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The effect of coconut water on the in vitro growth of *Phalaenopsis amabilis* (L.) Blume plantlets

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Abstract

Phalaenopsis amabilis is an Indonesian native orchid listed in Appendix II, therefore, appropriate cultivation strategies, including tissue culture techniques, are required to support its sustainable propagation. However, Murashige and Skoog (MS) medium does not contain sufficient natural growth-promoting substances; thus, organic supplementation such as coconut water is needed to enhance plantlet development. This study aimed to evaluate the effect of coconut water supplementation in MS medium and to determine the optimal concentration for promoting the growth of *P. amabilis* plantlets during the subculture stage. Plantlets were subcultured onto MS medium supplemented with coconut water at concentrations of 0%, 15%, and 30% using a Completely Randomized Design with five replications. Data were analyzed using ANOVA followed by Duncan's Multiple Range Test at a 5% significance level. Observations were conducted for two months on leaf emergence time, leaf length and width, number of leaves, shoot emergence time, number of shoots, root emergence time, number of roots, and leaf color. The results showed that 15% coconut water was the optimal concentration for improving plantlet growth, particularly in new leaf length (8 mm), number of shoots (1,66 shoots), and number of leaves (1,33 leaves). whereas 30% coconut water increased leaf width (3,2 mm). Overall, supplementation with 15% coconut water effectively enhanced the vegetative growth of *P. amabilis* plantlets, so it can be applied to successfully cultivate this plant.

Introduction

Orchids have great potential for cultivation due to their distinctive flower shapes, bright colors, varied sizes, and fragrant aroma. These flowers are able to maintain their beauty for several months and have high economic value. According to Presidential Decree No. 4 of 1993, the moon orchid was designated as the national flower under the name "Puspa Pesona". Globally, orchids consist of approximately 25,000 species and 800 genera. About half of them are found in Papua, while 2,000 species grow abundantly in Kalimantan, especially East Kalimantan. Other varieties are distributed across various islands in Indonesia (Lubis, 2010).

Phalaenopsis amabilis is an Indonesian native orchid classified under Appendix II, which means it is a species that may become endangered if traded freely or may be traded in accordance with applicable regulations. The growth of *P. amabilis* is relatively slow; therefore, a rapid propagation method through tissue culture is required. Tissue culture has the advantage of producing orchid seedlings in large quantities within a short time, so their availability can be maintained. However, it should also be noted that tissue culture requires high technical skills (Solihah, 2015 & CITES, 2018).

The success of tissue culture is influenced by the selection of appropriate media, the type of explant, the working environment, and the application of plant growth regulators. Murashige–Skoog (MS) medium is the most commonly used because it is rich in macro- and micronutrients, contains complete vitamins, and has high mineral salt and nitrate content (Kusuma, 2023). Saepudin (2020) stated that MS medium supports orchid growth faster because it contains higher concentrations of salts and nitrates compared to other media. This is why this medium was chosen for this study.

In addition, coconut water is needed as an organic supplements to promote the growth of *P. amabilis*. Coconut water contains nutrients such as electrolytes, sugars, and natural minerals including Na, Ca, Mg, Fe, Cu, and P. According to Tuhuteru (2012), Rupawan (2014), Apriliyania et al. (2021), and Chutimanukul *et al*. (2023), the use of coconut water as an organic supplement is an alternative to the use of synthetic plant growth regulators. Pratama (2018) showed that the application of coconut water significantly affected shoot development at 2, 4, 6, and 8 WAP in *Cymbidium* orchids. Saepudin (2020) and Inkiriwang (2016) showed that low concentrations of coconut water increased root growth, while high concentrations supported shoot and seedling growth. A concentration of 30% coconut water in MS medium supported the propagation of *Dendrobium* orchids. Based on research by Sundalangi (2023), the addition of coconut water to MS medium improved the growth of *Dendrobium* orchids.

