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ARTIKEL

THE GROWTH OF *Dendrobium spectabile* (Blume) Miq. ORCHID PLANTLET IN VACIN AND WENT (VW) MEDIUM WITH DIFFERENT CONCENTRATIONS OF COCONUT WATER

[*Pertumbuhan Planlet Anggrek Dendrobium spectabile (Blume) Miq. di Media Vacin and Went (VW) dengan Konsentrasi Air Kelapa yang Berbeda*]

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ABSTRACT

Dendrobium spectabile is an epiphytic orchid that grows slowly and experiences natural exploitation, so it needs to be conserved *ex-situ* through *in vitro* culture. One of the essential steps for *in vitro* culture is subculture to supply the nutrients needed by plantlets. Increasing the growth of these plantlets can be done by adding coconut water. This study aimed to determine the effect of adding coconut water and the optimal concentration of coconut water on the growth of *D. spectabile* plantlets in VW medium. The method used is growing plantlets in VW medium with different concentrations of coconut water. The research design was completely randomized with a single factor, the concentration of coconut water (0%, 5%, 10%, 15%, 20%). Data were analyzed by ANOVA at a 5% significance level. Plantlet growth was observed for two months. Parameters observed were new shoots, leaves, and roots emergence time; number of new shoots, leaves, and roots; and new leaves length. The addition of coconut water affected the growth of *D. spectabile* orchid plantlets. Five to ten percent of coconut water is the optimal concentration for shoots and leaves growth of *D. spectabile* orchid plantlets. Therefore, coconut water can increase the growth of *in vitro* plantlets.

Keywords: Coconut Water, *Dendrobium spectabile*, Plantlet Growth

ABSTRAK

Dendrobium spectabile merupakan anggrek epifit yang pertumbuhannya lambat dan mengalami eksploitasi di alam sehingga perlu dilakukan konservasi secara ex situ melalui kultur in vitro. Salah satu tahap penting pada kultur in vitro adalah subkultur yang bertujuan untuk menyuplai nutrisi yang dibutuhkan planlet. Peningkatan pertumbuhan planlet tersebut dapat dilakukan dengan menambahkan air kelapa. Penelitian ini bertujuan untuk mengetahui pengaruh penambahan air kelapa dan konsentrasi optimal air kelapa terhadap pertumbuhan planlet D. spectabile pada tahap subkultur di media VW. Metode yang digunakan adalah menumbuhkan planlet ke dalam media VW dengan konsentrasi air kelapa yang berbeda. Desain penelitian yang digunakan adalah Rancangan Acak Lengkap dengan faktor tunggal, yaitu konsentrasi air kelapa (0%, 5%, 10%, 15%, 20%). Data dianalisis dengan ANOVA pada taraf signifikansi 5%. Pertumbuhan planlet diamati selama 2 bulan. Parameter yang diamati berupa waktu muncul tunas, daun, dan akar baru; jumlah tunas, daun, dan akar baru; serta panjang daun baru. Penambahan air kelapa berpengaruh terhadap pertumbuhan planlet anggrek D. spectabile. Air kelapa sebanyak 5-10% merupakan konsentrasi yang optimal untuk pertumbuhan tunas dan daun pada planlet anggrek D. spectabile. Penggunaan air kelapa mampu meningkatkan pertumbuhan planlet secara in vitro.

Kata kunci: *Air Kelapa, Dendrobium spectabile, Pertumbuhan Planlet*

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INTRODUCTION

Dendrobium spectabile is classified as a curly orchid that has yellow sepals and petals with a maroon pattern. These characteristics make *D. spectabile* orchids highly valuable, which has caused their population in nature to decrease due to massive exploitation (Budiharta *et al*., 2011; Rahayu and Mulyani, 2020). According to CITES (2020), *D. spectabile* plants are included in the *Appendix II* category, a species that can be threatened with extinction if its trade is not strictly controlled, so *ex-situ* conservation of *D. spectabile* orchids is needed.

Conservation in orchids is practiced *in vitro* because it can produce a large number of plants in a relatively short time (Pratama and Nilahayati, 2018). This is because conventional propagation takes a very long time, about 4-5 years for orchids to flower (Vendrame *et al*., 2014). *In vitro* propagation methods are generally used to germinate orchid seeds due to the absence of endosperm, which functions as a food reserve. Germinated seeds will produce protocorms, form leaves and roots, and then become plantlets (Kartikaningrum *et al*., 2017). Plantlets will grow and develop due to the presence of nutrients in the culture medium so that their size increases and fills the culture bottle. In addition, it causes nutrients in the medium to decrease (Monthony *et al*., 2021).

