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The Effects of Benzyladenine and Tomato (*Solanum Lycopersicum* L.) Extract Concentrations on Shoot Induction of *Cattleya* sp. Orchid Under In Vitro Culture

Los efectos de las concentraciones de benciladenina y extracto de tomate (*Solanum lycopersicum* L.) sobre la inducción de brotes de *Cattleya* sp. orquídea bajo cultivo in vitro

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Abstract

The research aimed to investigate the effects of tomato extract and benzyladenine (BA) concentrations on the *in vitro* shoot growth of *Cattleya* sp. The study was carried out from March to July 2023 at the Tissue Culture Laboratory of the Hidayatul Islam Foundation, Medan, Indonesia. Four tomato extract concentrations (0 %, 5 %, 10 %, and 15 %) and three BA concentrations (0 ppm, 2 ppm, and 4 ppm) were employed based on the factorial Complete Randomized Design (CRD). Parameters observed include time of shoot emergence, number of shoots, number of leaves, plantlet height, number of roots, and time of root emergence. The Analysis of Variance (ANOVA) followed by the Tukey Multiple Comparison at $\alpha = 0.05$ was applied for the statistical analysis. The result showed that the fastest shoot emergence was found in B4T0 (BA ppm and 0 % tomato extract) treatment at 2 weeks after planting (WAP). Treatment B2T10 (2 ppm BA and 10 % tomato extract) had the highest number of leaves and shoots, 5 shoots and 17.67 leaves respectively. The highest plantlet was found in the B4T0 treatment (3.30 cm). This treatment also showed the fastest root emergence and the largest number of roots (5 WAP and 7 roots respectively). In conclusion, treatment B4T10 (4 ppm BA and 10 % tomato extract) was the most potential combination for *Cattleya sp. in vitro* propagation.

Keywords: *Cattleya sp., benzyladenine, Solanum lycopercicum, In vitro, bud*

Resumen

Este trabajo tuvo como objetivo investigar los efectos del extracto de tomate y las concentraciones de benciladenina (BA) en el crecimiento de brotes in vitro de *Cattleya* sp. El estudio se llevó a cabo de marzo a julio de 2023 en el Laboratorio de Cultivo de Tejidos de la Fundación Hidayatul Islam, Medan, Indonesia. Se emplearon cuatro concentraciones de extracto de tomate (0 %, 5 %, 10 %, 15 %) y tres concentraciones de BA (0 ppm, 2 ppm, 4 ppm) con base en el diseño factorial completo aleatorizado (CRD). Los parámetros observados

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incluyen el tiempo de aparición de los brotes, el número de brotes, el número de hojas, la altura de las plántulas, el número de raíces y el momento de la aparición de las raíces. Para el análisis estadístico se aplicó el Análisis de Varianza (ANOVA) seguido de la Comparación Múltiple de Tukey a $\alpha = 0.05$. El resultado mostró que la emergencia de brotes más rápida se encontró en el tratamiento B4T0 (BA ppm y 0 % extracto de tomate) a las 2 semanas después de la siembra (WAP). El tratamiento B2T10 (2 ppm de BA y 10 % de extracto de tomate) tuvo el mayor número de hojas y brotes, 5 brotes y 17.67 hojas respectivamente. La plántula más alta se encontró en el tratamiento B4T0 (3.30 cm). Este tratamiento también mostró la emergencia de raíces más rápida y el mayor número de raíces (5 WAP y 7 raíces respectivamente). En conclusión, el tratamiento B4T10 (4 ppm de BA y 10 % de extracto de tomate) fue la combinación más potencial para *Cattleya* sp. propagación in vitro.

Palabras clave: *Cattleya sp., Benzyladenine, Solanum lycopercicum, In vitro, brotes*

Introduction

Cattleya sp., also known as "the queen of orchids", has distinctive features of the flower including large, brightly colored, and aesthetically pleasing fragrance. It is widely cultivated due to its high economic value (Novianti et al., 2018). The demand for this species in the market keeps increasing; however, the conventional propagation results in a sluggish multiplication rate and protracted provision periods (Syamsiah et al., 2020). Consequently, the ability to supply planting materials is limited. The proliferation method, which is able to produce significant quantities, is imperative. This can be achieved through the *in vitro* tissue culture method, which is more efficient in time, energy, and space compared to conventional methods (Nurkapita et al., 2021, Harahap, 2023). Tissue culture has been widely adopted in the agricultural sector including horticulture.

