Jurnal Biodjati 10 (2): 393–409, November 2025
e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

The Effect of IAA, BAP, and Coconut Water on Tomato (*Solanum lycopersicum* L.) Organogenesis

Alba Wijaya Maharani[1], Ratih Restiani[*2], Aniek Prasetyaningsih[3]

Received: August 07, 2025
Revise from: September 12, 2025
Accepted: November 12, 2025

DOI: 10.15575/biodjati.v10i2.49410

[1,2,3]Biology Study Program, Faculty of Biotechnology, Universitas Kristen Duta Wacana, Jl. Dr. Wahidin Sudirohusodo, Yogyakarta, Special Region of Yogyakarta 55224, Indonesia.

e-mail:
[1]31210417@students.ukdw.ac.id,
*[*2]ratih.restiani@staff.ukdw.ac.id*
[3]aniek@staff.ukdw.ac.id

*****Corresponding author*

Abstract. *Tomato (Solanum lycopersicum* L.*) is a widely cultivated horticultural crop valued for its phytochemical and nutritional content. The increasing demand for high-quality seedlings has promoted the use of in vitro propagation, which offers greater efficiency in labor and land use, uniform seedling production, and independence from climate compared to conventional methods. This study aimed to investigate the effect of different concentrations of Plant Growth Regulators (PGRs) and coconut water as an alternative or supplement on the organogenesis of tomato hypocotyl explants. Tomato hypocotyl explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP (0.5, 1.0, and 1.5 ppm), IAA (0.1, 0.3, and 0.5 ppm), and coconut water (10%, 20%, and 30%). The experiment was arranged in a Completely Randomized Design (CRD) with four replications, consisting of a control, single-factor treatments, and combination treatments of BAP, IAA, and coconut water. Observations included the percentage and intensity of root, shoot, and callus formation, as well as shoot height. Data were analyzed using the non-parametric Kruskal–Wallis test, followed by Dunn's test. The results showed that roots, shoots, and callus successfully developed in treatments supplemented with 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA), and coconut water. The highest shoot growth, with a percentage of 87.7% and an average height of 5.75 cm, was observed in treatment K20, while the highest callus formation occurred in treatment K30. The best shoot and callus intensities were resulted in media with coconut water alone at concentrations of 10%, 20%, and 30%, whereas the highest root intensity was obtained in the BAP+IAA treatment without coconut water. The results indicate that coconut water is effective in supporting organogenesis, both directly through the formation of shoots and roots, and indirectly through callus formation that can subsequently develop into shoots and roots.*

Keywords: *BAP, coconut water, hypocotyl, IAA, organogenesis, Solanum lycopersicum* L

Citation
Maharani, A. W., Restiani, R., & Aniek, P. (2025). The Effect of IAA, BAP, and Coconut Water on Tomato (*Solanum lycopersicum* L.) Organogenesis. *Jurnal Biodjati*, 10(2), 393-409.

Jurnal Biodjati 10(2): 393–409, November 2025

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated horticultural commodities in the world due to its nutritional and phytochemical content, such as lycopene, vitamin C, beta-carotene, and phenolic compounds (Motlhalamme et al., 2025; Collins et al., 2022). In Indonesia, tomato demand continues to increase, with total production reaching 1,020,333 tons in 2019; however, production decreased by 2.14% between 2022 and 2023 (Kementerian Pertanian, 2024). Conventional cultivation faces several challenges, including limited agricultural land, climate change, pest and disease attacks, and declining soil fertility (Sunaryanti & Dwiyana, 2020; Sumberg & Giller, 2022).

In vitro culture offers an effective alternative for tomato propagation because it can produce high-quality seedlings in a shorter time, independent of climatic conditions, and with more efficient use of land and labor (Thakur et al., 2024; Hartmann et al., 2011). This technique involves growing plant cells, tissues, or organs under sterile and controlled conditions on nutrient media containing plant growth regulators (PGRs) (Norouzi et al., 2022; Vidyagina et al., 2021). In *in vitro* culture, selecting the appropriate explant is a crucial initial step to support optimal regeneration. Explants with active meristems, such as hypocotyls, have high cell division capability, enabling rapid and efficient proliferation, while also having the potential to produce pathogen-free plants (Alavijeh et al., 2025). The use of hypocotyls from seeds also opens opportunities for the emergence of new superior traits resulting from unique genetic interactions, which can be selected as prospective superior parent plants (Setyowidianto et al., 2017).

