



The Effect Of Tomato Extract (*Solanum lycopersicum* L.) and Coconut Water (*Cocos nucifera* L.) as Growth Regulators (PGR) in *Murashige And Skoog* (Ms) Media on the Growth of In Vitro Crossed *Cattleya* Orchid Tissue Culture

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Abstract

Cattleya orchids are highly prized ornamental plants that can be significantly propagated using in vitro tissue culture techniques. PGRs significantly influences the success of tissue culture. Synthetic PGRs are commonly used, but are relatively expensive and not environmentally friendly, so natural materials need to be used as alternatives. The purpose of this study was to determine how tomato extract (*Solanum lycopersicum* L.), coconut water (*Cocos nucifera* L.), and their combination as natural PGRs affect the growth of hybrid *Cattleya* orchid plantlets on Murashige and Skoog (MS) media. A Completely Randomized Design (CRD) was used to treat tomato extract (0%; 1%; 1.5%; 2%; 2.5%) and coconut water (0%; 5%; 10%; 15%; 20%) and their combinations. This study analyzed data based on parameters of plantlet height, number of leaves, number of roots, root length, and wet weight using ANOVA analysis followed by Duncan's test at the 5% level. The results showed that several growth parameters were affected such as plantlet height, number of roots, root length, and fresh weight showed significant effects. The number of leaves did not have a significant effect. Natural PGRs are generally considered a more cost-effective and environmentally friendly alternative, their effectiveness has not completely replaced synthetic PGRs.

Keywords: *Cattleya*, Coconut, Orchid, PGRs, Tomato.

1. Introduction

Orchids are ornamental plants with high aesthetic value, with a diversity of shapes, colors, and distinctive flower scents, making them a leading commodity in the ornamental plant industry, although orchids take a long time to grow (Ningsih *et al.*, 2021). There are approximately 25,000 to 30,000 orchid species worldwide, including those from the genus *Cattleya*. *Cattleya* orchids are among the plants with high commercial value, offering significant potential for development (T. I. S. Ningsih *et al.*, 2021). *Cattleya* orchids exhibit a wide variety of flower shapes, colors, and characteristics (Darlis *et al.*, 2021). Due to high market demand, hybrid *Cattleya* orchids have superior potential for development. Hybrid *Cattleya* orchids can boost propagation and trade efforts (Zulkaidhah *et al.*, 2019). However, *Cattleya* orchids are quite difficult to cultivate in Indonesia, one of which is through hydroponics because they are susceptible to fungal attacks if their roots are submerged in water for a long time (Hanoum, 2021). Orchids can be cultivated both vegetatively and generatively. To meet market demand, conventional vegetative orchid propagation methods such as stem cuttings, clump division, and splitting are also used. However, these methods often face obstacles and are ineffective (Darlis *et al.*, 2021). Furthermore, generative propagation cannot be carried out directly because orchid seeds lack endosperm, the nutrient reserves needed for growth. Therefore, in vitro generative propagation is a promising alternative for large-scale orchid seedling production. In tissue culture, the culture medium acts as a nutrient source that replaces the function of the endosperm, thus supporting optimal plant growth (Nuzullah & Firgiyanto, 2021).

Plant growth regulators (PGRs) are a key element in determining the success of the tissue culture process. The growth regulators commonly used are synthetic. However, due to the high cost and potential negative environmental impacts, more natural alternative plant growth regulators (PGRs) are needed (Sulaeman *et al.*, 2024). Natural plant growth regulators (PGRs) can be obtained from tomato extract (*Solanum lycopersicum* L.) and young coconut water (*Cocos nucifera* L.). Tomatoes (*Solanum lycopersicum* L.) are rich in compounds that can stimulate cell division, shoot formation, and root growth. Tomato extract contains complex compounds, including the hormones auxin and cytokinin,

which function as plant growth regulators. Administering these hormones at minimal concentrations can stimulate plant cell activity and grow more efficiently (Dewi *et al.*, 2021). The tomatoes used in the study were first ground to produce tomato extract, which can be used as a source of natural plant growth regulators (PGRs).

The coconut (*Cocos nucifera* L.) is a species of tree in the Palmae family with an upright trunk and is cultivated as a plantation commodity. The coconut plant has high utility value, as almost all its parts can be utilized for various purposes (Salsabila *et al.*, 2022). Coconut water can be used as a natural plant growth regulator (PGR) because it is affordable and readily available. Coconut water can support cytokinins, which act as plant growth regulators (PGR) (Ariyanti *et al.*, 2021). The coconut water used is obtained from young coconuts because it has a more diverse nutritional content compared to mature coconut water. Furthermore, mature coconut water tends to have a lower water volume due to the coconut's need for respiration and transpiration (I *et al.*, 2023). The purpose of this study was to test the effectiveness of tomato extract and coconut water as plant growth regulators in supporting the *in vitro* growth of hybrid *Cattleya* orchid tissue. It is hoped that the results of this research will produce a more cost-efficient and environmentally friendly source of natural plant growth regulators (PGRs) for the propagation of hybrid *Cattleya* orchids. Furthermore, this research may contribute to the development of more efficient tissue culture techniques for commercial cultivation of hybrid orchids. Therefore, the use of tomato extract and coconut water as natural plant growth regulators (PGRs) could be a potential step forward in plant biotechnology.

2. Literature Review

Cattleya orchids, belonging to the Orchidaceae genus, are among the most popular ornamental plants in the world. They are nicknamed the "Queen of Orchids" due to their large flowers and striking beauty (T. I. S. Ningsih *et al.*, 2021). Orchids are widely used as ornamental plants, herbal remedies, and as ingredients in beauty products (Hanoum, 2024; Hidayat & Pariyanto, 2021). The name *Cattleya* was given by John Lindley in 1824 in honor of William Catley, a 19th-century English horticulturist. Catley is known as the first collector of exotic orchids and successfully cultivated specimens of *Cattleya labiata*, thus immortalizing his name in the genus name (Hanoum, 2024). Some well-known species of the *Cattleya* genus include *Cattleya intemedia*, *Cattleya Brabantiae*, *Cattleya bicolor*, *Cattleya Clarkie*, *Cattleya lynn spencer walkeriana*, *Cattleya Corcovado*, *Cattleya bicolor brasiliensis*, *Cattleya gold digger-buttercup*, *Cattleya green emeralds*, and *Cattleya nobilis natalia* (Hanoum, 2024).

Cattleya is a type of epiphytic orchid with a sympodial growth pattern. It has double, slightly flattened, hard, and fleshy pseudobulbs with limited growth. Its root system is cylindrical, fleshy, and easily broken. It has a tapered tip that is smooth and slightly sticky, allowing the spongy roots to attach to solid substrates. Its leaves are thin and succulent, attached to the stem in a single leaf called a node. They are a long-lasting green color and do not fall off simultaneously (Hanoum, 2024; Sholiha, 2024). The distinctive feature of *Cattleya* orchids lies in their predictable flowering pattern based on leaf shape. Specimens with wide, short leaves tend to flower more frequently, while those with narrow, long leaves flower less frequently. *Cattleya* flowers are relatively large, with a combination of purple and orange (red tongue) and have a zygomorphic shape that can only be divided along one plane of symmetry. *Cattleya* orchids are classified as short-day plants, requiring a light intensity of around 20–40% to stimulate flowering. This plant grows optimally at temperatures of 25–35°C with 20–50% humidity. *Cattleyas* require shade and can thrive at altitudes of 0–500 meters above sea level. (Hanoum, 2024; Sholiha, 2024).

Crossbreeding is the process of obtaining new varieties by crossing two or more genetically distinct plants. Crossbreeding can occur naturally or with human assistance. If there are differences in plant characteristics, such as flower color, plant height, or plant shape, the crossbreeding is considered successful. Hybrid orchids are orchids produced through this process. This crossbreeding can produce hybrids between species, within the same species, or between different genera. This process creates a wide variety of traits, which can then be mass propagated using tissue culture methods. Hybrid orchids are one way to maintain plant quality to meet increasing market demand (Hartati & Cahyono, 2021; Zulkaidhah *et al.*, 2019). The crossbreeding process in orchids involves transferring pollinia or pollinaria to the stigma of a mature orchid flower. There are two types of crossbreeding: selfing and crossing. Selfing is the process of crossing by placing pollen on the stigma of a single flower, plant, or species. Crossing, on the other hand, involves placing pollen from the stigma of two different flowers (Dewanti, Wafa, Handoko, *et al.*, 2020).

Initiation is the process of taking explants from plants undergoing tissue culture (Hanifah *et al.*, 2024). Orchid initiation is carried out from the fruit of the plant, which can be achieved by sowing hundreds or even thousands of seeds contained within the fruit on Murashige and Skoog (MS) media, which will then develop into protocorm-like bodies (PLB) (Solichatun *et al.*, 2020). Orchid initiation through seeds or embryos is carried out because orchid seeds lack endosperm or food reserves and are very small. Therefore, orchid initiation from seeds has the potential to produce large numbers of uniform seedlings in a relatively short time (Dewanti, Wafa, Handoko, *et al.*, 2020).

