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Tissue Culture of Stevia (*Stevia rebaudiana* Bert.) with the Addition of *Aloe vera* Extract (*Aloe vera* (L). Burm.f) on Murashige and Skoog Medium (MS)

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Abstract

The increasing demand for natural, low-calorie sweeteners highlights the importance of Stevia rebaudiana Bertoni, which produces steviol glycosides that are sweeter than cane sugar and provide health benefits. Due to low seed viability, tissue culture is preferred for propagation, with its success dependent on growth regulators and nutrient availability. Additionally, incorporating plant-derived supplements like Aloe vera, rich in bioactive growth-promoting compounds, may enhance in vitro development. This study aimed to identify the optimal concentration of Aloe vera extract in Murashige and Skoog (MS) medium to enhance the in vitro growth of Stevia rebaudiana. The experiment was conducted using sterile culture bottles arranged in a Completely Randomized Design (CRD) with one experimental factor, namely Aloe vera extract concentration, consisting of five treatment levels: 0%, 5%, 10%, 15%, and 20%. Each treatment was replicated five times, resulting in a total of 25 culture bottles used in this study. Observations were carried out periodically based on several parameters, including shoot, root, stem, and leaf development, as well as overall plant viability and growth performance. The findings demonstrated that 10% Aloe vera extract was the most effective concentration for promoting shoot, stem, and leaf growth, while root growth showed no significant variation across treatments.

Keywords: *Aloe vera, stevia, tissue culture.*

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INTRODUCTION

Stevia rebaudiana Bertoni is a species of the Asteraceae family that contains natural sweetening compounds (Mirah et al., 2021). Stevia, which contains secondary metabolites of the steviol glycoside group, has been widely used as a natural sweetener. The sweetness of stevia is reported to be 70–400 times higher than that of cane sugar. *Stevia rebaudiana* produces steviol glycosides, including stevioside, rebaudioside, steviolbioside, rubusoside, and dulcoside (Adabiyah, 2019). Stevia plants have a zero glycemic index and exhibit antiseptic and antioxidant properties, giving them high commercial value. In addition, stevia can aid in managing diabetes and help control body weight (Mirah et al., 2021).

Stevia is classified as a “short-day plant,” meaning its growth and development are influenced by the length of daylight. The plant will flower when exposed to less than 13 hours of light and typically blooms around 60 days after planting (Edi et al., 2015). Stevia plants can produce steviol glycosides ranging from 4% to 20% of the dry leaf weight, depending on the cultivar and growing conditions. Steviol glycosides are synthesized in the endoplasmic reticulum and chloroplasts of the leaves. The sweet taste of *Stevia rebaudiana* is present in almost all parts of the plant, except for the roots (Sinta et al., 2018).

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Currently, stevia has become a popular commodity in many countries, including Malaysia, China, Japan, Taiwan, Brazil, Korea, Thailand, Peru, Uruguay, Paraguay, and Colombia. In these countries, stevia is traded both in local markets and as an export commodity. Japan is the largest consumer of stevia, accounting for approximately 40% of the sweetener market (Dimas et al., 2023). In Indonesia, research and development of *Stevia rebaudiana* have been conducted since 1984 at the Plantation Research Center, which is currently known as the Indonesian Research Institute for Plantation Biotechnology (Sidik et al., 2018). In recent decades, public awareness of environmental sustainability and health has increased, accompanied by a growing use of natural-based products (Kementan, 2015).

Stevia can be propagated using seeds in soil media; however, the low seed viability and vigor make this conventional method unsuitable for large-scale propagation. In contrast, tissue culture techniques allow the production of a large number of new plantlets in a relatively short period (Mirah et al., 2021). Propagation through tissue culture becomes more effective with the addition of external growth regulators to basal media such as Murashige and Skoog (MS). This medium is suitable for various plant species as it contains a complete range of macro and micronutrients (Pratama, 2018). A study by Arlianti et al. (2017) reported that the addition of the growth regulator NAA was effective in inducing *Stevia rebaudiana* in vitro, but this was not followed by successful acclimatization.