The initial step of *in vitro* propagation is the shoot induction, which significantly influences the formation of the plantlet (Akbar, 2017). Planting media is a pivotal factor in determining the success of tissue culture methods. Murashige and Skoog (MS) media is often used for tissue culture as it contains almost all of the essential elements (Nurilmala, 2018). In order to promote the quantity, growth, and quality of the plantlet, enrichment of planting media with supplemental substances is essential (Ningsih & Rohmawati, 2019). Plant growth regulator (PGR) is one of the substances that are often times incorporated into planting media. PGR is an organic compound that has the ability to affect and promote the growth and development of the plant (Ningsih & Rohmawati, 2019).

Naturally occurring growth regulators, auxins, are found in organic materials like tomatoes (*Solanum lycopersicum* L Tomato extract is a natural organic material with nutritional content that can be used in tissue culture media (Dewi et al., 2021). Ripe tomatoes contain active cytokinin hormones (Bhojwani & Razdan, 1983). Exogenous cytokinin levels cause cell division in meristem tissue to continue to increase its activity (Sari et al., 2019). In addition to cytokinin content, ripe tomatoes also contain the hormone auxin which can stimulate organogenesis, embryogenesis and shoot growth (Heriansyah & Indrawanis, 2020; Dwiyani et al., 2009). Tobing (2019), stated that the addition of tomato extract to in vitro culture media plays a role in increasing shoot height caused by cell elongation, in addition, the concentration of auxin in tomato extract has an effect on the growth of Dendrobium lineale orchid leaves.

Previous research showed that tomato extract promotes shoot growth, somatic embryogenesis, and organogenesis in micropopulations of diverse plant species (Serliana & Linda, 2017), for example in for example in *Dendrobium striaenopsis* (Dewi et al., 2021), Potato (*Solanum tuberosum* L. (Sari et al., 2019). Another substance that promotes plant growth is cytokinin. A study by Rugayah et al. (2021) showed that benzyladenine (BA), a cytokinin releaser, had a beneficial effect on seedling propagation by promoting stem development. Nonetheless, the effect of those PGRs on *Cattleya sp.* orchid has not been reported.

This study aimed to understand the effect of *in vitro* planting media enrichment with tomato extract and BA on the shoot induction of *Cattleya sp.* Furthermore, it was carried out to identify the most suitable tomato extract and BA

concentration, which positively affects the shoot formation. This study is useful in the development of *in vitro* tissue culture propagation of *Cattleya sp.*

Materials and methods

The study was carried out from March to July 2023 at the Tissue Culture Laboratory, Hidayatul Islam Foundation, Medan, Indonesia. A factorial Completely Randomized Design (CRD) was applied. Shoots of *Cattleya sp.* orchid plantlets were grown in MS media enriched by the combination of BA and tomato extract as follows (Table 1):

Table 1: Treatments of tomato extract with BA

PGR BA (ppm) \ Tomato extract (%)	0	5	10	15
0	B0T0	B0T5	B0T10	B0T15
2	B2T0	B2T5	B2T10	B2T15
4	B4T0	B4T5	B4T10	B4T15

Description:
B0T0 : 0 ppm BA and 0 % tomato extract
B0T5 : 0 ppm BA and 5 % tomato extract
B0T10 : 0 ppm BA and 10 % tomato extract
B0T15 : 0 ppm BA and 15 % tomato extract
B2T0 : 2 ppm BA and 0 % tomato extract
B2T5 : 2 ppm BA and 5 % tomato extract
B2T10 : 2 ppm BA and 10 % tomato extract
B2T15 : 2 ppm BA and 15 % tomato extract
B4T0 : 4 ppm BA and 0 % tomato extract
B4T5 : 4 ppm BA and 5 % tomato extract
B4T10 : 4 ppm BA and 10 % tomato extract
B4T15 : 4 ppm BA and 15 % tomato extract

Tomato extract

To obtain extract of tomatoes, they were washed clean, rinsed with sterile distilled water 3 times, then cut into pieces, then blended until smooth without adding water. After the tomatoes become pureed, then filtered, then we get tomato extract. To determine 0 %, 5 %, 10 %, 15 % tomato extract by making 0 mL, 5 mL of tomato solution in 100 mL of media solution, and so on for 10 % and 15%.

Tissue culture tools and culture bottles are washed clean and autoclaved until they reach 121 °C for 1 hour.

Volume of Plant Growth Regulator Stock

1 gram (1000 mg) of plant growth regulator (PGR) BA was taken and dissolved in one liter of distilled water (1000 mL) to obtain a PGR BA of 1000 ppm, then 0, 2 and 4 mL of the solution were taken and dissolved in 1000 mL of distilled water (MS medium) each to obtain concentrations of 0, 2 and 4 ppm of PGR BA respectively.