Plant growth regulators (PGRs) play an important role in regulating regeneration, including callus induction, shoot formation, and rooting (Wulandari, 2018). In this study, BAP, IAA, and coconut water were used. BAP stimulates cell division and shoot formation, while IAA promotes cell elongation, division, tissue differentiation, and root development (Pratiwi et al., 2024; Pudjiwati & Rindiani, 2022). The balance ratio between the two plays a crucial role in determining the direction of explant growth; therefore, selecting the appropriate combination is key to achieving optimal regeneration. For example, Andriani et al. (2023) reported that the use of BAP can increase the number of shoots as the explant ages. The cytokinin type BAP has several advantages, including stability, lower cost compared to other cytokinins, resistance to oxidation, and effectiveness in stimulating shoot formation (Agustina et al., 2020). Unlike synthetic PGRs, coconut water contains a complex mixture of natural phytohormones such as cytokinins (zeatin, kinetin, zeatin riboside), auxins, gibberellins, and abscisic acid, as well as minerals and vitamins that work synergistically to regulate explant growth (Prando et al., 2014; Mardhikasari et al., 2019). The addition of coconut water as a natural source of phytohormones such as cytokinins, auxins, and gibberellins, as well as vitamins and amino acids, can enhance growth responses due to its ability to act synergistically with synthetic PGRs. Moreover, the use of coconut water offers added value in terms of cost efficiency and sustainability, given its abundance and environmentally friendly nature. Several previous studies have investigated in vitro tomato regeneration using various types of explants and combinations of synthetic and natural PGRs. Research by Septiawati et al. (2021) on potato plants, which belong to the same family as tomato,

using shoot tip meristem explants, showed that the addition of coconut water could promote faster, longer, and more abundant root formation. However, to date there is no study has combined 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA), and coconut water as sources of PGRs in *in vitro* tomato culture using hypocotyl explants. This research highlights an opportunity to explore the effectiveness of this combination in supporting both direct and indirect organogenesis in tomato. According to previous research by Bello et al. (2024), supplementing the basal medium with 15% coconut water promoted multiple shoot induction in cotyledon explants of *Hibiscus sabdariffa*. Meanwhile, 5% coconut water effectively stimulated root formation in hypocotyl and cotyledonary node explants, as well as leaf formation in cotyledonary nodes. Furthermore, different concentrations of coconut water used in *in vitro* culture also induced callus formation. This study is important because the combination of BAP, IAA, and coconut water has not been widely explored in tomato *in vitro* culture, particularly using hypocotyl explants. Optimizing this combination has the potential to produce a more effective medium that supports balanced shoot and root formation. In addition, the use of coconut water as a natural source of phytohormones offers an environmentally friendly and cost-effective alternative to the exclusive use of synthetic plant growth regulators. Considering that tomato is one of the most important horticultural crops with high economic value, the development of an efficient and sustainable medium formulation is expected to support the propagation of superior plantlets and further applications of tissue culture in research and production.

MATERIALS AND METHODS

Materials

The materials used were divided into biological and chemical components. Biological materials included young coconut water and tomato seeds obtained from PT. East-West Seed Indonesia. Chemical materials consisted of Murashige and Skoog (MS) medium (Conner et al., 2025), sucrose, Indole-3-acetic acid (IAA), 6-Benzylaminopurine (BAP), distilled water, commercial agar, pH buffer solutions (HCl or NaOH), 30% bleach solution containing 5.5% (w/v) sodium hypochlorite, and 70% ethanol. The equipment used included an autoclave (Hirayama Manufacturing Corporation, Tokyo, Japan), oven (Memmert), laminar air flow cabinet (Labolytic), litmus paper (Mquant®), analytical balance (AND Electronic Balance), magnetic stirrer (Labinco), measuring cylinders (Herma), Erlenmeyer flasks (Pyrex), glass funnels, beakers (Iwaki), Petri dishes (Normax), graduated pipettes (HBG), micropipettes (Socorex Acura Micropipette Adjustable) with blue tips, stove (Maspion), culture bottles, filter paper, aluminum foil, plastic wrap, cotton (Onemed), rubber bands, umbrella paper, dropper pipettes, forceps, scalpels, nitrile gloves, hairnets, masks, Bunsen burner, matches, and labeling paper (Debnath et al., 2018).