However, no studies have yet been conducted on the effect of coconut water on the growth of *P. amabilis* plantlets during the subculture stage. Therefore, this study aims to determine the effect of coconut water on the growth of *P. amabilis* plantlets subcultured on MS medium. The results of this study are expected to be applied to promote the successful cultivation of *P. amabilis* orchid and prevent it from facing the threat of extinction.

Materials and methods

This study was conducted in the Plant Tissue Culture Room of the Structure and Function of Plant Biology Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, from September 2023 to April 2024.

1. Materials

This study used materials consisting of one-year-old *P. amabilis* orchid plantlets, young coconut water, ready-to-use Murashige–Skoog (MS) medium produced by Plant Cell Technology (PCT), sucrose, gelzan, pH meter, culture bottles, aluminum foil, magnetic stirrer, ruler, camera, Image Raster application, and Leaf Color Chart.

2. Method and Research Design

This study employed a Completely Randomized Design (CRD) with a single factor, namely different concentrations of coconut water (0%, 15%, and 30%), with five replications. After two mouths of observation, the data were analyzed using Analysis of Variance (ANOVA) to determine the effect of coconut water on the growth of the *P. amabilis* planlets. If ANOVA showed significant effect, the Duncan's Multiple Range Test (DMRT) was used to perform pairwise comparison of group means at a significance level of 5%.

3. Procedures

The study was conducted as follows: coconut water from young green coconuts at concentrations of 0%, 15%, and 30% was prepared and added to Murashige–Skoog (MS) medium. Sucrose (3 g) was then added to the medium solution. The solution was stirred until completely dissolved, and the pH was adjusted to ±6-7. Gelzan (0.20 g) was added, mixed, and heated until boiling. The medium was then poured into culture bottles and tightly sealed with aluminum foil. One-year-old orchid plantlets were subsequently subcultured onto the treatment media. Cultures were incubated at room temperature (24–26°C) under approximately 1000 lux light intensity, equivalent to a 40-Watt fluorescent (TL) lamp. Observations were conducted weekly for two months, with the following parameters:

a. Leaf emergence time
   New leaves emerging are observed every two days and documented with a camera.
b. Leaf length and leaf width
   Leaf length and width were measured during the final week of observation. Measurements were taken using a ruler placed against the outside of the bottle. The photos of the leaves were then analyzed using the Image Raster application to determine the length and width of the leaves.

c. Number of leaves
   The number of new leaves is counted once a week and documented using a camera.
d. Shoot emergence time
   New shoots emerging at the plant base are observed every two days and documented with a camera.
e. Number of shoots
   The number of new shoots is counted once a week and documented using a camera.
f. Root emergence time
   New roots emerging at the plant base are observed every two days and documented with a camera.
g. Number of roots
   The number of new roots is counted once a week and documented using a camera
h. Leaf color
   Leaf color was observed using the Leaf Color Chart

Results and discussion

1. Leaf Length and Leaf Width of New *P. amabilis* Leaves

The study showed that the application of coconut water in MS medium at concentrations of 0%, 15%, and 30% had a significant effect on the growth of the length and width of new leaves of *P. amabilis* orchid plantlets (Figure 1).