In the implementation of media preparation, both MS media (Murashige and Skoog) and PGR BA, were taken from the stock made. BA PGR Stock concentrates 100 ppm, namely:

BA PGR 100 mg/1000 mL sterile aquadest = 100 ppm BA PGR or BA PGR 10 mg per 100 mL sterile aquadest = 100 ppm BA PGR

Example: To make 0.5 liter media with 2 ppm BA PGR

$$\mathbf{V1\ N1 = V2\ N2}$$
$$500*2 = V2*100$$
$$V2=10\ mL$$

Description:

V 1 = Volume of solution to be made
V2 = Volume of BA stock to be pipetted
N1 = Concentration of desired PGR BA compound
N2 = Stock concentration (100 ppm)

How to make MS media:

In this study, there are compounds that were weighed direcly, namely A stock (NH_4NO_3) and B stock (KNO_3). There are compounds that were made in stock form with a strenghth of 200 x (stock of C, D, E, F), specifically vitamins with a strength of 250 x.

To make the medium, we take the stock, according to the dosage, for that we need 5 $mL.L^{-1}$ of stock C, D, E, F. Then pipetted vitamints 4 $mL.L^{-1}$ media. And weigh Myoinositol as much as 0.1 $gr.L^{-1}$ media, sugar (sucrose) 30 $gr.L^{-1}$ media and 7 $gr.L^{-1}$ agar for 1 liter media.

Work sequence:

1. All ingredients were mixed except agar
2. BA was added PGR according to treatment
3. Tomato extract was added according to treatment
4. Then sterile distilled water up was added to 1L

5. The pH (5.8-6) was measured. If the pH was too low, a few drops of 1N KOH were added, if it was too high, 1N HCL was added, and the pH was remeasured until it reached the standard value.
6. Then agar was cooked until boiling.
7. Poured into each sterilized culture flask, capped with heat resistant plastic and labeled.
8. Then sterilized in the autoclave at 121 °C, for 15 minutes.

For each treatment, three individual plantlets were used as replication. The observation parameters included time of shoot emergence, number of shoots, number of leaves, plantlet height, time of shoot emergence, and number of roots. Observation was carried out weekly except for plantlet height which was observed at 16 weeks after planting (WAP). Analysis of Variance (ANOVA) was performed using Statistical Package for the Social Sciences (SPSS) statistical software (SPSS Inc., IBM, Chicago, USA). The post hoc was carried out according to Tukey Multiple Comparison at $\alpha = 0.05$.

Results and discussion

Time of Shoot Emergence and Number of Shoots

The effect of the combination of BA and tomato extract on the time of shoot emergence and number of shoots is presented in Table 2. The result indicated that the B4T0 treatment (4 ppm BA without tomato extract) resulted in the fastest shoot emergence (2 WAP), significantly different from B0T10, B0T15, B2T15, B4T5, and B4T15. Treatment B0T15 (0 ppm BA and 15 % tomato extract) produced the longest time of shoot emergence (6.67 WAP). Our result was partially in line with Annas (2019), which showed that BA treatment resulted in faster shoot emergence of *Punica granatum* L. In this study, the 4 ppm BA treatment (the highest concentration of BA tested) showed the fastest shoot emergence. However, the application of 15 % tomato extract in the absence of BA resulted in the longest time of shoot emergence, in contrast with Serliana & Linda (2017), which reported that giving tomato extract could stimulate shoot growth into black orchids (*Coelogyne pandurata* L.).

The BA, which is a PGR in the cytokinin group, functions to stimulate the rate of emergence and formation of shoots. The auxin contained in tomato extract is useful in the formation of shoot primordial cells which cause cell elongation (Heriansyah & Indrawanis, 2020). The B4T15 treatment has a long shoot emergence (4.67 WAP). It might be due to the cytokinin and auxin concentration being too high so it showed an inhibition effect. A study by Setiawati et al. (2018), showed that the 3 ppm BA and tomato extract resulted in the slowest shoot emergence time. The results of this research were in line with Oktaviana & Riza Linda (2015), which reported that 15 % of tomato extract treatment resulted in the longest treatment for shoot induction. PGRs are non-nutrient organic compounds that in low concentrations promote growth, but in high concentrations may inhibit plant growth and development (Maharani et al., 2018). Adding auxin at a certain concentration might encourage cell elongation; however, auxin at a high concentration might cell elongation (Atiek et al., 2022).

