



Reny Herawati &lt;reny.herawati@unib.ac.id&gt;

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## Letter of Acceptance - Oral Presentation

1 pesan

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**ISEPROLOCAL Universitas Bengkulu** <iseprolocal@unib.ac.id>  
Kepada: reny.herawati@unib.ac.id

28 Agustus 2020 pukul 13.46

Dear Mrs. Reny Herawati

We are pleased to inform you that your paper has been accepted for an oral presentation in The 2nd Iseprolocal 2020, and I herewith attached a Letter of Acceptance. **The deadline for full paper submission is September 20<sup>th</sup>, 2020.**

A Whatsapp group is organized for the purpose of sharing further information and communication (<https://chat.whatsapp.com/BUF1Hv0rvLO5SBnERgvgVj>).

Hope to see you at the seminar.

Best regards,

Dr. Nurmeiliasari,

Secretary



**LOA ISEPROLOCAL\_Reny Herawati.pdf**  
307K



Reny Herawati &lt;reny.herawati@unib.ac.id&gt;

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## Today Reminder: 2nd ISEPROLOCAL 2020

1 pesan

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**ISEPROLOCAL Universitas Bengkulu** <iseprolocal@unib.ac.id>  
Bcc: reny.herawati@unib.ac.id

8 Oktober 2020 pukul 05.34

Dear,  
**The 2ND ISEPROLOCAL participants**

It is a friendly reminder of the 2nd ISEPROLOCAL 2020, 8th October 2020. The details of the 2nd ISEPROLOCAL 2020 are listed below:

**I. The keynote speaker presentation session**, from 09.00 a.m. to 12. a.m.

The link will be opened on Oct 8, 2020, at 08:45 a.m. (Jakarta Time). Please check the time zone carefully as it may be different from your local time. To join, simply click the link below.

LINK ZOOM: <https://zoom.us/j/99299764419>

or type Zoom ID: 992 9976 4419

and if it is possible to join Live Youtube: <https://www.youtube.com/watch?v=SUy292sJ1mQ>

It is recommended to join 15 minutes prior to the meeting. After the session ends, the participants leave the meeting. There are different links to the parallel sessions (see book of abstracts).

**II. Break** 12-13.15 p.m. (Jakarta time). The participants may prepare for the next session. There are 16 rooms with different zoom meeting IDs.

**III. Parallel sessions.**

The presenters and participants are given links to the meeting of parallel sessions. Please check the abstract book to find a meeting ID and passcode to enter the room.

There is an award to the best presenter in each room. Presenters are assessed and judged for their performance and quality of work. **We recommend that all participants use a virtual background** (attached files). After the parallel session, all participants leave the meeting and join **a closing ceremony when the best presenters are announced**.

**IV. Closing**

The closing of the event using a zoom webinar LINK ZOOM: <https://zoom.us/j/99299764419> or type Zoom ID: 992 9976 4419 will be held at 3.45 p.m. (Jakarta time). The committee will open 10 minutes before the closing ceremony. The best presenter of each room will be announced.

We hope you enjoy the scientific sharing session during The 2nd Iseprolocal 2020. Meet and greet scientists from many different countries and start to create a global good network.

Regards,  
The committee

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**ABSTRACTS BOOK 2ND ISEPROLOCAL 2020 8th Oct 2020.pdf**  
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Reny Herawati &lt;reny.herawati@unib.ac.id&gt;

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**LETTER OF CONFIRMATION (LoC) The 2nd Iseprolocal**

2 pesan

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**noreply@unib.ac.id** <noreply@unib.ac.id>

30 November 2020 pukul 14.08

Balas Ke: noreply@unib.ac.id

Kepada: reny.herawati@unib.ac.id

Dear,  
Reny Herawati

Herewith, we send the Letter of Confirmation (LoC) of your manuscript. You can also check/print the screen of OCS : <https://semcon.unib.ac.id/index.php/iseprolocal/iseprolocal/login> on your conference system.

Best regards,

Agustin Zarkani, Ph,D

NOTE: this is no-reply e-mail. Please contact us by e-mail: [iseprolocal@unib.ac.id](mailto:iseprolocal@unib.ac.id) or visit the website: [iseprolocal.unib.ac.id](https://iseprolocal.unib.ac.id)



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**Reny Herawati LoC Iseprolocal.pdf**  
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**noreply@unib.ac.id** <noreply@unib.ac.id>

30 November 2020 pukul 23.07

Balas Ke: noreply@unib.ac.id

Kepada: reny.herawati@unib.ac.id

Dear,  
Reny Herawati

Due to some grammatically errors with the first LoC, herewith, we re-sent the Letter of Confirmation (LoC) of your manuscript. You can also check/print the screen of OCS : <https://semcon.unib.ac.id/index.php/iseprolocal/iseprolocal/login> on your conference system.

[Kutipan teks disembunyikan]



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Reny Herawati <reny.herawati@unib.ac.id>

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## Iseprolocal Manuscript Revision

1 pesan

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**ISEPROLOCAL Universitas Bengkulu** <iseprolocal@unib.ac.id>  
Kepada: reny.herawati@unib.ac.id

31 Januari 2021 pukul 21.59

Dear, Mrs. Reny Herawati

Your manuscript has been reviewed by reviewer. If you have fix it, please send back soon. Thank you.

Best Regards,

-Iseprolocal committee



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# Addition of Coconut Water and Banana Extract on MS Media to Stimulate PLB (Protocorm Like Bodies) Regeneration of *Dendrobium gatton sunray*

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## ABSTRACT

Organic material is widely used for propagation of orchids, namely coconut water, extracts of tomatoes, bananas, bean sprouts and potatoes which can be added to certain media as a provider of nutrients, amino acid minerals, growth regulators. This study aims to determine the effect of the concentration of organic matter in coconut water and banana extract on MS media on the regeneration of PLB (protocorm like bodies) *Dendrobium gatton sunray*. The study was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Bengkulu University, from September to December 2019. This study used a Complete Randomized Block Design (RCBD) with two factors. The first factor was the concentration of coconut water with 3 levels namely: A0 = 0 (Control), A1 = 75 ml/l, A2 = 150 ml/l, and the second factor was the concentration of banana extract with 5 levels namely: B0 = 0 (Control), B1 = 75 g/l, B2 = 150 g/l. The results showed that coconut water did not affect all observed variables, however the banana extract treatment had a significant effect on all observed variables except for the number of shoots. There was an interaction between the two treatments, namely the variable plantlet weight. Orthogonal polynomial analysis showed that the interaction pattern of coconut water and banana extract treatment occurred in treatment 0-50, where the meeting point of the two treatments, namely the variable shoot length, leaf length, shoot number, and plantlet weight. Whereas in the variable number of leaves, the interaction occurs in the 100-150 treatment.

**Keywords:** coconut water, bananas extracts, *Dendrobium gatton sunray*, MS media

## 1. INTRODUCTION

*Dendrobium* is a type of orchid that is favored by orchid enthusiasts because it has a variety of forms, shapes, and even sizes, it is even easy to maintain and cultivate, and the price is relatively affordable. In addition, *Dendrobium* also has other features such as the freshness of long-lasting flowers, flexible flower stalks [1] so that it is widely used as an ornamental plant in the form of cut flowers that can decorate spaces with good quality [2]. According to Suryana [3] orchids had entered the international market for export to destination countries, including Japan, the Netherlands, Korea and Singapore. The Indonesian Statistics Agency [4] notes that the value of orchid production in Indonesia from 2016 to 2017 had increased between 19,978,078 stalks to 20,045,577 stalks with an average export value of 120,560 US dollars. Financially the orchid farming gives a profit, where in one growing season it provides a profitability (ROI) of 70% of the funds invested. Value added along the marketing chain show a reasonable profit for the business (farmers and

traders) as well, so that this commodity business has good prospects [5].

In general, orchids are only able to reproduce in nature at less than 1%, this is because orchid seeds are very small and do not have an endosperm so that orchids are symbiotic with mycorrhizae in nature. The role of mycorrhizae as a supplier of carbon sources for orchid seed germination in general has been replaced by tissue culture media. Optimization of tissue culture techniques continues to be carried out in an effort to accelerate the propagation of orchids, this technique only requires a small part of the plant to obtain the same plantlets as the parent [6].

The orchid seeds will grow into protocorms in the germination medium. Protocorm can form a secondary protocorm (protocorm like body/PLB). In vitro regeneration of PLB *Dendrobium* for shoot multiplication is generally carried out on media enriched with growth regulators and vitamins. One of the keys to success is the use of media types and growth regulators at the right concentration. The composition of the media used greatly determines the growth rate of protocorms and plantlets and is useful for increasing the quality and quantity of seedlings [7]. The composition of the media needed in

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tissue culture must contain nutrients that are useful for plants, namely consisting of carbohydrates, sugars and vitamins. Tissue culture media usually uses artificial media compositions, namely Murashige and Skoog and Knudson C media, either half or full concentration. The composition of the media is a determining factor in the success of in vitro orchid culture. Growth and reproduction rates of many species of orchids enhanced by adding some organic supplements such as coconut water (CW) or potato extract to culture medium [8-9]. Growth regulators are usually added to the medium to promote growth of explants. The growth regulators commonly used are auxins and cytokinins. The composition of the media can also be obtained from organic materials which already have natural nutrients and hormones in the form of auxins and cytokinins known as phytohormones. Complex organic materials can be used as additives that can encourage the development and growth of propagation explants in tissue culture [10;9]. The addition of organic materials such as extracts bananas, potato pulp and vegetable substances others have content high carbohydrate can increase growth and differentiation of cells in plants [11].

Organic materials that are widely used for orchid propagation are coconut water, tomato extracts, bananas, bean sprouts and potatoes which can be added with certain media as a provider of nutrients, amino acids, and growth regulators for orchid growth. Coconut water has been widely used as an additive in tissue culture media. The content of coconut water is in the form of active ingredients that are useful for the growth of plantlets which are rich in vitamins, sugars, amino acids and phytohormones [12-14]. Nurfadilah et al. [15] research showed that the combination of Ambon banana and BAP on concentration  $10^{-6}$  M BAP and 2.5% banana extract Ambon produces the best growth on time of emergence of shoots, number of shoots and number of leaves. This research was conducted to modify the MS media with added coconut water and banana extract to stimulate the regeneration of PLB *Dendrobium gaton sunray*.

## 2. MATERIAL AND METHODS

The research was conducted at the Laboratory of Agricultural Cultivation, Division of Tissue Culture and Biotechnology, Faculty of Agriculture, Bengkulu University. This research was conducted from September to December 2019. The materials used were PLB which had formed 2-3 mm buds as plantlets, banana extract, coconut water, Murashige & Skoog (MS) media, sugar, 70% alcohol, alcohol. 96%, and aquadest.

The design method used was a factorial completely randomized design (CRD) with 2 factors, namely: the first factor was coconut water with 3 levels, namely: A0 = 0 (Control), A1 = 75 ml, L-1, A2 = 150 ml.L- 1. The second factor was banana extract with 3 levels, namely: B0 = 0 (Control), B1 = 75 g.L-1, B2 = 150 g.L-1. Each treatment combination was repeated 5 times. One experimental unit consisted of one culture bottle planted with 3 PLBs.

$\frac{1}{2}$  MS medium was added to treatment, added sugar as much as 30 g / l, gelatin as much as 7 grams / l, then the media pH was measured 5.8. The media was cooked until the gelatin was dissolved and well blended, then immediately poured into a sterile bottle with a volume of 20 ml bottle-1, then the media was sterilized by autoclave at a temperature of 121 ° C, 15 psi pressure for 15 minutes. The explants used in the study were not uniformed into other media and were directly planted in the experimental media. The explants were removed from the bottle and selected for uniform growth. The selected explants consisted of 2 explants per bottle which were sterile, then the bottles were covered with plastic and tied with a rubber band and given a tight wrap. The culture bottles were maintained in the culture room at a temperature of 19-20 ° C with  $\pm$  1,000 lux irradiation for 12-16 hours / day.

Observations were made one week after planting (WAF) to 15 weeks (WAF). Every day the plants were checked for contaminants and browning, while the last week observations were made on the variables of the number of leaves, number of roots, length of leaves, length of shoots, number of shoots, and weight of plantlets.

Data were analyzed using analysis of variance at the 5% level, and further tests with LSD at the 5% level. The orthogonal polynomial test was used to determine the treatment and interaction patterns. Data were analyzed using SAS version 9.1.

## 3. RESULTS AND DISCUSSION

Variance analysis showed that coconut water had no effect on all observed variables, however banana extract had a significant effect on all observed variables except for the number of shoots (Table 1). There was an interaction between the two treatments, namely the plantlet weight.

The orthogonal polynomial analysis showed that the pattern of coconut water on the leaf length was very significant in a quadratic curve, while the banana extract pattern was very significant quadratic on the variable shoot length, leaf length, root length, number of leaves, number of roots, and plantlet weight, except that the number of shoots tended to be linear. (Table 1). Even though there was only an interaction on plantlet weight, the interaction pattern between the addition of coconut water and banana extract tended to be quadratic in the variables of shoot length, number of leaves, number of shoots, number of leaves, and plantlet weight (Table 1).

### 3.1. The effect of coconut water to MS media on the regeneration of PLB (Protocorm Like Bodies) *Dendrobium sp*

The effect of coconut water on PLB (Protocorm Like Bodies) regeneration was not significantly different in all observed variables. However, the pattern of coconut water tends to be quadratic in leaf length (Figure 1) with the regression equation  $Y = -0.005X^2 + 0.008X + 3.667$  and the value of  $R^2 = 1$ . The addition of coconut water up to 150 ml to the media had not shown a significant effect,

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Comment [u6]: Any reference? You can add Vargas et al (2014) .. DOI: 10.2134/agronj13.0405

Comment [u7]: is it licensed ? If not, it is better to drop it.

because the curve tends to increase and has not shown an optimal point, however, it was estimated that the optimal

point was achieved in the addition of 150-200 ml/l coconut water.

**Table 1** Analysis of variance and orthogonal polynomials on the addition of coconut water and banana extract to MS media to stimulate the regeneration of PLB (Protocorm Like Bodies) *Dendrobium gaton* sunray

| Source of variance                     | F value      |             |             |                 |                |                |                 |
|----------------------------------------|--------------|-------------|-------------|-----------------|----------------|----------------|-----------------|
|                                        | Shoot length | Leaf length | Root length | Number of shoot | Number of leaf | Number of root | Weight plantlet |
| Coconut water                          | 0.07         | 0.48        | 0.22        | 0.11            | 0.27           | 0.17           | 0.14            |
| Banana extract                         | 1.56*        | 1.41*       | 0.94*       | 0.39            | 0.94*          | 1.71*          | 1.48*           |
| C. water x B. extract                  | 0.59         | 0.63        | 0.19        | 0.48            | 0.46           | 0.08           | 1.10*           |
| C. water linier                        | 0.07         | 0.46        | 0.09        | 0.10            | 0.10           | 0.16           | 0.29            |
| C. water kuadratic                     | 0.06         | 0.50*       | 0.34        | 0.12            | 0.44           | 0.19           | 0.01            |
| B. extract linier                      | 1.49*        | 0.15        | 0.12        | 0.76*           | 1.06*          | 0.09           | 0.26            |
| B. extract kuadratic                   | 1.63*        | 2.67*       | 1.77*       | 0.01            | 0.82*          | 3.33*          | 2.69*           |
| C. water linier x B. extract linier    | 0.93*        | 0.59*       | 0.07        | 0.13            | 0.03           | 0.14           | 1.40*           |
| C. water linier x B. extract kuadratic | 0.03         | 0.56*       | 0.53*       | 0.14            | 0.01           | 0.04           | 1.43*           |
| C. water kuadratic x B. extract linier | 1.33*        | 0.98*       | 0.02        | 1.06*           | 1.39*          | 0.06           | 1.43*           |

Note: \*significant different at F test 5%

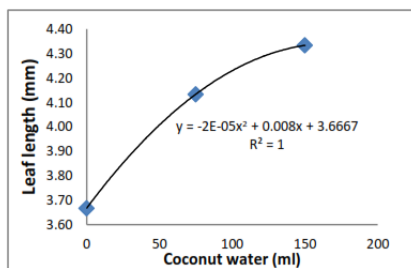


Figure 1 Respon of coconut water on the leaf length

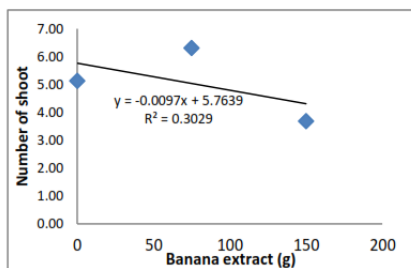


Figure 2 Respon of Banana extract on the number of shoot

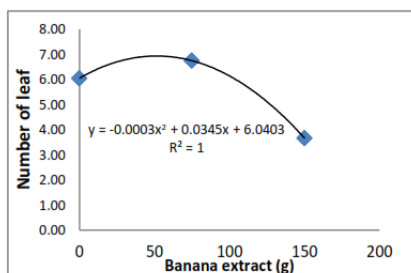


Figure 3 Respon of Banana extract on the number of leaf

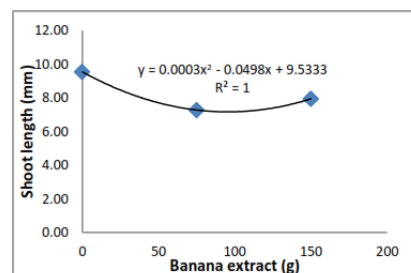


Figure 4 Respon of Banana extract on the shoot length

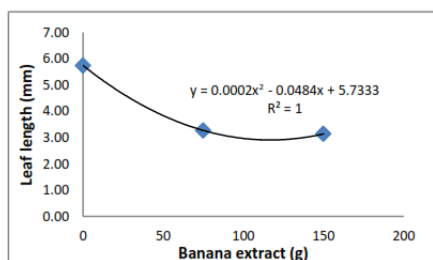


Figure 5 Respon of Banana extract on the leaf length

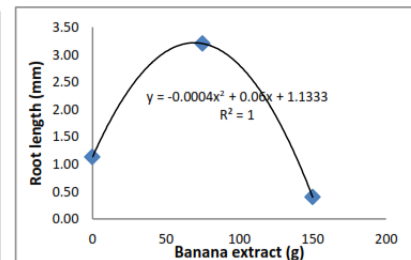


Figure 6 Respon of Banana extract on the root length

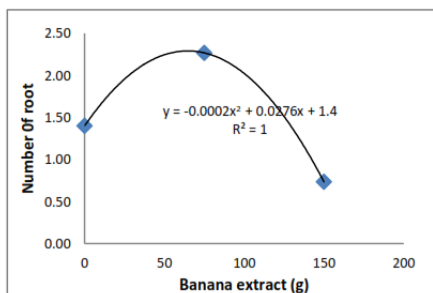


Figure 7 Respon of Banana extract on the number of root

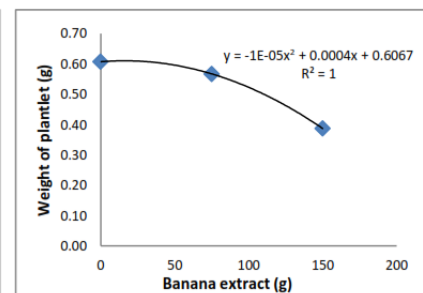


Figure 8 Respon of Banana extract on the weight of plantlet

Similar research by Pranata et al. [18] showed that the addition of 22.5% coconut water gave the best shoot length, then added with the addition of 15% coconut water. In addition, refers to Erfa et al. [19] in their research reported that the addition of coconut water up to 150 ml in the Phalaenopsis orchid growing medium resulted in better seedling height growth. Kristina et al. [16] stated that coconut water contains IAA (auxin) and cytokinins which were very good for stimulating plant growth and shoot growth. Coconut water has been used in tissue culture methods because of its growth regulating properties and cytokinin activity that supports cell division and promoting rapid growth [17]. The higher coconut water concentrations promoted better growth and shoot propagation in some orchid species, and the coconut water effect depending on the species and the explants [8-9].

