Jurnal Ilmu Pertanian Indonesia (JIPI), Juli 2025
(Indonesian Journal of Agricultural Sciences)
ISSN 0853-4217 EISSN 2443-3462

Vol. 30 (3): 533–539
http://journal.ipb.ac.id/index.php/JIPI
DOI: 10.18343/jipi.30.3.533

Effect of Different Coconut Water Concentrations on the Growth of *Dendrobium spectabile* (Blume) Miq. Planlet on MS Media

Faradita Azzahra Santosa, Nintya Setiari*, Yulita Nurchayati

(Received January 2024/Accepted May 2025)

ABSTRACT

***Dendrobium spectabile* is an orchid with unique flowers that are vulnerable to exploitation. Tissue culture is employed to conserve this orchid, where the type of medium and organic supplements significantly affect plantlet growth. *Murashige and Skoog* (MS) media was added with coconut water as an exogenous hormone source. This study aimed to determine the effect of adding coconut water and determine the optimal concentration to increase the growth of *D. spectabile* at the subculture stage. The method involved subculturing *D. spectabile* plantlets *in vitro* in MS media supplemented with coconut water at concentrations of 0%, 5%, 10%, 15%, and 20%. The research design used a completely randomized design (CRD), with coconut water concentration and ten replicates. Data were analyzed using ANOVA and Duncan's Multiple Range Test at a 5% significance level. Plantlet growth was observed for two months, and the parameters observed were the emergence of new shoots, leaves, and roots; the number of new shoots, leaves, and roots; and the length of new leaves. The results showed that the addition of coconut water to the subculture medium can increase the leaf growth of *D. spectabile* orchid plantlets but does not increase shoot and root growth. Coconut water 10% was the most optimal concentration for the number of new leaves and new leaf length. The addition of 15% coconut water was the most optimal concentration for plantlet new leaf emergence. Coconut water plays a vital role in *in vitro* culture, supporting the various growth processes of *D. spectabile* plantlets.**

Keywords: ***Dendrobium spectabile, ex situ conservation, organic supplement, subculture***

INTRODUCTION

Dendrobium spectabile is a curly-shaped orchid found in Papua, Papua New Guinea, and the Solomon Islands (Deswiniyanti 2015). *D. spectabile* is listed in CITES Appendix II, meaning that the international trade of this orchid species is strictly controlled due to excessive exploitation, which has led to a decline in its population in the wild (CITES 2020). This highlights the importance of conservation efforts for orchids through *ex situ* cultivation. The problem faced in ex-situ orchid conservation is that orchids have a slow growth rate (Zhang *et al*. 2018). Therefore, a plant propagation technique that allows for relatively rapid growth, such as plant tissue culture, commonly referred to as in vitro techniques, is needed. The subculture stage is an important stage in plant propagation through tissue culture. Subculture is the process of replacing the old nutrient media with fresh culture media, ensuring that the nutritional needs for plantlet or seedling growth are met (Elfiani and Jakoni 2015)

The selection of the right media and organic supplements will help in the growth and development of orchids during the subculture stage. Murashige and Skoog (MS) media is a growth medium that contains a complete set of macro- and micronutrients, as well as vitamins. However, MS medium lacks hormone composition as a growth regulator; therefore, exogenous supplementation is required. One source of hormones as exogenous growth regulators is coconut water. MS media enriched with coconut water is believed to optimize the growth of orchid plantlets (Pratama and Nilahayati 2018).

Coconut water is a liquid endosperm derived from coconuts (*Cocos nucifera*) and contains vitamins, organic compounds, sucrose, and hormones from the cytokinin and auxin groups that support plantlet growth and development (Saepudin *et al*. 2020). According to Santoso *et al*. (2020), the addition of 15% coconut water to subculture media resulted in the best growth of protocorms and shoots of *Phalaenopsis* sp. orchids. However, there has been limited research, especially regarding the addition of coconut water during the subculture stage, to support the growth of *D. spectabile* orchids. Therefore, this study was conducted to determine the effect of adding coconut water at different concentrations and to identify the optimal concentration for enhancing the growth of *D. spectabile* orchid plantlets during the subculture stage.

Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang 50275, Indonesia
* Corresponding Author:
Email: nintyasetiari@lecturer.undip.ac.id

METHODS

Research Time and Place

This research was conducted for 2 months, from April 2022 to June 2022. The work was conducted at the Tissue Culture Laboratory, Plant Structure and Function Biology, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, Central Java.

