective of The CABE 2021 is to promote effective interaction and cooperation among scientists and technicians who are involved in agriculture research and development in the world with the view of encouraging and facilitating research activity, implementing research findings, sharing of information and publication of research results. The CABE 2021 focuses on both theory, design and applications. In addition to the technical sessions, there will be invited sessions, panel sessions and keynote addresses.

At the moment, we are facing a new situation that has never happened before, the Global Pandemic caused by Coronavirus Disease of 2019 (Covid-2019). This issue has affected the lives of people globally, Including the lives of academics in education. The Covid-19 pandemic is an unprecedented phenomenon for us all. The situation is continually evolving, and we must face new challenges every day. With the appeal above, the International conference on Conference on Climate Change, Agriculture, Biodiversity, and Environtment Study has been switch into virtually mode. Originally the coference was planned in a physical conference. However, until mid-September 2021, the conditions for Covid-19 were not normal. The participants really need the publication results as an annual performance report. In this case, all participants refuse if the conference is postponed. At 23-24 December 2021, all participants were invited virtually for preparation and simulation. In the conference day, all committee were organizing the conference in virtually using zoom application from Tarakan, Kalimantan, Indonesia. The structure were similar with the physical conference as indicated in the following conference program. The keynote speakers session was cunducted in the morning and continued with parallel sessions after lunch break. In the parallel session, each participant was preset their paper for 15 minutes including questions and answers. The CABE 2021 were attended around 170 audience with 121 presenters from academicians, students, scientists, and other related professionals.

Our special thank also goes to all individuals and organizations such as the international program committees (IPC), the conference organizers, the reviewers, and the authors, for their contribution in making CABE 2021 not only a successful international conference but also as a memorable gathering event. We are also grateful for the support of the publication service of IOP. We hope that it should give you a beautiful memory to bring home in addition to new insights and friends gathered during the conference. We are truly grateful for your contribution and interest. We hope that you will get pleasure from CABE 2021 in this beautiful city, Tarakan, Indonesia.

Best regards,
Dr. Ratno Achyani, S.Pi, M.Si (General Chair of CABE 2021)

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- **Type of peer review:** Double Anonymous
- **Conference submission management system:** Edas
- **Number of submissions received:** 191
- **Number of submissions sent for review:** 191
- **Number of submissions accepted:** 121
- **Acceptance Rate (Submissions Accepted / Submissions Received × 100):** 63.4
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Influence of volume medium on growth and ginsenoside level in adventitious root culture of *Panax ginseng* CA Meyer

Influence of volume medium on growth and ginsenoside level in adventitious root culture of *Panax ginseng* CA Meyer

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Abstract. Ginseng (*Panax ginseng*, Family Araliaceae) is a traditional herbal plant that is pretty well known and has been widely used in various countries, such as Korea, China, and Japan. Ginseng contains ginsenoside secondary metabolites that have been shown to have therapeutic effects, such as antioxidant, anti-inflammatory, anti-allergic, anti-diabetic, and anti-cancer. Ginseng production by traditional cultivation methods is long and produces inconsistent results. Therefore, in vitro culture is an alternative method to produce ginseng and ginsenoside consistently. In 2018, PT Bintang Todjoe collaborated with the University of Surabaya (UBAYA) and the Hanbang-Bio Inc. (holding company of Kyung Hee University) to establish the Kalbe Ubaya Hanbang-Bio Laboratory (KUH Lab). From previous studies, the dry weight achieved was only 109.758 g, which did not reach the target (120 grams). Therefore, the media was modified by adding media volume from 13L to 15L. The increase in media volume increased fresh weight to 2728.7 g, dry weight to 137.6 g, and yield up to 5%. However, this increase in media volume has not increased ginsenoside levels.

Keywords: ***Adventitious root culture, ginsenoside, media volume, Panax ginseng.***

1. Introduction

Ginseng (*Panax ginseng*, Family Araliaceae) is a traditional herbal plant that is quite well known and has been widely used in various countries, such as Korea, China, and Japan [1]. Panax means "all-healing" and is believed to cure all diseases in humans. Ginseng contains a secondary metabolite known as ginsenosides. Ginsenosides have been shown to have therapeutic effects, such as antioxidant, anti-inflammatory, anti-allergic, anti-diabetic, and anti-cancer [2]. In addition, ginseng can also treat *neurodegenerative diseases* [3], cardiovascular diseases [4], and liver disorders [5]. There have been more than 38 ginsenosides that have been identified [6] Ginseng has also been used in functional foodsand dietary supplements [7][8].