Several factors, including hormones, genetic factors, the physiology of the donor tissue, growth conditions of the donor plant, the types of growth regulators, and the availability of nutrients in the medium influence the success of tissue culture (Amien et al., 2020). The growth of explants also strongly depends on the type and concentration of growth regulators added to the medium. Growth regulators play a role in promoting plant growth and development through mechanisms of stimulation, inhibition, or modification of growth and developmental processes (Fauzi, 2021). The activity of growth regulators in plant development is influenced by their type, concentration, chemical structure, as well as the genotype and physiology of the plant. Commonly used growth regulators include Benzyl Amino Purine (BAP), Kinetin, Thidiazuron (TDZ), Naphthaleneacetic Acid (NAA), and 2,4-Dichlorophenoxyacetic Acid (2,4-D). Endogenous growth regulators produced by plant tissues can interact with exogenous regulators added to the medium, resulting in the formation of organs such as shoots or roots (Lestari, 2010).

Growth regulators can influence plant growth according to the desired outcome. When plants produce hormones in insufficient amounts, growth regulators can assist in enhancing the activity of existing phytohormones or substitute their function. Additionally, extra components derived from other plants can be incorporated into the medium to stimulate tissue culture growth, one of which is *Aloe vera* (Fauzi, 2021). The gel from *Aloe vera* leaves consists of 96% water and 4% solids, containing 75 different compounds, including enzymes, sugars, minerals, hormones (auxins and gibberellins), and fatty acids. These nutrients play a role in influencing plant growth and development. Research results Fauzi (2021) has shown that the addition of 10% *Aloe vera* gel can significantly increase the height of mung bean plants. However, no significant changes were observed in leaf number, leaf area, or stem diameter across all treatments.

Based on this information, *Aloe vera* extract has the potential to be used as a supplement to stimulate the growth of *Stevia rebaudiana*. To date, *Aloe vera* extract has not been tested for its ability to promote stevia growth using tissue culture techniques. Therefore, this study was conducted by adding *Aloe vera* (*Aloe vera*) extract to the tissue culture medium of *Stevia rebaudiana* Bert. in vitro. The study aimed to determine the optimum concentration of *Aloe vera* extract in MS medium at 0%, 5%, 10%, 15%, and 20%.

MATERIALS AND METHODS

Time and Place

The study was conducted at the UPT Nursery for Food Crops, Horticulture, and Animal Husbandry under the Food Security and Agriculture Office of Bandung City, located at Jl. Cigagak, Cipadung, Cibiru District, Bandung City, West Java 40174, and at the Chemistry Laboratory of Muhammadiyah University of Bandung. The research was carried out over a period of five months, from January to May 2025.

Material and Equipment

The materials used in this study included MS medium, sugar, agar, *Aloe vera*, stevia plants (*Stevia rebaudiana* Bert.), liquid detergent, benlox fungicide, 70% and 96% alcohol, 70% ethanol, sterile distilled water, NaOCl, and Tween 20. Stevia explants were obtained from the UPT TPHP Dispangtan Seedling

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Center, Bandung City. The equipment used to support this research included an autoclave, culture bottles, measuring cylinders, beakers, stirrers, Laminar Air Flow (LAF) cabinet, rotary evaporator, containers, blender, sterile tissue, magnetic stirrer, hot plate stirrer, spatula, analytical balance, stove, pan, petri dishes, tweezers, scalpel, scissors, and bunsen burner.

Research Method

The experiment was conducted using culture bottles arranged in a Completely Randomized Design (CRD) with one factor, namely *Aloe vera* concentrations, consist of 5 levels: 0%, 5%, 10%, 15%, 20%. Each treatment was replicated five times, resulting in a total of 75 culture bottles used in the study.

Research Implementation

Preparation of *Aloe vera* Extract Solution

Aloe vera extract was prepared using the maceration method as described by Firmansyah & Setyaningsih (2018). The *Aloe vera* leaves used were 10 months old. The leaves were cleaned, and the skin and flesh were separated. A total of 500 grams of *Aloe vera* flesh was blended and then placed in a container. Subsequently, 1000 mL of 70% ethanol was added, and the mixture was left to stand for 3 days. The solution was then evaporated using a rotary evaporator at 50°C until the ethanol completely evaporated.