Initiation in orchids can be done from several parts, namely (1) Initiation from the stem shoot section as a source of callus induction explants which is then isolated by the shoot bud with the callus cut longitudinally (transversely) with a thickness of 0.5 - 1.0 mm and then cultured or planted on solid agar media (Rachmawati *et al.*, 2014). (2) Initiation from the rachis section which is planted on solid agar media with a horizontal position and the flower bud slices are in the upper position. Generally there are 1 - 4 rachis in each bottle, this depends on the size of the culture bottle used. The culture bottle will be incubated for 1 - 1.5 months in dark conditions and will be moved to bright conditions 16 hours

photoperiod under fluorescent lights intensity $\sim 13 \mu\text{mol} / \text{m}^2 / \text{s}$ until the embryo becomes mature for ± 3 months. At this stage it will produce 3 - 12 embryos from 1 embryo based on the variety response (Winarto, 2017). (A – D) Orchid initiation from the shoot section. (A) Orchid shoot, (B – C) Isolation of orchid shoot, (D) Shoot culture on solid agar media (Rachmawati *et al.*, 2014). (E – J) Orchid initiation from the rachis section. (E) Rachis as the source of explants, (F) Cutting flower buds, (G) Selected rachis, (H) Separation of flower sheaths, (I) Rachis ready for culture (Winarto, 2017). (K – L) Orchid initiation from seed fruit (Mercado & Jaimes, 2022; Schvambach *et al.*, 2022).

Plant tissue culture is a method of multiplying or duplicating plant cells or tissues using nutrient solutions in a controlled, aseptic environment. Tissue culture is based on plant totipotency, the ability of plants to reproduce and regenerate into whole plants. A growing medium containing macronutrients, micronutrients, organic supplements or vitamins, a carbon source, and plant growth regulators (PGRs) is crucial for optimal tissue culture growth (Maisyarah & Silvia, 2021). One of the advantages of plant tissue culture is that it can produce genetically uniform plants. All the resulting plants will be genetically identical because the tissue used to initiate the culture is taken from a single plant. This uniformity gives the plants advantages, such as greater resistance to disease and pests. Plant tissue culture is a fast and effective method, capable of producing hundreds to thousands of plants within a few months. Compared to conventional methods, this is much faster, as plant propagation takes several years to produce mature plants (Ferris, 2023). In the medical world, plant tissue culture techniques are also used as an efficient method for producing medicinal compounds derived from plants (Maisyarah & Silvia, 2021).

Cattleya orchid growth through tissue culture techniques generally begins with a mother plant that produces fruit containing seeds. Based on research by Pinto *et al.* (2023) with *Lycaste virginalis* orchids, the fruit seeds generally have 6 longitudinal veins with a color that is considered ripe yellowish green. The seeds themselves generally have fine grains with a golden yellow color and have a sand-like texture. The shape of the seeds is oval scobiform with embryos in the form of prolate spheroids. The seeds then germinate to form Protocorm-like bodies (PLB), which are spherical structures formed by explant tissue or callus in vitro techniques (Malahayati *et al.*, 2022). Over time, the protocorms develop into mini plantlets with leaf and root primordia, which then undergo multiplication so that the number of plantlets increases. Eventually, they will have complete leaves and roots and the plantlets are ready to be transferred to a new medium. In the final stage, the young plants are removed from the culture bottle and planted in a natural medium such as coconut fiber or moss to adapt to the outside environment and grow into mature orchid plants.

The success rate of tissue culture is influenced by several factors, including explants, the media used, and environmental conditions. An explant is a small piece of plant tissue or organ. Careful explant selection is crucial for the success of tissue culture. Explants that are too small reduce their viability and increase the risk of failure. Conversely, explants that are too large are more susceptible to contamination and can easily curl, reducing contact with the medium. The ideal size ranges from 0.5 to 1 cm, but this can vary depending on the plant part used and the type of plant being cultured (Kurnia *et al.*, 2022). Furthermore, the general explant and plant genotype influence the success rate of tissue culture (Apriliyani & Wahidah, 2021). The use of young plant tissue has a higher regeneration rate because it contains actively dividing cells and must be free from contaminants (Sugiari *et al.*, 2020; Tuwo *et al.*, 2022). Tissue culture in orchids can use plant organ explants or from seeds where seed germination can become Protocorm-like bodies (PLB), then can grow into shoots that form green leaf buds, then leaves and roots will grow from the shoots which are usually called plantlets (Dewanti, Wafa, Handoko, *et al.*, 2020).

The growing medium used in tissue culture propagation is crucial for meeting the plant's nutritional needs for optimal survival and growth. The growing medium should include essential amino acids, inorganic salts, vitamins, buffer solutions, and glucose as the primary energy source. The media must be prepared using appropriate proportions to achieve optimal results. There are two types of growing media: solid and liquid. Solid growing media can be used to grow protocorm-like bodies (PLB) until plantlets form, while liquid growing media can be used for growing explants that will produce tissue, particularly for white callus-like growth (Apriliyani & Wahidah, 2021). There are various types of tissue culture media, such as Vacin and Went (VW), Murashige and Skoog (MS), and Knudson C (KC), which have similar properties, but differ in their composition. Murashige and Skoog (MS) media generally contains macronutrients and micronutrients in the form of organic salts at specific concentrations and ratios. Furthermore, this medium contains sources of carbohydrates, water, amino acids, vitamins, and plant growth regulators (PGRs). Murashige and Skoog (MS) medium is often used because it contains relatively high levels of nitrogen and inorganic salts compared to other growing media. Furthermore, this medium is compatible with various plant species, making it a commonly used medium (Apriliyani & Wahidah, 2021).

In vitro culture environments are influenced by various factors, such as light, temperature, humidity, and medium pH (Apriliyani & Wahidah, 2021; Kurnia *et al.*, 2022). One of the main factors in the tissue culture process is lighting in the culture room, which can impact plant growth. Lighting from white fluorescent lamps, commonly applied in tissue culture, can trigger photochemical reactions that lead to the formation of formaldehyde and iron (Fe) deficiency in the culture medium components. Light plays a major role in regulating the direction of explant morphogenesis (Simamora *et al.*, 2021). Generally, the light intensity required in an incubation room is 600–1000 lux (Muchsin *et al.*, 2022). Meanwhile, the temperature in the incubation room is generally controlled at 25–28°C to ensure optimal conditions for plant growth (Sulichantini *et al.*, 2024). The pH of the medium used for orchids is generally between 5.6 and 5.8 (Idly & Syamsuddin, 2023). Furthermore, the humidity of the incubation room is an important factor that can influence growth. The humidity level is generally 70%. If the incubation room has a humidity level below 50%, the growing medium will generally dry out more easily, and if the humidity level is above 50%, the percentage of contamination

caused by microorganisms generally increases (Muchsin *et al.*, 2022). Research by Dewi *et al.* (2024) found that the size of the bottle used for tissue culture also affects media availability, space volume, and oxygen supply. This is closely related to the humidity within the bottle. If gas exchange is too high and the medium is too dense, humidity can decrease, causing the medium to dry out quickly. The bottle size needs to be adjusted to the size of the plantlets used to create a supportive environment for plantlet growth. The tissue culture bottles used must be transparent, withstand high temperatures and pressure, be resistant to chemical fuels, and be non-toxic. The bottle surface must be smooth and sterile to avoid potential contamination.

Plant growth regulators (PGRs), also known as plant hormones, are natural or synthetic organic compounds that control physiological processes in plants and are active at low concentrations. Plant growth regulators (PGRs) fall into five main groups: auxins, gibberellins, cytokinins, ethylene, and abscisic acid. Plant growth regulators (PGRs), or plant hormones, are closely related to the regulation of growth, development, and responses to stimuli. The use of PGRs offers various benefits, including improving root systems, particularly accelerating root growth in young plants. Furthermore, these substances can enhance vegetative growth, stimulate even fruit ripening, prevent leaf, flower, and fruit shedding, and expand photosynthetic pathways (Ayda *et al.*, 2023). The hormone auxin plays a role in accelerating cell enlargement and contributing to the differentiation of transport tissues such as the phloem and xylem. Gibberellins function as stimulants in cell division, while cytokinins, when combined with auxins in certain ratios, can accelerate cell division and the formation of plant structures (morphogenesis). Furthermore, ethylene is known as a hormone that plays a role in accelerating fruit ripening and can also stimulate the growth and development of plant organs. On the other hand, abscisic acid is known as a growth-inhibiting hormone because it can reduce the rate of division and elongation at the growing point and plays a role in maintaining seed dormancy (Yuda Purwana, Delvi Maretta, 2020). In tissue culture, commonly used growth hormones are derived from auxins and cytokinins. Some commonly used auxin growth regulators include 2,4-dichlorophenoxyacetate (2,4-D), naphthalene acetic acid (NAA), indole butyric acid (IBA), and indole acetic acid (IAA). The most commonly used cytokinin growth regulators are benzyl aminopurine (BAP) and kinetin (KIN) (Maisyarah & Silvia, 2021).

Several types of organic materials in the form of extracts are frequently used in plant tissue culture to support the growth and development of explants. Some commonly used ingredients include extracts from tomatoes, corn, bananas, and coconut water. Tomato extract is known to contain various compounds such as vitamins, fats, and natural growth regulators (PGRs), particularly cytokinins. Furthermore, tomatoes are a horticultural commodity rich in nutrients and have long been widely used by the public for various purposes. Per 100 grams of tomatoes, there are 3.6 grams of carbohydrates, 1 gram of protein, and 0.2 grams of fat. Furthermore, tomatoes contain 10 grams of calcium, 0.6 grams of iron, 10 grams of magnesium, and 16 grams of phosphorus. Tomatoes have a relatively high water content, with 94 grams of water per 100 grams of fruit. Tomatoes are also a good source of vitamins, containing 1700 IU of vitamin A, 0.1 grams of vitamin B1 (thiamine), 0.02 grams of vitamin B2 (riboflavin), 21 grams of vitamin C, and 0.6 grams of niacin (Daroini *et al.*, 2023). Tomato extract is a natural ingredient that contains various nutrients that can be utilized by plants, especially in tissue culture media. Tomato extract contains plant growth regulators (PGRs), which play a significant role in plant physiological processes, including chlorophyll formation. One of the hormones it contains, cytokinin, can stimulate cell division in meristem tissue. Furthermore, ripe tomatoes also contain auxin. This hormone's role includes stimulating organogenesis, embryogenesis, and shoot growth. Tomato extract has been used as a supplement in in vitro culture media for several orchid species. The auxin hormone contained in tomatoes can contribute to shoot height growth through cell elongation (L. K. Dewi *et al.*, 2021).