At some stage, the plantlets need to be subcultured from the old medium to the new medium to supply the required nutrients regularly. One of the basal media in tissue culture for *Dendrobium* orchid propagation is *Vacin and Went* (VW). The VW medium contains macro and micro nutrients with a composition suitable for the growth needs of orchid plants. However, the media composition does not contain hormones, so modifications are made by adding organic supplements in the form of coconut water to increase plantlet growth (Krisdianto *et al*., 2020).

Coconut water is a liquid endosperm containing hormones, amino acids, organic acids, several vitamins, and minerals. One of these minerals is nitrogen which promote vegetative growth in plants, such as roots and leaves (Leghari *et al*., 2016). Hormones contained in coconut water include cytokinin and auxin. Cytokinin stimulates cell division and morphogenesis, while auxin enhances root formation and elongation (Ahmed *et al*., 2016; Srinivasa *et al*., 2018). Research conducted by Zahara *et al*. (2017) showed that VW medium added with 10% coconut water produced the best growth for the number and length of roots, and the number of leaves in *Phalaenopsis hybrid 'Pink'* orchid plantlets. Coconut water with a concentration of 10% added to MS medium can increase the growth of shoots and roots of *Dendrobium anosmum* orchid plantlets (Tuhuteru *et al*., 2012).

The growth of *D. spectabile* orchid plantlets at the subculture stage with the addition of coconut water on VW medium to increase its growth has yet to be widely done. Therefore, this study was conducted to determine the effect of the addition of coconut water and the optimal concentration of coconut water on the growth of *D. spectabile* plantlets in *Vacin and Went* (VW) medium.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Biology of Plant Structure and Function, Department of Biology, Faculty of Science and Mathematics, Diponegoro University from April to August 2022.

The materials used were *D. spectabile* orchid plantlets (± 1 cm in height) from seeds and had 2–3 leaves, coconut water from young green coconut fruits, instant VW medium, gelzan, sucrose, distilled water, NaOH, and HCl solutions.

Coconut Water Preparation

Coconut water was filtered and the volume was measured according to the treatments, which were 0%, 5%, 10%, 15%, and 20% concentrations. Then, the volume of coconut water was calculated using the formula:

$$\text{Concentration (\%)} = \frac{\text{Volume (mL)}}{100\ \text{mL}}$$

Medium Preparation

The culture medium made was 1 L for all treatments, so 500 mL of distilled water was poured into an erlenmeyer. The instant VW medium of as much as 1.67 g and 30 g sucrose were put into the erlenmeyer and then homogenized with a magnetic stirrer. Then coconut water was added to the volume according to the treatment and homogenized again. The acidity of the culture medium solution was adjusted to approximately 6. After the pH was measured, gelzan and distilled water were added until the volume reached 1000 mL, homogenized, and heated on a hotplate until boiled. The medium solution was poured into culture bottles. The bottles were covered with aluminium foil and sterilized by autoclaving for 15 minutes at 121℃ with a pressure of 15 psi or 1 atm. The sterilized medium was incubated for one week.

Subculture Treatment

Plantlets were removed using sterile forceps and then placed on sterile petridishes. Plantlets that were still attached to the old medium were carefully separated. Afterward, the plantlets were planted in medium bottles according to the treatment. Each bottle was planted with one plantlet. The bottle was then covered with sterile aluminium foil, sealed with plastic wrap, and incubated in a room with a temperature of 24–26℃ and 24 hours of photoperiod using a white light fluorescent TL lamp with an intensity of 600 *lux*.

Growth Observation

The parameters observed in this study were new shoots, leaves, and roots emergence time every two days for two months of observation, as well as the number of new shoots, new leaves, and new roots, also the length of new leaves, every week for two months of observation.

Research Design and Data Analysis

This study used a Completely Randomised Design (CDR) with a single factor of five different concentrations of coconut water. Each treatment consisted of 10 replicates. The treatments were P0 (0% or control), P1 (5%), P2 (10%), P3 (15%), and P4 (20%).