Preparation of Treatment Media

The medium used was Murashige and Skoog (MS) medium. MS medium and sugar were dissolved in 200 mL of distilled water. *Aloe vera* extract was added to the MS medium solution according to the respective concentrations: 0%, 5%, 10%, 15%, and 20% (v/v). After homogenization, the pH was measured and adjusted to 5.8. Agar was added to the medium and heated while stirring until boiling. The medium was then poured into culture bottles at a volume 0f 20 ml each, closed, wrapped, and labeled. The media were sterilized using an autoclave at 121°C and 1 atm pressure for 30 minutes. The sterilized media were stored on storage racks at room temperature for 7 days. The media could be used if no contamination was observed.

Surface Sterilization

The surface sterilization of stevia plants followed the method of (Cahyono & Ningsih, 2023). Explants were rinsed under running water, then washed with 2 mL/L liquid detergent for 10 minutes and rinsed three times with sterile distilled water. Subsequently, explants were soaked in 1.5% benlox fungicide solution for 10 minutes, rinsed three times with sterile distilled water, then soaked in 70% alcohol for 1 minute and rinsed again three times. The next soaking was in 10% NaOCl with 10 drops/L of Tween 20 for 5 minutes, followed by soaking in 5% NaOCl with 10 drops/L of Tween 20 for 5 minutes. Finally, explants were rinsed three times with sterile distilled water before being planted on the medium.

Planting Stevia in the Growth Medium

The planting process was carried out inside a Laminar Air Flow (LAF) cabinet under aseptic conditions. The sterilized stevia explants were then trimmed, leaving 0.5 cm of the petiole. Subsequently, the explants were planted on MS medium supplemented with *Aloe vera* extract at various concentrations.

Observation

The growth of stevia was observed for 84 days after planting. The parameters measured and observed included: (1) the percentage of surviving plantlets, calculated on each observation day; (2) the time to shoot emergence, observed after the day of stevia planting; (3) the time to root emergence, observed after the day of stevia planting; (4) the number of roots, counted by recording the roots on each plantlet from the beginning of observation; (5) plantlet height, measured using a ruler from the surface of the medium to the growth point; and (6) the number of leaves, counted based on the number of leaves appearing on the plantlet. Observations of leaf number were conducted every six days.

Data Analysis

The data obtained from the observation of the number of surviving plantlets were analyzed using the Kruskal-Wallis test to determine differences between treatments at a 95% confidence level, utilizing the SPSS (Statistical Package for the Social Sciences) application.

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RESULT AND DISCUSSION

Number of Surviving Planlets

The percentage of surviving plantlets was observed on the final day of observation, which was day 84. Contamination occurred as early as the first week after initiation in both the control and the treatment with 5% Aloe vera extract, specifically on the fifth and sixth days. Based on the results, the number of surviving plantlets across all treatments did not differ significantly ($p>0.05$) (Figure 1.). This indicates that the addition of *Aloe vera* extract at concentrations ranging from 5% to 20% was not able to maintain the survival rate of stevia plantlets above 60%. This outcome contrasts with the findings of Sumaryono & Sinta (2011), who reported that MS medium supplemented with 0.1 mg/L paclobutrazol maintained a 100% survival rate of stevia plantlets.

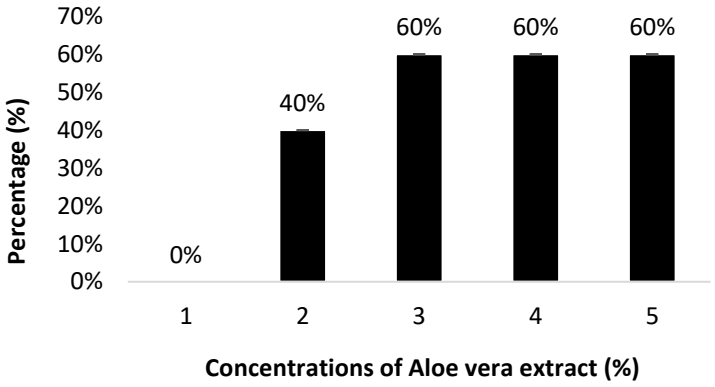

Figure 1. Percentage of Surviving Stevia Plantlets (*Stevia rebaudiana* Bert.) at *Various Aloe vera* Extract Concentrations