Coconut water is the clear liquid inside the endosperm (kernel) of the coconut (*Cocos nucifera*) and is commonly consumed fresh in tropical environments. Indonesia is the world's largest coconut producer and plays a significant role in the Indonesian economy (Al Banna *et al.*, 2023). Based on maturity, coconut water is categorized into three categories: young or immature coconuts (IMC), aged 5-6 months and containing less flesh (like jelly); mature coconuts (MC), aged 8-9 months, with a thin layer of flesh (2-4 mm); and over-mature coconuts (OMC), aged >12 months, with a dense layer of flesh (approximately 10 mm). Young coconut water is widely consumed globally as a refreshing beverage and boasts health benefits, largely due to its composition (Azra *et al.*, 2023).

Analysis of the chemical composition of coconut water indicates the presence of several types of plant growth regulators (PGRs). These compounds contain numerous organic compounds, such as 1,3-diphenylurea, which has cytokinin activity, which can induce cell division and organ differentiation. Zeatin glucuronide, zeatin riboside, high levels of potassium and potassium, protein, and potassium (potassium) in coconut water can stimulate rapid growth. Coconut water contains quite high levels of cytokinins, with cytokinins such as kinetin and zeatin in young coconut water reaching 273.62 mg/L and 290.47 mg/L, respectively. The auxin IAA concentration is 198.55 mg/L. Coconut water can stimulate differentiation and cell division (Kusnadi, 2019; Latifah *et al.*, 2017). Good-quality coconut water typically comes from young coconuts with white flesh and a soft texture. Coconut water is susceptible to damage after exposure to heat and open air. Young coconut water contains sugar, electrolytes, minerals, growth hormones, and amino acids, which are some of the nutrients contained in young coconut water, which also contains 7.27% carbohydrates, 0.2% protein, and 0.15% fat. Young coconut water contains several forms of sugar, such as sorbitol, fructose, and glucose. Young coconut water tends to be sweeter than regular coconut water because it has a higher sugar content. Meanwhile, mature coconut water is known to contain only small amounts of vitamins. Some of the vitamins contained in it include riboflavin at 0.01 mg/100 ml, pantothenic acid 0.52 mg/100 ml, vitamin C between 0.7–3.7 mg/100 g,

biotin 0.02 mg/100 ml, nicotinic acid 0.64 mg/100 ml, and folic acid at 0.003 mg/100 ml. Therefore, young coconut water was chosen as the material used in this study (I *et al.*, 2023).

3. Materials and Methods

3.1. Materials

This research was conducted using various laboratory equipment that supports the tissue culture process starting from the sterilization stage, making planting media, as well as planting and maintaining plantlets in the incubation room. At the sterilization stage, a hand sprayer was used, then at the planting media making stage, tools were used, including a hand sprayer, micropipette, micropipette tips, shaker, autoclave, blender, pH meter, beaker glass, spatula, Erlenmeyer flask. At the planting stage, a hand sprayer, laminar air flow cabinet (LAFB), petri dish, tweezers, scalpel blade, bunsen burner, culture bottle were used. The tools used in the incubation room for culture results include a storage rack for cultured explants, namely a ruler, cardboard, camera, air conditioner (AC), fluorescent lamp, lux meter and thermohygrometer.

This research was conducted using hybrid *Cattleya* orchids C4 (Purple *Cattleya* X Yellow *Cattleya*) and C5 (White *Cattleya* Spotted Purple X Purple) as the main materials obtained from the Tissue Culture Laboratory of the UPTD Horticultural Seed Center of West Java Province. In addition, several supporting materials were also used such as tomato extract, young coconut water, sterile water, 70% alcohol, methanol, stock solutions [A, B, C, D, E, F, G, H], HCL and NaOH as pH regulators of the media, agar powder [Swallow], granulated sugar, aluminum foil, heat-resistant plastic, rubber bands, plastic wrap (Darlis *et al.*, 2021).

3.2. Methods

The procedures carried out in this research consist of several stages, namely the selection of plant parts (explants) used, the sterilization process for the media and tools and materials to prevent contamination, the preparation of tomato extract and coconut water which are used as natural growth regulators (PGRs), planting explants in culture media, and the maintenance and observation stages to monitor the development of explants during the research.

3.2.1. Sterilization of Tools and Work Environment

Sterilization involves washing tissue culture equipment such as tweezers, scalpel blades, scissors, petri dishes, and culture bottles with soap and rinsing with clean water. After the equipment has been washed, it is then wrapped in paper and placed in an oven at 121°C for 1 hour. The culture bottles are then placed in an autoclave at 121°C for 1 hour, then transferred to the oven (Lengkong *et al.*, 2023).

3.2.2. Making Tomato and Coconut Water Extract

At this stage, tomatoes were selected for use in the study, characterized by their red skin and ripeness. They were washed with clean water and weighed. They were then thinly sliced and pureed without adding water using a blender. The pulp was separated from the pureed tomatoes by filtering. Afterward, sterilized bottles were filled with the filtered tomato extract until concentrations of 0%, 1%, 1.5%, 2%, and 2.5% were achieved. Meanwhile, the researchers diluted the coconut water to concentrations of 0%, 10%, 15%, and 20% (Ayuningtias *et al.*, 2024).

3.2.3. Media Preparation and Media Sterilization

Five treatments of growing media concentrations were created with three different factors by dividing the media into three parts, each containing five Erlenmeyer flasks, each containing 200 ml of the tomato extract and coconut water concentrations used. All ingredients were then weighed using an analytical balance: 30 g/L of sugar, 6 g/L of agar, 20 ml/L of stock solutions A and B, and 5 ml/L of solutions C to F. Add agar and sugar by dissolving them in 3 liters of distilled water and stir thoroughly using a spatula.

After dissolving, the tomato extract and coconut water were homogenized with the specified concentrations, namely tomato extract 0% (T0), 1% (T1), 1.5% (T2), 2% (T3), 2.5% (T4), young coconut water 0% (T0), 5% (T1), 10% (T2), 15% (T3), and 20% (T4), as well as a combination of tomato extract and coconut water, namely tomato extract 0% + coconut water 0% (T0), tomato extract 1% + coconut water 10% (T1), tomato extract 1.5% + coconut water 15% (T2), tomato extract 2% + coconut water 20% (T3), tomato extract 2.5% + coconut water 5% (T4). Then the media solution was homogenized again with a shaker. After the solution was homogeneous, it was heated to boiling and poured into a 20 ml culture bottle, then sterilized using an autoclave.

3.2.4. Preparation and Planting of Explants

In this stage, the hybrid *Cattleya* orchids C4 (Purple *Cattleya* X Yellow *Cattleya*) and C5 (White *Cattleya* with Purple Spots X Purple) were planted in a Laminar Air Flow Cabinet (LAFC). The LAFC was sterilized by spraying with 70% alcohol and exposed to UV light for 1 hour before planting. Planting equipment, including a Bunsen burner, tweezers, scissors, and scalpel blades, was also sterilized with 70% alcohol before use.

Remove the explants from the bottles using tweezers and place them in prepared Petri dishes. Plant them close to the Bunsen burner to minimize contamination. The plantlets were weighed using an analytical balance before planting. Plant two explants in each culture bottle. Next, tightly cover the culture bottles with plastic wrap and a rubber band. After the entire planting process is complete, store the culture bottles in the incubator and observe their growth (Rohman *et al.*, 2023).

3.2.5. Observation

There were five treatment levels with five replications and one seedling per replication, using a Completely Randomized Design (CRD). Weekly observations included plantlet height, root length, root count, leaf count, and initial and final wet weight. Plantlet height and root length were measured using a ruler, while leaf count and root count were directly observed. Initial wet weight was measured by weighing the plantlets before planting using an analytical balance, while final wet weight was obtained by weighing the plantlets after three months of planting using an analytical balance in a laminar air flow cabinet (LAFC).

4. Results and Discussion

4.1 The Effect of Tomato Extract and Coconut Water on the Growth of Hybrid *Cattleya* Orchids

4.1.1 Amount of Leaves

Based on the results of the Shapiro–Wilk normality test, the distribution of leaf number data showed variations between treatments. Most treatments had a significance value >0.05 , indicating a normal distribution. However, some showed non-normal distributions. This indicates that the data generally did not fully meet the assumption of normality. The results of the homogeneity of variance test using the Levene's Test showed a significance value of 0.060 based on the mean (>0.05), indicating that the variance between treatment groups was homogeneous. This was further supported by the significance value based on the median of 0.947, further confirming that the variance between groups was relatively uniform.