The data were analyzed using Statistical Product and Service Solution (SPSS) version 20.0 with Analysis of Variance (ANOVA) at 5% significance. The results of ANOVA were followed to Least Significance Different (LSD) further test if there was a significantly different effect.

RESULTS

Shoot and Leaf Growth

The ANOVA results showed that the treatment of coconut water addition with different concentrations on *D. spectabile* orchid significantly affected the parameters of new shoots emergence time (Figure 1) and the number of new shoots (Figure 2).

In leaf growth, the parameters observed were new leaves emergence time, number of new leaves, and length of new leaves. The addition of coconut water with different concentrations on VW medium has a significant effect on the new leaves emergence time (Figure 1), the number of new leaves (Figure 2), and the length of new leaves (Figure 3) on *D. spectabile* orchid plants according to ANOVA.

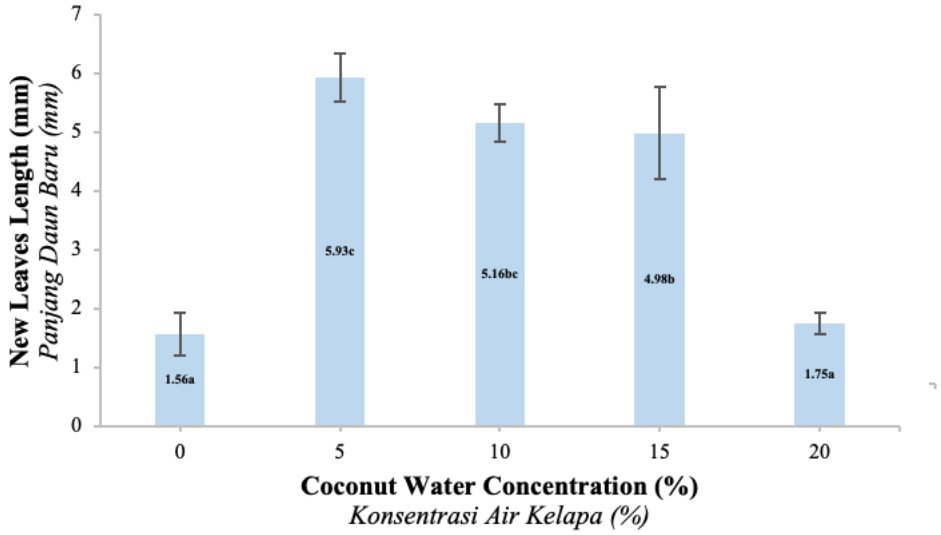

Figure 1. New shoots and new leaves emergence time on *D. spectabile* orchid for eight weeks with various coconut water concentration treatments. Data with different notations indicate significantly different results according to the LSD test at 5% significance (*Waktu muncul tunas baru dan daun baru pada anggrek D. spectabile selama 8 minggu dengan perlakuan berbagai konsentrasi air kelapa. Data dengan notasi yang berbeda menunjukkan hasil yang berbeda nyata berdasarkan uji LSD pada signifikansi 5%*).