Using traditional cultivation methods, the production of ginseng and ginsenosides is a very long process. It takes about 4-6 years to get ginseng ready to harvest. In addition, ginseng production with traditional cultivation is influenced by environmental factors, such as pests and diseases. The content of ginsenosides also varies broadly, depending on the age of the plant, season, and extraction method. Therefore, plant tissue culture techniques become an alternative method to produce ginseng and ginsenosides consistently [9]. Within vitro culture, parameters such as stirring, aeration, temperature,

and pH can be controlled. In addition, plant tissue culture techniques can reduce production costs and shorten the time from cultivation to harvest. A protocol has been developed to produce adventitious roots culture of ginseng on a bioreactor scale [6].

Adventitious roots are the root that grows from plantorgans other than roots. Adventitious roots are strongly influenced by stress, the presence of nutrients, injury, and phytohormones [10]. Induction of it is carried out by giving a high concentration of auxin while the cytokinin concentration was low. Auxin will induce and increase the number of roots but does not cause root elongation. Roots will elongate when the concentration of auxin in the medium is low. IndoleButyric Acid (IBA) is the most common type of auxin used for root induction in vivo and in vitro. Besides IBA, other types of auxin can be used to induce adventitious root growth, namely Indole Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA) [11]. Adventitious root culture has rapid growth and is genetically stable, making it the most promising technique for biomass production. Root culture can also produce secondary metabolite yields more consistently than cell suspension culture, making it suitable for producing secondary metabolites. In addition, adventitious root culture can use liquid media, so it is straightforward to produce commercially [12].

In 2018, PT Bintang Todjoe in collaboration with the University of Surabaya (UBAYA) and the Hanbang-Bio Inc. established the Kalbe Ubaya Hanbang-Bio Laboratory (KUH Lab). From previous studies, media using formulation B with a volume of13L produced a dry weight of ginseng as much as 109,758 g. Although the dry weight of ginseng formulation B was higherthan formulation A, this value did not meet the target where each bioreactor produced a dry weight greater than 120 g. Therefore, optimization was carried out on several factors, one of which was the ginseng growth medium. It is expected that the increase in media volume can increase the dry weight of ginseng.

Plant growth in vitro has 3 stages: the lag, exponential, and stationary stages. This *lag* stage is where plant cells adapt to the new medium. The exponential stage is where plant cells have adapted and divide rapidly. The stationary stage is where the growth of plant cells has slowed down and is replaced by the accumulation of secondary metabolites [13]. At KUH Lab, ginseng production uses a batch system so that the carbon source (sugar) and the N source (KNO3) will run out at some point. When the source of C and source of N is almost depleted, ginseng begins to enter the stationary stage where atthis stage the speed of cell division has decreased, or even cell division has ceased to occur in ginseng, replaced by theformation of ginsenoside. Therefore, an experiment was conducted to increase the volume of the ginseng growth medium from 13L to 15L. It is hoped that ginseng can prolong the exponential stage with more nutrients so that the dry weight produced can reach the target.

2. Material and Method

In this study, adventitious roots used were adventitious root cultures obtained from the Hanbang-Bio laboratory, Kyung Hee, University, South Korea.

2.1. The standard procedure of large-scale production of Panax ginseng adventitious root cultures

Large-scale production was carried out using bioreactor 18 L (BR-BIO180, Hanbang-Bio Inc., South Korea). The bioreactor was filled with 12 L of Reverse Osmosis (RO) water using Pure RO II. After adding RO water, 1 L of media concentrate (*Schenk Hildebrandt* medium + auxin) was added to the bioreactor (final media volume was 13L). The bioreactor was sterilized using an autoclave Hankuk HK- AC200P for 70 minutes at 121°C, 1.5 atm. The sterilized bioreactor was kept for 4 days to cool down and ensure no contamination. 150 g of adventitious roots were inoculated into the bioreactor. The bioreactor is connected to an air compressor to provide aeration and agitation. Adventitious roots were incubated for 7 weeks at a temperature of 24-25°C with a humidity level of 40-50%.