Tools and Materials

The materials used in this study included Murashige and Skoog (MS) media, distilled water, NaOH solution, HCl solution, tissue, solidifying agent (agar), sucrose, coconut water, and approximately 1-year-old *D. spectabile* orchid plantlets.

Preparation of Coconut Water and Media Making

The coconut water used was from young green coconut fruit. Coconut water was filtered using sterile filter paper to separate the residue. Furthermore, coconut water was measured according to the treatment.

The initial steps for preparing 1 L of media were as follows: 4.43 gL^{-1} ready-to-use MS media and 30 gL^{-1} sucrose were placed into an Erlenmeyer flask and homogenized. Next, coconut water was added to the media according to the treatment. The pH was measured using a *pH stick* until it reached ± 6. Gelzan (2 gL^{-1}) and distilled water were added, homogenized, and heated until completely dissolved. The media solution was placed in culture bottles and covered with aluminum foil. The bottles containing the media were sterilized in an autoclave for 15 minutes at 121°C. Sterilized bottles were placed on culture racks in an incubator.

Subculture Process

Subculturing began with the planlet removed from the bottle, and the planlet was planted into the treatment media, with each bottle containing one planlet. The bottle was then covered with sterile aluminum foil and sealed with plastic wrap. Subculture bottles were incubated in a room with 600 lx TL lighting, and the temperature of the culture room was set at 25°C.

Observations and Data Analysis

Plantlet growth was observed for 2 months. The parameters observed were the time to appearance of new shoots, leaves, and roots; the number of new shoots, leaves, and roots; and the length of new leaves. This study used a completely randomized design (CRD) to determine the differences in orchid planlet growth due to the application of different coconut water concentrations. The concentrations of coconut water were 0%, 5%, 10%, 15%, and 20%. Data were analyzed using analysis of variance (ANOVA) at a 95% confidence level. If there is a significant difference, further tests are carried out using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Time of Emergence and Number of New Shoots

The shoot growth response of orchid plantlets subcultured into new media with the addition of coconut water is shown in Figure 1 and 2. The ANOVA results indicated that the addition of coconut water at different concentrations to *D. spectabile* orchid plantlets had no significant effect on the time of new shoot emergence or the number of new shoots. This is probably because the endogenous hormones contained in the planlets

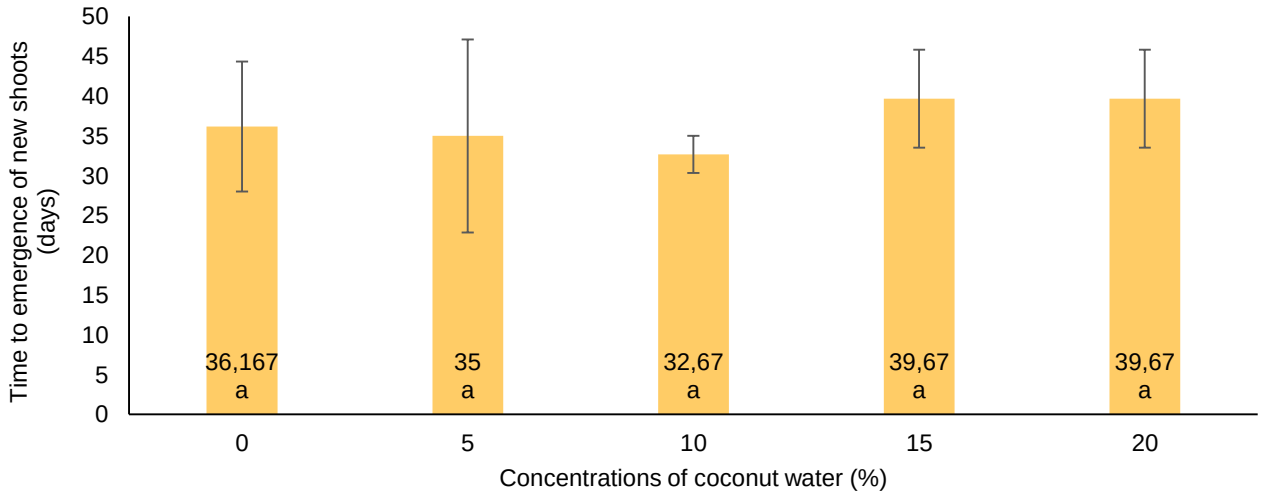

Figure 1 Time to emergence of new shoots (days) of orchid planlets D. spectabile for 8 weeks in the treatment of various concentrations of coconut water. Data followed by different letters indicate significantly different results based on DMRT test at 5% significance.