Table 2: The effect of benzyladenine and tomato extract enrichment on the time of shoot emergence and number of shoots

Treatment	Time of shoot emergence (weeks)	Number of shoots
B0T0	2.33 ± 0.58 de	1.67 ± 0.58 c
B0T5	3.33 ± 0.58 bcde	2.00 ± 0.00 c
B0T10	4.33 ± 0.58 bc	2.33 ± 0.58 bc
B0T15	6.67 ± 0.58 a	1.67 ± 0.58 c
B2T0	2.33 ± 0.58 de	2.00 ± 0.00 c
B2T5	3.33 ± 0.58 bcde	2.00 ± 0.00 c
B2T10	2.67 ± 0.58 de	5.00 ± 1.00 a
B2T15	3.67 ± 0.58 bcd	2.00 ± 0.00 c
B4T0	2.00 ± 0.00 e	4.00 ± 1.00 ab
B4T5	4.33 ± 0.58 bc	2.33 ± 0.58 bc
B4T10	3.00 ± 0.00 cde	3.33 ± 0.58 abc
B4T15	4.67 ± 0.58 e	1.67 ± 0.58 c

Numbers are the average of three replications ± standard deviation. Numbers followed by the same letters in the same column indicate no significant difference according to Tukey Multiple Comparison at $\alpha = 0.05$.

The highest number of shoots at 16 WAP was found in the B2T10 treatment (5.00 ± 1 shoots), while the lowest was in B4T15 (1.67 ± 0.58 shoots).

The B2T10 treatment was significantly different from all treatments except B4T0, and B4T10. It indicated that the treatment could provide cytokinin and auxin at optimum concentrations and in balance with the endogenous PGR content of the *Cattleya sp.* explants as reported by (Kartiman et al., 2018). The results of this research are in line with Yachya et al. (2022) and Wijaya & Sudrajad (2019), which stated that the optimal concentration for promoting the number of shoots was 2 ppm. The BA found in this study was able to accelerate shoot cell division through increased cell proliferation, including apical and axillary meristem activity (Kieber & Schaller, 2018).

Treatment B4T0 produced a high number of shoots (4.00 ± 1 shoots), not significant to the highest treatment (B2T10). Our result was in line with Harahap (2012), which stated that treatment with a BA concentration of 4 ppm produced the most shoots, indicating that BA, which is a PGR of the cytokinin group, functions to stimulate the shoot's formation. Nonetheless, when combined with high concentrations of auxin, the number of shoots decreased as showed in B4T15 treatment. The decrease might be due to the exceed of cytokines and auxin concentrations, which inhibited shoot growth. The results of the research are in line with research by Wijaya & Sudrajad (2019), which stated that giving BA 4 ppm and IBA 1 ppm resulted in the least shoot growth. Another study by Oktaviana & Riza Linda (2015) showed a similar result where 15 % tomato extract lowered the number of shoots. The combination of cytokine and auxin in a high concentration might inhibit shoot growth (Figure 1).

Number of Leaves and Planlet Height

The effect of BA and tomato extract concentrations on the number of leaves and plantlet height is presented in Table 3. The highest number of leaves was found in the B2T10 treatment (17.67 ± 1.53) (Figure 2A), while the lowest number of shoots was produced by the B0T5 (5.33 ± 0.58). Treatment B2T10 was significantly different from other treatments except B4T0. The result was in accordance with previous research by Wijaya & Sudrajad (2019), which found that the highest number of leaves was found in the 2 ppm BA treatment. The treatment might have appropriate BA concentration, thereby stimulating the growth and development of shoots.

The accumulation of cytokinin in the apical meristem promotes leaf development. Stopping the apical dominance and encouraging the growth of axillary buds and the appearance of new axillary buds, cytokinins can greatly