In this study, contamination occurred as early as the first week after initiation in both the control and the treatment with 5% *Aloe vera* extract. The contaminated explants were separated from the culture rack and immediately discarded. However, in treatments with higher concentrations of *Aloe vera* extract, contamination appeared later, emerging two weeks after initiation. This indicates that *Aloe vera* extract at concentrations between 10% and 20% may possess antimicrobial properties that help delay contamination. The explants were removed and discarded in a separate area. Then, the area where the bottles had been placed was cleaned and disinfected with a disinfectant solution. The uncontaminated cultures were subsequently checked regularly to ensure that no contamination occurred. According to Wijaya & Masfufatun (2022), *Aloe vera* contains active compounds from 12 types of anthraquinones that function as antibacterial and antifungal agents. Suryati et al. (2017) also demonstrated in their research that *Aloe vera* contains active compounds such as tannins, flavonoids, and saponins, which act as antibacterial agents. Saponins are alkaloid compounds that have the potential to damage bacterial DNA and RNA, while tannins work by inactivating adhesins, thereby preventing bacteria from attaching to host epithelial cells. The flavonoid content in *Aloe vera* can also cause cell lysis and inhibit cell wall formation, thus playing a role in killing and inhibiting the growth of bacteria (Suryati et al., 2017). Additionally, *Aloe vera* contains various nutrients such as minerals, enzymes, fatty acids, sugars, and plant hormones, including auxins and gibberellins, which can influence plant growth and development. These hormones play key roles in regulating processes like cell elongation, division, and differentiation, thereby promoting overall plant health and vigor (Fauzi, 2021).

In this study, browning was observed in all treatments. Browning is a common problem in plant tissue culture, especially when explants are taken from woody plants or plants containing high levels of extractive compounds such as alkaloids. Admojo & Indrianto (2016) stated that browning is caused by the production and accumulation of phenolic compounds, which arise due to wounding of the explant. This condition is generally triggered by the activity of the enzyme Polyphenol Oxidase (PPO). Wounded plant tissues are prone to metabolic imbalance, increased production of Reactive Oxygen Species (ROS), loss of cell membrane integrity, and lipid peroxidation. The peroxidation process promotes excessive accumulation of phenolic compounds, leading to browning. Changes in membrane permeability also trigger the release of enzymes and substrates from the cytosol, ultimately resulting in the formation of brown pigments. In this study, the wounding treatment of stevia explants prior to initiation is consistent with the findings of Admojo & Indrianto (2016) who linked tissue wounding to increased incidence of

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browning. This enzymatic browning process is also commonly observed in various plant tissues and food products, where PPO catalyzes the oxidation of phenolic substrates, resulting in brown coloration.

The browning phenomenon has a negative impact on explant growth. If not controlled, browning can inhibit explant regeneration or even cause plant tissue death. This occurs because the oxidation of phenolic compounds leads to the formation of brown pigments, which can be toxic to the tissue and disrupt normal cellular functions, ultimately reducing the viability and growth potential of the explant (Helena et al., 2022). Several strategies can be applied to reduce browning, including the addition of antioxidant compounds such as ascorbic acid, controlling culture conditions by storing explants in the dark, performing repeated subcultures, and supplementing the culture medium with activated charcoal and sucrose (Admojo & Indrianto, 2016).

In addition, in this study, some explants experienced bacterial and fungal contamination on the 7th day after initiation, which occurred in the control treatment (0%) and in the treatment with the addition of *Aloe vera* extract at a concentration of 5%. This contamination can occur even though all research protocols had been sterilized beforehand. Contamination in these treatments may lead to non-uniform data variation, as the presence of bacteria and fungi can disrupt explant growth and affect the consistency of experimental results. Andriani & Heriansyah (2021) stated that contamination can originate from the explants themselves, culture bottles, and tools that are not sufficiently sterile, as well as from an unhygienic work environment or incubation room. Even with sterilization protocols in place, microorganisms such as bacteria and fungi can still contaminate the culture medium or explants, leading to inconsistent experimental results and affecting the growth and development of the explants.