The ANOVA analysis results showed that differences in orchid species, natural growth regulators (PGRs), and concentration variations, as well as the interaction between these factors, had no effect on leaf number, with a significance value of 0.457 (>0.05) and a coefficient of determination (R^2) of 0. Orchid species had no significant effect on leaf number, with a significance value of 0.104 (>0.05). The concentration factor also had no significant effect, with a significance value of 0.489 (>0.05). Furthermore, the interaction between orchid species and concentration also showed no significant effect, with a significance value of 0.784 (>0.05). It is suspected that the growth of leaf number in hybrid *Cattleya* orchid plantlets is more influenced by plant genetic factors and environmental conditions than by the nutrients contained in Murashige and Skoog (MS) media and natural plant growth regulators (PGRs) used. Hormones contained in tomato extract, coconut water, and a combination of the two have not been able to provide an effect on the growth of hybrid *Cattleya* orchids. Plants tend to maintain a relatively constant number of leaves as a form of adaptation to the environment. There are combinations of treatment factors that do not have significant values, this is suspected due to the low variation in data on the number of leaves parameter, so that the interaction cannot be calculated statistically (Andrade, 2024; Telaumbanua *et al.*, 2025).

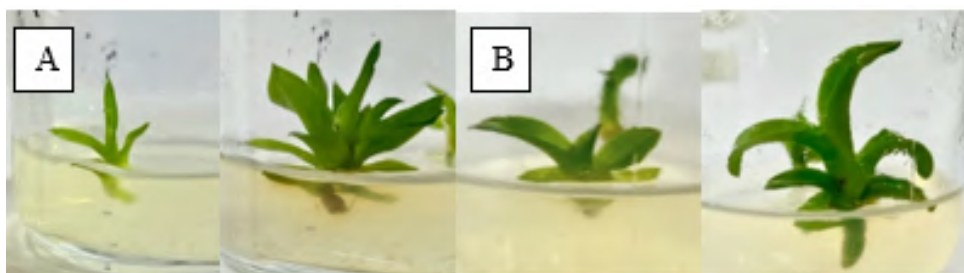


Figure 1 : Hybrid *Cattleya* orchid explants on leaf number parameters

Table 1 : Amount of Leaves ANOVA

Souce	Type III Sum of Square	.df	Mean Square	F	Sig.	Partial Eta Square
Corrected Model	1293.392 ^a	25	51.736	1.014	0.457	0.196
Intercept	19847.025	1	19847.025	388.864	<0.001	0.789
Orchid	136.900	1	136.900	2.682	0.104	0.025
PGR	0.000	0				0.000
Concentration	434.800	9	48.311	0.947	0.489	0.076
Orchid × PGR	0.000	0				0.000
Orchid × Concentration	281.500	9	31.278	0.613	0.784	0.050
PGR × Concentration	0.000	0				0.000
Orchid × PGR × Concentration	0.000	0				0.000
Error	5308.000	104	51.038			
Total	27215.000	130				
Corrected Total	6601.392	129				

a. R Square = 0.196 (Adjusted R Squared = 0.003)

4.1.2 Height of Planlets

Based on the results of the Shapiro–Wilk normality test, the distribution of plantlet height data showed variation between treatments. Most treatments had a significance value ≥ 0.05 , but several treatments showed a non-normal distribution. This indicates that the overall data did not fully meet the assumption of normality. The results of the homogeneity of variance test using the Levene's Test showed a significance value of 0.011 based on the mean (<0.05), indicating that the variance between treatment groups was not homogeneous. However, the median approach obtained a significance value of 0.869 (>0.05), indicating that the difference in variance was not significant, and the analysis could still be continued using ANOVA.

The ANOVA analysis results showed that the overall model had a very significant effect on plantlet height, with a significance value <0.001 and a coefficient of determination (R^2) of 0.774. The orchid species factor significantly affected plantlet height, with a significance value of 0.013 and an F-value of 6.355. The concentration factor had a highly significant effect with a significance value of <0.001 and an F-value of 17.216. Furthermore, the interaction between orchid species and concentration also showed a highly significant effect with a significance value of <0.001 and an F-value of 11.413. Based on the DMRT test results, the highest average plantlet height was found in the m3u2 treatment (C5 hybrid *Cattleya* (White *Cattleya* with Purple Spots X Purple) + a combination of 2% tomato extract and 20% coconut water), with an average value of 20.42 mm. Meanwhile, the lowest average value was found in the k4u1 treatment (C4 hybrid *Cattleya* (Purple *Cattleya* X Yellow *Cattleya*) + 20% coconut water), at 11.50 mm.

Based on the average height of hybrid *Cattleya* orchid plantlets, the highest average was found in the m3u2 treatment (C5 hybrid *Cattleya* (White Spotted *Cattleya* X Purple *Cattleya*) + a combination of 2% tomato extract and 20% coconut water). This is thought to be due to the hormonal balance of cytokinin and auxin. The cytokinin hormone contained in coconut water can help stimulate cell division and elongation and support shoot formation, resulting in improved extract growth (Latifah *et al.*, 2017). However, the role of cytokinin in cell elongation is influenced by the presence of other phytohormones, particularly auxin.

The treatment with the lowest average was the k4u1 treatment (C4 hybrid *Cattleya* (Purple *Cattleya* X Yellow *Cattleya*) + 20% coconut water). This condition may be due to the use of coconut water as a natural growth regulator (PGR) alone at high concentrations, which causes hormonal imbalance and ineffective cell elongation. The auxin hormone content in coconut water is relatively low, so the presence of tomato extract is thought to act as a source of auxin through its content of the amino acid tryptophan (Rahmawati & Widoretno, 2024). Tryptophan is an important compound in the biosynthesis of IAA (Indole Acetic Acid), which plays a role in cell elongation and stem growth. Furthermore, auxin plays a role in regulating plant responses to various stressors; it is used to mitigate the negative effects of stress on plant growth and development (Gao & Zhuang, 2024). (Gao & Zhuang, 2024). So the combination of concentrations in the m3u2 treatment (C5 hybrid *Cattleya* (White Spotted *Cattleya* X Purple) + a combination of 2% tomato extract and 20% coconut water) allows for a better hormonal balance so that the height growth of the plantlets is maximized. Treatments u1 (C4 hybrid *Cattleya* orchid) and u2 (C5 hybrid *Cattleya* orchid) with the addition of 1.5% tomato extract produced the highest average plantlet lengths, at 16.9 mm and 18.4 mm, respectively. This shows that the concentration of tomato extract 1.5% is the best concentration of tomato extract in increasing the height of plantlets, then in the u1 treatment (C4 hybrid *Cattleya* orchid) with the addition of a combination produced the highest average plantlet at a concentration of 1% tomato extract + 10% coconut water, namely 17.3 mm and in the u2 treatment (C5 hybrid *Cattleya* orchid) produced the highest average plantlet at a concentration of 2% tomato extract + 20% coconut water, namely 20.4 mm. According to Telaumbanua (2022), a higher concentration of coconut water causes the height of orchid plantlets to increase, while tomato extract contains a variety of complex phytohormones and in low concentrations has a more efficient stimulant effect so that growth is more optimal (Agustin *et al.*, 2020). Different results were also found in the coconut water treatments, each with a different concentration. U1 (C4 hybrid *Cattleya*

orchid) produced a superior growth rate of 15.8 mm at a 15% concentration, while U2 (C5 hybrid *Cattleya* orchid) produced a superior growth rate of 17.72 mm over the control. This is likely due to the different plant genotypes of U1 (C4 hybrid *Cattleya* orchid) and U2 (C5 hybrid *Cattleya* orchid), requiring different hormone concentrations to stimulate organogenesis (Zakiyah *et al.*, 2025). According to Ashar *et al.* (2023), the commonly used coconut water concentration ranges from 2 to 15%. This aligns with the results of research on U1 (C4 hybrid *Cattleya* orchid), which showed that a 15% concentration provided the best growth results.

The control treatment, using pure Murashige and Skoog (MS) medium, for U2 (C5 *Cattleya* orchid), is thought to have sufficient levels for plantlet height growth. The provision of macronutrients, micronutrients, and vitamins needed can be provided according to the plant's growth stage (Lubis & Harahap, 2024). Murashige and Skoog (MS) media contains relatively high levels of nitrogen and inorganic salts compared to other types of growing media. Nitrogen (N) is needed by orchids to stimulate cell elongation during the vegetative period. Nitrogen is the main macronutrient essential for plants as a major component of various compounds in the plant body. Growing plants require nitrogen to form new cells (Apriliyani & Wahidah, 2021; Ningsih *et al.*, 2024).

Table 2 : Height of Planlet ANOVA

Souce	Type III Sum of Square	.df	Mean Square	F	Sig.	Partial Eta Square
Corrected Model	663.217 ^a	25	26.529	14.256	<0.001	0.774
Intercept	29168.101	1	29168.101	15674.320	<0.001	0.993
Orchid	11.827	1	11.827	6.355	0.013	0.058
PGR	0.000	0				0.000
Concentration	288.336	9	32.037	17.216	<0.001	0.598
Orchid × PGR	0.000	0				0.000
Orchid × Concentration	191.143	9	21.238	11.413	<0.001	0.497
PGR × Concentration	0.000	0				0.000
Orchid × PGR × Concentration	0.000	0				0.000
Error	193.532	104	1.861			
Total	31678.310	130				
Corrected Total	856.749	129				

a.R Squared = 0.774 (Adjusted R Squared = 0.720)