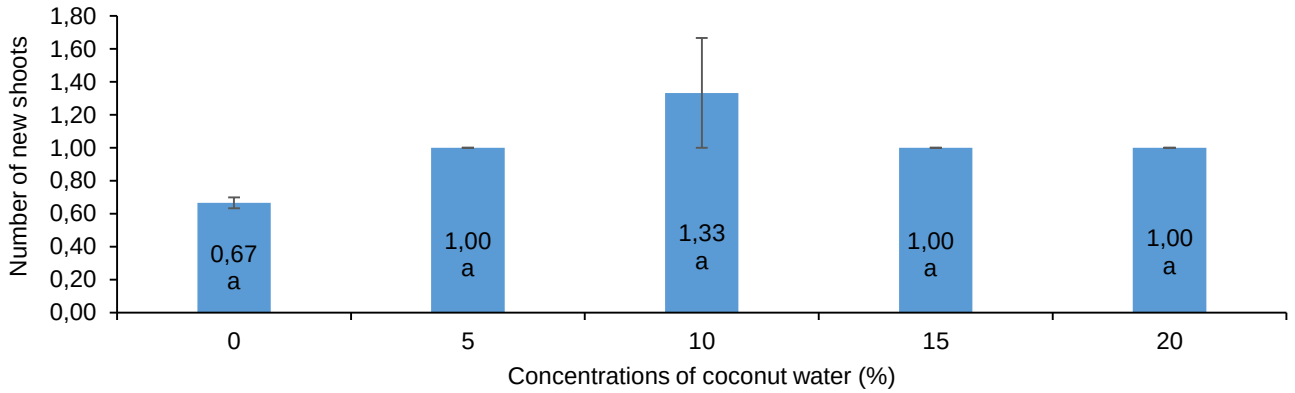

Figure 2 Number of new shoots of D. spectabile orchid planlets for 8 weeks in the treatment of various concentrations of coconut water. Data followed by different letters indicate significantly different results based on DMRT test at 5% significance.

were still sufficient for growth, so that the addition of hormones through coconut water had no effect. According to Rohmah *et al*. (2021), the addition of exogenous hormones does not affect plant growth if the endogenous hormones in the plant are sufficient. These results are like those of Nandariyah *et al*. (2021), that the addition of coconut water at different concentrations to MS media did not affect the time to emerge or the number of new shoots in garlic plants. However, new shoots tended to emerge the fastest (32.67 d) and in the greatest number (1.33 shoots) in plantlets supplemented with 10% coconut water.

Shoot growth was the fastest and most abundant in plantlets subcultured on MS media supplemented with 10% coconut water. This is probably because the endogenous hormone content in the plant is still sufficient for the needs of shoot growth, so the exogenous hormones needed tend to be low. Shoot growth was inhibited in media supplemented with high concentrations of coconut water (15% and 20%), as indicated by the long shoot emergence time (Figure 3 D-E). It is suspected that the addition of coconut water with a high concentration contains higher levels of cytokinins, which affects the balance of the ratio between exogenous and endogenous hormones. This is in accordance with the statement of Srinivasa *et al*. (2018), that the addition of coconut water as a source of organic supplements with high concentrations can inhibit shoot growth because it has an impact on the imbalance between exogenous hormones and endogenous hormones.

A coconut water concentration of 0% (control) produced the fewest shoots (Figure 3A). This is because the planting media without the addition of coconut water do not have exogenous cytokinin content, which cannot encourage planlets to produce more shoots. Although it is suspected that the endogenous cytokinin content in the plantlets is sufficient for shoot growth, the addition of coconut water as an additional cytokinin source with the right

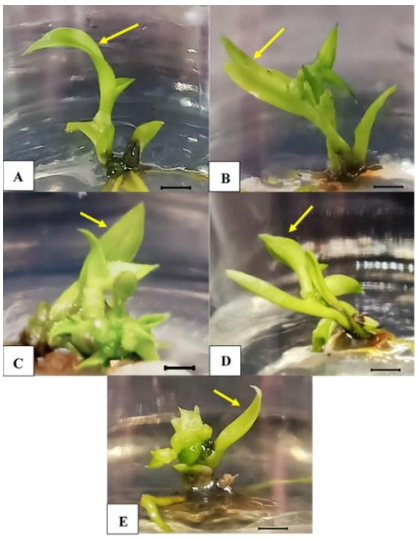

Figure 3 Growth of new leaves (indicated by arrows) on D. spectabile planlets treated with various concentrations of coconut water at week 8; A. 0%; B. 5%; C. 10%; D. 15%; E. 20%

concentration can be a driving factor that can produce faster and more shoot growth. This is in accordance with Pratama and Nilahayati (2018), who stated that coconut water added to the growth medium can increase the activity of endogenous cytokinin because coconut water contains cytokinin, which accelerates cell division and affects the increase in the number of shoots formed.