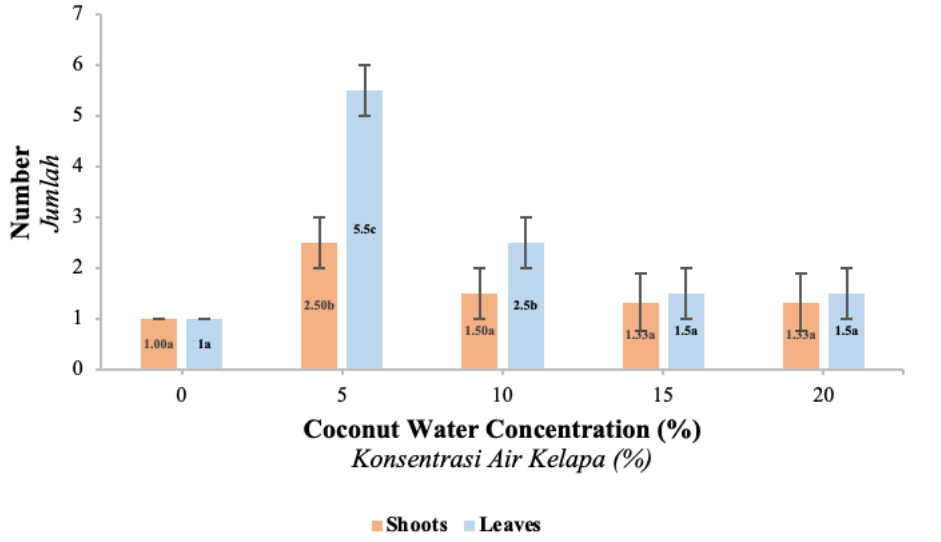

Figure 2. Number of new shoots and new leaves on *D. spectabile* orchid for eight weeks with various coconut water concentration treatments. Data with different notations indicate significantly different results according to the LSD test at 5% significance *(Jumlah tunas baru dan daun baru pada anggrek D. spectabile selama 8 minggu dengan perlakuan berbagai konsentrasi air kelapa. Data dengan notasi yang berbeda menunjukkan hasil yang berbeda nyata berdasarkan uji LSD pada signifikansi 5%).*

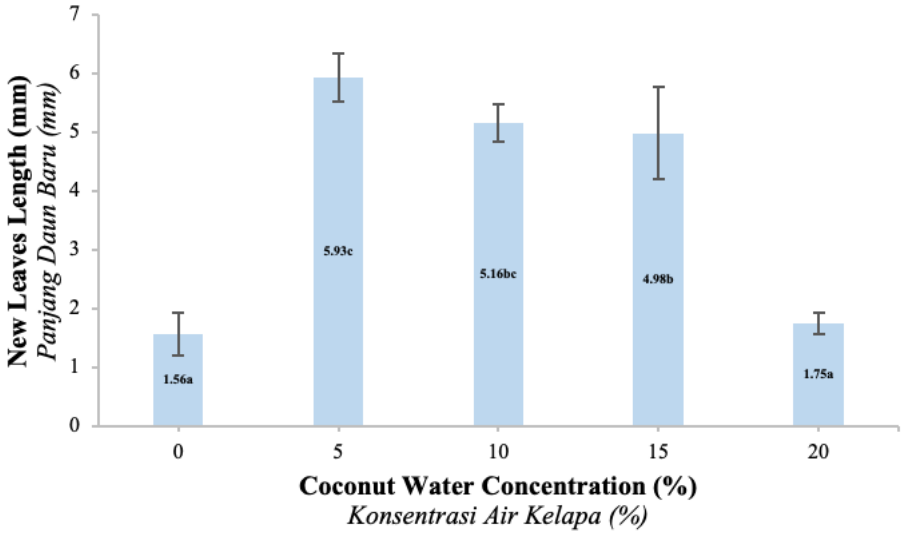

Figure 3. New leaves length on D. spectabile orchid after eight weeks with various coconut water concentration treatments. Data with different notations indicate significantly different results according to the LSD test at 5% significance (*Panjang daun baru pada anggrek D. spectabile setelah 8 minggu dengan perlakuan berbagai konsentrasi air kelapa. Data dengan notasi yang berbeda menunjukkan hasil yang berbeda nyata berdasarkan uji LSD pada signifikansi 5%*).

Root Emergence Time and Number of New Roots

The addition of coconut water to *D. spectabile* plants at various concentrations had no effect on the parameters of new root emergence time or new root number (Table 1). This finding can be confirmed by the absence of any new roots in the plants treated with different concentrations of coconut water (0%-20%), making the ANOVA test unable to be conducted.

Table 1. Mean time to root emergence (days) and the number of new roots of *D. Spectabile* orchid for eight weeks in various coconut water concentrations (*Rerata waktu muncul akar (hari) dan jumlah akar baru anggrek D. spectabile selama 8 minggu pada perlakuan berbagai konsentrasi air kelapa*).

Coconut Water Concentration *(Konsentrasi Air Kelapa)* (%)	**Observation Parameters** *(Parameter Pengataman)*	
	New Roots Emergence Time (days) *(Waktu Muncul Akar Baru) (hari)*	**Number of New Roots** *(Jumlah Akar Baru)*
0	N	N
5	N	N
10	N	N
15	N	N
20	N	N

Note: N: None; Data cannot be conducted ANOVA test
(Keterangan: N: Tidak ada; Data tidak dapat dilakukan uji ANOVA)