Table 3 : Average Growth Parameter of Plantlet Height

Treatment	Average of Height Plants (mm)	
	C4 Orchid	C5 Orchid
Control 0%	14 ^{bcd}	17,72 ^{ijk}
Tomato Extract 1%	13,8 ^{bcd}	12,8 ^{ab}
Tomato Extract 1.5%	16,9 ^{ghij}	18,4 ^{ik}
Tomato Extract 2%	16 ^{efghi}	11,9 ^a
Tomato Extract 2.5%	14,2 ^{bccde}	11,8 ^a
Control 0%	14 ^{bcd}	17,72 ^{ijk}
Coconut Water 5%	13,9 ^{bcd}	16,2 ^{fghi}
Coconut Water 10%	15,6 ^{defgh}	16,2 ^{fghi}
Coconut Water 15%	15,8 ^{defghi}	14,4 ^{bcddef}
Coconut Water 20%	11,5 ^a	16,4 ^{ghi}
Controll 0%	14 ^{bcd}	17,72 ^{ijk}
Mix (T) 1% + (C) 10%	17,3 ^{hij}	13 ^{abc}
Mix (T) 1,5 % + (C) 15 %	16,7 ^{ghij}	19,4 ^{kl}
Mix (T) 2% + (C) 20%	16,9 ^{ghij}	20,4 ^l
Mix (T) 2,5% + (C) 5%	15 ^{cdefg}	14 ^{bcd}



Figure 2 : Hybrid *Cattleya* orchid explants on plantlet height parameters (A) first week (2) second week

4.1.3 Number and Length of Roots

Based on the statistical test results, the root number parameter showed varying data distributions across treatments based on the Shapiro–Wilk normality test. Some treatments had significance values ≥ 0.05 , indicating a normal distribution, while others had significance values less than 0.05, indicating that the data generally did not fully meet the assumption of normality. Meanwhile, for the root length parameter, the Shapiro–Wilk normality test results indicated that all treatment groups had significance values greater than 0.05, indicating a normal distribution. The homogeneity of variance test using the Levene's Test showed that the root number had a significance value based on the mean of 0.001 (<0.05), indicating that the variance between treatment groups was not homogeneous. However, using the median approach, a significance value of 0.910 (>0.05) was obtained. Meanwhile, for root length, the significance value based on the mean was <0.001 , indicating that the variance between groups was not homogeneous. However, based on the median, a significance value of 0.479 (>0.05) was obtained, indicating that the variance was relatively homogeneous, allowing the analysis to proceed.

The results of the ANOVA analysis of root number showed a highly significant effect on root number ($p < 0.001$). The coefficient of determination (R^2) was 0.613. Orchid species had the greatest effect on root number with an F value of 40.444, followed by concentration with an F value of 3.742. The interaction between orchid species and concentration also had a highly significant effect with an F value of 5.228. For root length, the data also showed a highly significant effect ($p < 0.001$) with a coefficient of determination (R^2) of 0.629. Orchid species, plant growth regulators (PGRs), and concentration each had a highly significant effect on root length. Furthermore, the interaction between orchid species and PGRs and the interaction between orchid species and concentration also showed a highly significant effect on this parameter. The results of observations on the growth of hybrid *Cattleya* orchid roots show that roots are formed at the base of the stem of hybrid *Cattleya* orchid plantlet explants in the form of small protrusions with a whitish light green color which increase in length over time Figure 4 8. The emergence of roots in tissue culture indicates that the explants grown in vitro are in healthy condition because basically the roots play a role in nutrient absorption (Maharani *et al.*, 2025). Root formation in this study occurred in all trial media, namely Murashige and Skoog media with tomato extract, Murashige and Skoog media with coconut water, and Murashige and Skoog media with Mix (tomato extract and coconut water).

Further DMRT test results showed that the m4u2 treatment (Cattleya Orchid C5 + Mix (T) 2.5% + (K) 5%) produced the highest average root length, at 27.6 mm, significantly different from the other treatments. The lowest root length was obtained in the control treatment, at 1.50 mm. The highest average root number was obtained in the k3u1 treatment (C4 orchid + 15% coconut water), at 4.8 mm. The lowest average root number was obtained in the k4u2 treatment (C5 orchid + 20% coconut water), at 1.4 mm. For root length, the highest result was obtained in the m4u2 treatment (Cattleya Orchid C5 + Mix (T) 2.5% + (K) 5%). This combination concentration was the best combination for root length. This is suspected to be due to the higher auxin-to-cytokinin ratio that stimulates root formation (Fauziah *et al.*, 2021). Tomato extract contains vitamin B1, which stimulates root growth (Rahmawati & Widoretno, 2024). Meanwhile, for root growth, the highest yield was obtained in the k3u1 treatment (C4 orchid + 15% coconut water). Coconut water also contains cytokinins, which aid cell division through protein synthesis, and auxins, which aid cell elongation. This hormone combination is thought to promote root growth (Yahya *et al.*, 2024). The lowest root length was achieved in the control treatment, presumably due to the absence of natural growth regulators (PGRs) that support root growth. The control medium was supplemented with basic nutrients from Murashige and Skoog (MS) medium. However, the macro- and micronutrients contained in Murashige and Skoog (MS) are thought to require the addition of growth regulators (PGRs) to the tissue culture medium to support root growth (Fatana *et al.*, 2024). Meanwhile, the lowest average root number was obtained in the k4u2 treatment (C5 orchid + 20% coconut water). This could be caused by potential osmotic stress or hormonal imbalance at higher levels. Excessive hormone levels can further suppress root initiation by inhibiting the auxin-mediated pathway, leading to poor root primordia formation or complete inhibition of rhizogenesis. Although cytokinins are the main component, coconut water also contains indole-3-acetic acid (IAA), the main type of auxin in plants. Auxin plays a central role in the formation of primary, lateral, and adventitious roots. Initially, auxin is located at the shoot tip and is then transported to the root tip, where it promotes cell division and elongation, as well as root growth.

The highest average root counts for u1 (C4 hybrid *Cattleya* orchid) and u2 (C5 hybrid *Cattleya* orchid) were achieved in the 15% coconut water treatment, at 4.8 and 2.4, respectively. This suggests that 15% coconut water is the best concentration for increasing root count. Meanwhile, for the root length parameter, u1 (C4 hybrid *Cattleya* orchid) produced the highest average at 15% concentration, at 20 mm, and u2 (C5 hybrid *Cattleya* orchid) produced the highest average at 10% coconut water, at 21.6 mm. This may be due to the different genotypes of the same plant, resulting in different concentrations of hormones required to promote organogenesis, including rooting (Zakiyah *et al.*, 2025). In the combination of plant growth regulators (PGRs), the number of roots of u1 (C4 hybrid *Cattleya* orchid) produced the highest average at a concentration of 2.5% tomato extract + 5% coconut water of 3.6. However, the combination concentration of 1% tomato extract + 10% coconut water showed the same highest average as the combination of 2.5% tomato extract + 5% coconut water of 3.6. This can be interpreted that the combination of 1% tomato extract + 10% coconut water and 2.5% tomato extract + 5% coconut water is a sufficient combination concentration that can support root growth. Meanwhile, in the root length parameter, treatment u1 (C4 hybrid *Cattleya* orchid) produced the highest average at a concentration of 1.5% tomato extract + 15% coconut water of 14.4 mm and u2 (C5 hybrid *Cattleya* orchid)

produced the highest average at a concentration of 2.5% tomato extract + 5% coconut water of 27.6 mm. The number of roots given a combination of higher cytokinin and auxin concentrations did not significantly differ. Administering higher cytokinin concentrations compared to auxin can inhibit explant root formation. High cytokinin content compared to auxin suppresses root development in explants. Higher auxin concentrations play a crucial role, as cytokinin tends to suppress root formation in plantlets (Fitria *et al.*, 2023).

The number of roots in u1 (C4 hybrid *Cattleya* orchid) obtained at a concentration of 1.5% was 3.4, and u2 (C5 hybrid *Cattleya* orchid) obtained at a concentration of 2% was 3.0. The increase in root number may be due to the tomato extract containing the hormone auxin, which can support root elongation. This helps root primordia cells grow and elongate. Calcium and phosphorus content can also support root growth. Calcium supports the stimulation of root hair formation, while phosphorus plays a role in stimulating the formation of plantlet roots (Ayuningtias *et al.*, 2024). Meanwhile, the root length parameter, u1 (C4 hybrid *Cattleya* orchid) and u2 (C5 hybrid *Cattleya* orchid) obtained the highest averages at a concentration of 1.5% of 15.8 and 18 mm, respectively. This can be interpreted that 1.5-2% tomato extract is the best concentration of tomato extract in root growth. Auxin in tomato extract supports the elongation of the root tip meristem, this is because there is an increase in sugar content in the cell vacuole which will increase osmotic pressure and decrease pH which causes the cell wall to have an elastic and regular arrangement (Ayuningtias *et al.*, 2024).