In this study, fungal contamination was indicated by the appearance of hyphae on the surface of the media and explants, while brownish colonies identified bacterial contamination. This observation aligns with the findings of Wati et al. (2020), who also described similar visual markers for fungal and bacterial contamination in plant tissue cultures. The presence of hyphae typically signals fungal growth, whereas brownish colonies are characteristic of bacterial contamination. Both of these can adversely affect the success of tissue culture experiments.

Time of Shoot Emergence

Observing the time of shoot emergence was carried out to determine the effectiveness of tissue culture and the composition of the growth medium in producing shoot. The fastest shoot emergence time was observed in the control treatment and the treatment with the addition of 10% *Aloe vera* extract, both occurring on day 7 (Figure 2). Conversely, the longest shoot emergence time was found in the treatment with 5% *Aloe vera* extract concentration, which was on day 14. This indicates that adding *Aloe vera* extract at certain concentrations can accelerate shoot emergence, while at a lower concentration (5%) it actually delays the process. This factor is important to consider in developing tissue culture media to achieve optimal regeneration results.

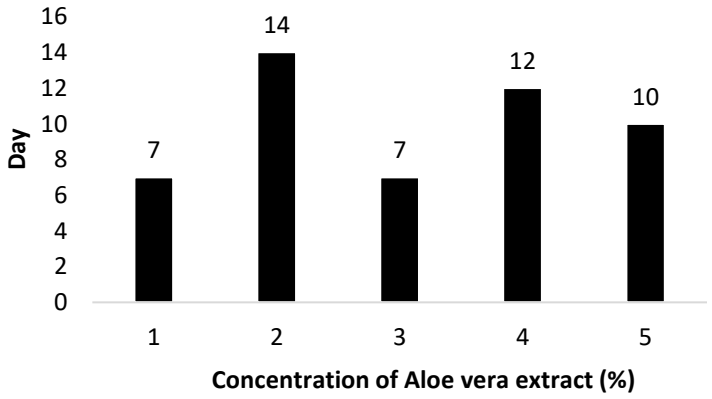

Figure 2. Average Shoot Emergence Time of Stevia (*Stevia rebaudiana* Bert.) at Various Concentrations of *Aloe vera* Extract

Overall, the shoot emergence time of stevia in this study showed a relatively long duration. Based on Asmono & Lestari (2020), the shoot emergence time of stevia in tissue culture typically occurs around 2 days after culture initiation. Similarly, the study Hadiyana et al. (2015) The fastest shoot emergence was observed at 4 days after planting, while the longest was at 20 days. In this study, the MS medium (control)

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was not sufficient to support stevia growth and required plant growth regulators (PGRs) to stimulate shoot emergence in less than 7 days. However, the addition of *Aloe vera* extract also did not result in faster shoot emergence compared to the control. This may be due to the auxin and gibberellin hormones in the *Aloe vera* extract not matching the needs of the stevia plant, resulting in no significant improvement in shoot growth (Arlianti et al., 2017). The growth of shoots indicates successful regeneration of explants carried out in vitro.

Root Growth Time and Number of Roots

Based on the results of the study, the growth and increase in the number of roots in stevia plants with the addition of *Aloe vera* extract did not show any root development up to day 84 in all treatments. Generally, stevia tissue cultures are grown on full-strength MS medium supplemented with plant growth regulators (PGRs) or plant extracts, such as NAA (Parnidi & Ridhawati, 2020) and vitamins as well as glycine (Ermayanti et al., 2017) to stimulate growth, especially root development. This indicates that the nutritional and hormonal needs of stevia in tissue culture cannot be met by using only full-strength MS basal medium. This condition aligns with the research findings, where the control treatment containing only full-strength MS medium also failed to promote root growth.