### 3.2. The effect of banana extract on MS media on the regeneration of PLB Dendrobium gattong sunray

The effect of banana extract on PLB regeneration is presented in Table 2. The results showed that although it was significantly different in the F test, the average between treatments did not show a significant difference. The addition of banana extract 75 g / l had the highest root length with an average of 3.2 mm, the number of leaves was 6.74, and the number of roots was 2.27 (Table 2). Giving banana extract to the media because it was a source of carbohydrates and contains several minerals and various vitamins such as vitamin C and especially thiamine. Refer to Kasutjaniangati and Irawan's [20] research showed that Ambon banana extract has a good effect on the root length of Dendrobium.

The orthogonal polynomial analysis showed that the banana extract response was very significant with a quadratic pattern on the variable shoot length, leaf length, root length, number of leaves, number of roots, and plantlet weight (Table 1), except that the number of shoots tended to be linear (Figure 2). This indicated that the

optimum point has not been reached for the variable number of shoots.

The addition of banana extract forms a quadratic pattern on the number of leaves with the equation  $Y = -0.0003X^2 + 0.035 + 6.04$  ( $R^2 = 1$ ), and reaches an optimum point in the addition of 50-100 mg of banana extract (Figure 3). The same pattern was also found in root length (Figure 6), number of roots (Figure 7), and plantlet weight (Figure 8). Different patterns were found in shoot length (Figure 4) and leaf length (Figure 5), where it reached the lowest point at the addition of 50-100 mg of banana extract. Refer to Hendaryono [21] in his study on banana extract 300 g/l resulted in a lower number of leaves.

The highest average shoot length, leaf length, and plantlet weight without banana extract treatment (B0) were 9.53, 5.73, and 0.61 respectively, while in treatment (B1) the root length, number of leaves and number of roots, respectively amounting to 3.20, 6.74, and 2.27 (Table 2). Banana extract apart functions as a coenzyme for several reactions in metabolism and also plays a role in the metabolism of energy derived from carbohydrates. Giving Ambon banana fruit extract in the sub-culture plantlet can stimulate the growth of Dendrobium. Banana fruit also contains natural hormones auxins and gibberellins which can stimulate growth plantlets [20;22].

### 3.3. The interaction effect of coconut water and banana extract on MS media on the regeneration of PLB Dendrobium gattong sunray

The interaction effect of coconut water and banana extract on plantlet weight variables is presented in Table 3. The best treatment for plantlet weight variables was A1B1 (75 ml coconut water + 75 mg banana extract) which was 0.90 g and the lowest treatment was A2B2 which was 0.16 g. Based on orthogonal polynomial analysis, it showed that although there was only an interaction on plantlet weight, the interaction pattern between coconut water and banana extract tends to be quadratic in the variables shoot length, number of leaves, number of shoots, number of leaves, and plantlet weight (Table 1).

**Comment [u8]:** is it interaction effect or combination?

**Table 2** Effect of banana extract on the Shoot length, Leaf length, Root length, Number of leaf, Number of root, and Weight plantlet

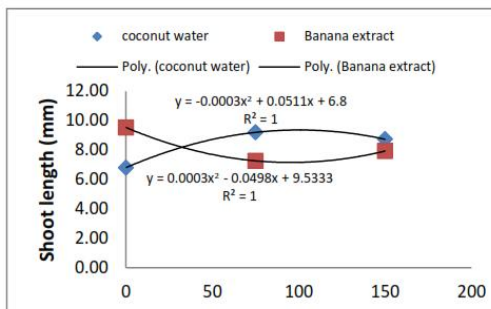
| Treatment | Means of treatment |             |             |                |                |                 |
|-----------|--------------------|-------------|-------------|----------------|----------------|-----------------|
|           | Shoot length       | Leaf length | Root length | Number of leaf | Number of root | Weight plantlet |
| B0        | 9.53a              | 5.73a       | 1.13a       | 6.04a          | 1.40a          | 0.61a           |
| B1        | 7.27a              | 3.27a       | 3.20b       | 6.74a          | 2.27a          | 0.57a           |
| B2        | 7.93a              | 3.13a       | 0.40a       | 3.66b          | 0.73a          | 0.39a           |

Note: LSD 5%

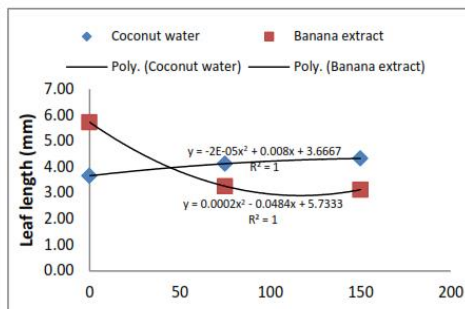
**Table 3** Effect of coconut water and banana extract on the weight plantlet

| Treatment | Means of weight plantlet (g) |
|-----------|------------------------------|
| A0B0      | 0.62a                        |
| A0B1      | 0.28c                        |
| A0B2      | 0.54a                        |
| A1B0      | 0.68a                        |
| A1B1      | 0.90b                        |
| A1B2      | 0.46a                        |
| A2B0      | 0.52a                        |
| A2B1      | 0.52a                        |
| A2B2      | 0.16c                        |

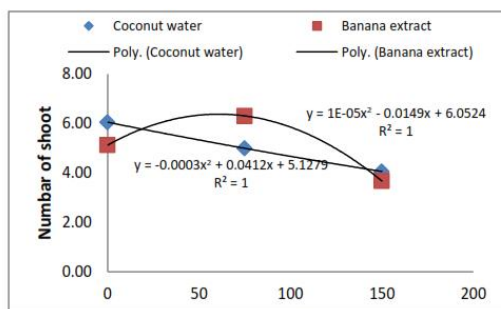
Note: LSD 5%



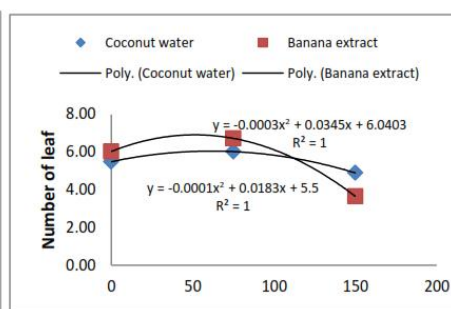
**Figure 9** Respon of coconut water and banana extract on the shoot length



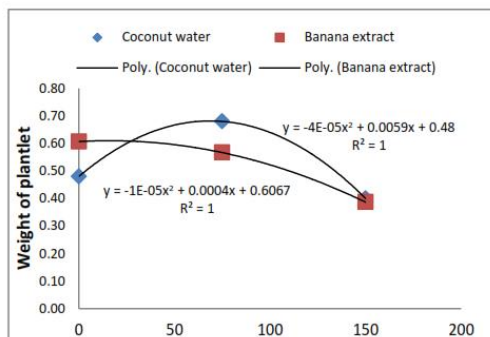
**Figure 10** Respon of coconut water and banana extract on the leaf length



**Figure 11** Respon of coconut water and banana extract on the number of shoot



**Figure 12** Respon of coconut water and banana extract on the number of leaf



**Figure 13** Respon of coconut water and banana extract on the weight of plantlet

The orthogonal polynomial analysis showed the interaction pattern of coconut water and banana extract treatments occurred in treatment 0-50, where the meeting point of the two treatments, namely the shoot length variable (Figure 9), leaf length (Figure 10), number of

shoots (Figure 11), and plantlet weights (Figure 13). Meanwhile, the variable number of leaves requires quite a lot of coconut water and banana extract, namely 100-150 to get the interaction between the two treatments (Figure 12). This showed that there was a different interaction

**Comment [u9]:**

- What is "numbar"?

- What is the title and unit of the X axis ?

- It seems no need to put the decimal in the axis

**Comment [u10]:** It would be better to put some explanation of the meaning or the merit of the finding.  
What is the importance of the meeting point?  
What is the implication of the meeting point in the media formulation?

response to the observed variables. MS is one of the media that is often used in the process of in vitro propagation or tissue culture. This is because MS media contains vitamin B1 which is very useful for plant growth. MS media has this feature because MS media contains high levels of nitrate, potassium, ammonium and the amount of inorganic nutrients for the cell multiplication, so it is thought to strongly influence the number of shoots of *Dendrobium* sp. Razdan [23] stated that MS medium was commonly used for shoot induction and contains appropriate nutrients to support optimal growth of plants in vitro. According to Suhartanto. et al. [24] that bananas are often used as a source of organic matter in tissue culture media, because 100 grams of banana flesh contain protein (1%), fat (0.3%), carbohydrates (27%), energy (116 -128 kcal), minerals (Ca 15 mg, K 380 mg, Fe 0.5 mg, Na 1.2 mg, and Vitamins (Vit. A 0.3 mg, Vit. B1 0.1 mg, B2 0.1 mg, B6 0.7 mg, Vit. C. Hadi's research (2006) states that the

#### 4. CONCLUSION

Coconut water did not affect all observed variables, however the banana extract treatment had a significant effect on all observed variables except for the number of shoots. There was an interaction between the two treatments, namely the variable plantlet weight. Orthogonal polynomial analysis showed that the interaction pattern of coconut water and banana extract treatment occurred in treatment 0-50, where the meeting point of the two treatments, namely the variable shoot length, leaf length, shoot number, and plantlet weight. Whereas in the variable number of leaves, the interaction occurs in the 100-150 treatment.

#### REFERENCES

- [1] D. Puchooa, Comparison of different culture media for the in vitro culture of *Dendrobium* (Orchidaceae). *Int. J. Agric. Biol.* 6 (2004) 884-888. DOI: 1560-8530/2004/06-5-884-888
- [2] Talukder et al., In vitro root formation on orchid plantlets with IBA and NAA. *Progress. Agric.* 13(1&2)(2002) 25-28.
- [3] A. Suryana, Prospects and Directions for Orchid Agribusiness (Prospek dan Arah Pengembangan Agribisnis Anggrek). Development. Agricultural Research and Development Agency. Jakarta, 2005.
- [4] The Indonesian Statistics Agency, Statistik-tanaman-hias-indonesia-2017. <https://www.bps.go.id/site/publication/2018/10/05/statistik-tanaman-hias-indonesia-2017>. Jun 2019 17:45:28 GMT.
- [5] K.B. Andri, W.J.F.A. Tumbuan, Potential development of orchid flower agribusiness in Batu City, East Java (Potensi pengembangan agribisnis bunga anggrek di kota batu Jawa Timur). *Jurnal LPPM Bidang EkoSosBudKum.* 2(1)(2015) 19-30.
- [6] Indonesian Environmental and Forestry Research and Development Center, Plant Propagation Through Tissue Culture (Perbanyakan Tanaman Melalui Kultur Jaringan). Makassar. Bogor. Bogor. 304 p. 2013.
- [7] D. Widiastoety, A. Santi, N. Solvia, Effect of myoinositol and activated charcoal on growth of *dendrobium* orchid plantlets in in vitro culture (Pengaruh Myoinositol dan Arang Aktif terhadap Pertumbuhan Planlet Anggrek *Dendrobium* dalam Kultur In vitro). *J. Hort.* 22(3) (2012) 205.
- [8] W. Pakum, S. Watthana, K. Srimuang, A. Kongbangkerd, Influence of Medium Component on In vitro Propagation of Thais Endangered Orchid: *Bulbophyllum nipondhii* Seidenf, *Plant Tissue Cult. & Biotech.*, 26(1) (2016) 37- 46. <https://doi.org/10.3329/ptcb.v26i1.29765>
- [9] T. Punjansing, M. Nakkuntod, S.Homchan, P. Inthima, A. Kongbangkerd, Influence of Organic Supplements on Shoot Multiplication Efficiency of *Phaius tankervilleae* var. Alba. *Int. J. of Agric. and Biosyst. Eng.* 13(4) (2019) 105-109.
- [10] S. Rahmah, T. Rahayu, A. Hayati, Study Adding Organic Substances on VW Media for Organogenesis the *Dendrobium* Orchid in Vitro. *e-Jurnal Ilmiah SAINS ALAMI (Known Nature)* 1(1) (2018) 3 - 103
- [11] Djajanegara, Utilization of banana and coconut water waste as a medium for tissue culture for the Moon Orchid (*Phalaenopsis amabilis*) Type 229

**Comment [u11]:** It would be better to put the practical meaning of this statistical term. What is the "interaction" and what is "meeting point" in the practical tissue culture?

(Pemanfaatan limbah buah pisang dan air kelapa sebagai bahan media kultur jaringan anggrek bulan (*Phalaenopsis amabilis*) Tipe 229). *J. Teknologi Lingkungan* 11(3) (2010) 373-380.

[12] M. Humaira, Z. Thomy, E. Harnelly, The effect of giving coconut water and banana pulp to MS medium on the growth of rabbit orchid plantlets (*Dendrobium antennatum* Lindl.) In vitro (Pengaruh pemberian air kelapa dan bubur pisang pada media ms terhadap pertumbuhan plantlet anggrek kelinci (*dendrobium antennatum* Lindl.) secara in vitro). *Proc. of Biotic Nasional Seminar Nasional*, 2015.

[13] J. Pratama, Modification of MS Media with Addition of Coconut Water for Subculture I *Cymbidium* Orchids (Modifikasi Media MS Dengan Penambahan Air Kelapa Untuk Subkultur I Anggrek *Cymbidium*). *Jurnal Agrium* 15(2) (2018) 91-109

[14] N.P.Y.A. Dewi, The Effect of Giving Coconut Water on Embryo Development in *Dendrobium anosmum* Lindl (Pengaruh Pemberian Air Kelapa terhadap Perkembangan Embrio pada *Dendrobium anosmum* Lindl). *JBE* 4 (1) (2019) 22-28.

[15] Nurfadilah, Mukarlina, E. Rusmiyanto P.W, The multiplication of black orchids (*Coelogyne pandurata* Lindl) on murashige skoog (MS) media with the addition of Ambon banana extract and benzyl amino purine (BAP) (Multiplikasi anggrek hitam (*Coelogyne pandurata* Lindl ) pada media murashige skoog (MS) dengan penambahan ekstrak pisang ambon dan benzyl amino purin (BAP)). *Protobiont* 7(3) (2018) 47 –53.

[16] N.N. Kristina, Syahid, S. Fatimah, The Effect of Coconut Water on In Vitro Shoot Multiplication, Rhizome Production, and Xanthorrhizol Content of Temulawak in the Field (Pengaruh Air Kelapa Terhadap Multipikasi Tunas In Vitro, Produksi Rimpang, dan Kandungan Xanthorrhizol Temulawak di Lapangan). *Jurnal Litri* 18 (3) (2012) 125-134.

[17] J. Yong, L. Ge, Y.F. Ng, S. Tan, The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water, *Molecules* 14(12) (2009) 5144-5164. <https://doi.org/10.3390/molecules14125144>

[18] M.G. Pranata, A. Yunus, B. Pujiasmanto, The effect of NAA concentrations and coconut water on the multiplication of ginger (*Curcuma xanthorrhiza* Roxb.) In vitro (Pengaruh konsentrasi naa dan air kelapa terhadap multiplikasi temulawak (*Curcuma xanthorrhiza* Roxb.) secara in vitro). *J. of Sustainable Agric.* 30(2) (2015)

[19] L. Erfa, Ferziana, Growth of *Phalaenopsis* orchid seedling into plantlets in subculture II media with addition of tripton and atonic (Pertumbuhan seedling anggrek *Phalaenopsis* menjadi plantlet pada media subkultur II dengan penambahan tripton dan atonik). *Jurnal Penelitian Pertanian Terapan*. Vol. 12. (Spec. Ed.) (2012) 52-58.

[20] Kasutjiningati, R. Irawan, Alternative media for in vitro orchid propagation (*Phalaenopsis amabilis*) (Media alternatif perbanyakan in vitro anggrek Bulan (*Phalaenopsis amabilis*)). *J. Agroteknos.* 3(3) (2013) 184-189.

[21] D.P.S. Hendaryono, Orchid nursery in bottles (Pembibitan anggrek dalam botol). Kanisius: Yogyakarta. 2000

[22] S. Damiska, R.S. Wulandari, H. Darwati, Addition of Yeast and Corn Seed Extract to In-Vitro Growth of Mangosteen Shoots (Penambahan Ragi dan Ekstrak Biji Jagung terhadap Pertumbuhan Tunas Manggis Secara In-Vitro). *J Hutan Lestari* 3(1) (2015) 35-42.