Table 4 : Number of Roots ANOVA

Source	Type III Sum of Square	.df	Mean Square	F	Sig.	Partial Eta Square
Corrected Model	106.223 ^a	25	4.249	6.576	<0.001	0.613
Intercept	905.858	1	905.858	1401.924	<0.001	0.931
Orchid	26.133	1	26.133	40.444	<0.001	0.280
PGR	0.000	0	-	-	-	0.000
Concentration	26.600	11	2.418	3.742	<0.001	0.284
Orchid × PGR	0.000	0	-	-	-	0.000
Orchid × Concentration	30.400	9	3.378	5.228	<0.001	0.311
PGR × Concentration	0.000	0	-	-	-	0.000
Orchid × PGR × Concentration	0.000	0	-	-	-	0.000
Error	67.200	104	0.646	-	-	-
Total	1089.000	130	-	-	-	-
Corrected Total	173.423	129	-	-	-	-

a.R squared = 0.613 (Adjusted R Squared = 0.519)

Table 5 : Average Growth Parameter of Number of Roots

Treatment	Average Number of Roots	
	C4 Orchid	C5 Orchid
Control 0%	2 ^{abc}	1,8 ^{abc}
Tomato Extract 1%	2,2 ^{abc}	2 ^{abc}
Tomato Extract 1.5%	3,4 ^{defg}	2,8 ^{bcde}
Tomato Extract 2%	3 ^{cdef}	3 ^{cdef}
Tomato Extract 2.5%	2,4 ^{abcde}	2 ^{abc}
Control 0%	2 ^{abc}	1,8 ^{abc}
Coconut Water 5%	3,4 ^{defg}	1,6 ^{ab}
Coconut Water 10%	4,4 ^{gh}	1,6 ^{ab}
Coconut Water 15%	4,8 ^h	2,4 ^{abcde}
Coconut Water 20%	2,8 ^{bcde}	1,4 ^a
Controll 0%	2 ^{abc}	1,8 ^{abc}
Mix (T) 1% + (C) 10%	3,6 ^{efg}	1,8 ^{abc}
Mix (T) 1,5 % + (C) 15 %	3 ^{cdef}	2 ^{abc}
Mix (T) 2% + (C) 20%	1,6 ^{ab}	4 ^{fgh}
Mix (T) 2,5% + (C) 5%	3,6 ^{efg}	2,4 ^{abcde}

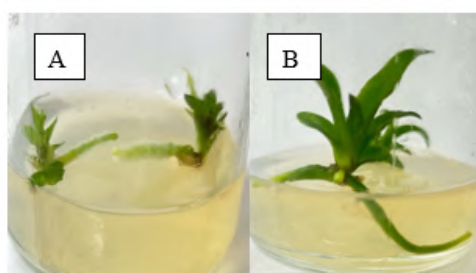
Table 6 : Length of Roots ANOVA

Source	Type III Sum of Square	df	Mean Square	F	Sig.	Partial Eta Square
Corrected Model	4246.920 ^a	25	169.877	7.050	<0.001	0.629
Intercept	23244.238	1	23244.238	964.616	<0.001	0.903
Orchid	379.684	1	379.684	15.757	<0.001	0.132
PGR	0.000	0	—	—	—	0.000
Concentration	1334.618	10	133.462	5.539	<0.001	0.347
Orchid × PGR	0.000	0	—	—	—	0.000
Orchid × Concentration	1705.506	10	170.551	7.078	<0.001	0.405
PGR × Concentration	0.000	0	—	—	—	0.000
Orchid × PGR × Concentration	0.000	0	—	—	—	0.000
Error	2506.076	104	24.097	—	—	—
Total	29655.690	130	—	—	—	—
Corrected Total	6752.996	129	—	—	—	—

a.R Squared = 0.629 (Adjusted R Squared = 0.540)

Table 7 : Average Growth Parameter of Length of Roots

Treatment	Length(mm)	
	C4 Orchid	C5 Orchid
Control 0%	1,5 ^a	6,72 ^{abc}
Tomato Extract 1%	9 ^{bcd}	9,1 ^{bcd}
Tomato Extract 1.5%	15.8 ^{edfgh}	18 ^{fgh}
Tomato Extract 2%	14.8 ^{edfgh}	8,9 ^{bcd}
Tomato Extract 2.5%	8,8 ^{bcd}	16,9 ^{efgh}
Control 0%	1,5 ^a	6,72 ^{abc}
Coconut Water 5%	14 ^{cdefg}	18,4 ^{fg}
Coconut Water 10%	9,8 ^{bcd}	21,6 ^{hi}
Coconut Water 15%	20 ^{gh}	17,4 ^{fgh}
Coconut Water 20%	15,5 ^{edfgh}	18,2 ^{fgh}
Control 0%	1,5 ^a	6,72 ^{abc}
Mix (T) 1% + (C) 10%	12,2 ^{bcd}	6,2 ^{ab}
Mix (T) 1,5 % + (C) 15 %	14,4 ^{edfgh}	15,2 ^{edfgh}
Mix (T) 2% + (C) 20%	9,8 ^{bcd}	10 ^{bcd}
Mix (T) 2,5% + (C) 5%	5,2 ^{ab}	27,6 ⁱ

**Figure 3** : Hybrid *Cattleya* orchid explants on root parameters (A) Root length (B) Number of roots

4.1.4 Wet Weight

Based on the statistical analysis of the wet weight difference parameter, a normality test using the Shapiro-Wilk method showed that the data average was normally distributed. The homogeneity test showed a significance value of 0.011, indicating that the data variance was not homogeneous. The ANOVA results showed that the plant growth regulator (PGR), concentration, and the interaction between the factors had a very significant effect on the difference value, with a significance value of <0.001 and a coefficient of determination (R^2) of 0.856. The orchid species factor had no significant effect on the difference parameter, with a significance value of 0.273 (>0.05). Conversely, the plant growth regulator (PGR) and concentration factors each had a very significant effect, with a significance value of <0.001

and F values of 22.986 (PGR) and 17.030 (concentration), indicating that both factors had a strong influence on the difference value. The interaction between the factors showed a significant effect in this study. The interaction between orchid species and PGR had a significant effect, with a significance value of 0.039 and an F value of 3.456. In addition, the interaction between orchid species and concentration, as well as the interaction between PGR and concentration, had a very significant effect with a significance value of <.001 and F values of 13.055 and 13.147, respectively. The three-way interaction between orchid species, PGR, and concentration also had a very significant effect with a significance value of <.001 and F value of 14.771.

DMRT results showed that the m3u2 treatment [C5 Orchid + Mix (2% Tomato Extract + 20% Coconut Water)] provided the highest average weight gain with an average difference of 1.57 grams. This indicates that the combination of 2% tomato extract + 20% coconut water is the best concentration for increasing wet weight. Meanwhile, k3u2 [C5 Orchid + 15% Coconut Water] achieved the lowest wet weight gain, at 0.06 grams.

The wet weight of the tomato extract and coconut water combination given in the m3u2 treatment demonstrated a synergistic effect on the nutrient and growth regulator content of both organic materials. Physiologically, wet weight in plants consists of two components: water and carbohydrates (Tambun *et al.*, 2023). Increasing leaf number and plant height will affect wet weight, which is inseparable from the role of roots in absorbing water and nutrients from the media (Tambun *et al.*, 2023). The number of leaves significantly influences fresh weight. This aligns with research by Polii (2009) that found that increased leaf growth affects the fresh weight of plants because leaves act as sinks for plants. Leaves are plant organs that contain water, thus increasing fresh weight (Dewi *et al.*, 2023).

Coconut water not only contains sugars, organic acids, amino acids, and vitamins, but also growth hormones such as cytokinins, auxins, and gibberellins, as well as other compounds that aid germination. Coconut water contains carbohydrates, mostly composed of natural sugars, such as glucose, fructose, and sucrose, which are the dominant types. Sucrose plays an important role in cells, such as producing energy during respiration, regulating membrane stabilization, helping plants produce new cells, regulating osmotic pressure, and increasing the tissue's ability to absorb water from the medium into the plant (Arif, 2025).

Tomato extract contains vitamin C and phenolic compounds. Phenolic compounds and vitamin C are antioxidants that act as free radical scavengers. Phenolic compounds act as antioxidants and protect roots from oxidative or biotic stress. Phenolic compounds are excellent oxygen radical scavengers because the electron reduction potential of phenolic radicals is lower than that of oxygen radicals, and also because phenoxyl radicals are generally less reactive than oxygen radicals. Phenolic compounds in optimal amounts can stimulate root growth by triggering cell division and root growth (Rahmawati & Widoretno, 2024).

The k3u2 treatment [C5 orchid + 15% coconut water] resulted in the lowest growth. This may be due to drought stress, which reduces the rate of water uptake by plant roots, which disrupts plant growth, especially in developing tissues (Zalzabila *et al.*, 2023). Furthermore, organic compound oxidation triggers browning in the culture medium. Coconut water, rich in organic compounds and minerals, is thought to increase tissue metabolic activity, triggering the release of phenolic compounds from explants, which then undergo oxidation and cause browning in the culture medium. Compared to tomato extract, coconut water is generally not an effective ingredient in inhibiting browning because it lacks antioxidants capable of optimally inhibiting phenolic oxidation. However, tomato extract contains complex compounds capable of preventing browning (Helena *et al.*, 2022).

Table 4.9 shows that in the combination treatment u1 (C4 hybrid *Cattleya* orchid) the highest average yield was obtained at a concentration of 1% tomato extract + 20% coconut water (0.97 grams), and u2 (C5 hybrid *Cattleya* orchid) the highest average yield was obtained at a concentration of 2% tomato extract + 20% coconut water (1.5 grams). In the tomato extract treatment, u1 (C4 hybrid *Cattleya* orchid) the highest average yield was obtained at a concentration of 2.5% tomato extract (0.59 grams), and u2 (C5 hybrid *Cattleya* orchid) the highest average yield was obtained at a control concentration of 0% (0.4 grams). In the coconut water treatment, the highest average yield for u1 (*Cattleya* orchid C4) was 0.66 grams at a 10% concentration, and for u2 (*Cattleya* orchid C5) the highest average yield was 0.95 grams at a 20% coconut water concentration.

Based on this data analysis, wet weight results in highly variable growth. This may be due to different plant genotypes, resulting in different concentrations of hormones required to stimulate organogenesis, including rooting (Zakiyah *et al.*, 2025). However, hybrid *Cattleya* orchid plantlets still showed an increase in wet weight. This is likely due to the auxin contained in tomato extract, which aids nutrient absorption from the medium. Nutrients are absorbed into the phloem and xylem vessels. Furthermore, tomato extract increases photosynthesis, produces chlorophyll, and generates sufficient food and glucose reserves to support plantlet development, thereby increasing plant fresh weight (Ayuningtias *et al.*, 2024).