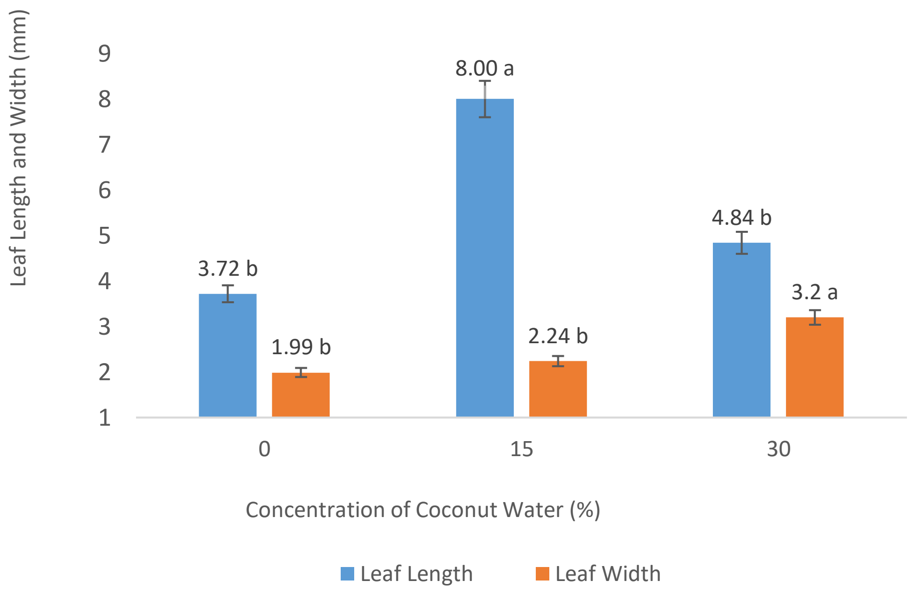

Figure 1. Length and width of new leaves of *Phalaenopsis amabilis* after treatment with coconut water at concentrations of 0%, 15%, and 30% for two months.

Treatment with a 15% coconut water concentration affected leaf length while a 30% concentration affected leaf width (Figure 1), because the added coconut water contains cytokinin and auxin hormones that can stimulate cell division and cell elongation. Cytokinins stimulate cell division by increasing protein synthesis, especially phosphatase enzymes that regulate protein activity in cells, including Cyclin-Dependent Kinases (CDKs) that regulate the cell cycle. When phosphatase enzyme activity increases, it causes the release of one phosphate group bound to CDKs, which is called dephosphorylation. Through dephosphorylation, enzymes regulate protein activity, such as Cyclin-Dependent Kinases (CDKs). Then, after phosphatase enzymes carry out dephosphorylation, only one phosphate group remains bound to CDKs, and this will accelerate entry into the mitotic phase. Activation of CDK proteins in mitosis, such as cyclins, can form active complexes. Active CDK–cyclin complexes can direct cells to the next stages of the cell cycle, including prophase, metaphase, anaphase, and telophase, including cell division.

The mechanism of auxin stimulates proteins located in the plasma membrane of plant cells to pump H^+ ions into the cell wall, which will activate enzymes and function to break hydrogen cross-links in cellulose molecules that form the cell wall. This process allows water to enter through osmosis into cell elongation, so that elongation affects the length of new leaves.

A 15% coconut water concentration is sufficient to support leaf length growth. Coconut water, in addition to containing cytokinin and auxin hormones, also contains nutrients such as nitrogen that support leaf length and width growth (Ariyanti, 2018). Saepudin (2020) stated that nitrogen is an essential nutrient for leaf growth and development by playing a role in chlorophyll and protein synthesis. In addition, nitrogen is required for amino acid synthesis, which becomes the basic material for protein formation. Thus, nitrogen availability also affects cell elongation and cell division, which ultimately affects leaf size.

An increase in leaf length and width contributes to a leaf surface area that serves as the site of photosynthesis, resulting in the production of more photosynthates. This is supported by Sari et al. (2019), who state that larger leaf size increases photosynthesis as a source of energy to promote plant growth and development. Coconut water at a 15% concentration resulted in the longest leaves, which increased the overall leaf size.

2. Root Length of *P. amabilis*

The study showed that the application of coconut water in MS medium at concentrations of 0%, 15%, and 30% had a significant effect on the root length growth of *P. amabilis* orchid plantlets (Figure 2).