Research Design

This study was an experimental research designed using a Completely Randomized Design (CRD) with a factorial design, aimed at determining the effect of specific concentrations of IAA, BAP, and coconut water on the organogenesis of tomato (S*olanum lycopersicum* L.) hypocotyl explants. Each treatment was replicated four times.

Jurnal Biodjati 10(2): 393–409, November 2025

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Treatment Combinations

The combinations of BAP, IAA, and coconut water at various concentrations are presented in the following table 1.

Table 1. Combinations and concentrations of IAA, BAP, and coconut water

Concentrations BAP & IAA (ppm)	Concentrations coconut water (%)			
	0	10	20	30
0	(Control)	K10	K20	K30
0.5+0.1	0.5+0.1	0.5+0.1 (K10)	0.5+0.1 (K20)	0.5+0.1 (K30)
1+0.3	1+0.3	1+0.3 (K10)	1+0.3 (K20)	1+0.3 (K30)
1.5+0.5	1.5+0.5	1.5+0.5 (K10)	1.5+0.5 (K20)	1.5+0.5 (K30)

Procedure

Preparation of Murashige and Skoog (MS) medium and treatment media. The preparation of Murashige and Skoog (MS) medium was carried out for tomato seed germination. To prepare 200 mL of MS medium, 0.886 g of instant MS powder and 1.6 g of sucrose were added into an Erlenmeyer flask. The mixture was dissolved in 200 mL of distilled water and homogenized using a magnetic stirrer. The pH of the medium was adjusted to a range of 5.7–5.8; if the pH exceeded this range, 1 N HCl was added, whereas if it was below the range, 1 N NaOH was added until the desired pH was achieved. The medium solution was then heated on a stove, and 1.6 g of agar was added while continuously stirring to prevent clumping. The medium was poured into culture bottles (200 mL per bottle) and immediately sealed with aluminum foil and parchment paper, tied securely, and labeled according to the treatment. The media were sterilized using an autoclave at 1 atm pressure and 121°C for 15 minutes. For the preparation of treatment media, BAP, IAA, and coconut water were added, with the amount of distilled water adjusted according to each treatment to maintain a final medium volume of 200 mL. Coconut water was filtered before being added to the medium to avoid contamination by debris, and only young coconut water was used.

Sterilization of Tomato Seed Explants

Tomato seeds were obtained from PT. East-West Seed Indonesia Ltd. Seeds were germinated by soaking them in warm water for 5 minutes. The seeds were surface-sterilized using 30% clorox containing 5.5% (w/v) sodium hypochlorite (NaOCl) for 5 minutes, followed by rinsing three times with sterile distilled water for 1 minute each. Additional surface sterilization was carried out using 70% (v/v) ethanol for 1 minute, followed by another three rinses with sterile distilled water for 1 minute each. Sterilized seeds were then inoculated onto MS medium for germination and incubated for 10 days (Sivankalyani et al., 2014; Setiaji et al., 2020).

Inoculation of Hypocotyl Explants

In vitro-germinated tomato seedlings were aseptically removed using sterile forceps and placed in sterile petri dishes. The hypocotyl segment was cut into 1 cm pieces using a sterile scalpel. Culture bottles containing the medium were briefly flamed at the mouth using a bunsen burner. The sterilized hypocotyl explants were transferred into the medium, then closed with aluminum foil, wrapped with plastic wrap, and labeled according to the treatment.