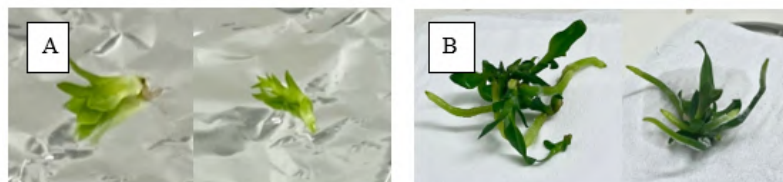


Figure 4 : Hybrid *Cattleya* orchid explants on wet weight parameters (A) first week (B) last week

Table 8 : Wet Weight ANOVA

Souce	Type III Sum of Square	.df	Mean Square	F	Sig.	Partial Eta Square
Corrected Model	11.477 ^a	25	0.459	12.188	0.000	0.854
Intercept	14.020	1	14.020	372.195	0.000	0.877
Orchid	0.002	1	0.002	0.063	0.803	0.001
PGR	0.000	0	-	-	-	0.000
Concentration	5.041	9	0.560	14.870	0.000	0.720
Orchid × PGR	0.000	0	-	-	-	0.000
Orchid × Concentration	3.437	9	0.382	10.138	0.000	0.637
PGR × Concentration	0.000	0	-	-	-	0.000
Orchid × PGR × Concentration	0.000	0	-	-	-	0.000
Error	1.959	52	0.038			
Total	28.573	78				
Corrected Total	13.436	77				

a.R Squared = 0.854 (Adjusted R Suared = 0.784)

Table 9 : Average Growth Parameter of Wet Weight

Treatment	Average of Wet Weight(gram)	
	C4 Orchid	C5 Orchid
Control 0%	0,36 ^{abcd}	0,40 ^{abcd}
Tomato Extract 1%	0,14 ^a	0,15 ^a
Tomato Extract 1.5%	0,12 ^a	0,11 ^a
Tomato Extract 2%	0,41 ^{abcd}	0,10 ^a
Tomato Extract 2.5%	0,59 ^{cde}	0,26 ^{abc}
Control 0%	0,36 ^{abcd}	0,4 ^{abcd}
Coconut Water 5%	0,10 ^a	0,14 ^a
Coconut Water 10%	0,66 ^{def}	0,54 ^{bcd}
Coconut Water 15%	0,36 ^{abcd}	0,06 ^a
Coconut Water 20%	0,24 ^{abc}	0,95 ^{fg}
Controll 0%	0,36 ^{abcd}	0,4 ^{abcd}
Mix (T) 1% + (C) 10%	0,97 ^{fg}	0,7 ^a
Mix (T) 1,5 % + (C) 15 %	0,53 ^{bcd}	1,16 ^g
Mix (T) 2% + (C) 20%	0,9 ^{efg}	1,57 ^h
Mix (T) 2,5% + (C) 5%	0,21 ^{ab}	0,20 ^{ab}

4.1.5 Environment Factors

4.1.5.1 Temperature

Temperature is an environmental factor that plays a crucial role in the growth of tissue culture explants. This study showed that the average temperature was $22.73 \pm 1.64^{\circ}\text{C}$.

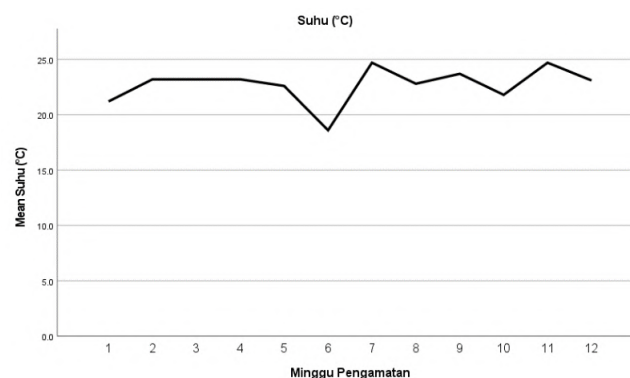
**Figure 5 : Temperature of Incubation Room Graph**

Table 10 : Temperature

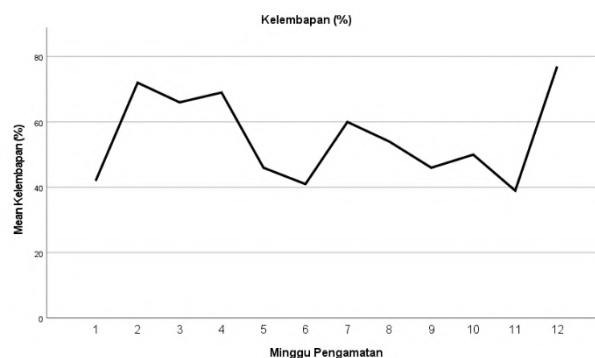
Weeks	Temperature (°C)
1	21,2°C
2	23,2°C
3	23,2°C
4	23,2°C
5	22,6°C
6	18,6°C
7	24,7°C
8	22,8°C
9	23,7°C
10	21,8°C
11	24,7°C
12	23,1°C
Average	22,73 ± 1,64°C

4.1.5.2 Humidity

Humidity is an environmental factor that influences the growth of tissue culture explants. In this study, the humidity of the incubation room was measured using a thermohygrometer, showing an average humidity of $55.16 \pm 13.22\%$.

Table 11 : Humidity

Weeks	Humidity (%)
1	42 %
2	72 %
3	66 %
4	69 %
5	46 %
6	41 %
7	60 %
8	54 %
9	46 %
10	50 %
11	39 %
12	77 %
Average	55,16 ± 13,22 %

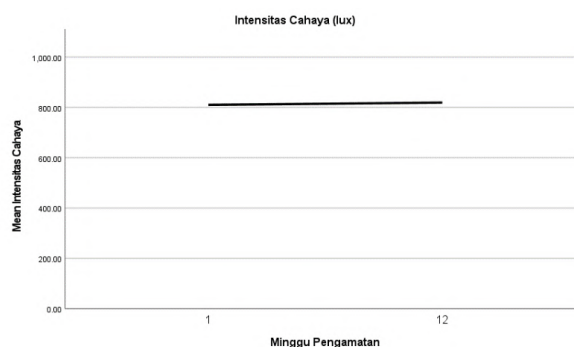
**Figure 6 : Humidity of Incubation Room Graph**

4.1.5.3 Light Intesity

In this study, fluorescent lamps were used as the light source, which were used constantly throughout the incubation period. The light intensity ranged from 810 to 819 lux, with an average of 814.5 ± 6.36 lux.

Table 12 : Light Intesity

Weeks	Light Intesity
1	810 lux
12	819 lux
Average	814,5 ± 6,36 lux

**Figure 7 : Light Intensity of Incubation Room Graph**

Based on observations, environmental factors were observed to influence the environmental conditions of hybrid *Cattleya* orchid explant culture. An average temperature of 22.73°C, below the optimal range, resulted in lower plantlet metabolic activity. This condition impacts water requirements and humidity balance within the culture chamber. According to Sulichantini *et al.* (2024), incubation is generally controlled at 25–28°C to ensure optimal conditions for plant growth. This indicates that the study's temperature was below optimal conditions. Incubation at temperatures lower than those required for optimal growth will reduce metabolic activities such as respiration, water loss, wilting, and decreased metabolic activity (Benelli *et al.*, 2022). However, the study results showed that the plantlets used still showed growth; plants can grow at varying temperature requirements.

The average humidity was 55.16%, indicating that humidity conditions were still moderate and could support explant growth during the culture process. According to Sholiha (2024), *Cattleya* orchids can grow optimally with air humidity of 20–50%. According to Muchsin *et al.* (2022), incubation rooms with humidity <50% generally dry out the growing medium more easily. If humidity is >50%, the percentage of contamination caused by microorganisms generally increases. Furthermore, the incubation room exhibits significant fluctuations, suspected to be due to temperature changes. As temperatures decrease, the rate of water evaporation from the medium and the environment also decreases, resulting in increased relative humidity.

The relatively stable light intensity of 814.4 lux indicates that light is not the primary cause of environmental fluctuations. Stable light intensity plays a role in maintaining photosynthesis. This is in line with research by Muchsin *et al.* (2022), which found that the light intensity required in an incubation room is generally 600–1000 lux. Inappropriate light intensity can inhibit energy absorption by chlorophyll, which reduces photosynthetic efficiency and directly affects growth in various plant species. The process of plant photosynthesis is greatly aided by sunlight (Saputri *et al.*, 2025). Although leaves contain chlorophyll, a green pigment that absorbs light energy, photosynthesis cannot occur without sunlight. This light triggers chemical reactions in the chloroplasts that convert carbon dioxide and water into glucose and oxygen. In other words, light is an absolute requirement for photosynthesis, as well as accelerating it. Plants grown in environments with adequate lighting typically have bright green leaves, strong stems, and upright growth. Conversely, plants grown in environments with low light tend to experience etiolation, meaning abnormal and weak growth, which can even lead to death (Saputri *et al.*, 2025).

The temperature and humidity fluctuations that occurred during the study were thought to be caused by the incubation chamber's poorly controlled environmental conditions, such as the influence of outside room temperature, the frequency of opening the chamber or incubation equipment, and the limitations of the automatic temperature control system. Despite the fluctuations in temperature and humidity, stable light intensity helped maintain the photosynthesis process, allowing the plantlets to continue growing. This indicates that these three environmental factors collectively influence the microenvironmental conditions of the culture, but remain within the growth tolerance limits of hybrid *Cattleya* orchid plantlets.