Time of Emergence, Number, and Length of New Leaves

ANOVA showed that the application of coconut water in the growth medium significantly influenced the time of new leaf emergence in *D. spectabile*. The DMRT test results showed that 15% coconut water tended to cause leaves to appear faster than other treatments (Figure 4). It is suspected that coconut water contains hormones that can encourage

endogenous hormone production, resulting in leaf organogenesis. Kalve *et al*. (2014) explained that leaf formation begins in the rib zone (RZ) of the SAM (shoot apical meristem) with WUSCHEL factor transcripts (WUS), which play a role in the process of cell division activated by cytokinin hormones. The process of cell division can continue because WUS can induce the expression of CLAVATA3 (CLV3), while suppressing the response of regulatory proteins that can interfere with cytokinin signaling. However, WUS must be stopped so that cells that have divided can differentiate. Therefore, the process of inhibiting WUS is carried out by CLV3, which binds to CLV1, so that cells in the CZ are pushed to the peripheral zone (PZ) to differentiate. Cell differentiation can occur with the help of the hormone auxin, which is translocated by the transmembrane protein PIN-FORMED1 (PIN1) to the PZ, which eventually forms leaf primordia and develops into leaves.

The addition of coconut water at a higher concentration (20%) slowed down the time of leaf emergence, presumably because the hormone content in coconut water works more effectively at low concentrations. According to Boyola *et al*. (2024), the application of hormones as growth regulators must be at the right concentration because if it is too high, it can inhibit plant growth, whereas if it is too low, the effect becomes invisible. This is in accordance with the results of the study by Lewar *et al*. (2023), which showed that the higher the concentration of coconut water added, the slower the speed of seed growth, because coconut water contains hormones that actively work at low concentrations.

The 10% coconut water added to MS media produced the highest number of new leaves in orchid planlets (Figure 5). This is because coconut water contains nitrogen, magnesium, and manganese, which play a role in the formation of vegetative organs (Dyarta and Dewanti 2023). Saepudin *et al*. (2012) stated that

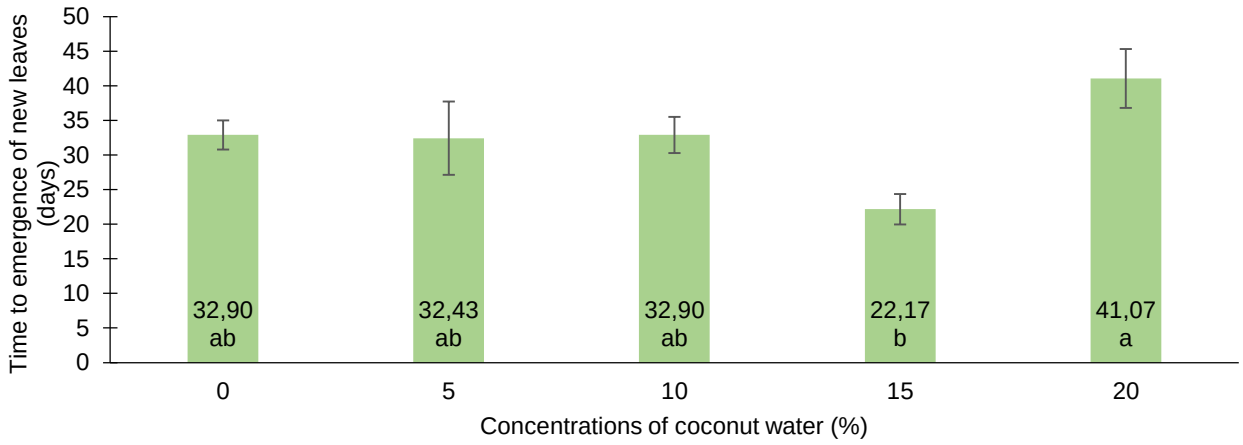

Figure 4 Time to emergence of new leaves of *D. spectabile* orchid planlets for 8 weeks in the treatment of various concentrations of coconut water. Data followed by different letters indicate significantly different results based on DMRT test at 5% significance.