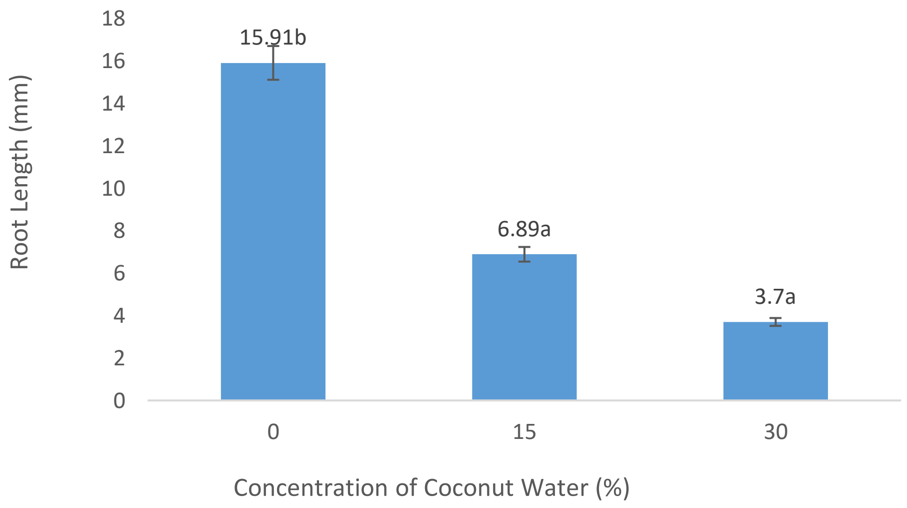

Figure 2. Root length of *P. amabilis* orchid plantlets after treatment with coconut water at concentrations of 0%, 15%, and 30% for two months.

The roots of *P. amabilis* orchid plantlets 0% coconut water treatment were longer (15.91 mm) compared to those in 15% and 30% coconut water treatments (6.89 mm and 3.7 mm, respectively) (Figure 2). This was due to the sufficient endogenous hormones in the plant at the 0% coconut water treatment, allowing the plant to promote root growth without the addition of coconut water, so the plant was able to stimulate root length growth. The roots of the plant at the 0% concentration appeared earlier, namely in the 4th week, which provided better nutrition. The addition of coconut water at concentrations of 15% and 30% inhibited root elongation, presumably due to excess exogenous auxin and cytokinin in the plant. An increase in hormone levels in plants causes a hormonal imbalance that affects the directional growth.

Cytokinins and auxins antagonistically influence the direction of plant growth. If auxin levels are higher, root growth is promoted while shoot growth is inhibited. In contrast, if cytokinin levels are higher, then root growth is inhibited. According to Seliem *et al.* (2025), cytokinins are usually not used at the rooting stage because they can inhibit root formation and interfere with root growth. In addition, cytokinins can also inhibit the effect of auxin on root initiation in tissue culture in certain species.

The addition of coconut water may disrupt the balance of hormone concentrations in the plant, causing root growth to be inhibited. Endogenous hormones work optimally at low concentrations to support plant growth. When coconut water is added, the auxin content in the cell cytoplasm increases. The increased auxin concentration due to the addition of coconut water, which is rich in auxin, can produce excessive or abnormal growth responses, which may cause root growth to slow down.

3. Number of New Shoots, Leaves, and Roots of *P. amabilis*

The study showed that the application of coconut water in MS medium at concentrations of 0%, 15%, and 30% had no significant effect on the growth of the number of shoots, leaves, and roots of *P. amabilis* (Figure 3).