Observation

Observations were conducted periodically for 6 weeks after inoculation of the explants into the culture medium. The observation period of six weeks was chosen because explants generally require this duration to exhibit clear morphological responses such as callus, shoot, or root formation. According to Yaroshko et al. (2023), *in vitro* tomato cultures showed complete shoot formation within 4–6 weeks after inoculation. Similarly, Nyamah et al. (2018) reported that tomato explants from three cultivars exhibited significant morphogenetic development after six weeks of incubation, indicating that this time frame is optimal for evaluating the effectiveness of treatments on plant regeneration. Parameters observed included the time of callus/shoot/root formation, the number and morphology of shoots, roots, and callus formed, the number/percentage of explants producing roots, shoots, or callus, and the number/percentage of surviving explants.

Data Analysis

Data analysis was performed using R software. The initial stage included normality and homogeneity tests to determine the appropriate statistical test. If the data were normally distributed and homogeneous, analysis of variance (ANOVA) was conducted to determine the effect of treatments. If the data did not meet the homogeneity assumption, the non-parametric Kruskal–Wallis test was used as an alternative. Furthermore, if significant differences were detected, post hoc analysis was performed using Dunn's test to identify treatment pairs that differed significantly.

RESULTS AND DISCUSSION

Effect of IAA, BAP, and Coconut Water Combinations on Tomato Organogenesis

In this study, the application of plant growth regulators IAA, BAP, and coconut water resulted in the formation of roots, shoots, and callus. Plant growth regulators (PGRs) function as organic compounds that play a significant role in regulating cell division, differentiation, and in vitro tissue growth (Mehbub et al., 2022). Indole-3-acetic acid (IAA) is an auxin hormone that stimulates the formation of adventitious roots and induces callus in plant explants. High auxin concentrations in the culture medium stimulate root formation in explants (Cavallaro et al., 2022). Specifically, IAA acts through three main mechanisms: biosynthesis, transport, and signal transduction. IAA is synthesized from tryptophan via the TAA/YUC pathway and is transported in a polar manner by AUX/LAX (influx), PIN (efflux), and ABCB proteins, creating a concentration gradient that directs plant organ growth and differentiation. Within the cell, IAA binds to TIR1/AFB receptors, triggering the degradation of Aux/IAA proteins, which allows ARF transcription factors to activate target genes such as *SAUR* and *GH3* that regulate cell division, stem elongation, and root development (Zhang et al., 2022). As shown in Figure 1, the application of IAA in the culture medium induced the formation of shoots, callus, and roots in tomato hypocotyl explants.

Cytokinins also play a critical role in explant growth and development by stimulating shoot formation, cell division, and callus proliferation. Specifically, benzylaminopurine (BAP) functions as a synthetic cytokinin that stimulates explant growth by activating cytokinin receptors in

the cell membrane, inducing the expression of genes such as *CYCD3* and *PCNA* that promote cell division, and supporting cell differentiation through regulation of *WUSCHEL* and *SHOOT MERISTEMLESS* genes. At a high cytokinin-to-auxin ratio, BAP induces shoot formation, suppresses apical dominance, and enhances metabolic activity and protein synthesis essential for organ morphogenesis in plants (Mok et al., 2000).

Complex organic subtances such as coconut water also serves as an alternative source of growth-promoting substances because it is rich in metabolic precursors such as sugars, amino acids, and essential minerals that not only provide nutrition but also support hormone activity in the culture medium. Vitamin C functions as an antioxidant that protects cultured cells from oxidative stress, which is crucial in the in vitro plantlet regeneration process as it helps maintain cell stability and viability. In addition, components such as Ca, Na, Fe, K, P, and vitamin C act as growth regulators for explants, supporting development and enhancing the success of plant tissue culture. Moreover, coconut water contains natural phytohormones such as auxins (IAA), cytokinins (zeatin, kinetin), and gibberellins, which can stimulate cell division and tissue differentiation. The hormonal balance in coconut water plays a crucial role in determining growth direction; at an optimal concentration 10%, these natural hormones can induce active cell division without differentiation, leading to callus formation. The organic components in coconut water also enhance cellular metabolic activity, provide energy and biosynthetic precursors for cell proliferation, and stimulate gene expression related to cell division and callus formation. Therefore, coconut water functions not only as a nutrient source but also effectively supports cell proliferation and callus induction (Prando et al., 2014; Mardhikasari et al., 2019; Shayanthavi et al., 2025).