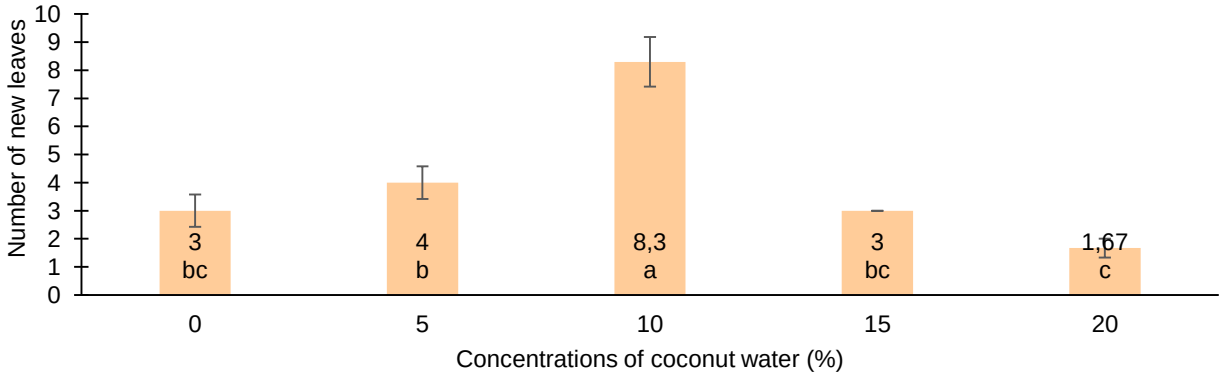

Figure 5 Number of new leaves of D. spectabile orchid planlets for 8 weeks in the treatment of various concentrations of coconut water. Data followed by different letters indicate significantly different results based on DMRT test at 5% significance.

the N element plays a role in the process of synthesizing amino acids and proteins which are then used in the process of plant growth and development. Potassiu (K) acts as an activator of various enzymes. The elements Mg and Mn in coconut water are chlorophyll components that are useful in photosynthesis.

The longest new leaves were produced on plants treated with 10% coconut water (Figure 6). This is because coconut water contains the hormone cytokinin, which can stimulate leaf elongation. Nursetiadi *et al*. (2016) suggested that the addition of cytokinin can promote leaf elongation, but the addition of high concentrations of cytokinin will inhibit the growth of leaf length. Auxin is another hormone present in coconut water besides cytokinin. Auxin triggers leaf elongation and encourages the development of meristems that have the potential to form young leaves. The mechanism of auxin in cell elongation begins with the proton pump transferring H^+ with the help of ATP at the plasma membrane. H^+ is directed to the cell wall, where it forms H^+–ATPase to acidify the extracellular matrix. When the matrix is acidic, the ion pump activates the expansin enzyme, which breaks the hydrogen cross-links that make up the cell wall, loosening the cell wall, increasing water absorption into the cell, and increasing turgor pressure, making the cell elastic and increasing its length (Dünser and Kleine-Vehn 2015).

The results showed that the addition of 20% coconut water resulted in the slowest growth in leaf growth parameters (time of emergence, number, and length of new leaves) (Figure 3E). This is probably because 20% coconut water is too high for *D. spectabile* planlet leaf growth. The hormone content in coconut water is more effective at low concentrations; therefore, if given at excessive concentrations, it can inhibit planlet growth. Coconut water of 15% produced the fastest leaf emergence time, while 10% coconut water was the best treatment that produced the most optimal number and length of leaves. This is thought to be due to the role of each hormone in coconut water, which optimally works at certain phases. According to Salamah *et al*. (2022), coconut water contains more cytokinin (5.8 mg/L) than auxin (0.07 mg/L) and a small amount of gibberellin. In the leaf initiation phase, cell differentiation occurs, which requires a balanced working interaction between cytokinin and auxin to reach the division phase. During the growth phase, a higher ratio of cytokinin is required than other hormones. Therefore, coconut water at a concentration of 10% is sufficient to meet the cytokinin needs of *D. spectabile* plantlets for leaf growth.

Time of Emergence and Number of New Roots

ANOVA test results showed that the addition of coconut water to the growth medium of *D. spectabile* orchid planlets did not significantly affect the time of new root emergence and the number of new roots. It is suspected that the nutrients and endogenous hormones in the plantlets are sufficient to stimulate root organogenesis. Dasuha (2022) and Nandariyah *et al*. (2021) reported that the addition of coconut water had no effect on the time of root emergence of garlic plants and the number of roots in orchid plants, because endogenous hormones and nutrients were sufficient for the needs of planlets in the growth process (Rohmah *et al*. 2021).