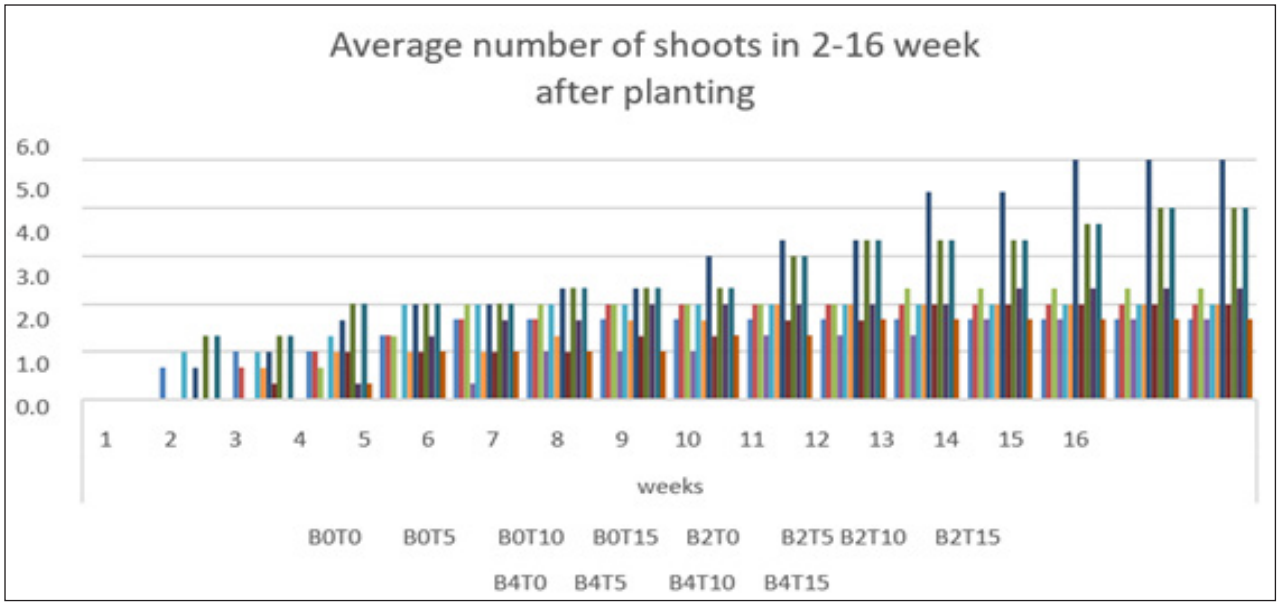

Figure 1: Graph of the effect of BA and tomato extract treatment on the number of shoots of *Cattleya* sp. at 2-16 MST.

increase the number of axillary buds. The more axillary buds that appear, the greater the number of nodes in the explant, so the increase in the number of shoots is directly proportional to the increase in the number of nodes (Yusnita et al., 2019). This research is also in line with Dewi et al. (2021), which stated that a 10 % concentration of tomato extract treatment produces the best number of leaves; presumably due to the natural auxin produced by tomato extract working synergistically with the cytokinins in stimulating cell division.

Table 3: The effect of benzyladenine and tomato extract enrichment on the number of leaves and plantlet height

Treatment	Number of Leaves	Plantlet Height (cm)
B0T0	9.00 ± 1.00 cd	2.23 ± 0.35 bcd
B0T5	5.33 ± 0.58 e	1.77 ± 0.15 cd
B0T10	9.33 ± 1.15 cd	1.87 ± 0.25 cd
B0T15	6.33 ± 0.58 de	1.57 ± 0.15 d
B2T0	10.33 ± 1.53 c	2.20 ± 0.30 bcd
B2T5	7.00 ± 1.00 de	2.13 ± 0.21 bcd
B2T10	17.67 ± 1.53 a	2.57 ± 0.35 abc
B2T15	8.33 ± 0.58 cde	1.97 ± 0.31 cd
B4T0	15.67 ± 1.15 ab	3.30 ± 0.46 a
B4T5	8.00 ± 1.00 cde	1.97 ± 0.21 cd
B4T10	13.67 ± 1.53 b	2.87 ± 0.31 ab
B4T15	6.67 ± 1.15 de	1.73 ± 0.15 d

Numbers are the average of three replications ± standard deviation. Numbers followed by the same letters in the same column indicate no significant difference according to Tukey Multiple Comparison at α = 0.05.

In high concentrations of BA and tomato extract, B4T15 treatment, the average number of leaves decreased to 6.67 leaves (Figure 2B). This shows that the addition of BA and tomato extract exceeded the optimal concentration that inhibited leaf growth and development. BA and tomato extract both contain exogenous cytokinins, and explants also contain endogenous cytokinins. The explant accumulates endogenous cytokinin along with the given (exogenous) cytokinin, disrupting the desired balance of cytokinin. Apart from that, differences in growth phases, physiological conditions, and the plant's ability to absorb hormones influence the plant's growth response, especially the number of leaves. It is suspected that the combination of PGRs contains too much cytokinin but is unable to grow leaves optimally (Karyaningtyas et al., 2023).