[23] M.K. Razdan, Introduction to Plant Tissue. 2nd Edition. Qxford & IBH Publishing Co. Pvt. Ltd. New Delhi. 2003.

[24] M.R. Suhartanto, Sobir, H. Harti, Healthy Technology for Banana Cultivation: From Seed to Post Harvest (Teknologi Sehat Budidaya Pisang: dari Benih sampai Pasca Panen). Pusat Kajian Hortikultura Tropika, LPPM-IPB. 2012.

Comment [u12]: C



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## Re: Iseprolocal Manuscript Revision\_RenyH

2 pesan

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**Reny Herawati** <reny.herawati@unib.ac.id>

1 Februari 2021 pukul 00.10

Kepada: ISEPROLOCAL Universitas Bengkulu <iseprolocal@unib.ac.id>

Cc: umisalamah@unib.ac.id

Dear Iseprolocal Committee,

I have made revisions according to reviewer suggestions, please kindly find the attached file.  
Thank you for your attention

Best regards,  
Reny et al.



**Revised\_266-422-1-SP(1)\_rev1.docx**

548K

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**ISEPROLOCAL Universitas Bengkulu** <iseprolocal@unib.ac.id>

2 Februari 2021 pukul 14.03

Kepada: Reny Herawati <reny.herawati@unib.ac.id>

Cc: umisalamah@unib.ac.id

Well received with thanks.

[Kutipan teks disembunyikan]

# Addition of Coconut Water and Banana Extract on MS Media to Stimulate PLB (Protocorm Like Bodies) Regeneration of *Dendrobium gatton sunray*

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## ABSTRACT

Organic material is widely used for propagation of orchids, namely coconut water, extracts of tomatoes, bananas, bean sprouts and potatoes which can be added to certain media as a provider of nutrients, amino acid minerals, and growth regulators. This study aims to determine the effect of the concentration of organic matter in coconut water and banana extract on MS media on the regeneration of PLB (protocorm like bodies) *Dendrobium gatton sunray*. The study was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Bengkulu University, from September to December 2019. This study used a Complete Randomized Block Design (RCBD) with two factors. The first factor was the concentration of coconut water with 3 levels namely: A0 = 0 (Control), A1 = 75 ml.L<sup>-1</sup>, A2 = 150 ml.L<sup>-1</sup>, and the second factor was the concentration of banana extract with 5 levels namely: B0 = 0 (Control), B1 = 75 g.L<sup>-1</sup>, B2 = 150 g.L<sup>-1</sup>. The results showed that coconut water did not affect all variables, however the banana extract had a significant effect on all variables except for the number of shoots. There was an interaction between the two treatments, namely the variable plantlet weight. Orthogonal polynomial analysis showed that there was an interaction between the two treatments, where coconut water and banana extract had a good response on the plantlet growth when given together as much as 75 ml. L<sup>-1</sup> (A1) and 75 g.L<sup>-1</sup> (B1). The concentration of both treatment was lower than it was given individually.

**Keywords:** coconut water, bananas extracts, *Dendrobium gatton sunray*, MS media

## 1. INTRODUCTION

*Dendrobium* is a type of orchid that is favoured by orchid enthusiasts because it has a variety of forms, shapes, and even sizes, it is even easy to maintain and cultivate, and the price is relatively affordable. In addition, *Dendrobium* also has other features such as the freshness of long-lasting flowers, flexible flower stalks [1] so that it is widely used as an ornamental plant in the form of cut flowers that can decorate spaces with good quality [2]. According to Suryana [3] orchids had entered the international market for export to destination countries, including Japan, the Netherlands, Korea and Singapore. The Indonesian Statistics Agency [4] notes that the value of orchid production in Indonesia from 2016 to 2017 had increased between 19,978,078 stalks to 20,045,577 stalks with an average export value of 120,560 US dollars. Financially the orchid farming gives a profit, where in one growing season it provides a profitability (ROI) of 70% of the funds invested. Value added along the marketing chain show a reasonable profit for the business (farmers and traders) as well, so that this commodity business has good prospects [5].

In general, orchids are only able to reproduce in nature at less than 1%, this is because orchid seeds are very small and do not have an endosperm so that orchids are symbiotic with mycorrhizae in nature. The role of mycorrhizae as a supplier of carbon sources for orchid seed germination in general has been replaced by tissue culture media. Optimization of tissue culture techniques continues to be carried out in an effort to accelerate the propagation of orchids, this technique only requires a small part of the plant to obtain the same plantlets as the parent [6].

The orchid seeds will grow into protocorms in the germination medium. Protocorm can form a secondary protocorm (protocorm like body/PLB). In vitro regeneration of PLB *Dendrobium* for shoot multiplication is generally carried out on media enriched with growth regulators and vitamins. One of the keys to success is the use of media types and growth regulators at the right concentration. The composition of the media used greatly determines the growth rate of protocorms and plantlets and is useful for increasing the quality and quantity of seedlings [7]. The composition of the media needed in tissue culture must contain nutrients that are useful for plants, namely consisting of carbohydrates, sugars and vitamins. Tissue culture media usually uses artificial

media compositions, namely Murashige and Skoog and Knudson C media, either half or full concentration. The composition of the media is a determining factor in the success of in vitro orchid culture. Growth and reproduction rates of many species of orchids enhanced by adding some organic supplements such as coconut water (CW) or potato extract to culture medium [8-9]. Growth regulators are usually added to the medium to promote growth of explants. The growth regulators commonly used are auxins and cytokinins. The composition of the media can also be obtained from organic materials which already have natural nutrients and hormones in the form of auxins and cytokinins known as phytohormones. Complex organic materials can be used as additives that can encourage the development and growth of propagation explants in tissue culture [10;9]. The addition of organic materials such as extracts bananas, potato pulp and vegetable substances others have content high carbohydrate can increase growth and differentiation of cells in plants [11].

Organic materials that are widely used for orchid propagation are coconut water, tomato extracts, bananas, bean sprouts and potatoes which can be added with certain media as a provider of nutrients, amino acids, and growth regulators for orchid growth. Coconut water has been widely used as an additive in tissue culture media. The content of coconut water is in the form of active ingredients that are useful for the growth of plantlets which are rich in vitamins, sugars, amino acids and phytohormones [12-14]. Nurfadilah et al. [15] research showed that the combination of Ambon banana and BAP on concentration  $10^{-6}$ M BAP and 2.5% banana extract Ambon produces the best growth on time of emergence of shoots, number of shoots and number of leaves. This research was conducted to modify the MS media with added coconut water and banana extract to stimulate the regeneration of PLB *Dendrobium gattsoni* sunray.

## 2. MATERIAL AND METHODS

The research was conducted at the Laboratory of Agricultural Cultivation, Division of Tissue Culture and Biotechnology, Faculty of Agriculture, Bengkulu University. This research was conducted from September to December 2019. The materials used were PLB which had formed 2-3 mm buds as plantlets, banana extract, coconut water, Murashige & Skoog (MS) media, sugar, 70% alcohol, alcohol. 96%, and aquadest.

The design method used was a factorial completely randomized design (CRD) with 2 factors, namely: the first factor was coconut water with 3 levels, namely: A0 = 0 (Control), A1 = 75 ml.L<sup>-1</sup>, A2 = 150 ml.L<sup>-1</sup>. The second factor was banana extract with 3 levels, namely: B0 = 0 (Control), B1 = 75 g.L<sup>-1</sup>, B2 = 150 g.L<sup>-1</sup>. Each treatment combination was repeated 5 times. One experimental unit consisted of one culture bottle planted with 3 PLBs.

½ MS medium was added to treatment, added sugar as much as 30 g.L<sup>-1</sup>, gelatin as much as 7 g.L<sup>-1</sup>, then the media pH was measured 5.8. The media was cooked until the gelatin was dissolved and well blended, then

immediately poured into a sterile bottle with a volume of 20 ml bottle<sup>-1</sup>, then the media was sterilized by autoclave at a temperature of 121 ° C, 15 psi pressure for 15 minutes. The explants used in the study were not uniformed into other media and were directly planted in the experimental media. The explants were removed from the bottle and selected for uniform growth. The selected explants consisted of 2 explants per bottle which were sterile, then the bottles were covered with plastic and tied with a rubber band and given a tight wrap. The culture bottles were maintained in the culture room at a temperature of 19-20 ° C with  $\pm 1,000$  lux irradiation for 12-16 hours / day.

Observations were made one week after planting (WAF) to 15 weeks (WAF). Every day the plants were checked for contaminants and browning, while the last week observations were made on the variables of the number of leaves, number of roots, length of leaves, length of shoots, number of shoots, and weight of plantlets.

Data were analyzed using analysis of variance at the 5% level, and further tests with LSD at the 5% level. The orthogonal polynomial test was used to determine the treatment and interaction patterns [16].

## 3. RESULTS AND DISCUSSION

Variance analysis showed that coconut water had no effect on all observed variables, however banana extract had a significant effect on all observed variables except for the number of shoots (Table 1). There was an interaction between the two treatments, namely the plantlet weight.

The orthogonal polynomial analysis showed that the pattern of coconut water on the leaf length was very significant in a quadratic curve, while the banana extract pattern was very significant quadratic on the variable shoot length, leaf length, root length, number of leaves, number of roots, and plantlet weight, except that the number of shoots tended to be linear. (Table 1). Even though there was only an interaction on plantlet weight, the interaction pattern between the addition of coconut water and banana extract tended to be quadratic in the variables of shoot length, number of leaves, number of shoots, number of leaves, and plantlet weight (Table 1).

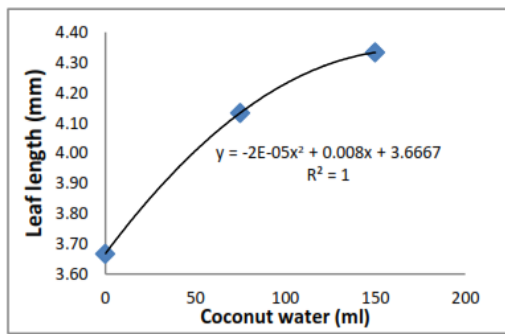
### 3.1. The effect of coconut water to MS media on the regeneration of PLB (Protocorm Like Bodies) *Dendrobium sp*

The effect of coconut water on PLB (Protocorm Like Bodies) regeneration was not significantly different in all observed variables. However, the pattern of coconut water tends to be quadratic in leaf length (Figure 1) with the regression equation  $Y = -0.005X^2 + 0.008X + 3.667$  and the value of  $R^2 = 1$ . The addition of coconut water up to 150 ml to the media had not shown a significant effect, because the curve tends to increase and has not shown an optimal point, however, it was estimated that the optimal point was achieved in the addition of 150-200 ml/l coconut water.

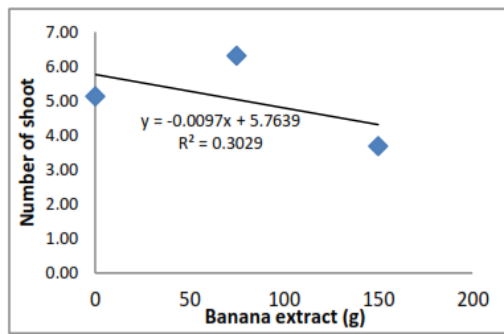
**Table 1** Analysis of variance and orthogonal polynomials on the addition of coconut water and banana extract to MS media to stimulate the regeneration of PLB (Protocorm Like Bodies) *Dendrobium gattongi* sunray

| Source of variance                     | F value      |             |             |                 |                |                |                 |
|----------------------------------------|--------------|-------------|-------------|-----------------|----------------|----------------|-----------------|
|                                        | Shoot length | Leaf length | Root length | Number of shoot | Number of leaf | Number of root | Weight plantlet |
| Coconut water                          | 0.07         | 0.48        | 0.22        | 0.11            | 0.27           | 0.17           | 0.14            |
| Banana extract                         | 1.56*        | 1.41*       | 0.94*       | 0.39            | 0.94*          | 1.71*          | 1.48*           |
| C. water x B. extract                  | 0.59         | 0.63        | 0.19        | 0.48            | 0.46           | 0.08           | 1.10*           |
| C. water linier                        | 0.07         | 0.46        | 0.09        | 0.10            | 0.10           | 0.16           | 0.29            |
| C. water kuadratic                     | 0.06         | 0.50*       | 0.34        | 0.12            | 0.44           | 0.19           | 0.01            |
| B. extract linier                      | 1.49*        | 0.15        | 0.12        | 0.76*           | 1.06*          | 0.09           | 0.26            |
| B. extract kuadratic                   | 1.63*        | 2.67*       | 1.77*       | 0.01            | 0.82*          | 3.33*          | 2.69*           |
| C. water linier x B. extract linier    | 0.93*        | 0.59*       | 0.07        | 0.13            | 0.03           | 0.14           | 1.40*           |
| C. water linier x B. extract kuadratic | 0.03         | 0.56*       | 0.53*       | 0.14            | 0.01           | 0.04           | 1.43*           |
| C. water kuadratic x B. extract linier | 1.33*        | 0.98*       | 0.02        | 1.06*           | 1.39*          | 0.06           | 1.43*           |

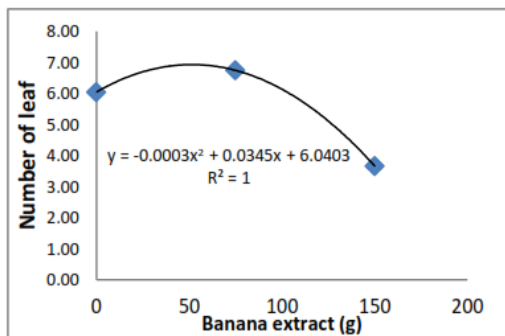
Note: \*significant different at F test 5%



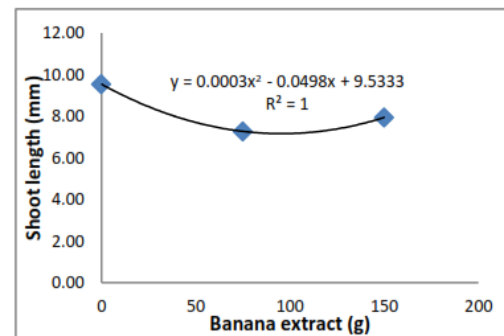
**Figure 1** Respon of coconut water on the leaf length



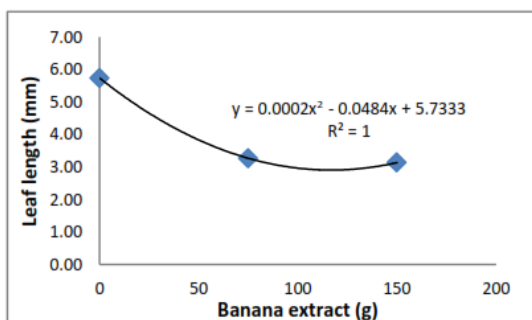
**Figure 2** Respon of Banana extract on the number of shoot



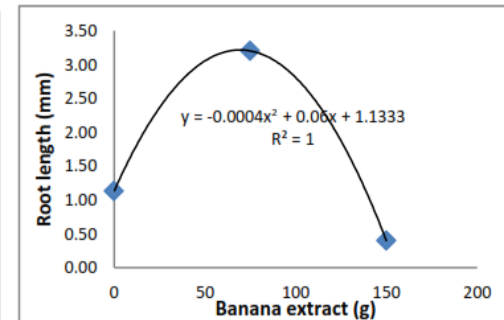
**Figure 3** Respon of Banana extract on the number of leaf



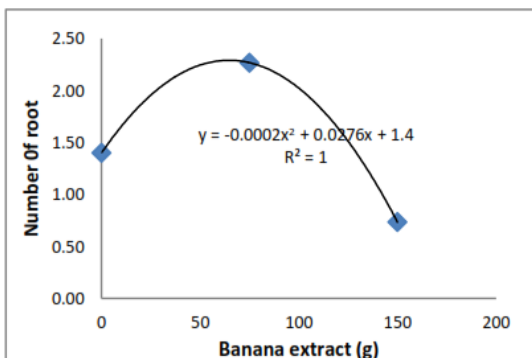
**Figure 4** Respon of Banana extract on the shoot length



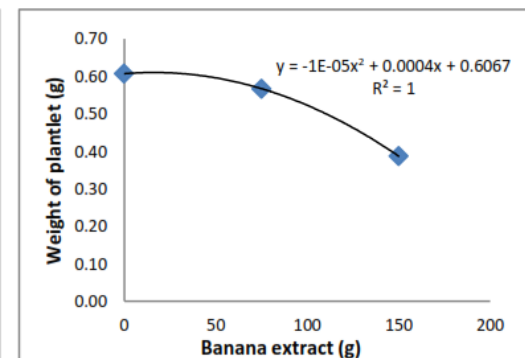
**Figure 5** Respon of Banana extract on the leaf length



**Figure 6** Respon of Banana extract on the root length



**Figure 7** Respon of Banana extract on the number of root



**Figure 8** Respon of Banana extract on the weight of plantlet

Similar research by Pranata et al. [17] showed that the addition of 22.5% coconut water gave the best shoot length, then added with the addition of 15% coconut water. In addition, refers to Erfa et al. [18] in their research reported that the addition of coconut water up to 150 ml in the Phalaenopsis orchid growing medium resulted in better seedling height growth. Kristina et al. [19] stated that coconut water contains IAA (auxin) and cytokinins which were very good for stimulating plant growth and shoot growth. Coconut water has been used in tissue culture methods because of its growth regulating properties and cytokinin activity that supports cell division and promoting rapid growth [20]. The higher coconut water concentrations promoted better growth and shoot propagation in some orchid species, and the coconut water effect depending on the species and the explants [8-9].

### 3.2. The effect of banana extract on MS media on the regeneration of PLB Dendrobium gaton sunray

The effect of banana extract on PLB regeneration is presented in Table 2. The results showed that although it was significantly different in the F test, the average between treatments did not show a significant difference. The addition of banana extract 75 g L<sup>-1</sup> had the highest root length with an average of 3.2 mm, the number of leaves was 6.74, and the number of roots was 2.27 (Table 2). Giving banana extract to the media because it was a source of carbohydrates and contains several minerals and various vitamins such as vitamin C and especially thiamine. Refer to Kasutjaniangati and Irawan's [21] research showed that Ambon banana extract has a good effect on the root length of Dendrobium.