DISCUSSION

Shoot and Leaf Growth

The treatment of 5% coconut water causes the plants to emerge more new shoots and new leaves the fastest (Figure 1) and the highest number of new shoots and new leaves (Figure 2). It is assumed that coconut water contains cytokinin and auxin hormones in the right concentration, which can activate transcription factors so that there is gene expression that plays a role in cell division and differentiation in the meristem, which results in the formation of new shoots and leaves increased in *D. spectabile* orchid plants. According to Ljung (2013), hormones at low concentrations that bind to their specific receptors will activate transcription factors so that gene expression that leads to plant growth can occur. Cytokinins contained in coconut water will activate phosphatase enzymes to reduce phosphate groups that bind to Cyclin-Dependent Kinases (CDKs) proteins so that these proteins can accelerate cell division, resulting in faster emergence of shoots and leaves. Therefore, the number of shoots and leaves increases (Lipavská *et al*., 2011).

The formation of new shoots (Figure 4) in *D. spectabile* orchids involves two hormones that regulate meristem, specifically cytokinin and auxin. Cytokinin regulates the process of cell division, while auxin plays a role in the process of organ initiation. The mechanism of shoot formation starts with cytokinin regulating the expression of WUSCHEL transcription factor (WUS), resulting in cell division in the meristem's Organizing Center (OC). The WUS will trigger the expression of the CLAVATA3 (CLV3) gene, which plays a role in determining cell identity. Furthermore, the CLV3 gene will interact with CLAVATA1 (CLV1) in the Central Zone (CZ) to inhibit the expression of the WUS gene so that the cells that have determined their identity will be pushed toward the Peripheral Zone (PZ) to undergo differentiation to form shoot primordia. Afterward, the transmembrane protein PIN-FORMED1 (PIN1), an auxin exporter translocates auxin to the PZ so there is a high concentration of auxin. The auxin will trigger shoot initiation in cells located in the PZ, which is characterized by the formation of shoot primordia that eventually develop into shoots (Lee *et al*., 2019).

The number of leaves (Figure 2) in *D. spectabile* plants increased due to the formation of new leaves. Based on Kalve *et al*. (2014), the process of leaf formation begins with the cytokinin hormone regulating the expression of the transcription factor WUSCHEL (WUS) in the Rib Zone (RZ). Then WUS will activate the CLAVATA3 (CLV3) gene in the Central Zone (CZ) to promote growth in the Shoot Apical Meristem (SAM). WUS also inhibit ARABIDOPSIS type-A RESPONSE REGULATORS (ARRs), which can interfere with cytokinin signaling so cell division can still occur. Furthermore, CLV3 binds to CLAVATA1 (CLV1), which inhibits WUS expression. As a result, cells in the CZ will head to the Peripheral Zone (PZ) to undergo differentiation to form leaf primordia which then develop into leaves. This can occur due to the presence of high concentrations of auxin in the PZ because auxin translocation is assisted by the PIN-FORMED1 (PIN1) transporter, so the number of leaves has increased.

The growth of shoots in *D. spectabile* plants, illustrated in Figure 4, and the growth of leaves in *D. spectabile*, as represented in Figure 5, are also influenced by coconut water, which contains several minerals such as nitrogen, phosphorus, and calcium that play a role in cell division, so the formation of shoots and leaves has increased. Shukla *et al*. (2014) explained that macronutrients in the form of N, P, and Ca play a role in cell division in a plant. The element N is a component of chlorophyll that is needed in the process of photosynthesis to produce carbohydrates. Then these carbohydrates will be used as a substrate for respiration to produce energy. The energy from respiration will later be used to promote cell division, followed by cell enlargement, and then cell differentiation occurs, which leads to leaf formation, so the number of leaves increases (Hassan *et al*., 2015). Moreover, increasing N levels in plants will increase cell division because nitrogen acts as a transcription factor to promote ADENOSINE PHOSPHATE-ISOPENTENYLTRANSFERASE3 (IPT3) gene expression, which plays a role in accelerating cytokinin synthesis so that shoots and leaves will form more rapidly (Sakakibara, 2021). Besides the nitrogen element, there is phosphorus which plays a role in the process of protein phosphorylation to activate the cyclin-CDK complex in the cell cycle so that cell division proceeds more rapidly (Sabu

et al., 2021). Coconut water added to VW medium also contains calcium. Calcium in the form of Ca^{2+} ions plays a role in neutralizing negatively charged DNA, so the shortening and thickening of chromatin threads into chromosomes in the mitotic phase of the cell cycle can occur (Fioreze *et al*., 2018).