High exogenous growth regulators and endogenous hormones inhibit cell division in explants. The results of this research are in line with research by Wijaya & Sudrajad (2019), which states that giving BA 4 ppm and IBA 1 ppm produces the lowest number of leaves. The results of this research are also in line with Oktaviana & Riza Linda (2015), who stated that 15 % tomato extract produced the smallest number of leaves. It is suspected that there was not an appropriate balance between endogenous PGR from explants and exogenous PGR from tomato extract and BAP. Exogenous hormone

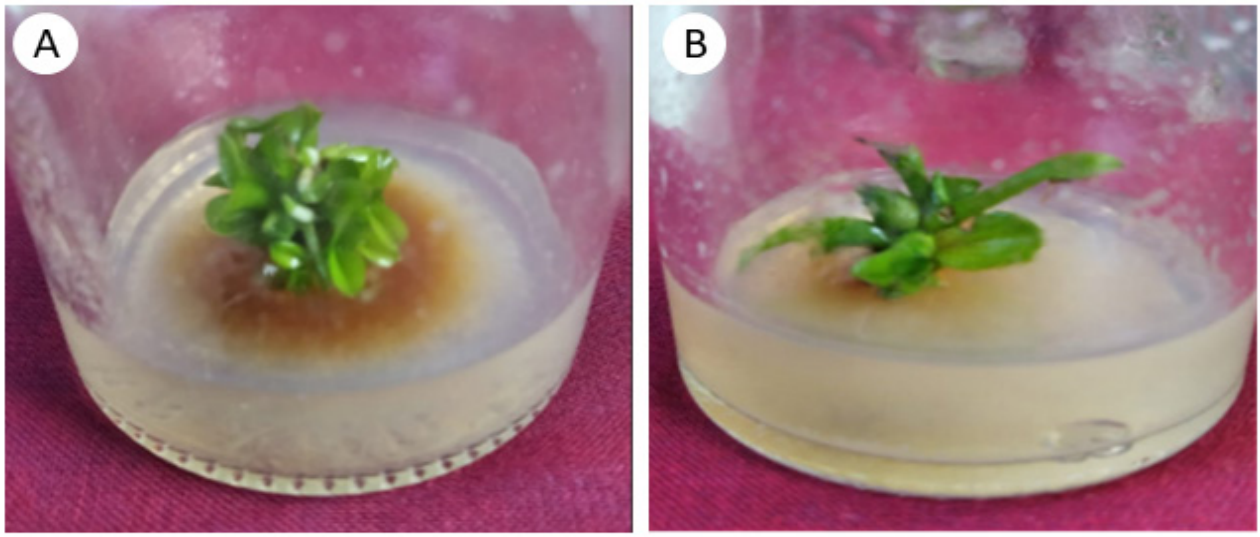

Figure 2: Shoots and leaves of the *Cattleya sp.* of B2T10 (A) and B4T15 (B) treatments at 16 WAP.

addition will change the balance of PGRs in plant cells. This concentration of endogenous PGRs also influences exogenous cytokinin and auxin work on the plantlet's growth.

The B4T0 treatment interaction produced the highest plantlet, namely 3.37 cm. The treatment was not significantly different from B4T10 (Figure 3A) and B2T10 but was significantly different from the other treatments. This is likely because it had the right amount of cytokinin and auxin hormones for the *Cattleya* sp. orchid. The addition of cytokinin at the right concentration can increase plantlet height because cytokinin stimulates cell division, which is followed by cell enlargement and elongation, which are stimulated by auxin. The G1/S and G2/M transitions in the cell cycle are controlled by cytokinins during the cell proliferation stage. Auxin stimulates cell wall stretching factors such as elastin to stimulate cell elongation to loosen the cell wall (Nazir et al., 2022).

The result was in line with Apriani et al. (2016), which states that BA 4 mg.L^{-1} produces optimal plantlet height. This is also in line with research by Dewi et al. (2021), which states that 10 % tomato extract produces the highest plantlet height, namely 1.40 cm. It is suspected that tomato extract contains the auxin and cytokinin hormones, which act as growth regulators. In low concentrations, these hormones can stimulate plant cells. so that growth can occur optimally. According to Barroroh & Umul (2005), giving tomato extract at a dose of 100 g.L^{-1} produces the highest *Cattleya* sp. orchid plant height compared to other doses.

On the other hand, B4T15 treatment yielded the lowest plantlet average of 1.73 cm (Figure 3B). The treatment was significantly different from the B2T10, B4T10, and B4T0 treatments but not significantly different from other treatments. The B4T15 treatment interaction showed a decrease in the average plantlet height, possibly because the combination of BA and tomato extract did not meet the needs of the *Cattleya sp.* orchid in increasing plantlet height. It is suspected that the hormone auxin is greater than cytokinin, so it can inhibit explant height. The right PGR concentration is necessary for plants to grow well because hormone concentrations that are too high or too low can inhibit growth. a study by (Ningsih & Rohmawati, 2019) showed that 20 % tomato extract leads to the. It was suspected that a high concentration of tomato extract would inhibit the growth of plantlets.