The orthogonal polynomial analysis showed that the banana extract response was very significant with a quadratic pattern on the variable shoot length, leaf length, root length, number of leaves, number of roots, and plantlet weight (Table 1), except that the number of shoots tended to be linear (Figure 2). This indicated that the optimum point has not been reached for the variable number of shoots.

The addition of banana extract forms a quadratic pattern on the number of leaves with the equation  $Y = -0.0003X^2 + 0.035 + 6.04$  ( $R^2 = 1$ ), and reaches an optimum point in the addition of 50-100 mg of banana extract (Figure 3).

The same pattern was also found in root length (Figure 6), number of roots (Figure 7), and plantlet weight (Figure 8). Different patterns were found in shoot length (Figure 4) and leaf length (Figure 5), where it reached the lowest point at the addition of 50-100 g of banana extract. Refer to Hendaryono [22] in his study on banana extract 300 g/l resulted in a lower number of leaves.

The highest average shoot length, leaf length, and plantlet weight without banana extract treatment (B0) were 9.53, 5.73, and 0.61 respectively, while in treatment (B1) the root length, number of leaves and number of roots, respectively amounting to 3.20, 6.74, and 2.27 (Table 2). Banana extract apart functions as a coenzyme for several reactions in metabolism and also plays a role in the metabolism of energy derived from carbohydrates. Giving Ambon banana fruit extract in the sub-culture plantlet can stimulate the growth of Dendrobium. Banana fruit also contains natural hormones auxins and gibberellins which can stimulate growth plantlets [21;23]

### 3.3. The interaction effect of coconut water and banana extract on MS media on the regeneration of PLB Dendrobium gaton sunray

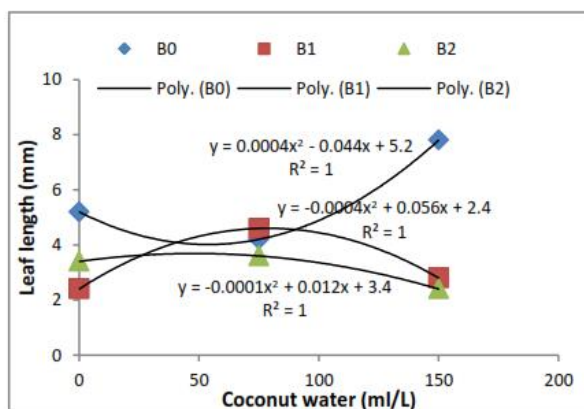
Based on orthogonal polynomial analysis, it showed that although there was only an interaction on plantlet weight, the interaction pattern between coconut water and banana extract tends to be quadratic in the variables shoot length, number of leaves, number of shoots, number of leaves, and plantlet weight (Table 1). The interaction effect between coconut water at various levels of banana extract was found in the variables of leaf length, root length, and plantlet weight (Figure 9-11). The effect of the interaction between coconut water at various levels of banana extract was found in the variables of leaf length, root length, and plantlet weight. Meanwhile, the effect of the interaction between banana extracts at various levels of coconut water was found in the variable shoot length, number of shoots, leaf length, number of leaves, and plantlet weight (Figure 12-16). Coconut water treatment gives a good response when given together with banana extract as much as 75 mg (B1) on the variable leaf length (Figure 9), root length (Figure 10), and plantlet weight (Figure 11), where coconut water reaches its optimum. at concentrations between 50-100 mL.L<sup>-1</sup>.

**Table 2** Effect of banana extract on the Shoot length, Leaf length, Root length, Number of leaf, Number of root, and Weight plantlet

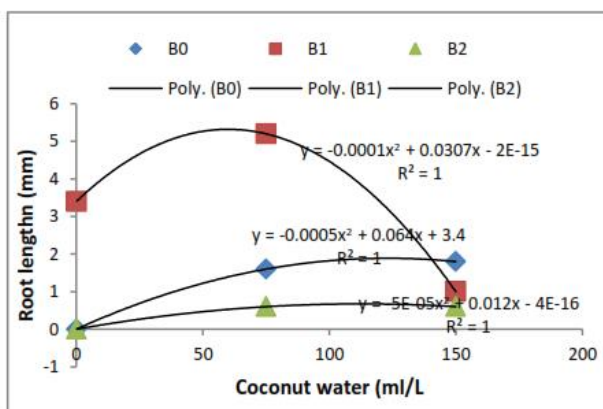
| Treatment | Means of treatment |             |             |                |                |                 |
|-----------|--------------------|-------------|-------------|----------------|----------------|-----------------|
|           | Shoot length       | Leaf length | Root length | Number of leaf | Number of root | Weight plantlet |
| B0        | 9.53a              | 5.73a       | 1.13a       | 6.04a          | 1.40a          | 0.61a           |

|    |       |       |       |       |       |       |
|----|-------|-------|-------|-------|-------|-------|
| B1 | 7.27a | 3.27a | 3.20b | 6.74a | 2.27a | 0.57a |
| B2 | 7.93a | 3.13a | 0.40a | 3.66b | 0.73a | 0.39a |

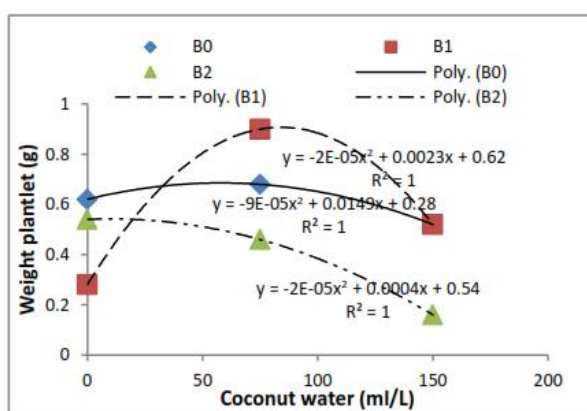
*Note: LSD 5%*



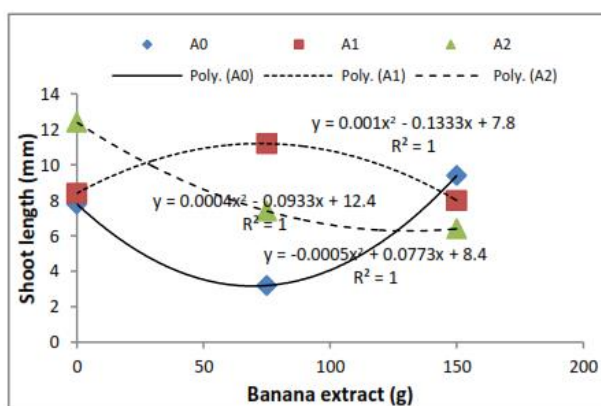
**Figure 9** Response of leaf length on the coconut water at the various levels of banana extract



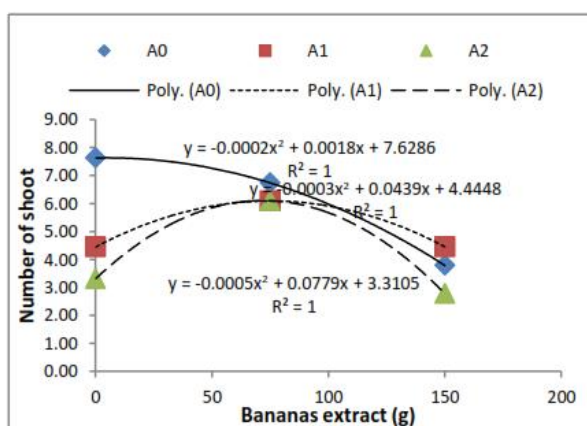
**Figure 10** Response of root length on the coconut water at the various levels of banana extract



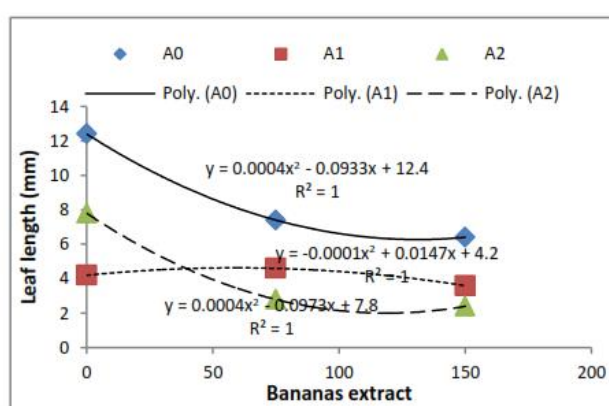
**Figure 11.** Response of weight plantlet on the coconut water at the various levels of banana extract



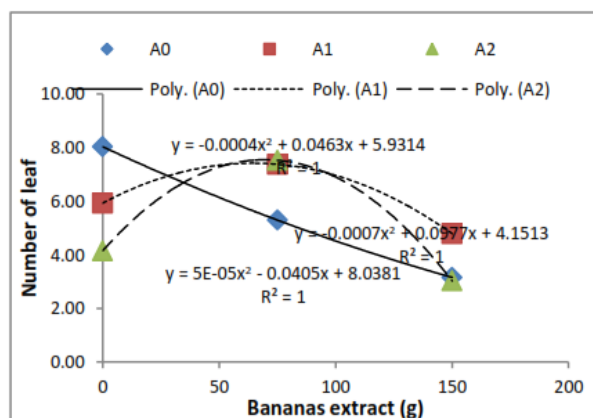
**Figure 12.** Response of shoot length on the banana extract at the various levels of coconut water



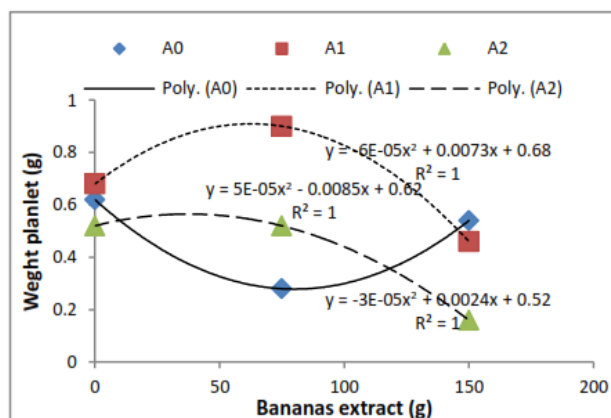
**Figure 13.** Response of number of shoot on the banana extract at the various levels of coconut water



**Figure 14.** Response of leaf length on the banana extract at the various levels of coconut water



**Figure 15.** Response of number of leaf on the banana extract at the various levels of coconut water



**Figure 16.** Response of weight plantlet on the banana extract at the various levels of coconut water

Otherwise, the banana extract treatment gave a good response at a coconut water concentration of 75 mL.L<sup>-1</sup> (A1) to the variable shoot length (Figure 12), number of shoots (Figure 13), leaf length (Figure 14), number of leaves (Figure 15), and plantlet weight (Figure 16), where banana extract reaches the optimum between 50-100 g. It was due to the presence of nutrients and growth regulators in coconut water as well as mineral content in bananas, causing interactions to spur plantlet growth, so that the concentration of both treatment was lower than given individually.

According to Suhartanto. et al. [24] that bananas are often used as a source of organic matter in tissue culture media, because 100 grams of banana flesh contain protein (1%), fat (0.3%), carbohydrates (27%), energy (116 -128 kcal), minerals (Ca 15 mg, K 380 mg, Fe 0.5 mg, Na 1.2 mg, and

#### 4. CONCLUSION

Coconut water did not affect all observed variables, however the banana extract treatment had a significant effect on all observed variables except for the number of shoots. There was an interaction between the two treatments, where coconut water and banana extract had a good response on the plantlet growth when given together as much as 75 mL.L<sup>-1</sup> (A1) and 75 g. L<sup>-1</sup> (B1). The concentration of both treatment was lower than it was given individually based on the results of previous studies.

#### REFERENCES

- [1] D. Puchooa, Comparison of different culture media for the in vitro culture of *Dendrobium* (Orhidaceae). *Int. J. Agric. Biol.* 6 (2004) 884-888. DOI: 1560-8530/2004/06-5-884-888
- [2] Talukder et al., In vitro root formation on orchid plantlets with IBA and NAA. *Progress. Agric.* 13(1&2)(2002) 25-28.

Vitamins (Vit. A 0.3 mg, Vit. B1 0.1 mg, B2 0.1 mg, B6 0.7 mg, Vit. C. Addition of BAP 2 mg.L<sup>-1</sup>, coconut water 150 mL.L<sup>-1</sup> and 50 g.L<sup>-1</sup> Ambon banana extract had the same effect on increase in the number of shoots, an average of 2 shoots of *Phalaenopsis amabilis* [21]. The highest number of shoots was produced in media supplemented with 50 mL.L<sup>-1</sup> coconut water and 50 g.L<sup>-1</sup> potato extract, while media supplemented with 150 mL.L<sup>-1</sup> coconut water and 50 potato extracts increased the number of leaves and the number of roots [9].

- [3] A. Suryana, Prospects and Directions for Orchid Agribusiness (Prospek dan Arah Pengembangan Agribisnis Anggrek). Development. Agricultural Research and Development Agency. Jakarta, 2005.
- [4] The Indonesian Statistics Agency, Statistik-tanaman-hias-indonesia-2017. <https://www.bps.go.id/site/publication/2018/10/05/statistik-tanaman-hias-indonesia-2017>. Jun 2019 17:45:28 GMT.
- [5] K.B. Andri, W.J.F.A. Tumbuan, Potential development of orchid flower agribusiness in Batu City, East Java (Potensi pengembangan agribisnis bunga anggrek di kota batu Jawa Timur). *Jurnal LPPM Bidang EkoSosBudKum.* 2(1)(2015) 19-30.
- [6] Indonesian Environmental and Forestry Research and Development Center, Plant Propagation Through Tissue Culture (Perbanyakan Tanaman Melalui Kultur Jaringan). Makassar. Bogor. Bogor. 304 p. 2013.

- [7] D. Widiastoety, A. Santi, N. Solvia, Effect of myoinositol and activated charcoal on growth of dendrobium orchid plantlets in in vitro culture (Pengaruh Myoinositol dan Arang Aktif terhadap Pertumbuhan Planlet Anggrek Dendrobium dalam Kultur In vitro). *J. Hort.* 22(3) (2012) 205.
- [8] W. Pakum, S. Watthana, K. Srimuang, A. Kongbangkerd, Influence of Medium Component on In vitro Propagation of Thais Endangered Orchid: *Bulbophyllum nipondhii* Seidenf, *Plant Tissue Cult. & Biotech.*, 26(1) (2016) 37- 46.  
<https://doi.org/10.3329/ptcb.v26i1.29765>
- [9] T. Punjansing, M. Nakkuntod, S.Homchan, P. Inthima, A. Kongbangkerd, Influence of Organic Supplements on Shoot Multiplication Efficiency of *Phaius tankervilleae* var. Alba. *Int. J. of Agric. and Biosyst. Eng.* 13(4) (2019) 105-109.
- [10] S. Rahmah, T. Rahayu, A. Hayati, Study Adding Organic Substances on VW Media for Organogenesis the Dendrobium Orchid in Vitro. *e-Jurnal Ilmiah SAINS ALAMI (Known Nature)* 1(1) (2018) 3 - 103
- [11] Djajanegara, Utilization of banana and coconut water waste as a medium for tissue culture for the Moon Orchid (*Phalaenopsis amabilis*) Type 229 (Pemanfaatan limbah buah pisang dan air kelapa sebagai bahan media kultur jaringan anggrek bulan (*Phalaenopsis amabilis*) Tipe 229). *J.Teknologi Lingkungan* 11(3) (2010) 373-380.
- [12] M.Humaira, Z. Thomy, E. Harnelly, The effect of giving coconut water and banana pulp to MS medium on the growth of rabbit orchid plantlets (*Dendrobium antennatum* lindl.) In vitro (Pengaruh pemberian air kelapa dan bubur pisang pada media ms terhadap pertumbuhan planlet anggrek kelinci (*Dendrobium antennatum* lindl.) secara in vitro). *Proc. of Biotic Nasional Seminar Nasional*, 2015.
- [13] J. Pratama, Modification of MS Media with Addition of Coconut Water for Subculture I Cymbidium Orchids (Modifikasi Media MS Dengan Penambahan Air Kelapa Untuk Subkultur I Anggrek Cymbidium). *Jurnal Agrium* 15(2) (2018) 91-109
- [14] N.P.Y.A. Dewi, The Effect of Giving Coconut Water on Embryo Development in Dendrobium anosmum Lindl (Pengaruh Pemberian Air Kelapa terhadap Perkembangan Embrio pada Dendrobium anosmum Lindl). *JBE* 4 (1) (2019) 22-28.
- [15] Nurfadilah, Mukarlina, E. Rusmiyanto P.W, The multiplication of black orchids (*Coelogyne pandurata* Lindl) on murashige skoog (MS) media with the addition of Ambon banana extract and benzyl amino purine (BAP) (Multiplikasi anggrek hitam (*Coelogyne pandurata* Lindl ) pada media murashige skoog (MS) dengan penambahan ekstrak pisang ambon dan benzyl amino purin (BAP)). *Protobiont* 7(3) (2018) 47 –53.
- [16] M. Vargas, B. Glaz, G. Alvarad, J. Pietragalla, A. Morgounov, Y. Zelenskiy, and J. Crossa, Analysis and Interpretation of Interactions in Agricultural Research. *Agronomy Journal*, 107(2)(2015)748.  
[doi:10.2134/agronj13.0405](https://doi.org/10.2134/agronj13.0405)
- [17] M.G. Pranata, A. Yunus , B. Pujiasmanto, The effect of NAA concentrations and coconut water on the multiplication of ginger (*Curcuma xanthorrhiza* roxb.) In vitro (Pengaruh konsentrasi naa dan air kelapa terhadap mult iplikasi temulawak (*curcuma xanthorrhiza* roxb.) secara in vitro). *J. of Sustainable Agric.* 30(2) (2015)
- [18] L. Erfa, Ferziana, Growth of Phalaenopsis orchid seedling into plantlets in subculture II media with addition of tripton and atonic (Pertumbuhan seedling anggrek Phalaenopsis menjadi plantlet pada media subkultur II dengan penambahan tripton dan atonik). *Jurnal Penelitian Pertanian Terapan*. Vol. 12. (Spec. Ed.) (2012) 52-58.
- [19] N.N. Kristina, Syahid, S. Fatimah, The Effect of Coconut Water on In Vitro Shoot Multipication, Rhizome Production, and Xanthorrhizol Content of Temulawak in the Field (Pengaruh Air Kelapa Terhadap Multipikasi Tunas In Vitro, Produksi Rimpang, dan Kandungan Xanthorrhizol Temulawak di Lapangan). *Jurnal Litri* 18 (3) (2012) 125-134.
- [20] J. Yong, L. Ge, Y.F. Ng, S. Tan, The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water, *Molecules* 14(12) (2009) 5144-5164. <https://doi.org/10.3390/molecules14125144>
- [21] Kasutjaningati, R. Irawan, Alternative media for in vitro orchid propagation (*Phalaenopsis amabilis*) (Media alternatif perbanyakan in vitro anggrek Bulan (*Phalaenopsis amabilis*)). *J. Agroteknos.* 3(3) (2013) 184-189.
- [22] D.P.S. Hendaryono, Orchid nursery in bottles (Pembibitan anggrek dalam botol). Kanisius: Yogyakarta. 2000

[23] S. Damiska, R.S. Wulandari, H. Darwati, Addition of Yeast and Corn Seed Extract to In-Vitro Growth of Mangosteen Shoots (Penambahan Ragi dan Ekstrak Biji Jagung terhadap Pertumbuhan Tunas Manggis Secara In-Vitro). *J Hutan Lestari* 3(1) (2015) 35-42..