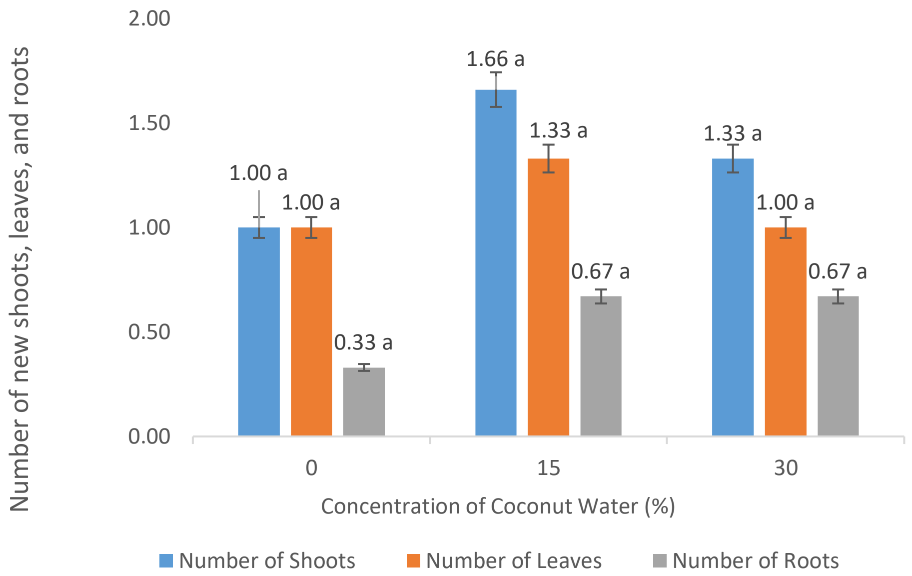

Figure 3. Number of new shoots, leaves, and roots of *P. amabilis* orchid plantlets after treatment with coconut water at concentrations of 0%, 15%, and 30% for two months.

Figure 3 shows that, although there was no statistically significant difference, the application of 15% coconut water tended to produce a greater number of shoots, leaves, and roots. The number of shoots, leaves, and roots in planlets treated with a higher concentration of coconut water (30%) tended to decrease. This is thought to be due to excessive coconut water causing an imbalance in hormone levels, which inhibited growth. A similar result was also reported in Santosa *et al.* (2025), showing that concentrations of 10% and 15% coconut water resulted in higher growth rates in *Dendrobium spectabile* planlets compared to the application of a higher concentration of coconut water (20%).

The application of 15% coconut water concentration tended to be effective in increasing the growth of new shoots, leaves, and roots in *P. amabilis* orchid plantlets because it contains cytokinin and auxin hormones. Cytokinins play an important role in shoot and leaf formation by stimulating cell division activity at the shoot tips. The cell division process stimulated by cytokinins enables the active formation of new shoots and assists differentiation in the Shoot Apical Meristem (SAM), whereas auxin hormone is used for root formation.

Plantlets treated with 15% coconut water showed better growth than other treatments, which may be associated with a higher cytokinin-to-auxin ratio at this concentration, promoting shoot development. (Saepudin, 2020). A 15% coconut water concentration tended to stimulate the development of shoots, leaves, and roots, thereby promoting the formation of more shoots, leaves, and roots. Growth influenced by coconut water concentrations of 0%, 15%, and 30% at the 8th week showed an increase that tended to be effective in the number of leaves (Figure 4). Tuhuteru (2012); Nana and Slamah (2014); Asmono *et al.* (2017) stated that the cytokinin content in coconut water affects protein synthesis and enzyme activity for cell differentiation in the formation of new shoots, leaves, and roots in *P. amabilis* orchids. A higher auxin-to-cytokinin ratio promotes root formation, while a higher cytokinin concentration influences shoot formation. Nutrient absorption through coconut water produces components that support the growth of new cells resulting from mitosis.

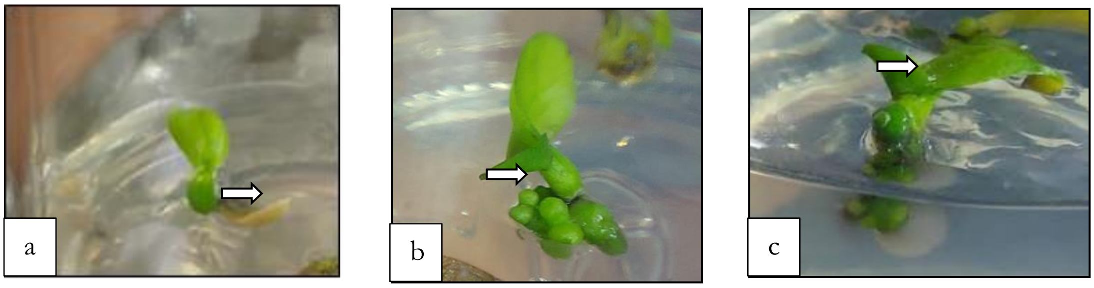

Figure 4. Growth of new leaves (arrows) in *P. amabilis* plantlets after treatment with coconut water at concentrations of (a) 0%, (b) 15%, and (c) 30% for two months.