2.2. Optimization of media volume for large-scale production of Panax ginseng adventitious root culture

Variations in the volume of media used were 13L and 15L media. The only difference lies in the volume of RO water used. In the control bioreactor, the volume of RO water is 12 L, while in the treatment bioreactor, the volume of RO water is 14 L. The bioreactor, which has been filled with RO water, is added with 1 L of media concentrate to have a final volume of 13L and 15L. The media

concentrate for the treatment is 15L more concentrated than the concentrated media for the 13L treatment. The 15L medium was not more dilute than the 13L medium. The 13L and 15L media were sterilized for 70 minutes at 121°C, 1.5 atm. Media that has been sterilized, allowed to stand for 4 days to cool down and ensure no contamination. As much as 150 g of adventitious roots were added to each medium inside the bioreactor connected to an air compressor. The adventitious roots were incubated for 7 weeks at 24-25°C with a 20-50% humidity level.

2.3. Harvesting and drying of adventitious roots

After the incubation period is complete, adventitious roots are harvested. The media is removed first, and then the adventitious roots are accommodated in a container. The adventitious roots were separated into small pieces and then cleaned using tap water 3 times. After being cleaned 3 times using tap water, the adventitious roots were cleaned again using RO water 1 time. Adventitious roots were drained by sitting for 1 hour to reduce water adhering to the roots. After being drained, the adventitious roots are put into a plastic container and stored in the refrigerator. The adventitious roots were dried using a Memmert UF750 oven, set to 60°C, 60% fat, and 60% flap. The drying duration depends on the wet weight of the adventitious roots. The heavier the adventitious roots, the longer the drying duration, and vice versa. After drying, the dry weight of the adventitious roots was weighed using Mettler Toledo MS16001L, and the yield was calculated. The yield calculation was carried out using the formula (1):

$$\%\ yield = \frac{dry\ weight}{fresh\ weight} \times 100\% \qquad (1)$$

2.4. Extraction of adventitious roots from Panax ginseng

Adventitious roots were extracted using the Soxhlet method. 5 g of dry adventitious roots were extracted using 300 mL 80% methanol for 2 hours. The extract was dried using a set of rotary evaporator (Rotavapor R-300, Buchi, Germany). The dry extract was dissolved in 20 mL of RO water. After that, the extraction was resumed using water-saturated butanol with a ratio of 1:1. The mixture of extract and butanol was centrifuged at 8000 rpm (1 rpm = 1/60 Hz) for 15 minutes. The organic phase was taken and accommodated in a 250 mL flask, and this stage was carried out until the organic phase became clear. Butanol extract was dried again using a rotary evaporator. The drying process was stopped when butanol was no longer smelled.

2.5. Analysis of ginsenosides

Analysis of ginsenosides was performed using HPLC Agilent Infinity II. Butanol dry extract was dissolved in 50 mL methanol with HPLC grade. The sample was filtered using a 0.2 m PTFE filter. The stationary phase used Kinetex C18 with a size of 50 mm × 4.6 mm, and the mobile phase used an eluent with a gradient of acetonitrile (A) and water. The eluent gradient was set as follows: 7 min 19 % A (isocratic); 7 to 11 minutes 19 to 29 % A (isocratic); 11 to 14 minutes 29 % A (isocratic); 30 to 31.5 minutes 70 to 90% A; 31.5 to 34 minutes 90% A; 34 to 34.5 minutes 90 to 19% A; 34.5 to 45 minutes 19 % A. 5 L of the sample was injected at a flow rate of 0.6 mL min^{-1}. The detector used is a Diode Array Detector (DAD) at a wavelength of 203 nm. Ginsenosides content was calculated by comparing the sample's peak area and the reference standard's peak area.

2.6. Statistical analysis

All data were statistically analyzed using IBM SPSS statistics 25 software. The data will be tested using One-Way ANOVA if the data is eligible for a parametric test or tested using Kruskal-Wallis if the data is not eligible for a parametric test. The data is stated to be significantly different if the P-value <0.05.

3. Result and Dissuccion

The presence of macronutrients and micronutrients in the growth media used for ginseng cultivation dramatically affects the growth and development of ginseng cultures, both in terms of biomass and bioactive compounds accumulation. High production of biomass and secondary metabolites are

influenced by nutrient conditions, including nitrogen, phosphate, potassium, and mainly sucrose, which are adequate in ginseng growth media so that ginseng can produce optimum growth. Sucrose is known as a carbon source that is widely used in plant culture cells. Cells use sucrose as a source of energy and biosynthesis. An adequate amount of sucrose in the nutrient media of adventitious root culture will help the growth of better ginseng root culture biomass [14] [15].