[24] M.R. Suhartanto, Sobir, H. Harti, Healthy Technology for Banana Cultivation: From Seed to Post Harvest (Teknologi Sehat Budidaya Pisang: dari Benih sampai Pasca Panen). Pusat Kajian Hortikultura Tropika, LPPM-IPB. 2012.



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Sincerely yours,

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# Addition of Coconut Water and Banana Extract on MS Media to Stimulate PLB (Protocorm Like Bodies) Regeneration of *Dendrobium gattongi*

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# Addition of Coconut Water and Banana Extract on MS Media to Stimulate PLB (Protocorm Like Bodies) Regeneration of *Dendrobium gaton sunray*

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## ABSTRACT

Organic material is widely used for propagation of orchids, namely coconut water, extracts of tomatoes, bananas, bean sprouts and potatoes which can be added to certain media as a provider of nutrients, amino acid minerals, growth regulators. This study aims to determine the effect of the concentration of organic matter in coconut water and banana extract on MS media on the regeneration of PLB (protocorm like bodies) *Dendrobium gaton sunray*. The study was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Bengkulu University, from September to December 2019. This study used a Complete Randomized Block Design (RCBD) with two factors. The first factor was the concentration of coconut water with 3 levels namely: A0 = 0 (Control), A1 = 75 ml/l, A2 = 150 ml/l, and the second factor was the concentration of banana extract with 5 levels namely: B0 = 0 (Control), B1 = 75 g/l, B2 = 150 g/l. The results showed that coconut water did not affect all observed variables, however the banana extract treatment had a significant effect on all observed variables except for the number of shoots. There was an interaction between the two treatments, namely the variable plantlet weight. Orthogonal polynomial analysis showed that the interaction pattern of coconut water and banana extract treatment occurred in treatment 0-50, where the meeting point of the two treatments, namely the variable shoot length, leaf length, shoot number, and plantlet weight. Whereas in the variable number of leaves, the interaction occurs in the 100-150 treatment.

**Keywords:** coconut water, bananas extracts, *Dendrobium gaton sunray*, MS media

## 1. INTRODUCTION

*Dendrobium* is a type of orchid that is favored by orchid enthusiasts because it has a variety of forms, shapes, and even sizes, it is even easy to maintain and cultivate, and the price is relatively affordable. In addition, *Dendrobium* also has other features such as the freshness of long-lasting flowers, flexible flower stalks [1] so that it is widely used as an ornamental plant in the form of cut flowers that can decorate spaces with good quality [2]. According to Suryana [3] orchids had entered the international market for export to destination countries, including Japan, the Netherlands, Korea and Singapore. The Indonesian Statistics Agency [4] notes that the value of orchid production in Indonesia from 2016 to 2017 had increased between 19,978,078 stalks to 20,045,577 stalks with an average export value of 120,560 US dollars. Financially the orchid farming gives a profit, where in one growing season it provides a profitability (ROI) of 70% of the funds invested. Value added along the marketing chain show a reasonable profit for the business (farmers and

traders) as well, so that this commodity business has good prospects [5].

In general, orchids are only able to reproduce in nature at less than 1%, this is because orchid seeds are very small and do not have an endosperm so that orchids are symbiotic with mycorrhizae in nature. The role of mycorrhizae as a supplier of carbon sources for orchid seed germination in general has been replaced by tissue culture media. Optimization of tissue culture techniques continues to be carried out in an effort to accelerate the propagation of orchids, this technique only requires a small part of the plant to obtain the same plantlets as the parent [6].

The orchid seeds will grow into protocorms in the germination medium. Protocorm can form a secondary protocorm (protocorm like body/PLB). In vitro regeneration of PLB *Dendrobium* for shoot multiplication is generally carried out on media enriched with growth regulators and vitamins. One of the keys to success is the use of media types and growth regulators at the right concentration. The composition of the media used greatly determines the growth rate of protocorms and plantlets and is useful for increasing the quality and quantity of seedlings [7]. The composition of the media needed in

tissue culture must contain nutrients that are useful for plants, namely consisting of carbohydrates, sugars and vitamins. Tissue culture media usually uses artificial media compositions, namely Murashige and Skoog and Knudson C media, either 30% or full concentration. The composition of the media is a determining factor in the success of in vitro orchid culture. Growth and reproduction rates of many species of orchids enhanced by adding some organic supplements such as coconut water (CW) or potato extract to culture medium [8-9]. Growth regulators are usually added to the medium to promote growth of explants. The growth regulators commonly used are auxins and cytokinins. The composition of the media can also be obtained from organic materials which already have natural nutrients and hormones in the form of auxins and cytokinins known as phytohormones. Complex organic materials can be used as additives that can encourage the development and growth of propagation explants in tissue culture [10;9]. The addition of organic materials such as extracts bananas, potato pulp and vegetable substances others have content high carbohydrate can increase growth and differentiation of cells in plants [11].

Organic materials that are widely used for orchid propagation are coconut water, tomato extracts, bananas, bean sprouts and potatoes which can be added with certain media as a provider of nutrients, amino acids, and growth regulators for orchid growth. Coconut water has been widely used as an additive in tissue culture media. The content of coconut water is in the form of active ingredients that are useful for the growth of plantlets which are rich in vitamins, sugars, amino acids and phytohormones [12-14]. Nurfadilah et al. [15] research showed that the combination of Ambon banana and BAP on concentration  $10^{-6}$ M BAP and 2.5% banana extract Ambon produces the best growth on time of emergence of shoots, number of shoots and number of leaves. This research was conducted to modify the MS media with added coconut water and banana extract to stimulate the regeneration of PLB *Dendrobium gaton sunray*.

## 2. MATERIAL AND METHODS

The research was conducted at the Laboratory of Agricultural Cultivation, Division of Tissue Culture and Biotechnology, Faculty of Agriculture, Bengkulu University. This research was conducted from September to December 2019. The materials used were PLB which had formed 2-3 mm buds as plantlets, banana extract, coconut water, Murashige & Skoog (MS) media, sugar, 70% alcohol, alcohol 96%, and agar.

The design method used was a factorial completely randomized design (CRD) with 2 factors, namely: the first factor was coconut water with 3 levels, namely: A0 = 0 (Control), A1 = 75 ml.L<sup>-1</sup>, A2 = 150 ml.L<sup>-1</sup>. The second factor was banana extract with 3 levels, namely: B0 = 0 (Control), B1 = 75 g.L<sup>-1</sup>, B2 = 150 g.L<sup>-1</sup>. Each treatment combination was repeated 5 times. One experimental unit consisted of one culture bottle planted with 3 PLBs.

½ MS medium was added to treatment, added sugar as much as 30 g / l, gelatin as much as 7 grams / l, then the media pH was measured 5.8. The media was cooked until the gelatin was dissolved and well blended, then immediately poured into sterile bottle with a volume of 20 ml bottle-1, then the media was sterilized by autoclave at a temperature of 121 ° C, 15 psi pressure for 15 minutes. The explants used in the study were not uniformed into other media and were directly planted in the experimental media. The explants were removed from the bottle and selected for uniform growth. The selected explants consisted of 2 explants per bottle which were sterile, then the bottles were covered with plastic and tied with a rubber band and given a tight wrap. The culture bottles were maintained in the culture room at a temperature of 19-20 ° C with ± 1,000 lux irradiation for 12-16 hours / day.

Observations were made one week after planting (WAF) to 15 weeks (WAF). Every day the plants were checked for contaminants and browning, while the last week observations were made on the variables of the number of leaves, number of roots, length of leaves, length of shoots, number of shoots, and weight of plantlets.

Data were analyzed using analysis of variance at the 5% level, and further tests with LSD at the 5% level. The orthogonal polynomial test was used to determine the treatment and interaction patterns. Data were analyzed using SAS version 9.1.

## 3. RESULTS AND DISCUSSION

Variance analysis showed that coconut water had no effect on all observed variables, however banana extract had a significant effect on all observed variables except for the number of shoots (Table 1). There was an interaction between the two treatments, namely the plantlet weight. The orthogonal polynomial analysis showed that the pattern of coconut water on the leaf length was very significant in a quadratic curve, while the banana extract pattern was very significant quadratic on the variable shoot length, leaf length, root length, number of leaves, number of roots, and plantlet weight, except that the number of shoots tended to be linear. (Table 1). Even though there was only an interaction on plantlet weight, the interaction pattern between the addition of coconut water and banana extract tended to be quadratic in the variables of shoot length, number of leaves, number of shoots, number of leaves, and plantlet weight (Table 1).

### 3.1. The effect of coconut water to MS media on the regeneration of PLB (Protocorm Like Bodies) *Dendrobium sp*

The effect of coconut water on PLB (Protocorm Like Bodies) regeneration was not significantly different in all observed variables. However, the pattern of coconut water tends to be quadratic in leaf length (Figure 1) with the regression equation  $Y = -0.005X^2 + 0.008X + 3.667$  and the value of  $R^2 = 1$ . The addition of coconut water up to 150 ml to the media had not shown a significant effect,

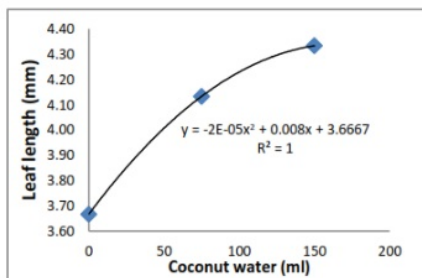
because the curve tends to increase and has not shown an optimal point, however, it was estimated that the optimal

point was achieved in the addition of 150-200 ml/l coconut water.

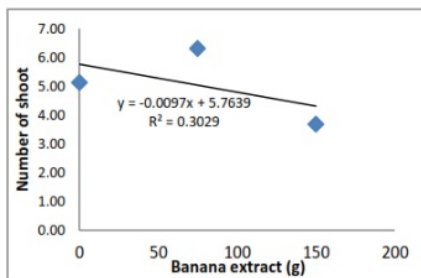
**Table 1** Analysis of variance and orthogonal polynomials on the addition of coconut water and banana extract to MS media to stimulate the regeneration of PLB (Protocorm Like Bodies) Dendrobium gattongi

| Source of variance                     | F value      |             |             |                 |                |                |                 |
|----------------------------------------|--------------|-------------|-------------|-----------------|----------------|----------------|-----------------|
|                                        | Shoot length | Leaf length | Root length | Number of shoot | Number of leaf | Number of root | Weight plantlet |
| Coconut water                          | 0.07         | 0.48        | 0.22        | 0.11            | 0.27           | 0.17           | 0.14            |
| Banana extract                         | 1.56*        | 1.41*       | 0.94*       | 0.39            | 0.94*          | 1.71*          | 1.48*           |
| C. water x B. extract                  | 0.59         | 0.63        | 0.19        | 0.48            | 0.46           | 0.08           | 1.10*           |
| C. water linier                        | 0.07         | 0.46        | 0.09        | 0.10            | 0.10           | 0.16           | 0.29            |
| C. water kuadratic                     | 0.06         | 0.50*       | 0.34        | 0.12            | 0.44           | 0.19           | 0.01            |
| B. extract linier                      | 1.49*        | 0.15        | 0.12        | 0.76*           | 1.06*          | 0.09           | 0.26            |
| B. extract kuadratic                   | 1.63*        | 2.67*       | 1.77*       | 0.01            | 0.82*          | 3.33*          | 2.69*           |
| C. water linier x B. extract linier    | 0.93*        | 0.59*       | 0.07        | 0.13            | 0.03           | 0.14           | 1.40*           |
| C. water linier x B. extract kuadratic | 0.03         | 0.56*       | 0.53*       | 0.14            | 0.01           | 0.04           | 1.43*           |
| C. water kuadratic x B. extract linier | 1.33*        | 0.98*       | 0.02        | 1.06*           | 1.39*          | 0.06           | 1.43*           |

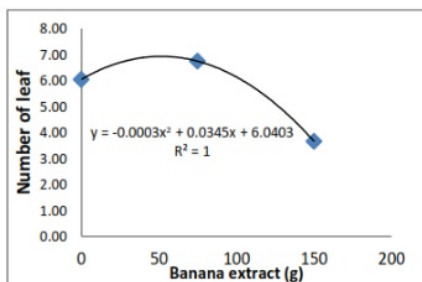
Note: \*significant different at F test 5%



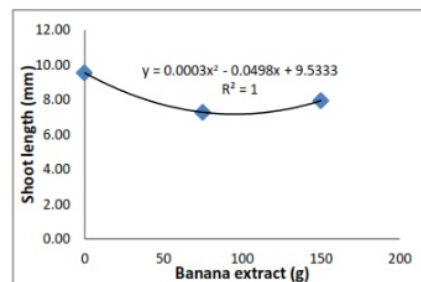
**Figure 1** Respon of coconut water on the leaf length



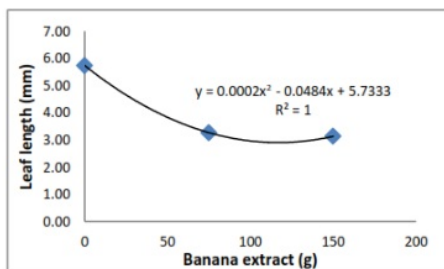
**Figure 2** Respon of Banana extract on the number of shoot



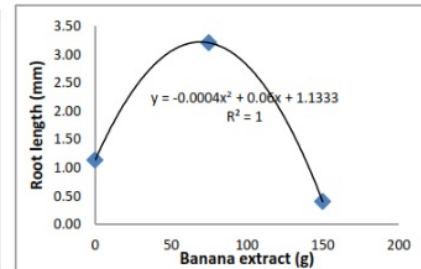
**Figure 3** Respon of Banana extract on the number of leaf



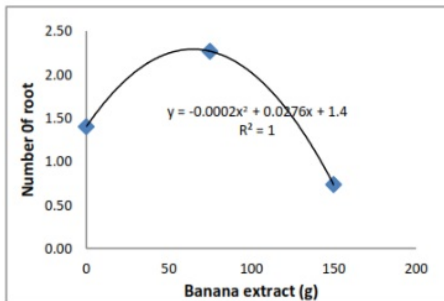
**Figure 4** Respon of Banana extract on the shoot length



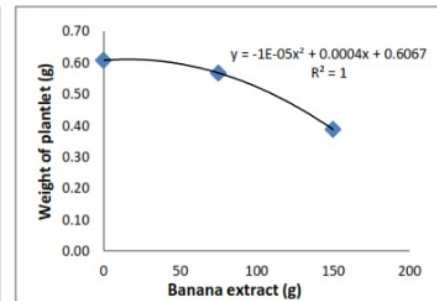
**Figure 5** Respon of Banana extract on the leaf length



**Figure 6** Respon of Banana extract on the root length



**Figure 7** Respon of Banana extract on the number of root



**Figure 8** Respon of Banana extract on the weight of plantlet

Similar research by Pranata et al. [18] showed that the addition of 22.5% coconut water gave the best shoot length, then added with the addition of 15% coconut water. In addition, [32] refers to Erfa et al. [19] in their research reported that the addition of coconut water up to 150 ml in the Phalaenopsis orchid growing medium resulted in better seedling height growth. Kristina et al. [16] stated that coconut water contains IAA (auxin) and cytokinins which were very good for stimulating plant growth and shoot growth. Coconut water has been used in tissue culture methods because of its growth regulating properties and cytokinin activity that supports cell division and promoting rapid growth [17]. The higher coconut water concentrations promoted better growth and shoot propagation in some orchid species, and the coconut water effect depending on the species and the explants [8-9].

### 3.2. The effect of banana extract on MS media on the regeneration of PLB Dendrobium gaton sunray

The effect of banana extract on PLB regeneration is presented in Table 2. The results showed that although it was significantly different in the F test, the average between treatments did not show a significant difference. The addition of banana extract 75 g / l had the highest root length with an average of 3.2 mm, the number of leaves was 6.74, and the number of roots was 2.27 (Table 2). Giving banana extract to the media because it was a source of carbohydrates and contains several minerals and various vitamins such as vitamin C and especially thiamine. Refer to Kasutjaniangati and Irawan's [20] research showed that Ambon banana extract has a good effect on the root length of Dendrobium.

The orthogonal polynomial analysis showed that the banana extract response was very significant with a quadratic pattern on the variable shoot length, leaf length, root length, number of leaves, number of roots, and plantlet weight (Table 1), except that the number of shoots tended to be linear (Figure 2). This indicated that the

optimum point has not been reached for the variable number of shoots.