Coconut water in MS medium is not only required in the tissue culture of orchid plantlets, but Plant Growth Regulators (PGRs) are also important components. Plant Growth Regulators (PGRs) are important in the process of preparing tissue culture media. PGRs and endogenous phytohormones play an important role in plantlet development, with the ability to increase the activity of other growth regulators such as auxin and cytokinin. Growth-regulating substances, namely cytokinins, have an important role in maintaining the size and activity of the Shoot Apical Meristem (SAM) and Root Apical Meristem (RAM). This is in line with the opinion of Kieber and Schaller (2014), who stated that cytokinin activity is an important factor in regulating cell division in the SAM.

4. Time of Emergence of Shoots, Leaves, and Roots of *P. amabilis*

The study showed that the application of coconut water in MS medium at concentrations of 0%, 15%, and 30% had a significant effect on the emergence time of shoots, leaves, and roots of *P. amabilis* (Figure 5).

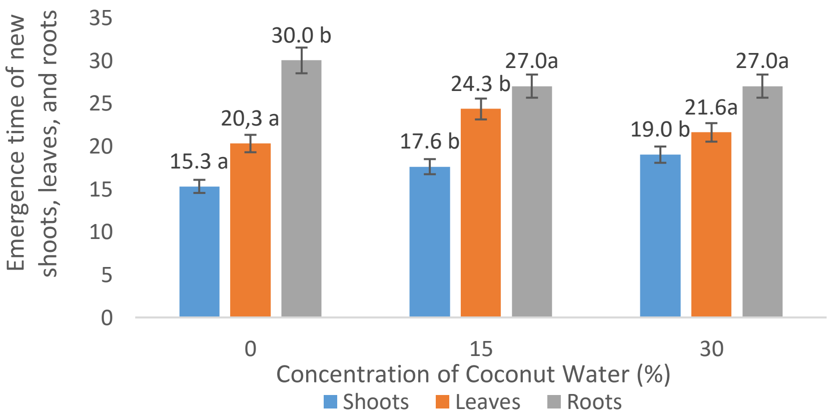

Figure 5. Emergence time of new shoots, leaves, and roots of *P. amabilis* orchid plantlets after treatment with coconut water at concentrations of 0%, 15%, and 30% for two months.

Figure 5 shows that the application of 0% coconut water accelerated the formation of shoots and leaves in *P. amabilis* orchids compared to the higher coconut water concentration (30%). This is presumably because the endogenous hormone content in the plant was already sufficient to stimulate the differentiation of meristematic cells.

Plantlets treated with coconut water concentrations of 15% and 30% showed slower shoot and leaf growth due to hormonal imbalance caused by the higher concentrations. It is presumed that the coconut water concentrations applied were too high and inhibited shoot and leaf growth due to the imbalance of plant hormones. Root growth was also affected, with the 0% concentration showing slower growth due to nutrient deficiency, while the 15% and 30% concentrations accelerated root growth with optimal nutrients and auxin availability.

Plant roots treated with the 0% concentration tended to show slower growth, presumably due to nutrient deficiency, lack of auxin hormone, and the absence of coconut water content, which inhibited root growth. Plants at concentrations of 15% and 30% showed faster root growth because higher concentrations of coconut water provided optimal nutrients and auxin for the plants (Mayura *et al.*, 2017). Yulia (2020) stated that the process of shoot and root formation is influenced by the cytokinin and auxin hormone content in the plantlets, as well as by the endogenous cytokinin or auxin concentration in the explants themselves. The balance between cytokinin and auxin hormones in tissue culture is very important for effective shoot growth. According to Lutfiani (2022), the rate of shoot growth can be increased through the appropriate relationship between natural hormones present in the explants and additional external plant growth regulators. The balance between exogenous auxin concentration and endogenous hormones in the medium allows physiological processes in the explants to proceed optimally, thereby effectively stimulating shoot growth. Therefore, this supports growth and differentiation in the meristematic regions of plants, including in tissue culture.