In the parameter of leaf length, coconut water at 5% concentration added to VW medium caused *D. spectabile* orchids to have the longest leaves (Figure 3). This is because coconut water contains the auxin hormone, which can stimulate cell expansion, resulting in increased leaf length. According to Du *et al*. (2020), auxin will activate the H+ proton pump in the plasma membrane causing H+ ions to be pumped to the cell wall. Furthermore, these ions will activate the expansin enzyme, which causes the broken hydrogen bonds that compose the cell wall to be broken and the cell wall structure to loosen. As a result, the pressure on the cell wall decreases, so water will enter the cell by osmosis and cell expansion occurs.

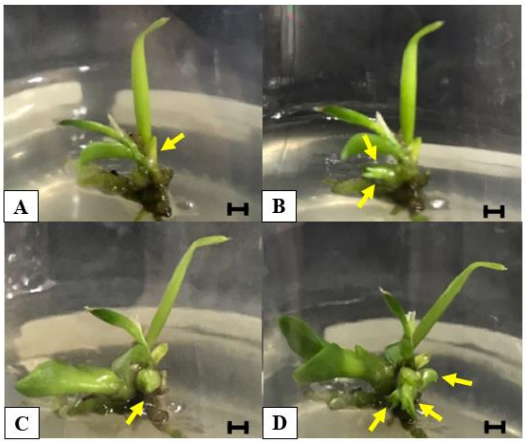

Figure 4. Growth of new shoots (pointed arrows) on *D. spectabile* plantlets treated with 5% coconut water; A. Week 2; B. Week 4; C. Week 6; D. Week 8 *(Pertumbuhan tunas baru (ditunjuk anak panah) pada planlet D. spectabile dengan perlakuan konsentrasi 5% air kelapa; A. Minggu ke-2; B. Minggu ke-4; C. Minggu ke-6; D. Minggu ke-8)* (Bar = 10 mm).

Coconut water with higher concentrations than 5% (Figure 1-3) added to VW medium will inhibit the growth of *D. spectabile* plants. This is likely due to the high concentration of coconut water added to the medium, and it is assumed that the hormones contained in the coconut water are also more highly concentrated. The hormone is a chemical signal that is needed in low concentrations to be able to stimulate the growth of shoots and leaves. Hormones used in high concentrations will inhibit the growth of shoots and leaves because they are chemical signals that compete with each other to bind to receptors, causing the signals to be ineffective, affecting gene expression, and inhibiting shoot and leaf growth (Ljung, 2013). Furthermore, it is known that hormones are chemical signals that play a role in activating genes related to the regulation of plant growth and development processes. Hormones need to be present in low concentrations and interact either synergistically or antagonistically in regulating plant growth and development processes (Agudelo-Morale *et al*., 2021).

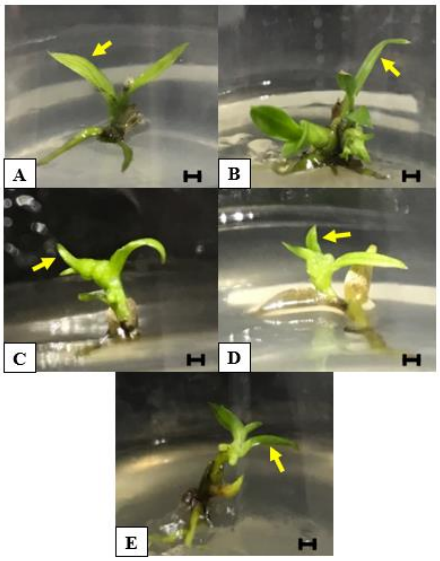

Figure 5. Leaf growth (pointed arrows) on D. spectabile plantlets treated with various concentrations of coconut water during week 8; A. 0%; B. 5%; C. 10%; D. 15%; E. 20% *(Pertumbuhan daun (ditunjuk anak panah) pada planlet D. spectabile dengan perlakuan berbagai konsentrasi air kelapa pada minggu ke-8; A. 0%; B. 5%; C. 10%; D. 15%; E. 20%)* (Bar = 10 mm).