The addition of banana extract forms a quadratic pattern on the number of leaves with the equation  $Y = -0.0003X^2 + 0.035 + 6.04$  ( $R^2 = 1$ ), and reaches an optimum point in the addition of 50-100 mg of banana extract (Figure 3). The same pattern was also found in root length (Figure 6), number of roots (Figure 7), and plantlet weight (Figure 8). Different patterns were found in shoot length (Figure 4) and leaf length (Figure 5), where it reached the lowest point at the addition of 50-100 mg of banana extract. Refer to Hendaryono [21] in his study on banana extract 300 g/l resulted in a lower number of leaves.

The highest average shoot length, leaf length, and plantlet weight without banana extract treatment (B0) were 9.53, 4.73, and 0.61 respectively, while in treatment (B1) the root length, number of leaves and number of roots, respectively amounting to 3.20, 6.74, and 2.27 (Table 2). Banana extract apart functions as a coenzyme for several reactions in metabolism and also plays a role in the metabolism of energy derived from carbohydrates. Giving Ambon banana fruit extract in the sub-culture plantlet can stimulate the growth of Dendrobium. Banana fruit also contains natural hormones auxins and gibberellins which can stimulate growth plantlets [20;22].

### 3.3. The interaction effect of coconut water and banana extract on MS media on the regeneration of PLB Dendrobium gaton sunray

The interaction effect of coconut water and banana extract on plantlet weight variables is presented in Table 3. The best treatment for plantlet weight variables was A1B1 (75 ml coconut water + 75 mg banana extract) which was 0.90 g and the lowest treatment was A2B2 which was 0.16 g. Based on orthogonal polynomial analysis, it showed that although there was only an interaction on plantlet weight, the interaction pattern between coconut water and banana extract tends to be quadratic in the variables shoot length, number of leaves, number of shoots, number of leaves, and plantlet weight (Table 1).

**Table 2** Effect of banana extract on the Shoot length, Leaf length, Root length, Number of leaf, Number of root, and Weight plantlet

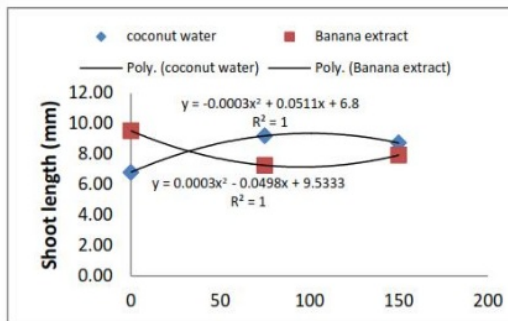
| Treatment | Means of treatment |             |             |                |                |                 |
|-----------|--------------------|-------------|-------------|----------------|----------------|-----------------|
|           | Shoot length       | Leaf length | Root length | Number of leaf | Number of root | Weight plantlet |
| B0        | 9.53a              | 5.73a       | 1.13a       | 6.04a          | 1.40a          | 0.61a           |
| B1        | 7.27a              | 3.27a       | 3.20b       | 6.74a          | 2.27a          | 0.57a           |
| B2        | 7.93a              | 3.13a       | 0.40a       | 3.66b          | 0.73a          | 0.39a           |

Note: LSD 5%

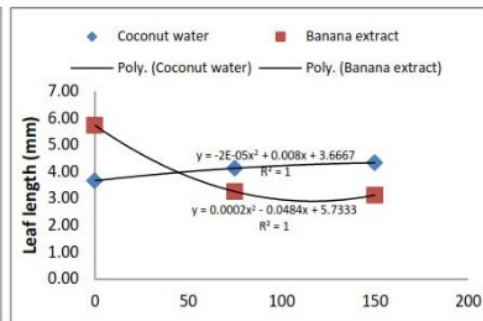
**Table 3** Effect of coconut water and banana extract on the weight plantlet

| Treatment | Means of weight plantlet (g) |
|-----------|------------------------------|
| A0B0      | 0.62a                        |
| A0B1      | 0.28c                        |
| A0B2      | 0.54a                        |
| A1B0      | 0.68a                        |
| A1B1      | 0.90b                        |
| A1B2      | 0.46a                        |
| A2B0      | 0.52a                        |
| A2B1      | 0.52a                        |
| A2B2      | 0.16c                        |

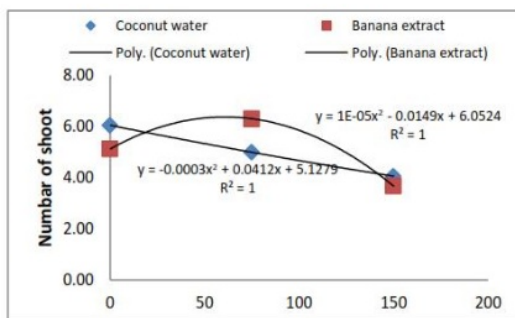
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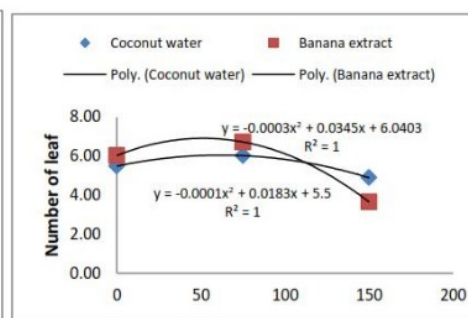
**Figure 9** Respon of coconut water and banana extract on the shoot length



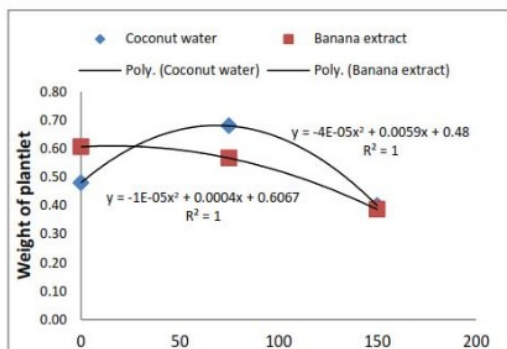
**Figure 10** Respon of coconut water and banana extract on the leaf length



**Figure 11** Respon of coconut water and banana extract on the number of shoot



**Figure 12** Respon of coconut water and banana extract on the number of leaf



**Figure 13** Respon of coconut water and banana extract on the weight of plantlet

The orthogonal polynomial analysis showed the interaction pattern of coconut water and banana extract treatments occurred in treatment 0-50, where the meeting point of the two treatments, namely the shoot length variable (Figure 9), leaf length (Figure 10), number of

shoots (Figure 11), and plantlet weights (Figure 13). Meanwhile, the variable number of leaves requires quite a lot of coconut water and banana extract, namely 100-150 to get the interaction between the two treatments (Figure 12). This showed that there was a different interaction

response to the observed variables. MS is one of the media that is often used in the process of in vitro propagation or tissue culture. This is because MS media contains vitamin B1 which is very useful for plant growth. MS media has this feature because MS media contains high levels of nitrate, potassium, ammonium and the amount of inorganic nutrients for the cell multiplication, so it is thought to strongly influence the number of shoots of *Dendrobium* sp. Razdan [23] stated that MS medium was commonly used for shoot induction and contains appropriate nutrients to support optimal growth of plants in vitro. According to Suhartanto, et al. [24] that bananas are often used as a source of organic matter in tissue culture media, because 100 grams of banana flesh contain protein (1%), fat (0.3%), carbohydrates (27%), energy (116 -128 kcal), minerals (Ca 15 mg, K 380 mg, Fe 0.5 mg, Na 1.2 mg, and Vitamins (Vit. A 0.3 mg, Vit. B1 0.1 mg, B2 0.1 mg, B6 0.7 mg, Vit. C. Hadi's research (2006) states that the

#### 4. CONCLUSION

Coconut water did not affect all observed variables, however the banana extract treatment had a significant effect on all observed variables except for the number of shoots. There was an interaction between the two treatments, namely the variable plantlet weight. Orthogonal polynomial analysis showed that the interaction pattern of coconut water and banana extract treatment occurred in treatment 0-50, where the meeting point of the two treatments, namely the variable shoot length, leaf length, shoot number, and plantlet weight. Whereas in the variable number of leaves, the interaction occurs in the 100-150 treatment.

#### REFERENCES

- [1] D. Puchooa, Comparison of different culture media for the in vitro culture of *Dendrobium* (Orchidaceae). *Int. J. Agric. Biol.* 6 (2004) 884-888. DOI: 1560-8530/2004/06-5-884-888
- [2] Talukder et al., In vitro root formation on orchid plantlets with IBA and NAA. *Progress. Agric.* 13(1&2)(2002) 25-28.
- [3] A. Suryana, Prospects and Directions for Orchid Agribusiness (Prospek dan Arah Pengembangan Agribisnis Anggrek). Development. Agricultural Research and Development Agency. Jakarta, 2005.
- [4] The Indonesian Statistics Agency, Statistik-tanaman-hias-indonesia-2017. <https://www.bps.go.id/site/publication/2018/10/05/statistik-tanaman-hias-indonesia-2017> Jun 2019 17:45:28 GMT.
- addition of banana pulp as much as 100 g / l in the medium combined with Vitabloom 2 g/l can increase the shoots and number of *Dendrobium* kanayou leaves. Addition of BAP 2 mg / l, coconut water 150 ml/l and 50 g/l Ambon banana extract had the same effect on increase in the number of shoots, an average of 2 shoots of *Phalaenopsis amabilis* [20]. highest number of shoots was produced in media supplemented with 50 ml /l coconut water and 50 g/l potato extract, while media supplemented with 150 ml coconut water and 50 potato extracts increased the number of leaves and the number of roots [9].
- [5] K.B. Andri, W.J.F.A. Tumbuan, Potential development of orchid flower agribusiness in Batu City, East Java (Potensi pengembangan agribisnis bunga anggrek di kota batu Jawa Timur). *Jurnal LPPM Bidang EkoSosBudKum.* 2(1)(2015) 19-30.
- [6] Indonesian Environmental and Forestry Research and Development Center, Plant Propagation Through Tissue Culture (Perbanyakan Tanaman Melalui Kultur Jaringan). Makassar. Bogor. Bogor. 304 p. 2013.
- [7] D. Widiastoety, A. Santi, N. Solvia, Effect of myoinositol and activated charcoal on growth of dendrobium orchid plantlets in in vitro culture (Pengaruh Myoinositol dan Arang Aktif terhadap Pertumbuhan Planlet Anggrek *Dendrobium* dalam Kultur In vitro). *J. Hort.* 22(3) (2012) 205.
- [8] W. Pakum, S. Watthana, K. Srimuang, A. Kongbangkerd, Influence of Medium Component on In vitro Propagation of Thais Endangered Orchid: *Bulbophyllum nipondhii* Seidenf, *Plant Tissue Cult. & Biotech.*, 26(1) (2016) 37- 46. <https://doi.org/10.3329/ptcb.v26i1.29765>
- [9] T. Punjansing, M. Nakkuntod, S.Homchan, P. Inthima, A. Kongbangkerd, Influence of Organic Supplements on Shoot Multiplication Efficiency of *Phaius tankervilleae* var. Alba. *Int. J. of Agric. and Biosyst. Eng.* 13(4) (2019) 105-109.
- [10] S. Rahmah, T. Rahayu, A. Hayati, Study Adding Organic Substances on VW Media for Organogenesis the *Dendrobium* Orchid in Vitro. *e-Jurnal Ilmiah SAINS ALAMI (Known Nature)* 1(1) (2018) 3 - 103
- [11] Djajanegara, Utilization of banana and coconut water waste as a medium for tissue culture for the Moon Orchid (*Phalaenopsis amabilis*) Type 229

11

(Pemanfaatan limbah buah pisang dan air kelapa sebagai bahan media kultur jaringan angrek bulan (*Phalaenopsis amabilis*) Tipe 229). *J. Teknologi Lingkungan* 11(3) (2010) 373-380.

[12] M. Humaira, Z. Thomy, E. Harnelly, The effect of coconut water and banana pulp to MS medium on the growth of rabbit orchid plantlets (*Dendrobium antennatum* Lindl.) *In vitro* (Pengaruh pemberian air kelapa dan bubur pisang pada media ms terhadap pertumbuhan planlet angrek kelinci (*dendrobium antennatum* Lindl.) secara *in vitro*). *Proc. of Biotic Nasional Seminar Nasional*, 2015.

[13] J. Pratama, Modification of MS Media with Addition of Coconut Water for Subculture I *Cymbidium Orchids* (Modifikasi Media MS Dengan Penambahan Air Kelapa Untuk Subkultur I Angrek *Cymbidium*). *Jurnal Agrium* 15(2) (2018) 91-109

[14] N.P.Y.A. Dewi, The Effect of Giving Coconut Water on Embryo development in *Dendrobium anosmum* Lindl (Pengaruh Pemberian Air Kelapa terhadap Perkembangan Embrio pada *Dendrobium anosmum* Lindl). *JBE* 4 (1) (2019) 22-28.

[15] Nurfadilah, Sukarlina, E. Rusmiyanto P.W, The multiplication of black orchids (*Coelogyne pandurata* Lindl) on murashige skoog (MS) media with the addition of Annona banana extract and benzyl amino purine (BAP) (Multiplikasi angrek hitam (*Coelogyne pandurata* Lindl) pada media murashige skoog (MS) dengan penambahan ekstrak pisang ambon dan benzyl amino purin (BAP)). *Protobiont* 7(3) (2018) 47 –53.

[16] N.N. Kristina, Syahid, S. Fatimah, The Effect of Coconut Water on In Vitro Shoot Multiplication, Rhizome Production, and Xanthorrhizol Content of Temulawak in the Field (Pengaruh Air Kelapa Terhadap Multiplikasi Tunas In Vitro, Produksi Rimpang, dan Kandungan Xanthorrhizol Temulawak di Lapangan). *Jurnal Litri* 18 (3) (2012) 125-134.

[35] J. Yong, L. Ge, Y.F. Ng, S. Tan, The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water, *Molecules* 14(12) (2009) 5144-5164. <https://doi.org/10.3390/molecules14125144>

[18] M.G. Pranata, A. Yunus, B. Pujiasmanto, The effect of NAA concentrations and coconut water on the multiplication of ginger (*Curcuma xanthorrhiza* Roxb.) *In vitro* (Pengaruh konsentrasi naa dan air kelapa terhadap multiplikasi temulawak (*Curcuma xanthorrhiza* Roxb.) secara *in vitro*). *J. of Sustainable Agric.* 30(2) (2015)

[19] L. Erfa, Ferziana, Growth of *Phalaenopsis* orchid seedling into plantlets in subculture II media with addition of tripton and atonic (Pertumbuhan seedling angrek *Phalaenopsis* menjadi plantlet pada media subkultur II dengan penambahan tripton dan atonik). *Jurnal Penelitian Pertanian Terapan*. Vol. 12. (Spec. Ed.) (2012) 52-58.

[20] Kasutjiani, R. Irawan, Alternative media for *In vitro* orchid propagation (*Phalaenopsis amabilis*) (Media alternatif perbanyakan *in vitro* angrek Bulan (*Phalaenopsis amabilis*)). *J. Agroteknos.* 3(3) (2013) 184-189.

[21] D.P.S. Hendaryono, Orchid nursery in bottles (Pembibitan angrek dalam botol). Kanisius: Yogyakarta. 2000

[22] S. Damiska, R.S. Wulandari, H. Darwati, Addition of Yeast and Corn Seed Extract to In-Vitro Growth of Mangosteen Shoots (Penambahan Ragi dan Ekstrak Biji Jagung terhadap Pertumbuhan Tunas Manggis Secara In-Vitro). *J Hutan Lestari* 3(1) (2015) 35-42.

[23] M.K. Razdan, Introduction to Plant Tissue. 2nd Edition. Qxford & IBH Publishing Co. Pvt. Ltd. New Delhi. 2003.

[24] M.R. Suhartanto, Sobir, H. Harti, Healthy Technology for Banana Cultivation: From Seed to Post Harvest (Teknologi Sehat Budidaya Pisang: dari Benih sampai Pasca Panen). Pusat Kajian Hortikultura Tropika, LPPM-IPB. 2012.

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# Addition of Coconut Water and Banana Extract on MS Media to Stimulate PLB (Protocorm Like Bodies) Regeneration of *Dendrobium gatton sunray*

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## ABSTRACT

Organic material is widely used for propagation of orchids, namely coconut water, extracts of tomatoes, bananas, bean sprouts and potatoes which can be added to certain media as a provider of nutrients, amino acid minerals, and growth regulators. This study aims to determine the effect of the concentration of organic matter in coconut water and banana extract on MS media on the regeneration of PLB (protocorm like bodies) *Dendrobium gatton sunray*. The study was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Bengkulu University, from September to December 2019. This study used a Complete Randomized Block Design (RCBD) with two factors. The first factor was the concentration of coconut water with 3 levels namely: A0 = 0 (Control), A1 = 75 ml.L<sup>-1</sup>, A2 = 150 ml.L<sup>-1</sup>, and the second factor was the concentration of banana extract with 5 levels namely: B0 = 0 (Control), B1 = 75 g.L<sup>-1</sup>, B2 = 150 g.L<sup>-1</sup>. The results showed that coconut water did not affect all variables, however the banana extract had a significant effect on all variables except for the number of shoots. There was an interaction between the two treatments, namely the variable plantlet weight. Orthogonal polynomial analysis showed that there was an interaction between the two treatments, where coconut water and banana extract had a good response on the plantlet growth when given together as much as 75 ml. L<sup>-1</sup> (A1) and 75 g.L<sup>-1</sup> (B1). The concentration of both treatment was lower than it was given individually.

**Keywords:** coconut water, bananas extracts, *Dendrobium gatton sunray*, MS media

## 1. INTRODUCTION

*Dendrobium* is a type of orchid that is favoured by orchid enthusiasts because it has a variety of forms, shapes, and even sizes, it is even easy to maintain and cultivate, and the price is relatively affordable. In addition, *Dendrobium* also has other features such as the freshness of long-lasting flowers, flexible flower stalks [1] so that it is widely used as an ornamental plant in the form of cut flowers that can decorate spaces with good quality [2]. According to Suryana [3] orchids had entered the international market for export to destination countries, including Japan, the Netherlands, Korea and Singapore. The Indonesian Statistics Agency [4] notes that the value of orchid production in Indonesia from 2016 to 2017 had increased between 19,978,078 stalks to 20,045,577 stalks with an average export value of 120,560 US dollars. Financially the orchid farming gives a profit, where in one growing season it provides a profitability (ROI) of 70% of the funds invested. Value added along the marketing chain show a reasonable profit for the business (farmers and traders) as well, so that this commodity business has good prospects [5].