4.1.6 Contaminations

Microbial contamination is the primary cause of plant culture loss. Microbial contamination can inhibit plantlet growth. Fungi and bacteria are the primary contaminants in tissue culture. This can occur due to unsterile air and poor laboratory conditions. Microbes can enter the internal media and external explants, culture bottles, or unsterile planting

tools, as well as through negligence (Sucahyo *et al.*, 2023). Microbes such as fungi and bacteria are commonly found in contaminated tissue culture media. Rapidly multiplying microbes will cover the growing media and the surface of the explants. Furthermore, microbes can invade explants through wounds caused by cutting and sterilization procedures, resulting in explant tissue death.

Contamination slows plant multiplication, causes root rot, and can lead to plant death. Bacteria contaminate plant tissue explants through the explants, the testing room, the operator, and inefficient sterilization methods. Contamination caused by bacteria can cause culture conditions such as watery and slimy, while contamination caused by fungi causes the culture to become damaged and cloudy whitish in color, the culture media becomes cracked. Bacteria that contaminate the culture in tissue culture media can be gram-negative bacteria or gram-positive bacteria (Sucahyo *et al.*, 2023).



Figure 8 : Contaminations

4.2 The Potential of Natural Plant Growth Regulators (PGRs) from Tomato Extract and Coconut Water as Alternatives to Synthetic Plant Growth Regulators (PGRs)

Based on research results, the use of natural plant growth regulators (PGRs) in the form of tomato extract, coconut water, and a combination (tomato extract + coconut water) showed varying effects on the growth parameters of hybrid *Cattleya* orchid plantlets, but still showed plantlet growth. Plant growth regulators (PGRs), such as tomato extract and coconut water, contain endogenous growth hormones in the form of auxins and cytokinins, and are supported by vitamins, sugars, and minerals. This combination allows for a more natural stimulation of cell division and elongation. In tissue culture, there are two important groups of plant growth regulators (PGRs): cytokinins and auxins. Research shows that growth parameters respond very differently to PGRs. Leaf number tends to be influenced by cytokinins, which stimulate division and shoot formation. Plantlet height is influenced by the interaction of cytokinins and auxins, which promote cell elongation. Root parameters are more influenced by auxins, which play a role in root elongation. Fresh weight, as measured by the accumulation of growth factors, is influenced by auxins and cytokinins. The interaction and balance between the plant growth regulators (PGRs) provided in the media and those produced endogenously by the cells determine the development direction of a culture, thus influencing growth and morphogenesis processes (Fauziah *et al.*, 2021).

According to IB1 & Ketut (2022), coconut water contains minerals including potassium, sodium, calcium, magnesium, iron, copper, phosphorus, sulfur, and chlorine. Young coconut water also contains 95.5%, 0.1% protein, less than 0.1% fat, 4.0% carbohydrates, 0.4% ash, and 2.2–3.4 mg/100 ml of vitamin C, and vitamin B complex consisting of nicotinic acid, pantothenic acid, biotin, folic acid, vitamin B1, and a little pyridoxine. Meanwhile, according to Siregar *et al.* (2025), green coconut water contains organic compounds such as protein and minerals, especially with high levels of K and Cl. It has about 95% water, 2.08% total sugar, 0.56% phosphoric acid, 6.60% potassium, and 0.62% minerals. In addition, green coconut water also contains sucrose, fructose, glucose, diphenylurea, zeatin, glucoside, zeatin riboside, high levels of K and Cl, protein, carbohydrates, minerals, vitamins, and a small amount of fat, calcium and phosphorus, and potassium.

There are 3.6 grams of carbohydrates, 1 gram of protein, and 0.2 grams of fat in every 100 grams of tomatoes. Furthermore, tomatoes contain 16 grams of phosphate, 10 grams of calcium, 0.6 grams of iron, and 10 grams of magnesium. There are 94 grams of water per 100 grams of tomatoes. Furthermore, tomatoes contain 1700 IU of vitamin A, 0.1 grams of vitamin B1 (thiamine), 0.02 grams of vitamin B2 (riboflavin), 21 grams of vitamin C, and 0.6 grams of niacin (Daroini *et al.*, 2023). However, the use of natural plant growth regulators (PGRs) has limitations. Hormone levels in natural materials are suspected to be non-standardized and can vary depending on the maturity level of the material and storage conditions. This results in inconsistent growth responses between treatments.

Synthetic plant growth regulators (PGRs) such as BAP, NAA, IAA, or 2,4-D contain adjustable hormones, making them easier to control in directing morphogenesis for shoot and root formation (Ashabi, 2024). Synthetic plant growth regulators (PGRs) can produce a more uniform and rapid response than natural plant growth regulators. Based on research by Septianingsih *et al.* (2024), the addition of 1 mg/l BAP + 50 ml/l coconut water, 5 mg/l BAP + 50 ml/l coconut water, and 3 mg/l BAP resulted in high shoot yields. However, the addition of coconut water did not significantly affect the in vitro growth of *Bucephalandra* sp. The concentration of coconut water used did not impact the growth of *Bucephalandra* sp.

From an economic and sustainability perspective, natural plant growth regulators (PGRs) have several advantages. Tomato extract and coconut water are more readily available, low-cost, and relatively more environmentally friendly than synthetic PGRs (Sulaeman *et al.*, 2024). Each plant species has a different ability to respond to the composition of

the media present in its environment. Therefore, natural PGRs can be said to have the potential as an alternative or partial substitute, but they cannot completely replace the effectiveness and consistency of synthetic PGRs in hybrid *Cattleya* orchid tissue culture.

Table 13 : Comparison Between Synthetic and Natural PGR

Aspect	Natural Plant Growth Regulators (PGRs)	Synthetic Plant Growth Regulators (PGRs)
Source	Natural organic ingredients (tomato extract and coconut water)	Synthetic chemical compounds (BAP, NAA, IAA)
Hormone content	Variable or not standardized	Standardized or measurable
Growth	Variable	More stable
Cost	Economical	More expensive
Availability	Easily obtained	More specifically obtained
Environmental impact	Environmentally friendly	Less environmentally friendly

4.3 Optimal Concentration of Tomato Extract and Coconut Water in Supporting the Growth of Hybrid *Cattleya* Orchid Plantlets

Based on research observations, the optimal concentrations of natural plant growth regulators (PGRs) in the form of tomato extract, coconut water, and a combination of the two varied depending on the growth parameters and genotype of the hybrid *Cattleya* orchid used. In general, tomato extract provided the best growth response at concentrations of 1.5%-2%, increasing both plantlet height and root number and length.

Preliminary tests using tomato extract at concentrations of 6%, 8%, 10%, and 12% yielded acidic and watery results, making them unsuitable for planting because tomatoes have a pH of 4–4.2 (Habibah *et al.*, 2015) and culture media solutions generally have a pH of 5.5–5.8 (Numba *et al.*, 2024). These concentrations are thought to be linked to the concept of a dose-response relationship, a principle that states that the toxic effect of a substance depends on the dose. Higher doses indicate potential damage (Arsyad *et al.*, 2024).

Coconut water has been shown to be effective in the range of 10%-15%, particularly on root growth and length. According to Ashar *et al.* (2023), the commonly used concentration of coconut water ranges from 2 to 15%. Research by Yasmint & Harahap (2025) found that coconut water at a dose of 50 ml/L had the most dominant effect on shoot number and plant height, while a dose of 100 ml/L had the best effect on leaf number. This finding is inconsistent with the results of the previous study.

Furthermore, the combination (tomato extract + coconut water) showed the best response at a concentration of 2% tomato extract + 20% coconut water on C4 hybrid *Cattleya* orchids (Purple *Cattleya* X Yellow *Cattleya*) and 1% tomato extract + 10% coconut water on C5 hybrid *Cattleya* orchids (White Spotted Purple *Cattleya* X Purple *Cattleya*). This condition may be due to the use of coconut water as a natural growth regulator (PGR) alone at high concentrations, which causes hormonal imbalance and ineffective cell elongation. The auxin hormone content in coconut water is relatively low, so the presence of tomato extract is thought to act as a source of auxin hormone through its tryptophan amino acid content (Rahmawati & Widoretno, 2024). According to Telaumbanua (2022), higher coconut water concentrations increase the height of orchid plantlets, while tomato extract contains a variety of complex phytohormones and, at low concentrations, has a more efficient stimulant effect, resulting in optimal growth (Agustin *et al.*, 2020).

5. Conclusion

Based on the research results, the use of natural growth regulators (PGRs) in the form of tomato extract, coconut water, and their combinations has been proven to affect the growth of hybrid *Cattleya* orchid plantlets in vitro, especially on the parameters of plantlet height, number of roots, and root length, although it did not show a significant difference in the number of leaves. Variations in treatment concentrations and interactions between factors also have a significant effect on several growth parameters, indicating the existence of certain optimal concentrations and the presence of specific plantlet responses to treatment combinations. However, in the number of leaves parameter, the administration of natural PGRs does not always result in better growth than the control, which indicates that the effectiveness of natural PGRs is parameter-specific and has not shown a consistent pattern of increase. In addition, compared to synthetic PGRs which have more measurable concentrations, natural PGRs have limitations in terms of stability and uniformity of hormone content, so that their influence on the morphogenesis process cannot always be controlled optimally. Furthermore, the results of the study showed that the optimal concentrations varied depending on the parameters observed, with optimal tomato extract in the range of 1.5%–2%, coconut water at 10%–15%, and the best combination

in the treatments of 2% + 20% (C4) and 1% + 10% (C5), which overall can support the growth and regeneration of plantlets more effectively.

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