Time of Root Emergence and Number of Roots

In this study, the BA and tomato extract did not have a significant effect on root emergence. The roots sprouted at 5.5 weeks in general. This is

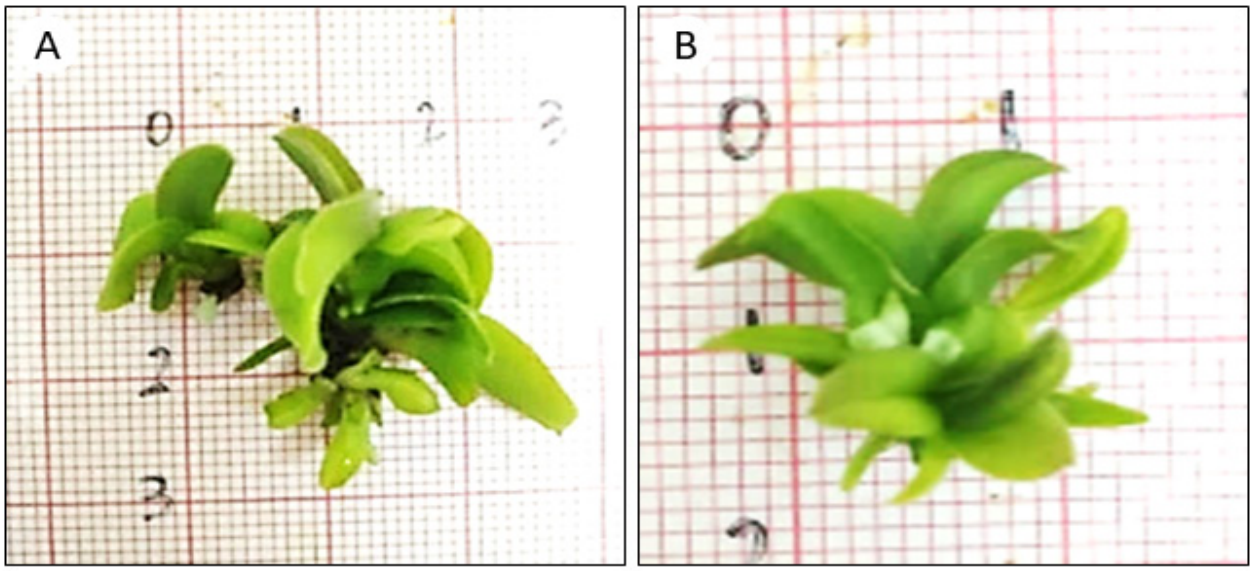

Figure 3: Plantlet height of the *Cattleya sp.* of B2T10 (A) and B4T15 (B) treatments at 16 WAP.

because explants naturally produce endogenous auxin to support root formation. Agriani (2010) stated that *in vitro* explants produced sufficient auxin naturally. The process of root elongation begins with stimulation by endogenous auxin. In general, the effect of cytokinin and auxin administration on the explants is not optimal, which is thought to be the reason for this. The optimal concentration of cytokinin and auxins cannot be determined with certainty because endogenous PGR sources in different plants can provide different effects. According to Hartman et al. (2010), different plants might respond differently to cytokinin and auxin. The administration of various concentrations of auxin does not show a response to all the observed variables as the endogenous auxin contained in the plant is sufficient. In this study, the average root emergence time in the B4T0 treatment (4 ppm BA and 0 % tomato extract) showed the fastest root emergence (5 WAP). The administration of MS media and 4 ppm BA was the appropriate concentration for *Cattleya* sp. orchids in terms of growth and root formation (Table 4).

The B4T0 treatment showed the highest number of roots (7.00 ± 1.73 roots) (Figure 4A), not significantly different from B2T0 (6.33 ± 0.58 roots) and B0T0 (6.33 ± 0.58 roots). The B0T15 treatment (0 ppm BA and 15 % tomato extract) showed no roots sprouted (Figure 4B).

Table 4: The effect of benzyladenine and tomato extract enrichment on the time of root emergence and number of roots

Treatment	Time of root emergence (weeks)	Number of roots
B0T0	5.67 ± 0.58 a	6.33 ± 0.58 ab
B0T5	8.00 ± 0.00 a	1.67 ± 0.58 cde
B0T10	7.00 ± 1.00 a	2.00 ± 0.00 cde
B0T15	0.00 ± 0.00 a	0.00 ± 0.00 e
B2T0	5.33 ± 0.58 a	6.33 ± 0.58 ab
B2T5	6.00 ± 0.00 a	3.00 ± 1.00 cd
B2T10	5.33 ± 0.58 a	3.33 ± 0.58 cd
B2T15	6.33 ± 5.51 a	0.67 ± 1.15 de
B4T0	5.00 ± 0.00 a	7.00 ± 1.73 a
B4T5	4.67 ± 4.16 a	1.33 ± 1.15 cde
B4T10	6.67 ± 0.58 a	4.00 ± 1.00 bc
B4T15	5.67 ± 4.93 a	1.33 ± 1.15 cde

Numbers are the average of three replications ± standard deviation. Numbers followed by the same letters in the same column indicate no significant difference according to Tukey Multiple Comparison at α = 0.05.