D. spectabile orchid plants supplemented with coconut water at 0% concentration (control) produced the longest shoot and leaf emergence time (Figure 1), the least number of shoots and leaves (Figure 2), and the shortest leaves (Figure 3). This is presumably because there is no supply of hormones derived from adding coconut water. Meanwhile, the concentration of endogenous plant hormones is too low to activate transcription factors and stimulate gene expression in response to plant needs, particularly in cell division, cell expansion, and cell differentiation required to initiate new shoots and leaves. This is in line with the statement of Shan *et al*. (2012) that adding exogenous hormones using coconut water is necessary for plants to increase their vegetative growth, although each plant produces endogenous hormones. Very low concentrations of endogenous hormones cannot bind to receptors, resulting in bonds between repressor proteins and transcription factors so that growth-related genes cannot be expressed and the formation of shoots and leaves is obstructed.

The research showed that generally, there is an influence on the time of shoot and leaf emergence, the number of shoots and leaves, and the length of new leaves of *D. spectabile* after being treated with coconut water on VW medium. Research conducted by Eriansyah *et al*. (2014) and Matloob *et al*. (2017) gave similar results, which is the addition of coconut water with various concentrations affected the time of shoot emergence on tapak dara (*Catharanthus roseus*) plants. Besides that, the number of shoots on glutinous bananas grown *in vitro* with 12% coconut water as an optimal concentration in emerging new shoots. The addition of coconut water with several concentrations influenced the time of leaf emergence and the number of new leaves of *D. anosmum* orchids cultured *in vitro*. The 5% concentration produced leaves with the fastest time and the highest number of leaves at 11 weeks after planting, which was 11.63 leaves (Tuhuteru *et al*., 2012). A previous study by Pakum *et al*. (2016) explained that 15% of coconut water added to *Bulbophyllum nipondhii* orchid plants is the best concentration because it can produce the longest average leaf, 10.4 mm.

Root Emergence Time and Number of New Roots

Coconut water added to *D. spectabile* plants with different concentrations did not affect root emergence time and the number of new roots (Table 1). It is suspected that the coconut water added to the medium contained less auxin than cytokinin for the formation of the roots. Therefore, it was unable to sufficiently supply the plant's necessity in activating transcription factors to express genes related to the formation of new roots, so the root formation was retarded. Pratama and Nilahayati (2018) elaborated that root formation can occur if the ratio of auxin to cytokinin hormones is high.

On the other hand, very low hormone concentrations cannot activate current transcription factors to express genes related to root formation. Thus, binding between transcription factors and repressor proteins so genes that play a role in root formation are not expressed, resulting in the inhibition of root growth (Shan *et al*., 2012; Gallei *et al*., 2020). According to Su *et al*. (2011), the process of lateral root formation can occur due to the transport of auxin to the pericycle cells with the help of the PIN-FORMED (PIN) transporter. Auxin signals will then be recognized by their specific receptors, resulting in the degradation of repressor proteins to activate transcription factors and trigger the expression of the LATERAL ORGAN BOUNDARIES DOMAIN 16/29 (LBD16/29) gene. The gene expression will promote asymmetric division in the pericycle cells. Contrarily, cytokinin inhibits the expression of the PIN transporter, thereby inhibiting auxin translocation. As a result, the divided pericycle cells will differentiate into lateral root primordia, which then develop into lateral root organs.

The addition of coconut water to VW medium in this study did not affect the time of root emergence and the number of new roots of *D. spectabile* orchid. These results contradict Karunarathna *et al*. (2022), which showed that the time of root emergence and the number of new roots of stevia (*Stevia rebaudiana*) plants grown *in vitro* were affected by applying coconut water with different concentrations. Coconut water at 5% concentration emerged roots with the fastest time (6.66 days) and the highest number of roots (9.83). This may occur because the genotypes of one plant differ, so that the hormones needed are also at different concentrations to promote organogenesis, including roots (Rachmi *et al*., 2020).

CONCLUSION

The addition of coconut water affects the growth of shoots and leaves of *D. spectabile* plantlets in VW medium but has no effect on root emergence time and the number of new roots. Coconut water at 5-10% is the optimal concentration for the growth of *D. spectabile* plantlets in VW medium.

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AUTHOR CONTRIBUTIONS

AZ: contributed to the research data collection, analyzed the data, article preparation, and manuscript revision. NS: corresponding author: conceptualized the research, helped draft the article, and finalized the manuscript; YN: helped draft the article, reviewer for this student research.

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