In this study, the addition of *Aloe vera* extract at concentrations ranging from 5% to 20% was not able to stimulate root growth in stevia. Root development in tissue-cultured plantlets is generally influenced by the ratio of plant growth regulators (PGRs), specifically a higher auxin level compared to cytokinin. A higher concentration of cytokinin than auxin usually inhibits root formation and growth in plantlets (Hartati et al., 2016). It is suspected that the cytokinin content in *Aloe vera* is greater than its auxin content, which is not suitable for the needs of stevia plants (Sempana et al., 2021). Therefore, the addition of *Aloe vera* extract was not effective in stimulating root growth in stevia.

Several factors can influence the inhibition of root growth and development in explants, including the explant genotype, the origin tissue of the explant, explant size, and explant age. These factors play a crucial role in determining the success of in vitro propagation, as each plant species has a different capacity for differentiation (Angelina et al., 2017). Moreover, the number of roots formed can reflect the plant's ability to expand its nutrient absorption capacity. A higher root number also indicates that the explants are in a healthy condition and capable of efficiently absorbing nutrients from the medium (Hartati et al., 2016).

One of the factors influencing root growth in explants is the selection of the optimal concentration of *Aloe vera* extract. The balance between auxin and cytokinin hormones generally determines the effectiveness of root development in plantlets (Hartati et al., 2016). In this study, the cytokinin concentration is estimated to be higher than that of auxin, as reflected by the absence of root growth in stevia explants. Cytokinins are known to promote shoot formation but can inhibit root development when present in higher concentrations relative to auxins. This hormonal imbalance likely contributed to the lack of root induction observed. Additionally, the pH of the medium and the agar concentration also play important roles in supporting plant growth. The culture medium is typically adjusted to a pH of around 5.8 to create optimal conditions for nutrient uptake and tissue development (Funnekotter et al., 2023). According to Yulis (2023), the density level of the medium also greatly affects the efficiency of plant growth; a medium that is too soft due to excess water content is more susceptible to contamination and cannot support optimal growth, while a medium that is too dense limits the absorption of water and nutrients by the plant. Therefore, proper pH adjustment and the appropriate addition of agar are very important to support explant growth optimally.

Plantlet Height

The application of *Aloe vera* extract resulted in non-significant differences in stevia plantlet height among all treatments and the control ($p > 0.05$) (Figure 3). Based on Figure 3, the average height of stevia planlets across all treatments did not show a significant difference ($p>0.05$). This indicates that the addition of *Aloe vera* extract has not been able to optimally stimulate planlet height growth. The availability of appropriate hormones generally influences plant height growth. The presence of auxin and cytokinin hormones, either endogenously or from supplementation with *Aloe vera* extract, can support the increase in planlet height.

The presence of hormones in *Aloe vera* extract is presumed to contribute to the increase in planlet height across all treatments compared to the control. The treatment with a 10% concentration showed the best results for the planlet height parameter. This may be due to a more optimal increase in metabolic

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activity. The combination of cytokinin and auxin in *Aloe vera* extract can stimulate cell division as well as determine the direction of cell differentiation in plants (Sulichantini, 2016).

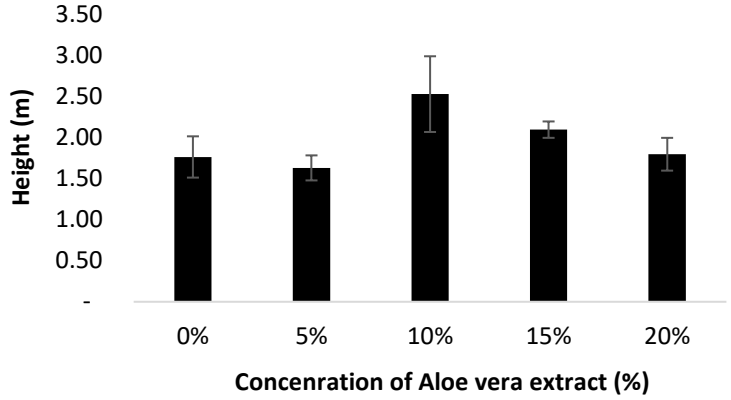

Figure 3. Average Plantlet Height of Stevia (*Stevia rebaudiana* Bert.) at Various Concentrations of *Aloe vera* Extract.