Figure 1. A) plantlets, B) callus, C) roots of tomatos (*Solanum lycopersicum* L.)

Effect of PGRs on Shoot Height

Observation of plantlet height was carried out as it reflects the growth activity and organ development, serving as an indicator of organogenesis. Measurements were taken at the same explant age by measuring the plantlet height from the base to the tip of the leaf.

Figure 2 shows that the single coconut water treatments K10 and K20 outperformed the other treatments, with mean shoot heights of 4.75 cm and 5.75 cm, respectively. K20 exhibited smaller variation, as indicated by the shorter error bars. Similarly, in other treatments, higher error bars reflected greater variation in plant height among replicates. The higher explant growth observed in K20 might be attributed to the function of coconut water as a plant growth regulator due to its zeatin content, a type of cytokinin. This compound supports various plant growth processes, including seed germination, tissue differentiation, cell division, and shoot growth stimulation. In addition, coconut water contains inositol, reduced nitrogen compounds, and amino acids, all of which contribute to shoot elongation (Ng et al., 2020). A balanced ratio between auxin and cytokinin can stimulate both cell division and differentiation. Cytokinins promote cell division by enhancing protein synthesis, whereas auxins promote cell elongation, leading to stem elongation. Auxins regulate cell elongation by activating specific proteins in the cell plasma membrane, enabling H^+ ions to enter the cell wall, causing cell expansion through osmosis. Following elongation, cells continue to enlarge through the resynthesis of cell wall and cytoplasmic components, underscoring the crucial role of auxins in the division of meristematic cells in developing tissues (Melianawati et al., 2025).

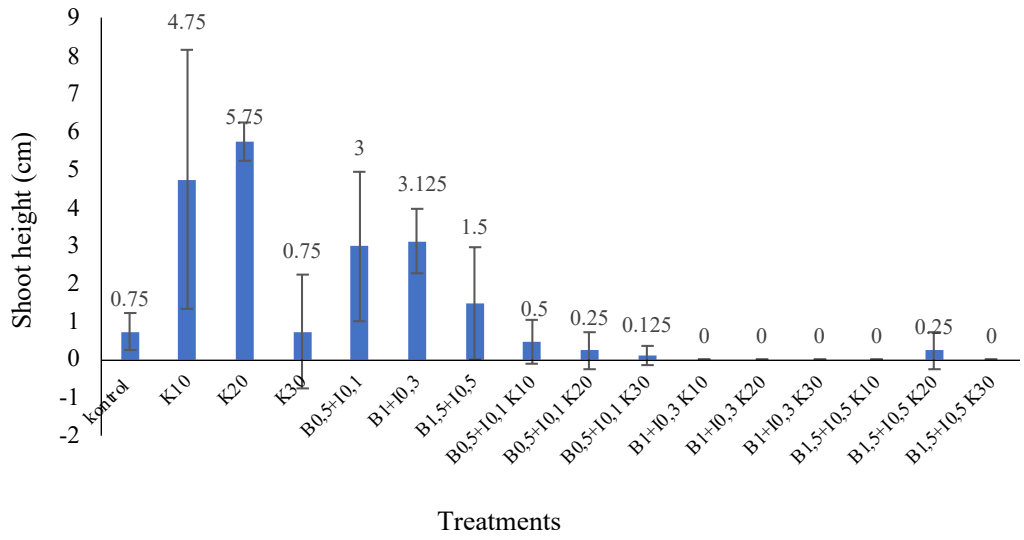


Figure 2. Average shoot height of tomato (y), treatment of growth regulator (x)

Table 3. Significance of shoot height based on Dunn's test results

Treatment	*p*-value
B1.5+I0.5 K10 - K20	0.05
B1.5+I0.5 K30 - K20	0.05
B1+I0.3 K10 - K20	0.05
B1+I0.3 K20 - K20	0.05
B1+I0.3 K30 - K20	0.05