Coconut water with 0% concentration (control) tended to produce roots with the fastest time and highest number (Figure 7 and 8). It is suspected that the ratio of endogenous auxin and cytokinin hormones in the plantlets is sufficient for root formation. According to Rohmah *et al*. (2021), if the ratio of endogenous hormones in the plant is sufficient, the addition of coconut water, which contains exogenous hormones, can change the ratio of endogenous hormones in the plant. Lateral root formation starts from the auxin signal, which is translocated by the auxin transporter to the pericellular cells, which are received by the auxin

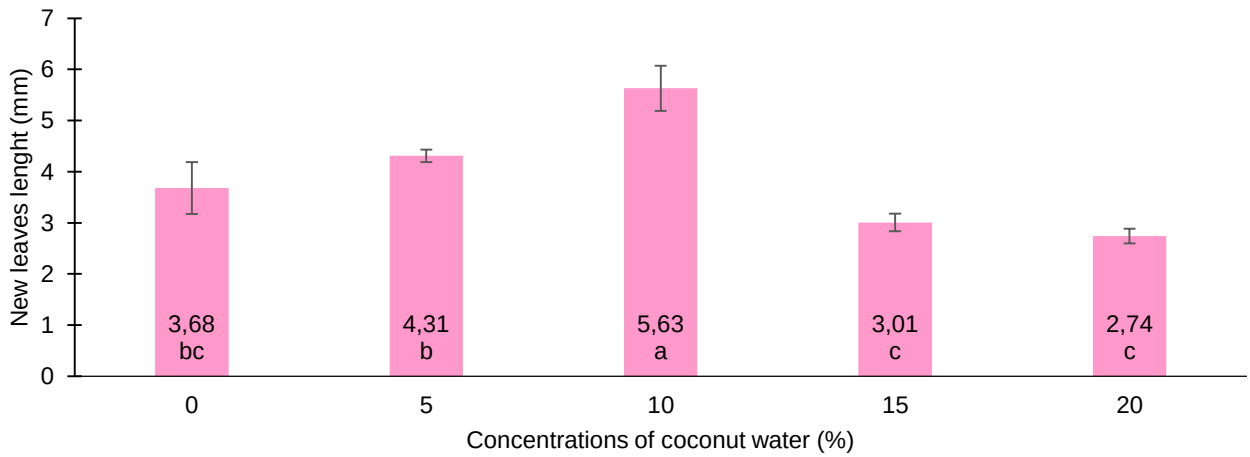

Figure 6 Length of new leaves (mm) of orchid planlets D. spectabile for 8 weeks in the treatment of various concentrations of coconut water. Data followed by different letters indicate significantly different results based on DMRT test at 5%

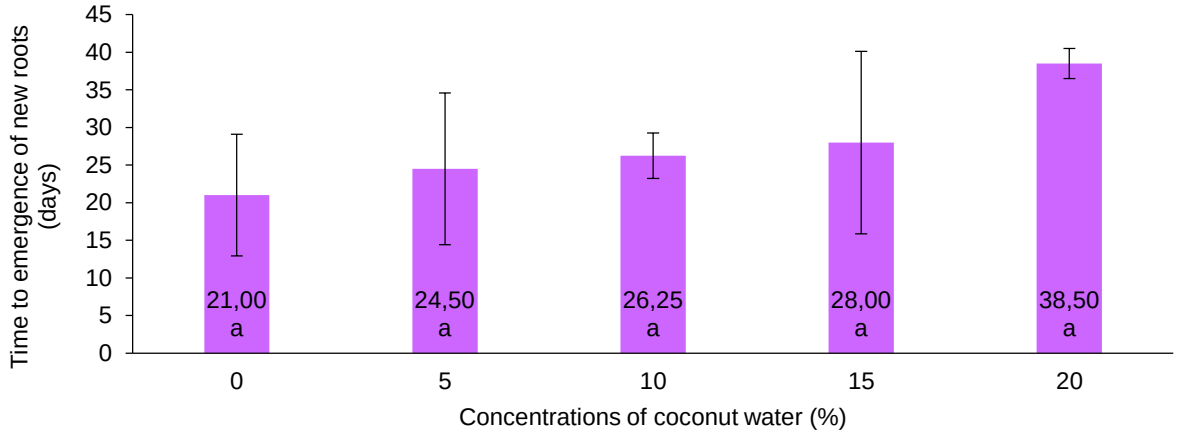

Figure 7 Time to emergence of new roots of D. spectabile orchid planlets for 8 weeks in the treatment of various concentrations of coconut water. Data followed by different letters indicate significantly different results based on DMRT test at 5% significance.