Aloe vera extract is known to contain auxin and cytokinin hormones, which play a role in supporting planlet height growth (Sempana et al., 2021). Auxin works by stimulating specific proteins on the plant cell plasma membrane to pump H^+ ions into the cell wall. This process triggers cell elongation by increasing the acidity of the cell wall, thereby activating certain enzymes that break some of the hydrogen cross-links in the cellulose chains that make up the cell wall. This condition allows plant cells to elongate as water enters through osmotic pressure. After elongation, the cells continue to grow through the resynthesis of cell wall materials and cytoplasm (Supriyanto et al., 2022).

In addition, the presence of cytokinin hormones can also stimulate stem elongation in stevia tissue culture. Cytokinins play a role in cell division, which ultimately contributes to stem height growth. The presence of cytokinins in *Aloe vera* extract aligns with the findings of (Hasanah, 2017), who used BAP as a synthetic cytokinin. The addition of BAP at a concentration of 0.5 ppm resulted in the highest average stevia stem height, measuring 32.071 mm over 6 weeks. This indicates that both BAP and natural cytokinins from plant extracts can enhance planlet height growth (Widiastoety, 2014). In addition to auxin hormones, the vitamin B1 content in *Aloe vera* also plays a role in supporting stem elongation by helping plants adapt to new environments. Vitamin B1 is thought to reduce stress caused by changes in the growing medium (Sempana et al., 2021).

Number of Leaves

Based on the research results, no leaves grew on full-strength MS medium without the addition of *Aloe vera* extract. This indicates that stevia's requirements in tissue culture cannot be met by basal MS medium alone. In this study, leaf growth in stevia only occurred in the treatment with the addition of 10% *Aloe vera* extract. Leaves appeared on day 56 after culture initiation, with a total of 10 leaves (Figure 4). Other treatments, including the control (0%) and concentrations of 5%, 15%, and 20%, did not show any leaf growth until the end of the observation period.

Figure 4. Number of leaves with the addition of 10% *Aloe vera* extract (treatment 2)

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This phenomenon indicates that a 10% concentration of *Aloe vera* extract is able to provide a balance of hormones and nutrients that supports leaf organ formation in stevia. The auxin and cytokinin hormones contained in *Aloe vera* extract are thought to play a role in stimulating cell division and differentiation processes, leading to leaf organ development. The presence of cytokinin in *Aloe vera* extract is suspected to be a key factor in leaf growth in stevia. This condition is in line with the findings of Marliana (2021), who reported that the application of 0.5 mL/L cinnamon extract resulted in an average of 13.4 stevia leaves, which was higher compared to other treatments. Similarly, the study by Firdaus & Asmono (2021) reported that the treatment of white light combined with kinetin 1 (2 ppm) as a synthetic cytokinin hormone produced up to 17.2 leaves on stevia plants. This further supports the role of cytokinins in promoting leaf growth in stevia tissue culture.

In contrast to the treatment with 10% *Aloe vera* extract, no leaf growth was observed with the addition of 5% *Aloe vera* extract. This is presumed to be because the 5% concentration is not effective enough to stimulate leaf formation in stevia. Meanwhile, at concentrations higher than 10%, namely 15% and 20%, it is suspected that hormonal imbalance occurs, preventing leaf growth in stevia. This condition is consistent with the statement by Yulia et al. (2020), who noted that increasing auxin concentrations beyond a certain level can inhibit leaf growth. Differences in the concentrations of plant growth regulators, each with varying abilities to stimulate explant growth, are one of the factors causing variation in the number of leaves observed in this study. A higher availability of cytokinins can promote faster growth and development of explants. This is in line with the general understanding that plant hormones, or phytohormones, in appropriate concentrations can either stimulate or inhibit plant growth and organ formation, depending on their type and balance (Lydianthy & Nihayati, 2019).

CONCLUSION

Based on the research results, it can be concluded that the addition of 10% *Aloe vera* extract is the optimum concentration capable of stimulating the growth of shoots, stems, and leaves of stevia. However, both MS medium without any addition and MS medium with various concentrations of *Aloe vera* extract have not been able to stimulate root growth in stevia.

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