In general, orchids are only able to reproduce in nature at less than 1%, this is because orchid seeds are very small and do not have an endosperm so that orchids are symbiotic with mycorrhizae in nature. The role of mycorrhizae as a supplier of carbon sources for orchid seed germination in general has been replaced by tissue culture media. Optimization of tissue culture techniques continues to be carried out in an effort to accelerate the propagation of orchids, this technique only requires a small part of the plant to obtain the same plantlets as the parent [6].

The orchid seeds will grow into protocorms in the germination medium. Protocorm can form a secondary protocorm (protocorm like body/PLB). In vitro regeneration of PLB *Dendrobium* for shoot multiplication is generally carried out on media enriched with growth regulators and vitamins. One of the keys to success is the use of media types and growth regulators at the right concentration. The composition of the media used greatly determines the growth rate of protocorms and plantlets and is useful for increasing the quality and quantity of seedlings [7]. The composition of the media needed in tissue culture must contain nutrients that are useful for

plants, namely consisting of carbohydrates, sugars and vitamins. Tissue culture media usually uses artificial media compositions, namely Murashige and Skoog and Knudson C media, either half or full concentration. The composition of the media is a determining factor in the success of in vitro orchid culture. Growth and reproduction rates of many species of orchids enhanced by adding some organic supplements such as coconut water (CW) or potato extract to culture medium [8-9]. Growth regulators are usually added to the medium to promote growth of explants. The growth regulators commonly used are auxins and cytokinins. The composition of the media can also be obtained from organic materials which already have natural nutrients and hormones in the form of auxins and cytokinins known as phytohormones. Complex organic materials can be used as additives that can encourage the development and growth of propagation explants in tissue culture [10;9]. The addition of organic materials such as extracts bananas, potato pulp and vegetable substances others have content high carbohydrate can increase growth and differentiation of cells in plants [11].

Organic materials that are widely used for orchid propagation are coconut water, tomato extracts, bananas, bean sprouts and potatoes which can be added with certain media as a provider of nutrients, amino acids, and growth regulators for orchid growth. Coconut water has been widely used as an additive in tissue culture media. The content of coconut water is in the form of active ingredients that are useful for the growth of plantlets which are rich in vitamins, sugars, amino acids and phytohormones [12-14]. Nurfadilah et al. [15] research showed that the combination of Ambon banana and BAP on concentration  $10^{-6}$ M BAP and 2.5% banana extract Ambon produces the best growth on time of emergence of shoots, number of shoots and number of leaves. This research was conducted to modify the MS media with added coconut water and banana extract to stimulate the regeneration of PLB *Dendrobium gattongi* sunray.

## 2. MATERIAL AND METHODS

The research was conducted at the Laboratory of Agricultural Cultivation, Division of Tissue Culture and Biotechnology, Faculty of Agriculture, Bengkulu University. This research was conducted from September to December 2019. The materials used were PLB which had formed 2-3 mm buds as plantlets, banana extract, coconut water, Murashige & Skoog (MS) media, sugar, 70% alcohol, alcohol. 96%, and aquadest.

The design method used was a factorial completely randomized design (CRD) with 2 factors, namely: the first factor was coconut water with 3 levels, namely: A0 = 0 (Control), A1 = 75 mL.L<sup>-1</sup>, A2 = 150 mL.L<sup>-1</sup>. The second factor was banana extract with 3 levels, namely: B0 = 0 (Control), B1 = 75 g.L<sup>-1</sup>, B2 = 150 g.L<sup>-1</sup>. Each treatment combination was repeated 5 times. One experimental unit consisted of one culture bottle planted with 3 PLBs.

$\frac{1}{2}$  MS medium was added to treatment, added sugar as much as 30 g.L<sup>-1</sup>, gelatin as much as 7 g.L<sup>-1</sup>, then the media pH was measured 5.8. The media was cooked until the gelatin was dissolved and well blended, then immediately poured into a sterile bottle with a volume of 20 ml bottle-1, then the media was sterilized by autoclave at a temperature of 121 ° C, 15 psi pressure for 15 minutes. The explants used in the study were not uniformed into other media and were directly planted in the experimental media. The explants were removed from the bottle and selected for uniform growth. The selected explants consisted of 2 explants per bottle which were sterile, then the bottles were covered with plastic and tied with a rubber band and given a tight wrap. The culture bottles were maintained in the culture room at a temperature of 19-20 ° C with  $\pm$  1,000 lux irradiation for 12-16 hours / day.

Observations were made one week after planting (WAF) to 15 weeks (WAF). Every day the plants were checked for contaminants and browning, while the last week observations were made on the variables of the number of leaves, number of roots, length of leaves, length of shoots, number of shoots, and weight of plantlets.

Data were analyzed using analysis of variance at the 5% level, and further tests with LSD at the 5% level. The orthogonal polynomial test was used to determine the treatment and interaction patterns [16].

## 3. RESULTS AND DISCUSSION

Variance analysis showed that coconut water had no effect on all observed variables, however banana extract had a significant effect on all observed variables except for the number of shoots (Table 1). There was an interaction between the two treatments, namely the plantlet weight. The orthogonal polynomial analysis showed that the pattern of coconut water on the leaf length was very significant in a quadratic curve, while the banana extract pattern was very significant quadratic on the variable shoot length, leaf length, root length, number of leaves, number of roots, and plantlet weight, except that the number of shoots tended to be linear. (Table 1). Even though there was only an interaction on plantlet weight, the interaction pattern between the addition of coconut water and banana extract tended to be quadratic in the variables of shoot length, number of leaves, number of shoots, number of leaves, and plantlet weight (Table 1).

### 3.1. The effect of coconut water to MS media on the regeneration of PLB (Protocorm Like Bodies) *Dendrobium sp*

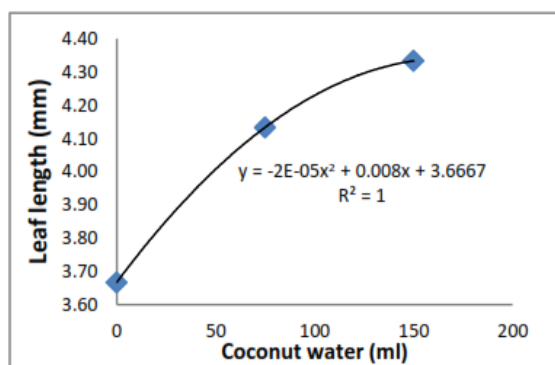
The effect of coconut water on PLB (Protocorm Like Bodies) regeneration was not significantly different in all observed variables. However, the pattern of coconut water tends to be quadratic in leaf length (Figure 1) with the regression equation  $Y = -0.005X^2 + 0.008X + 3.667$  and the value of  $R^2 = 1$ . The addition of coconut water up to 150 ml to the media had not shown a significant effect, because the curve tends to increase and has not shown an optimal point, however, it was estimated that the optimal

point was achieved in the addition of 150-200 ml/l coconut water.

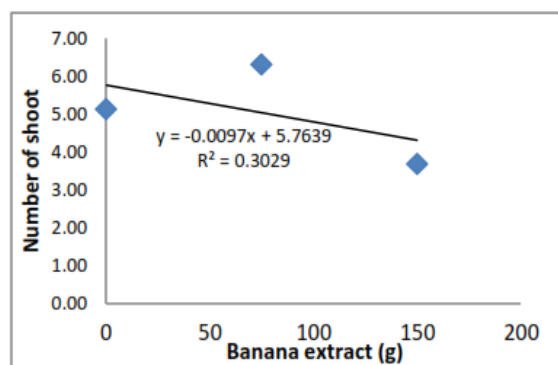
**Table 1** Analysis of variance and orthogonal polynomials on the addition of coconut water and banana extract to MS media to stimulate the regeneration of PLB (Protocorm Like Bodies) *Dendrobium gattongi* sunray

| Source of variance                     | F value      |             |             |                 |                |                |                 |
|----------------------------------------|--------------|-------------|-------------|-----------------|----------------|----------------|-----------------|
|                                        | Shoot length | Leaf length | Root length | Number of shoot | Number of leaf | Number of root | Weight plantlet |
| Coconut water                          | 0.07         | 0.48        | 0.22        | 0.11            | 0.27           | 0.17           | 0.14            |
| Banana extract                         | 1.56*        | 1.41*       | 0.94*       | 0.39            | 0.94*          | 1.71*          | 1.48*           |
| C. water x B. extract                  | 0.59         | 0.63        | 0.19        | 0.48            | 0.46           | 0.08           | 1.10*           |
| C. water linier                        | 0.07         | 0.46        | 0.09        | 0.10            | 0.10           | 0.16           | 0.29            |
| C. water kuadratic                     | 0.06         | 0.50*       | 0.34        | 0.12            | 0.44           | 0.19           | 0.01            |
| B. extract linier                      | 1.49*        | 0.15        | 0.12        | 0.76*           | 1.06*          | 0.09           | 0.26            |
| B. extract kuadratic                   | 1.63*        | 2.67*       | 1.77*       | 0.01            | 0.82*          | 3.33*          | 2.69*           |
| C. water linier x B. extract linier    | 0.93*        | 0.59*       | 0.07        | 0.13            | 0.03           | 0.14           | 1.40*           |
| C. water linier x B. extract kuadratic | 0.03         | 0.56*       | 0.53*       | 0.14            | 0.01           | 0.04           | 1.43*           |
| C. water kuadratic x B. extract linier | 1.33*        | 0.98*       | 0.02        | 1.06*           | 1.39*          | 0.06           | 1.43*           |

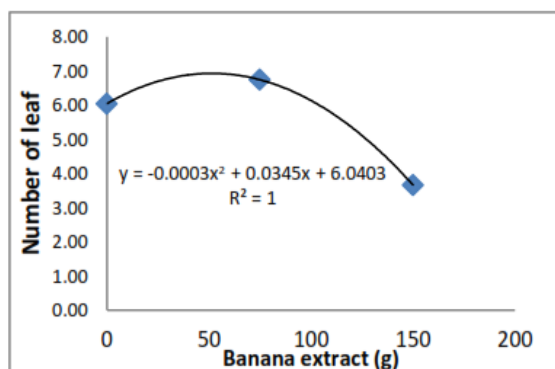
Note: \*significant different at F test 5%



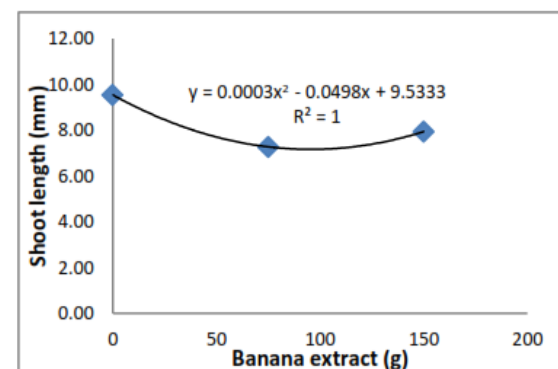
**Figure 1** Respon of coconut water on the leaf length



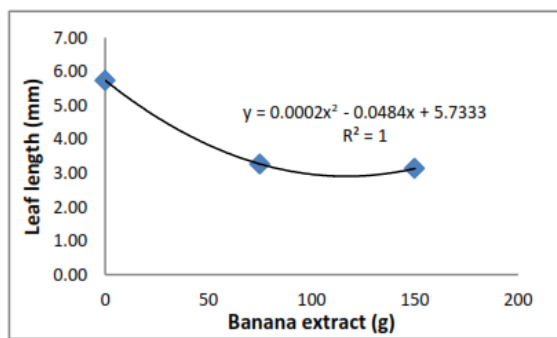
**Figure 2** Respon of Banana extract on the number of shoot



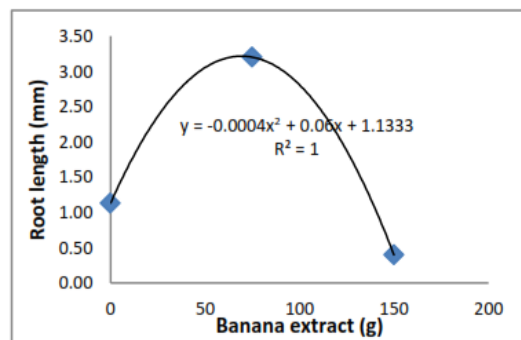
**Figure 3** Respon of Banana extract on the number of leaf



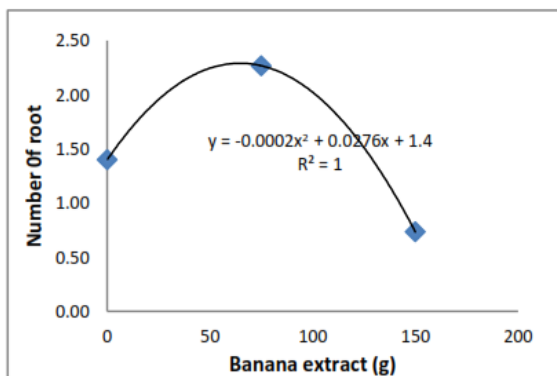
**Figure 4** Respon of Banana extract on the shoot length



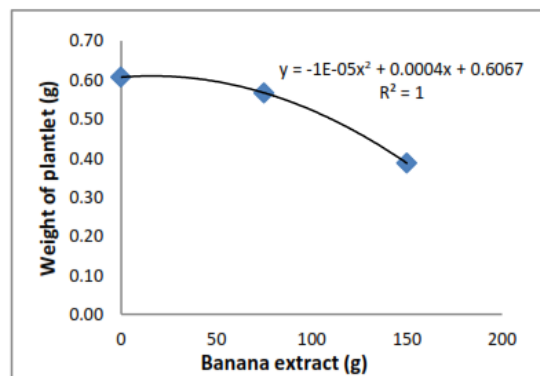
**Figure 5** Respon of Banana extract on the leaf length



**Figure 6** Respon of Banana extract on the root length



**Figure 7** Respon of Banana extract on the number of root



**Figure 8** Respon of Banana extract on the weight of plantlet

Similar research by Pranata et al. [17] showed that the addition of 22.5% coconut water gave the best shoot length, then added with the addition of 15% coconut water. In addition, refers to Erfa et al. [18] in their research reported that the addition of coconut water up to 150 ml in the Phalaenopsis orchid growing medium resulted in better seedling height growth. Kristina et al. [19] stated that coconut water contains IAA (auxin) and cytokinins which were very good for stimulating plant growth and shoot growth. Coconut water has been used in tissue culture methods because of its growth regulating properties and cytokinin activity that supports cell division and promoting rapid growth [20]. The higher coconut water concentrations promoted better growth and shoot propagation in some orchid species, and the coconut water effect depending on the species and the explants [8-9].

### 3.2. The effect of banana extract on MS media on the regeneration of PLB *Dendrobium gattton sunray*

The effect of banana extract on PLB regeneration is presented in Table 2. The results showed that although it was significantly different in the F test, the average between treatments did not show a significant difference. The addition of banana extract 75 g L<sup>-1</sup> had the highest root length with an average of 3.2 mm, the number of leaves was 6.74, and the number of roots was 2.27 (Table

2). Giving banana extract to the media because it was a source of carbohydrates and contains several minerals and various vitamins such as vitamin C and especially thiamine. Refer to Kasutjianingati and Irawan's [21] research showed that Ambon banana extract has a good effect on the root length of *Dendrobium*.

The orthogonal polynomial analysis showed that the banana extract response was very significant with a quadratic pattern on the variable shoot length, leaf length, root length, number of leaves, number of roots, and plantlet weight (Table 1), except that the number of shoots tended to be linear (Figure 2). This indicated that the optimum point has not been reached for the variable number of shoots.

The addition of banana extract forms a quadratic pattern on the number of leaves with the equation  $Y = -0.0003X^2 + 0.035 + 6.04$  ( $R^2 = 1$ ), and reaches an optimum point in the addition of 50-100 mg of banana extract (Figure 3). The same pattern was also found in root length (Figure 6), number of roots (Figure 7), and plantlet weight (Figure 8). Different patterns were found in shoot length (Figure 4) and leaf length (Figure 5), where it reached the lowest point at the addition of 50-100 g of banana extract. Refer to Hendaryono [22] in his study on banana extract 300 g/l resulted in a lower number of leaves.

The highest average shoot length, leaf length, and plantlet weight without banana extract treatment (B0) were

9.53, 5.73, and 0.61 respectively, while in treatment (B1) the root length, number of leaves and number of roots, respectively amounting to 3.20, 6.74, and 2.27 (Table 2). Banana extract apart functions as a coenzyme for several reactions in metabolism and also plays a role in the metabolism of energy derived from carbohydrates. Giving Ambon banana fruit extract in the sub-culture plantlet can stimulated the growth of *Dendrobium*. Banana fruit also contains natural hormones auxins and gibberellins which can stimulate growth plantlets [21;23]

### 3.3. The interaction effect of coconut water and banana extract on MS media on the regeneration of PLB *Dendrobium gattson sunray*

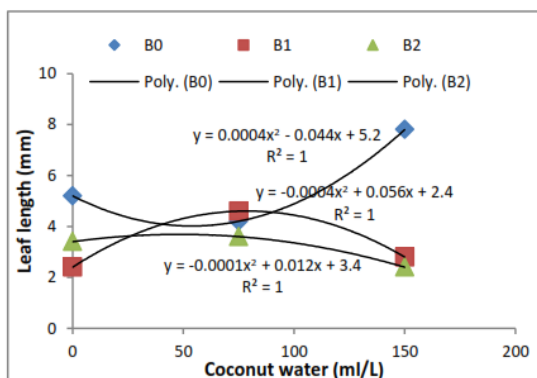
Based on orthogonal polynomial analysis, it showed that although there was only an interaction on plantlet weight, the interaction pattern between coconut

water and banana extract tends to be quadratic in the variables shoot length, number of leaves, number of shoots, number of leaves, and plantlet weight (Table 1). The interaction effect between coconut water at various levels of banana extract was found in the variables of leaf length, root length, and plantlet weight (Figure 9-11). The effect of the interaction between coconut water at various levels of banana extract was found in the variables of leaf length, root length, and plantlet weight. Meanwhile, the effect of the interaction between banana extracts at various levels of coconut water was found in the variable shoot length, number of shoots, leaf length, number of leaves, and plantlet weight (Figure 12-16). Coconut water treatment gives a good response when given together with banana extract as much as 75 mg (B1) on the variable leaf length (Figure 9), root length (Figure 10), and plantlet weight (Figure 11), where coconut water reaches its optimum. at concentrations between 50-100 ml.L<sup>-1</sup>.