In this study, the volume of growth medium for ginseng culture was increased from 13L to 15L. Changes in the volume of this growth medium were carried out to increase the dry weight of the ginseng root adventitious culture. Sivakumar et al. [16] (2005) reported that media volume could increase fresh and dry weight but decrease the percentage of ginsenosides. These results indicate that increasing media volume is suitable for increasing the growth of ginseng's adventitious root cultures, but not ginsenosides accumulation. It should be noted beforehand that ginsenosides accumulation and biomass growth from ginseng adventitious cultures were contradictory. When plant cultures are exponential, biomass growth and nutrients in the growth medium will increase, slowly decreasing.

In contrast, the accumulation of secondary metabolites occurs when the plant culture is stationary. The nutrients in the growth medium are limited and depleted, causing the plant culture to produce various secondary metabolites to survive. This also underlies the increase in media volume with the same concentration increasing the growth of ginseng root adventitious culture biomass because nutrients can prolong the exponential phase in ginseng root adventitious culture [17]. However, it shortened the stationary phase of ginseng root culture because the harvest time remained the same, 8 weeks.

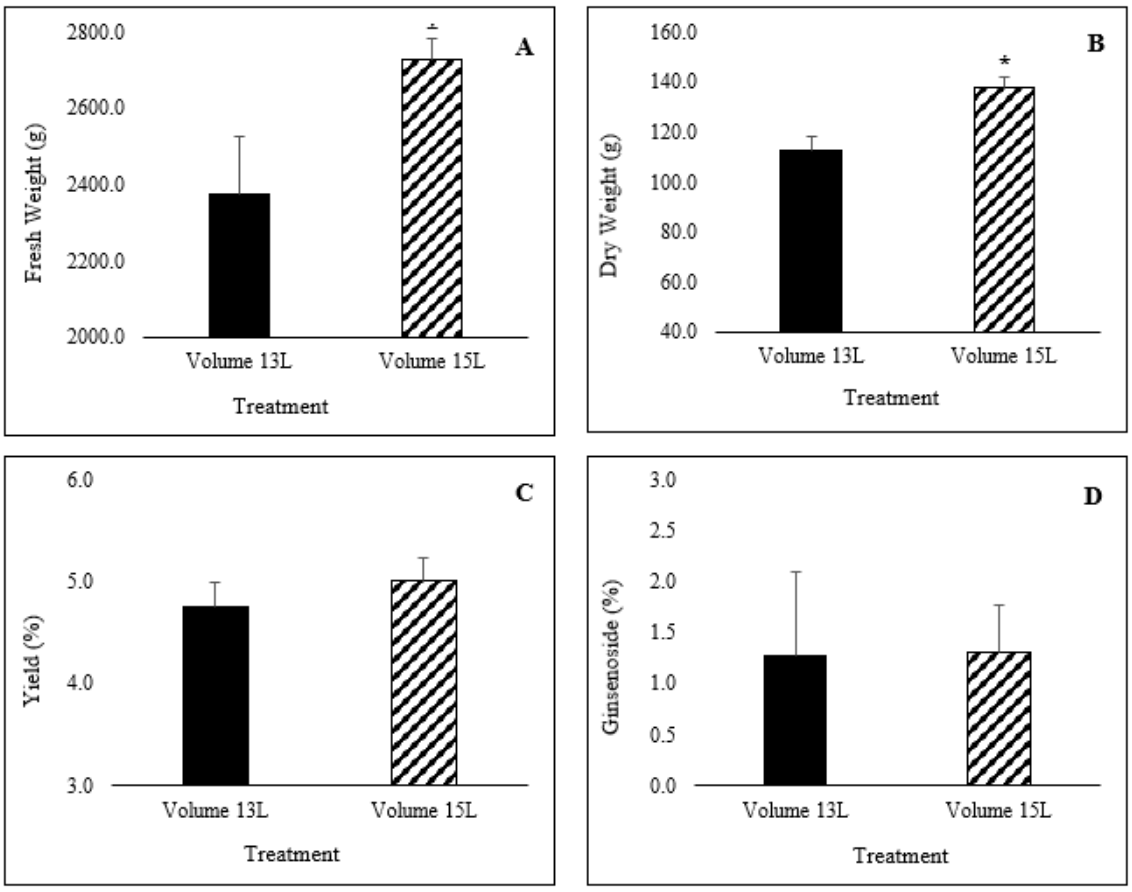


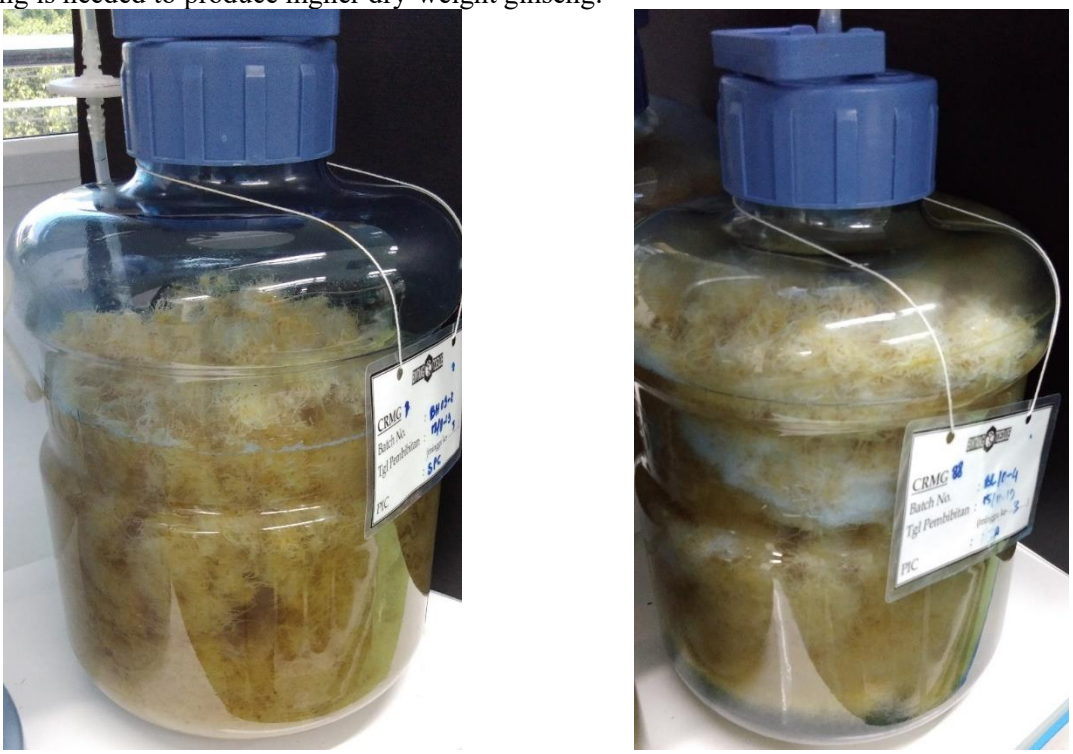
Figure 1. Barchart of the effect of media volume on fresh weight, dry weight, yield, and ginsenoside.

Significant differences are marked with (*) (P,0.05%). (A) Based on the ANOVA One Way test of fresh weight, each media volume has a significant difference. The average fresh weight for the growth medium volume of 13L reached 2372.4 grams, while the fresh weight for the growth medium volume