The application of coconut water at the appropriate concentration may accelerate the formation of shoots and leaves due to its content of cytokinin and auxin hormones, which play a role in the process of organogenesis. This is also explained by Santosa *et al.* (2025), who states that coconut water contains cytokinin, which activates enzymes during cell division, resulting in the formation of the Shoot Apical Meristem (SAM) as a leaf primordium, as well as auxin, which plays a role in the differentiation of the leaf primordium into a true leaf.

5. Leaf Color of *P. amabilis*

The observation results using the Leaf Color Chart (LCC) showed that the leaf color of *P. amabilis* orchids under the 15% coconut water treatment produced a dark green color. The 0% concentration produced the lightest green color, whereas the 30% coconut water concentration produced the darkest green color (Table 1).

Table 1. Average Leaf Color of New Leaves of *P. amabilis* after treatment with coconut water at concentrations of 0%, 15%, and 30% for two months.

Concentration of Coconut Water (%)	Leaf Color	Leaf Color Value	Leaf Color Chart
0	Light Green	2	
15	Dark Green	3	
30	Deep Green	4	

Table 1 shows that plantlets subcultured on MS medium supplemented with coconut water at concentrations of 15% and 30% had dark green and darker green leaf colors. This occurred because plants at concentrations of 15% and 30% had higher chlorophyll content, which resulted in darker leaf color; thus, cytokinins and auxins present in coconut water could stimulate chlorophyll formation. At the 0% concentration, the chlorophyll content in the leaves was lower, so endogenous auxin and cytokinin were already able to stimulate chlorophyll growth properly, and the resulting color tended to be lighter or greener. According to Mandal (2020), chlorophyll as an important green pigment in plant photosynthesis is formed rapidly and optimally, resulting in leaves that are greener than those that grow more slowly. However, leaves that grow larger and are formed earlier tend to have greener color and experience faster growth.

Plants with light green leaves indicate nitrogen (N) deficiency, whereas dark green leaves indicate that the plants have a sufficient nitrogen supply. One of the symptoms of nitrogen deficiency is a reduction in the green color of the leaves (chlorosis), which is usually distributed evenly throughout the leaves. The leaves become paler and yellowish, and if the nitrogen deficiency is severe, the leaves may die. According to the explanation Inaya *et al.* (2021), nitrogen is necessary for the synthesis of proteins and chlorophyll; therefore, deficiency of nitrogen causes the plant's leaves to turn greenish-yellow.

Conclusion

The results of this study indicate that the addition of coconut water to Murashige–Skoog (MS) medium affected the growth of *Phalaenopsis amabilis* plantlets, including new leaf length, leaf width, root length, and leaf emergence time. A concentration of 15% coconut water was the most effective in promoting plantlet growth, particularly in terms of new leaf length, number of shoots, number of leaves, number of roots, and leaf emergence time.

It can be concluded that, to prevent the extinction of *P. amabilis* orchids, cultivation can be carried out by culturing plantlets on MS medium supplemented with 15% coconut water, in order to achieve faster growth. Furthermore, additional research is needed on the acclimatization process so that seedlings produced in vitro can be successfully cultivated in the natural environment.

Author Statements

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Competing of interest: The authors declare that there are no conflicts of interest, either financial or personal, that could have influenced the results of this study.

Author's contributions: Elvina Adinda Triansyah, Nintya Setiari, Conducted the research, collected and analyzed the data, and prepared the manuscript. Nintya Setiari Designed the study and provided scientific guidance throughout the research design, implementation, data analysis, and manuscript preparation.. Yulita Nurchayati Provided conceptual and academic input on the research design and contributed to the improvement of the manuscript.

Generative AI: The authors declare that they did not use Generative AI assistance in the preparation of this manuscript.

Data availability: The data supporting the findings of this study are available from the authors and can be provided to researchers who meet the relevant criteria.

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