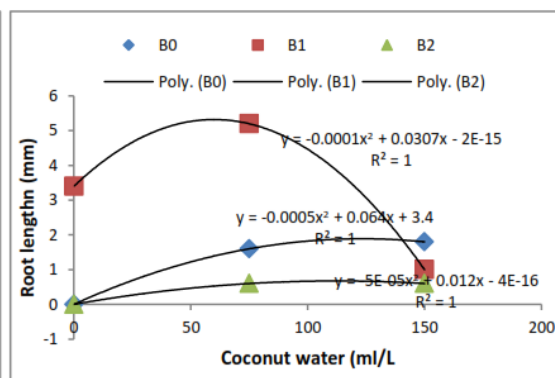
**Table 2** Effect of banana extract on the Shoot length, Leaf length, Root length, Number of leaf, Number of root, and Weight plantlet

| Treatment | Means of treatment |             |             |                |                |                 |
|-----------|--------------------|-------------|-------------|----------------|----------------|-----------------|
|           | Shoot length       | Leaf length | Root length | Number of leaf | Number of root | Weight plantlet |
| B0        | 9.53a              | 5.73a       | 1.13a       | 6.04a          | 1.40a          | 0.61a           |
| B1        | 7.27a              | 3.27a       | 3.20b       | 6.74a          | 2.27a          | 0.57a           |
| B2        | 7.93a              | 3.13a       | 0.40a       | 3.66b          | 0.73a          | 0.39a           |

Note: LSD 5%



**Figure 9** Response of leaf length on the coconut water at the various levels of banana extract



**Figure 10** Response of root length on the coconut water at the various levels of banana extract

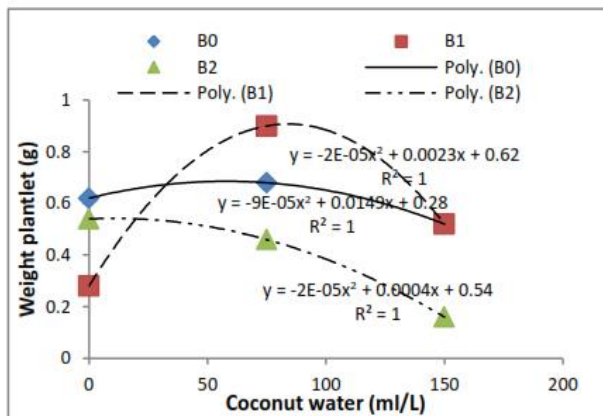


Figure 11. Response of weight plantlet on the coconut water at the various levels of banana extract

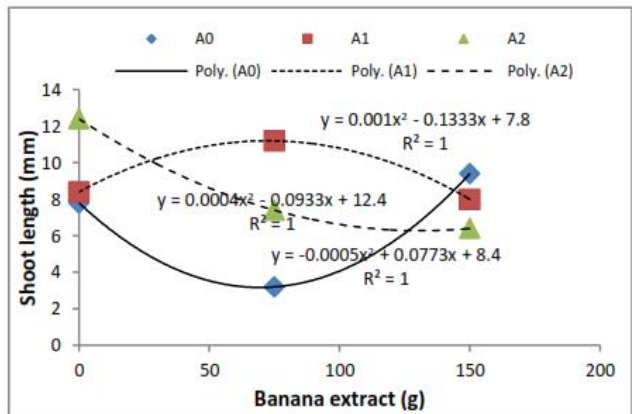


Figure 12. Response of shoot length on the banana extract at the various levels of coconut water

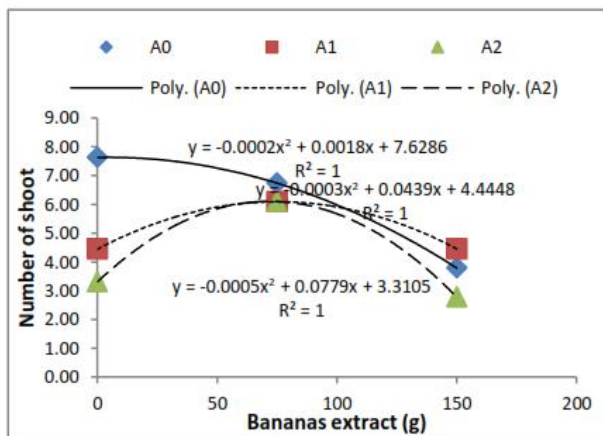


Figure 13. Response of number of shoot on the banana extract at the various levels of coconut water

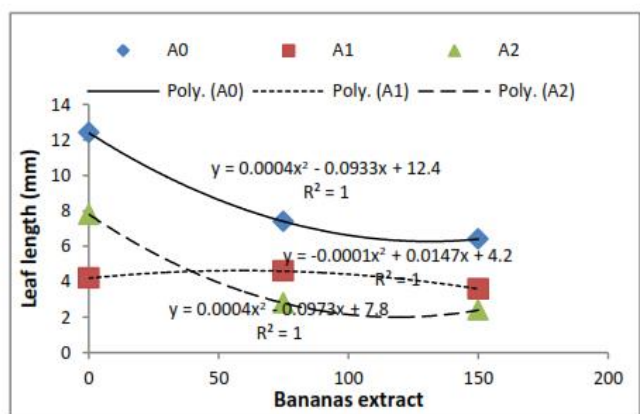


Figure 14. Response of leaf length on the banana extract at the various levels of coconut water

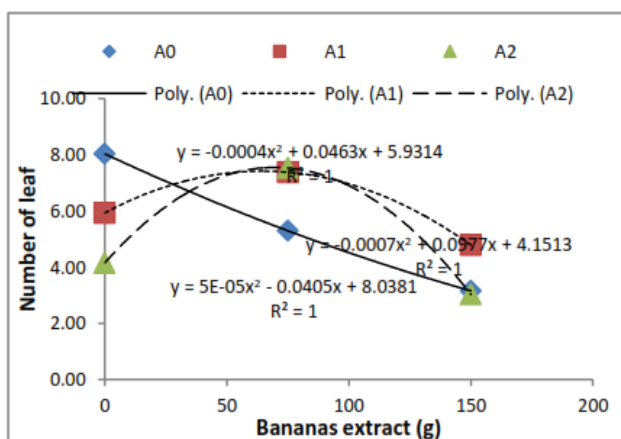


Figure 15. Response of number of leaf on the banana extract at the various levels of coconut water

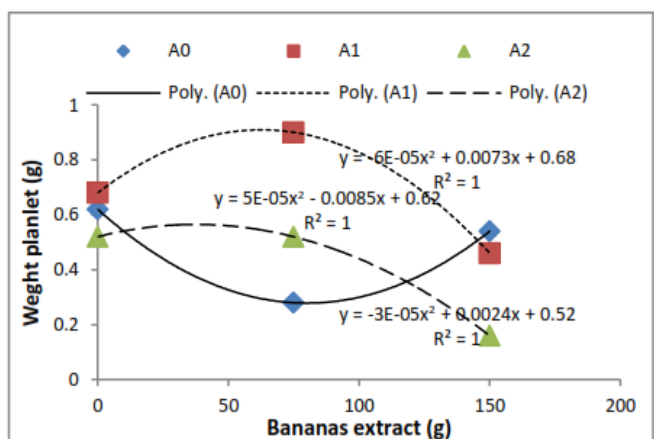


Figure 16. Response of weight plantlet on the banana extract at the various levels of coconut water

Otherwise, the banana extract treatment gave a good response at a coconut water concentration of 75 mL.L<sup>-1</sup> (A1) to the variable shoot length (Figure 12), number of shoots (Figure 13), leaf length (Figure 14), number of leaves (Figure 15), and plantlet weight (Figure 16), where banana extract reaches the optimum between 50-100 g. It was due to the presence of nutrients and growth regulators in coconut water as well as mineral content in bananas, causing interactions to spur plantlet growth, so that the concentration of both treatment was lower than given individually.

According to Suhartanto. et al. [24] that bananas are often used as a source of organic matter in tissue culture media, because 100 grams of banana flesh contain protein (1%), fat (0.3%), carbohydrates (27%), energy (116 -128 kcal), minerals (Ca 15 mg, K 380 mg, Fe 0.5 mg, Na 1.2 mg, and Vitamins (Vit. A 0.3 mg, Vit. B1 0.1 mg, B2 0.1 mg, B6 0.7 mg, Vit. C. Addition of BAP 2 mg.L<sup>-1</sup>, coconut water 150 mL.L<sup>-1</sup> and 50 g.L<sup>-1</sup> Ambon banana extract had the same effect on increase in the number of shoots, an average of 2 shoots of *Phalaenopsis amabilis* [21]. The highest number of shoots was produced in media supplemented with 50 mL.L<sup>-1</sup> coconut water and 50 g.L<sup>-1</sup> potato extract, while media supplemented with 150 mL.L<sup>-1</sup> coconut water and 50 potato extracts increased the number of leaves and the number of roots [9].

#### 4. CONCLUSION

Coconut water did not affect all observed variables, however the banana extract treatment had a significant effect on all observed variables except for the number of shoots. There was an interaction between the two treatments, where coconut water and banana extract had a good response on the plantlet growth when given together as much as 75 mL.L<sup>-1</sup> (A1) and 75 g. L<sup>-1</sup> (B1). The concentration of both treatment was lower than it was given individually based on the results of previous studies.

#### REFERENCES

- [1] D. Puchooa, 'Comparison of different culture media for the in vitro culture of *Dendrobium* (Orhidaceae)'. *Int. J. Agric. Biol.*, vol. 6, pp. 884-888, 2004, doi: 1560-8530/2004/06-5-884-888.
- [2] S. K. Talukder, K. M. Nasiruddin, S. Yesmin, R. Begum, and S. Sarker, S. 'In vitro Root formation on orchid plantlets with IBA and NAA'. *Progressive Agric.* Vol. 13, no.1-2, pp. 25-28, 2002.
- [3] A. Suryana, 'Prospects and Directions for Orchid Agribusiness (Prospek dan Arah Pengembangan Agribisnis Anggrek)'. *Dev. Agric. Res. and Dev.* Agency. Jakarta, 2005.
- [4] The Indonesian Statistics Agency, *Statistik-tanaman-hias-indonesia-2017*. Accessed: Oct. 2, 2020.[Online].Available: <https://www.bps.go.id/site/publication/2018/10/05/statistik-tanaman-hias-indonesia-2017>.Jun 2019 17:45:28 GMT.
- [5] K.B. Andri, W.J.F.A, 'Tumbuan, Potential development of orchid flower agribusiness in Batu City, East Java (Potensi pengembangan agribisnis bunga anggrek di kota batu Jawa Timur)'. *Jurnal LPPM Bidang EkoSosBudKom*. Vol. 2, no. 1, pp.19-30, 2015.
- [6] Indonesian Environmental and Forestry Research and Development Center, 'Plant Propagation Through Tissue Culture (Perbanyakan Tanaman Melalui Kultur Jaringan)'. *Makassar. Bogor*. Bogor, 2013.
- [7] D. Widiastoety, A. Santi, N. Solvia, 'Effect of myoinositol and activated charcoal on growth of dendrobium orchid plantlets in in vitro culture (Pengaruh Myoinositol dan Arang Aktif terhadap Pertumbuhan Planlet Anggrek Dendrobium dalam Kultur In vitro)'. *J. Hort.* Vol. 22, no. 3, pp. 205-209, 2012.
- [8] W. Pakum, S. Watthana, K. Srimuang, A. Kongbangkerd, 'Influence of medium component on in vitro propagation of Thais Endangered orchid: *Bulbophyllum nipondhii* Seidenf'. *Plant Tissue Cult. & Biotech.*, vol. 26, no. 1, pp. 37-46, 2016, doi: <https://doi.org/10.3329/ptcb.v26i1.29765>
- [9] T. Punjansing, M. Nakkuntod, S.Homchan, P. Inthima, A. Kongbangkerd, 'Influence of Organic Supplements on Shoot Multiplication Efficiency of *Phaius tankervilleae* var. Alba'. *Int. J. of Agric. and Biosyst. Eng*, vol 13, no. 4, pp.105-109, 2019.
- [10] S. Rahmah, T. Rahayu, A. Hayati, 'Study Adding Organic Substances on VW Media for Organogenesis the *Dendrobium* Orchid in Vitro'. *e-Jurnal Ilmiah SAINS ALAMI (Known Nature)*, vol. 1, no. 1, pp. 93 – 103, 2018.
- [11] Djajanegara, 'Utilization of banana and coconut water waste as a medium for tissue culture for the Moon Orchid (*Phalaenopsis amabilis*) Tipe 229 (Pemanfaatan limbah buah pisang dan air kelapa sebagai bahan media kultur jaringan anggrek bulan (*Phalaenopsis amabilis*) Tipe 229)'. *J.Teknologi Lingkungan*, vol. 11, no. 3, pp. 373-380, 2010.
- [12] M.Humaira, Z. Thomy, E. Harnelly, 'The effect of giving coconut water and banana pulp to MS medium

on the growth of rabbit orchid plantlets (*Dendrobium antennatum* Lindl.) In vitro (Pengaruh pemberian air kelapa dan bubur pisang pada media ms terhadap pertumbuhan planlet anggrek kelinci (*dendrobium antennatum* Lindl.) secara in vitro)'. *Proc. of Biotic Seminar Nasional*, pp. 326-330, 2015.

[13] J. Pratama, 'Modification of MS Media with Addition of Coconut Water for Subculture I *Cymbidium* Orchids (Modifikasi Media MS Dengan Penambahan Air Kelapa Untuk Subkultur I Anggrek *Cymbidium*)'. *Jurnal Agrium*, vol. 15, no. 2, pp. 91-109, 2018.

[14] N.P.Y.A. Dewi, 'The Effect of Giving Coconut Water on Embryo Development in *Dendrobium anosmum* Lindl (Pengaruh Pemberian Air Kelapa terhadap Perkembangan Embrio pada *Dendrobium anosmum* Lindl)'. *JBE*, vol. 4, no. 1. Pp. 22-28, 2019.

[15] Nurfadilah, E. Mukarlina, P.W. Rusmiyanto, 'The multiplication of black orchids (*Coelogyne pandurata* Lindl) on murashige skoog (MS) media with the addition of Ambon banana extract and benzyl amino purine (BAP) (Multiplikasi anggrek hitam (*Coelogyne pandurata* Lindl ) pada media murashige skoog (MS) dengan penambahan ekstrak pisang ambon dan benzyl amino purin (BAP)'. *Protobiont*, vol. 7, no. 3, pp. 47 – 53, 2018.

[16] M. Vargas, B. Glaz, G. Alvarad, J. Pietragalla, A. Morgounov, Y. Zelenskiy, and J. Crossa, 'Analysis and Interpretation of Interactions in Agricultural Research'. *Agronomy Journal*, vol. 107, no. 2, pp.748, 2015, doi:10.2134/agronj13.0405

[17] M.G. Pranata, A. Yunus , B. Pujiasmanto, 'The effect of NAA concentrations and coconut water on the multiplication of ginger (*curcuma xanthorrhiza* roxb.) In vitro (Pengaruh konsentrasi naa dan air kelapa terhadap multiplikasi temulawak (*curcuma xanthorrhiza* roxb.) secara in vitro)'. *Caraka Tani-J. of Sustainable Agric*, vol. 30, no. 2, pp. 62-68, 2015.

[18] L. Erfa, Ferziana, 'Growth of *Phalaenopsis* orchid seedling into plantlets in subculture II media

with addition of tripton and atonic (Pertumbuhan seedling anggrek *Phalaenopsis* menjadi plantlet pada media subkultur II dengan penambahan tripton dan atonik)'. *Jurnal Penelitian Pertanian Terapan*. Vol. 12. (Spec. Ed.), pp. 52-58, 2012.

[19] N.N. Kristina, Syahid, S. Fatimah, 'The Effect of Coconut Water on In Vitro Shoot Multiplication, Rhizome Production, and Xanthorrhizol Content of Temulawak in the Field (Pengaruh Air Kelapa Terhadap Multiplikasi Tunas In Vitro, Produksi Rimpang, dan Kandungan Xanthorrhizol Temulawak di Lapangan)'. *Jurnal Litri*, vol. 18, no. 3, pp. 125-134, 2012.

[20] J. Yong, L. Ge, Y.F. Ng, S. Tan, 'The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water'. *Molecules*, vol. 14, no. 12, pp. , 5144-5164, 2009, doi: <https://doi.org/10.3390/molecules14125144>

[21] Kasutjaniangati, R. Irawan, 'Alternative media for in vitro orchid propagation (*Phalaenopsis amabilis*) (Media alternatif perbanyakan in vitro anggrek Bulan (*Phalaenopsis amabilis*)'. *J. Agroteknos*, vol. 3, no. 3, pp. 184-189, 2013.

[22] D.P.S. Hendaryono, 'Orchid nursery in bottles (Pembibitan anggrek dalam botol)'. *Kanisius: Yogyakarta*. 2000

[23] S. Damiska, R.S. Wulandari, H. Darwati, 'Addition of Yeast and Corn Seed Extract to In-Vitro Growth of Mangosteen Shoots (Penambahan Ragi dan Ekstrak Biji Jagung terhadap Pertumbuhan Tunas Manggis Secara In-Vitro)'. *J Hutan Lestari*, vol. 3, no. 1, pp. 35-42, 2015.

[24] M.R. Suhartanto, Sobir, H. Harti, 'Healthy Technology for Banana Cultivation: From Seed to Post Harvest (Teknologi Sehat Budidaya Pisang: dari Benih sampai Pasca Panen)'. *Pusat Kajian Hortikultura Tropika, LPPM-IPB*. 2012.