of 15L reached 2728.7. grams. (B) Based on the ANOVA One Way test of dry weight, each media volume has a significant difference. The average dry weight for the growth medium volume of 13L reached 112.5 grams, while the dry weight for the growth medium volume of 15L reached 137.6 grams. (C) Based on the ANOVA One Way test of yield, there is no significant difference from each media volume. The average yield for the growth medium volume of 13L reached 4.8%, while the yield for the growth medium volume of 15L reached 5%. (D). There is no significant difference between media volumes based on the ANOVA One Way test of ginsenoside. The average ginsenoside for the growth medium volume of 13L reached 1.3%, while ginsenoside for the growth medium volume of 15L also reached 1.3%.

It can be seen from the graph that there was an increase in fresh weight and dry weight when the media volume was increased. The statistical tests between the two treatments also showed significantly different results, both for fresh weight and dry weight data. The average fresh weight and dry weight for the growth medium volume of 13L reached 2372.4 grams and 112.5 grams, respectively, with a standard deviation of 151.6 and 54.5, while the volume of growth medium 15L reached 2728.7. grams and 137.6 grams, with standard deviations of 6.0 and 4.5, respectively. The standard deviation obtained by both data shows that the data on 15L media volume is more uniform. This condition is more desirable because the resulting weights are more consistent. The increase in fresh and dry weight due to increased media volume follows the theory. An increase in nutrients in the volume of growth media can increase the ginseng adventitious root culture [18].