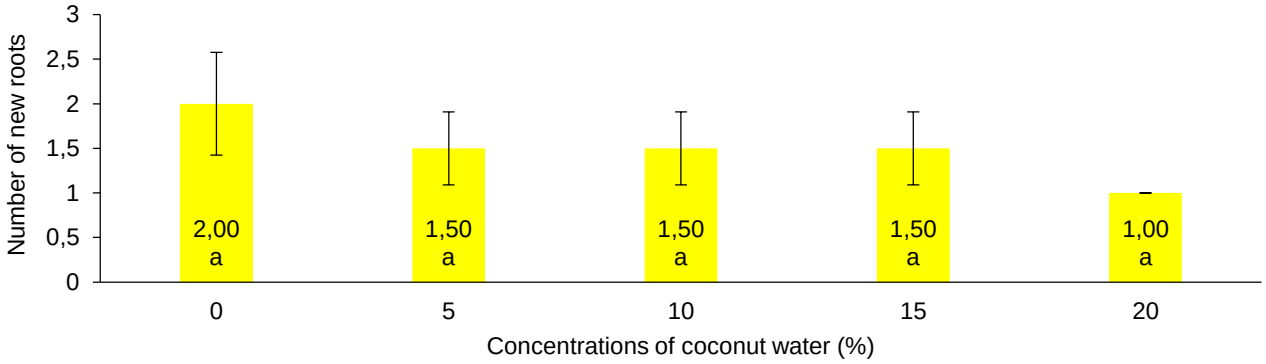

Figure 8 Number of new roots of D. spectabile orchid planlets for 8 weeks in the treatment of various concentrations of coconut water. Data followed by different letters indicate significantly different results based on DMRT test at 5% significance.

receptor. The auxin signal received by the receptor activates certain genes for asymmetric cell division in a periclinal or anticlinal manner, forming a collection of cells. Simultaneously, cytokinin hormones inhibit auxin transporter proteins to prevent auxin accumulation. The root primordium then increases in length, penetrating the primary root cortex, and lateral roots appear on the surface of the parent root (Su *et al*. 2011).

The 20% coconut water added to the growth medium showed the longest root growth (Figure 7) and the least amount (Figure 8). This is thought to be due to exogenously added hormones and nutrients from coconut water levels that are too high, resulting in inhibited formation. Singh (2018) stated that exogenously added hormones are needed to improve the growth process of plants, even though each plant already has endogenous hormones. According to Saepudin *et al*. (2020), increased vegetative growth in plants can occur if coconut water is added at low concentrations, and if at high concentrations, it can inhibit plant growth.

CONCLUSION

The addition of coconut water to the subculture media can increase the growth of *D. spectabile* planlet leaves but does not increase the growth of shoots and roots. A coconut water concentration of 10% was the optimal concentration for the growth of *D. spectabile* plantlets at the subculture stage in MS media, as determined by the number of new leaves and the length of new leaves, while 15% concentration was the optimal concentration of coconut water when new leaves appeared.

ACKNOWLEDGMENT

The authors are grateful to Diponegoro University for funding this research through the Riset Publikasi Internasional or RPI scheme No.569-110/UN7.D2/PP/VII/2022.

REFERENCES

Boyola KH, Hartati RM, Setyawati ER. 2024. Pengaruh macam dan konsentrasi zat pengatur tumbuh alami terhadap pertumbuhan stek mawar (*Rosa* sp.). *Agroforetech*. 4(2): 1676–1681.

Deswiniyanti NW. 2015. *In Vitro* Propagation of native orchid *Dendrobium spectabile* (Blume) Miq. *II International Orchid Symposium*. 1078: 69–73. https://doi.org/10.17660/ActaHortic.2015.1078.8

Dünser K. Kleine-Vehn J. 2015. Differential growth regulation in plants—the acid growth balloon theory. *Current opinion in plant biology*. 28: 55–59. https://doi.org/10.1016/j.pbi.2015.08.009.

Dyarta S, Dewanti P. 2023. Optimasi pertumbuhan planlet anggrek *Dendrobium gabriella surajaya* menggunakan media alternatif AB Mix dan air kelapa secara *in vitro*. *Agrin : Jurnal Penelitian Pertanian*. 27(2): 124–139. https://doi.org/10.20884/1.agrin.2023.27.2.743

Elfiani E, Jakoni J. 2015. Sterilisasi eksplan dan subkultur anggrek, sirih merah dan krisan pada perbanyakan tanaman secara *in vitro*. *Dinamika Pertanian*. 30(2): 117–124.