The B4T0 treatment (4 ppm BA and 0 % tomato extract) presumably could supply macro and micronutrient content in the MS media that stimulated the formation of *Cattleya sp.* orchid roots. The results were in line with Saepudin et al. (2020) which reported that MS media has nutrients and macros that are suitable for plant growth, including nitrogen (N) and calcium (Ca). The N nutrient has an important role in metabolic processes including photosynthesis. The translocation of photosynthate to the roots

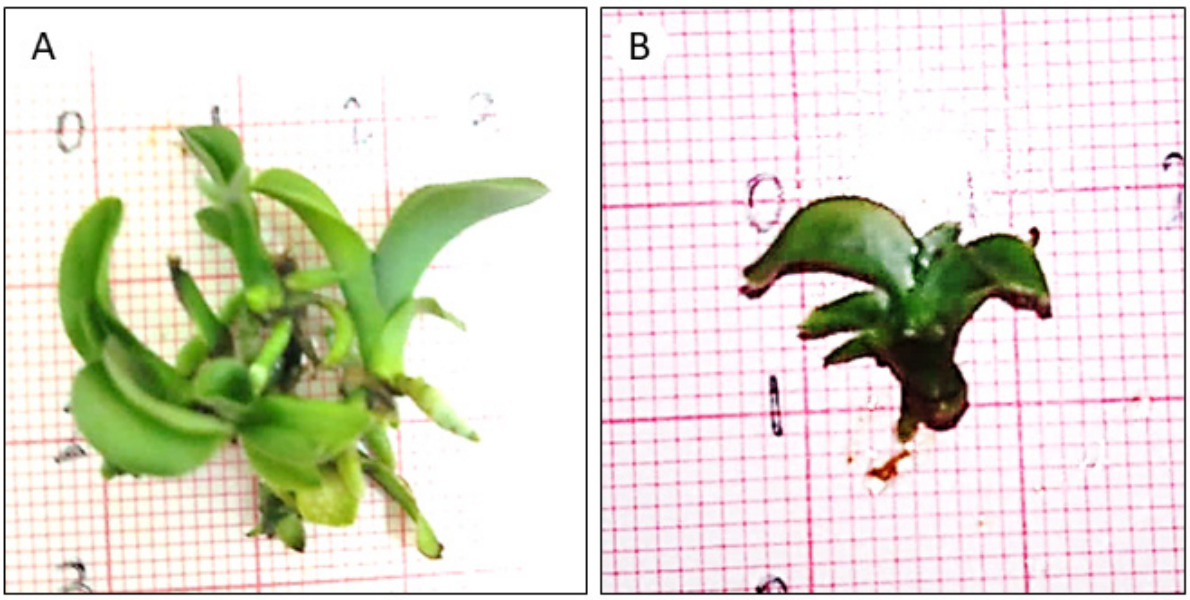

Figure 4: Number of roots of the *Cattleya sp.* of B4T0 (A) and B0T15 (B) treatments at 16 WAP.

promotes root system development. The Ca promotes root emergence through the regulation of cell membrane permeability. Calcium has an influence, especially in the formation of the tips of root hairs.

Conclusion

According to this study, BA had a significant effect on the time of shoot emergence, number of shoots, number of leaves, plantlet height, and number of roots. Tomato extract had a significant effect on the time of shoot emergence, number of shoots, number of leaves, plantlet height, and number of roots. Both BA and tomato extract had no significant effect on the time of root emergence. Treatment B4T10 (4 ppm BA and 10 % tomato extract) was the most potential combination for *Cattleya sp. in vitro* propagation.

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Conflicts of Interest

We state that this work has no affiliation or involvement in any organization, with any financial interests or any non-financial interests, and has no connection with the material discussed in this manuscript. This statement is written correctly and with full awareness.

Authors Contribution

FH: research team cordination, data analysis, editing draft report, preparing journals. AKBT: taking samples in the field, making madia, sterilization, planting explants. CS: management of research permits, observations, data analysis. AHD: Editing, draft report editing, report printing. SE: Data analysis, preparation for journal writing. N: planting explants, editing draft reports, printing report. E: Data analysis, preparation for journal writing. N: preparation for journal writing, proof reading

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