From figure 3, our result showed that the treatment with 13L media volume had a lower yield with an average of 4.8% compared to the treatment with 15L media volume with an average of 5%. However, this difference was not significantly different based on statistical tests. Both results also show the same standard deviation, which is 0.2 each. Higher yields are expected because less fresh-weight ginseng is needed to produce higher dry-weight ginseng.



A B

Figure 2. (A) Adventitious root of *Panax ginseng* grown in 13L media. (B). Adventitious root of *Panax ginseng* grown in 15L media. Adventitious root of *Panax ginseng* grown on 15L media seemed denser and almost filled the entire bioreactor compared to Adventitious root of *Panax ginseng* grown on 13L media.

The results of the percentage of ginsenoside data (Fig 4.) from the 13L and 15L media volume

treatments did not show a significant difference. Each treatment showed an average yield of ginsenoside in the range of 1.3%. The results that were not significantly different were due to the addition of media volume aimed at increasing the availability of nutrients, causing the ginseng adventitious root culture to have a more prolonged exponential phase, thereby increasing biomass but shortening the stationary phase is the phase of formation of ginsenoside compounds. Paek et al. [19] (2003) also reported that the high availability of sucrose in the growth medium could inhibit ginsenoside production. Some alternatives that are generally used to increase ginsenoside production are using abiotic[20][21][22][23] and biotic elicitors[24][25] and media modification with several heavy metals [26][27][28][29][30].

Table 1. Percentage of ginsenoside in media volume of 13L and 15L.

Ginsenoside	% Ginsenoside media volume 13L	% Ginsenoside media volume 15L
Rg1	0,12	0,11
Re	0,79	0,51
Rb1	0,09	0,10
Rb2	0,05	0,07
Rg2	0,08	0,13
Rh1	0,03	0,07
Ro	0,03	0,03
F1	0,08	0,04
F2	0,01	0,00
Rh2	0,02	0,04

Ginsenoside, which belongs to the saponin group, is the main bioactive component in ginseng. Ginsenoside contributes as a cardio-protective, increases the body's immune system, hepatoprotective, and other pharmacological effects. Ginsenoside is divided into several groups, and more than 30 kinds of ginsenoside have been isolated and identified. In general, ginsenosides are divided into 3 groups based on their structure, namely the Rb group (protopanaxadiols, which includes Ra1, Ra2, Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, etc.), the Rg group (protopanaxatriols, where among them are Rg1, Rg2, Re, Rf, Rh1, and others), and the Ro group (oleanolic acid). Besides that, ginsenosides are broadly also divided into major ginsenosides and minor ginsenosides. Major ginsenosides include Rb1, Rb2, Rc, Re and Rg1. These groups are the most dominant ginsenosides found from the total ginsenosides present. Meanwhile, minor ginsenoside (Rg3, Rh2, Rh1) is a type of ginsenoside present in small amounts but has superior pharmaceutical activity than major ginsenoside. *Panax ginseng* CA Meyer that we use is a type of mountain ginseng known to have a higher content of minor ginsenoside than major ginsenoside. Based on the results of HPLC analysis, it can be seen from table 1 that minor ginsenosides (Rh1 and Rh2) were most commonly found in 15L media. Moreover, minor ginsenoside is often expected to exist because it has active therapeutic effects such as anti-tumor activity, liver protection, allergic effects, and neurotherapeutic effects [31][32][33].

4. Conclusion

Increasing the volume of growth media for ginseng adventitious root culture from 13L to 15L increased fresh weight, dry weight, and yield. The average dry weight produced using 15L media volume was able to achieve the target set by the KUH lab, which was greater than 120 grams (137.6 grams). Therefore, the production media volume, initially 13L, is now changed to 15L. However, increasing media volume was not able to increase ginsenoside levels. Experiments to increase ginsenoside levels willbe conducted using the elicitors addition strategy.