Gupta P, Sharma S, Saxena S. 2015. Biomass yield and steviol glycoside production in callus and suspension culture of *Stevia rebaudiana* treated with proline and polyethylene glycol. *Applied biochemistry and biotechnology*. 176(3): 863–874. https://doi.org/10.1007/s12010-015-1616-0

Kalve S, De Vos D, Beemster GT. 2014. Leaf development: a cellular perspective. *Frontiers in Plant Science*. 5(362): 1–26. https://doi.org/10.3389/fpls.2014.00362

Lewar Y, Kumanireng KN, Hasan A. 2023. Kajian konsentrasi air kelapa muda sebagai organic priming terhadap viabilitas benih kacang merah yang terdeteriorasi. *Jurnal Pertanian Terapan*. 28(1): 119–130. https://doi.org/10.35726/jp.v28i1.6943

Nandariyah, Mahmudah LS, Arniputri RB, Sakya AT. 2021. The effect of NAA and coconut water combination on garlic (*Allium sativum* L.) tissue culture. *IOP Conference Series: Earth and Environmental Science*. 905(1): 012036. https://doi.org/10.1088/1755-1315/905/1/012036.

Nursetiadi EKA, Yuniastuti E, Putri RBA. 2016. Pengaruh macam media dan konsentrasi BAP terhadap multiplikasi tanaman manggis (*Garcinia mangostana*) secara *in vitro*. *Bioteknol*. 13: 63–72. https://doi.org/10.13057/biotek/c130203.

Pratama J, Nilahayati. 2018. Modifikasi media MS dengan penambahan air kelapa untuk subkultur I anggrek *Cymbidium*. *Agrium*. 15(2): 96–109. https://doi.org/10.29103/agrium.v15i2.1071.

Rohmah AS, Sasmita ER, Wahyurini E. 2021. Pertumbuhan berbagai macam bahan eksplan kentang atlantik secara *in vitro* dengan perlakuan IAA. *Agrosains: Jurnal Penelitian Agronomi*. 23(2): 72–79. https://doi.org/10.20961/agsjpa.v23i2.49027

Saepudin A, Yulianto Y, Aeni RN. 2020. Pertumbuhan eksplan *in vitro* anggrek hibrida dendrobium pada beberapa media dasar dan konsentrasi air kelapa. *Media Pertanian*. 5(2): 97–115. https://doi.org/10.37058/mp.v5i2.2451

Salamah DM, Sulandjari K, Rahayu YS. 2022. Pengaruh pemberian berbagai konsentrasi air kelapa muda terhadap pertumbuhan tunas stek batang ubi kayu (*Manihot esculenta* Crantz) pada varietas Gajah dan Karet. *Jurnal Ilmu Pertanian*. 7(1): 49–54. https://doi.org/10.35329/agrovital.v7i1.2125

Santoso,E, Rahayu T, Hayati A. 2020. Pengaruh air kelapa (*Cocos nucifera* L.) dengan medium VW terhadap pertumbuhan protocorm anggrek secara *in vitro*. *Jurnal Ilmiah Sains Alami*. 3(1): 37–43. https://doi.org/10.33474/j.sa.v3i1.7208.

Singh CR. 2018. Review on problems and its remedy in plant tissue culture. *Asian Journal of Biological Sciences*. 11(4): 165–172. https://doi.org/10.3923/ajbs.2018.165.172

Srinivasa R, Siddiqiu N, Mishra R. 2018. Effect of additive supplements in tissue culture of *Gloriosa superba* L. – a medicinally important plant. *Indian Journal of Applied and Pure Biology*. 33(1): 75–81.

Su YH, Liu YB, Zhang XS. 2011. Auxin-cytokinin interaction regulates meristem development. *Molecular Plant*. 4(4): 616–625. https://doi.org/10.1093/mp/ssr007.

Wang B, Smith SM, Li J. 2018. Genetic regulation of shoot architecture. *Annual review of plant biology*. 69: 437–468. https://doi.org/10.1146/annurev-arplant-042817-040422.

Zhang S, Yang Y, Li J, Qin J, Zhang W, Huang W, Hu H. 2018. Physiological diversity of orchids. *Plant Diversity*. 40: 196–208. https://doi.org/10.1016/j.pld.2018.06.003.