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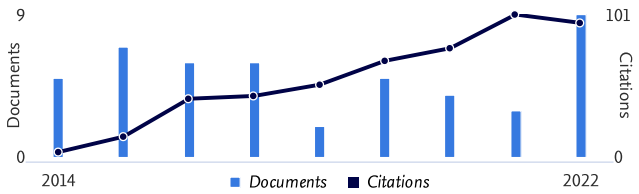

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Title	Only the chairs can edit	*Influence of Volume Medium on Growth and Ginsenoside Level in Adventitious Root Culture of Panax Ginseng CA Meyer*
Abstract	Only the chairs can edit	Ginseng (Panax ginseng, Family Araliaceae) is a traditional herbal plant that is pretty well known and has been widely used in various countries, such as Korea, China, and Japan. Ginseng contains ginsenoside secondary metabolites that have been shown to have therapeutic effects, such as antioxidant, anti-inflammatory, anti-allergic, anti-diabetic, and anti-cancer. Ginseng production by traditional cultivation methods is a very long process and produces inconsistent results. Therefore, in vitro culture is an alternative method to produce ginseng and ginsenoside consistently. In 2018, PT Bintang Todjoe collaborated with the University of Surabaya (UBAYA) and the Hanbang-Bio Inc. (holding company of Kyung Hee University) to establish the Kalbe Ubaya Hanbang-Bio Laboratory (KUH Lab). From previous studies, the dry weight that was achieved was only 109.758 g, which did not reach the target (120 grams). Therefore, the media was modified by adding media volume from 13L to 15L. The increase in media volume was able to increase fresh weight to 2728.7 g, dry weight to 137.6 g, and yield up to 5%. However, this increase in media volume has not succeeded in increasing the levels of ginsenoside.
Presenter(s)		presenter not specified
DOI	Only the chairs can edit	
Status		Accepted
Review manuscript		However, authors cannot upload: paper status <table><tr><th>Document (show)</th><th>Pages</th><th>File size</th><th>Changed</th></tr><tr><td></td><td>7</td><td>606,170</td><td>Nov 13, 2021 23:39 Asia/Jakarta</td></tr></table>
Final manuscript	Could upload until Nov 30, 2021 16:28 Asia/Jakarta.	However, authors cannot upload: final deadline
Stamped for attendee proceedings		However, authors cannot upload:

Personal notes

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Reviews

2 Meta Reviews

Review 1

Originality	Significance of Topic	Presentation	Recommendation
Weak Accept (6)	Accept (8)	Weak Accept (6)	Weak Accept (7)

Strengths/Weakness (What are the major reasons to accept/reject the paper? [Be brief.])

Strengths/Weakness:
- Paper quality must be proofread

- Format paper must be revised
- The research location only needs to be written once in one paper, namely in the method section. Delete all research locations except the method section
- Show the contribution of this paper by explaining the differences between the proposed method and the methods in the literature
- Explain what the advantages of the proposed method of previous research in the introduction.

Contribution/s & Detailed comments (What are the major issues addressed in the paper? Do you consider them important? Comment on the degree of novelty, creativity and technical depth in the paper. Please provide detailed comments that will be helpful to the TPC for assessing the paper, as well as feedback to the authors.)

Contribution/s & Detailed comments:
- The results is too shallow, it is need more results. The discussion must be revised by remove some literature review from discussion.
- Figure must be inserted in the text but not after references.
- Figures 1-4 can be combine into one Figure
- All papers submitted to the CABE 2021 must be written in English and formatted in the standard of the publisher format (All regular papers are limited to about six (6) to ten (10) pages

Review 2

Originality	Significance of Topic	Presentation	Recommendation
Weak Accept (6)	Weak Accept (6)	Weak Accept (6)	Weak Accept (7)

Strengths/Weakness (What are the major reasons to accept/reject the paper? [Be brief.])

Strengths/Weakness:
-This paper still needs to be improved in terms of its English grammar or structure.
-Figure quality must be improved
-Please check the paper format
-All Figure must be placed in the text
-The research topic is interested but the main results related to the topic are not provided in the results
-Used 'Fig." instead of "Figure".

Contribution/s & Detailed comments (What are the major issues addressed in the paper? Do you consider them important? Comment on the degree of novelty, creativity and technical depth in the paper. Please provide detailed comments that will be helpful to the TPC for assessing the paper, as well as feedback to the authors.)

Contribution/s & Detailed comments:
-Please indicate the measured data before processed
-Clearly explain about the meaning of all figure instead of explain of other results
-Please carefully check about the reference format. The allowed similarity is 15%