As shown in Table 3, based on Dunn's test results, treatment K20 showed a significant difference (P = 0.05) compared to treatments B1.5+I0.5 K10, B1.5+I0.5 K30, B1+I0.3 K10, B1+I0.3 K20, and B1+10.3 K30. This indicates that the application of 20% coconut water alone had a more significant effect on shoot growth compared to the combinations of coconut water with BAP and IAA. At the physiological level, this suggests that 20% coconut water provides a natural hormonal balance that promotes apical dominance and the differentiation of meristematic cells into new shoot tissues. The bioactive compounds and hormone precursors contained in coconut water at this concentration may also enhance enzymatic activity related to protein synthesis and the development of young tissues (shoot primordia), leading to faster and more efficient shoot initiation and elongation. Therefore, treatment K20 not only exhibited statistically significant results but also demonstrated an optimal physiological response for shoot formation and development.

Effect of BAP, IAA, and Coconut Water on Root, Shoot, and Callus Growth of *Solanum lycopersicum* L

The addition of BAP, IAA, and coconut water to MS medium influenced the formation of roots, shoots, and callus in hypocotyl explants of *Solanum lycopersicum* L. This study aimed to evaluate the effects of these treatments on enhancing the growth of the three organs. Observations of callus included several parameters, including the percentage of callus formation, as well as the morphological characteristics and intensity level of the callus formed.

As shown in Table 4, the percentages of root, shoot, and callus formation in tomato hypocotyl explants varied depending on the combination of plant growth regulators (PGRs) and coconut water. The highest root formation (100%) occurred in both the control treatment and the combination of BAP 0.5 mg/L + IAA 0.1 mg/L. The strong root formation in the control indicates that tomato hypocotyls possess high natural regenerative capacity, supported by sufficient endogenous auxin reserves. Wounding during explant preparation also stimulates auxin synthesis and redistribution to the injured area, activating root-inducing molecular pathways involving ARF, LBD, and WOX gene families. Environmental cues such as light further modulate auxin responses. Thus, endogenous signaling and stress conditions are adequate to trigger root formation even without exogenous hormones (Olatunji et al., 2017). This aligns with Ziarani (2025), who reported root development in hypocotyl explants on hormone-free MS medium after 21 days. The highest shoot formation was observed in the 20% coconut water treatment (K20) at 87.5%, followed by BAP 1 mg/L + IAA 0.3 mg/L and BAP 0.5 mg/L + IAA 0.1 mg/L, each at 50%. Coconut water contains essential amino acids (lysine, cysteine, histidine, methionine), vitamins, and minerals such as potassium, calcium, and magnesium, and is also rich in sugars (Michael, 2011). These sugars serve as carbon sources and osmotic regulators for explants (Cosic et al., 2020). Ario and Setiawan (2020) noted that high cytokinin concentrations can inhibit cell division or differentiation in tissue culture.

According to Singh et al. (2014), the optimal BAP concentration is 2 mg/L, and higher levels may reduce shoot production by inhibiting cell division.

The growth and development of explants in in vitro culture are key indicators for evaluating the effectiveness of the applied treatments. The observations included growth intensity and the morphological characteristics of the roots, shoots, and callus. Callus intensity was assessed using standard scoring criteria, while its morphology was evaluated based on texture and color. All assessments were conducted on the 40th day after planting. The application of BAP, IAA, and coconut water resulted in varying intensities of root, shoot, and callus formation. Based on Table 4, the highest root growth (100%) was observed in the control and in the B0.5 + I0.1 treatment. The greatest shoot growth occurred in the 20% coconut water treatment (K20), whereas callus formation was highest in the K30 treatment. These percentages only indicate the number of explants that responded to the treatment; therefore, intensity assessment was required to determine the degree of differentiation.

As shown in Table 5, the highest callus growth percentages were found in the single coconut water treatments (K10, K20, and K30), with respective values of 50%, 62.5%, and 75%. Qualitative observations in Table 5 also revealed high callus intensity in these treatments, characterized by larger callus volume and greater surface coverage on the explants. In contrast, although the B1 + I0.3 and B1.5 + I0.1 K20 treatments each reached 50% callus formation, their callus intensity was lower than that of the single coconut water treatments. This occurs because coconut water provides a more stable and supportive environment for tissue development. Its natural content of cytokinins,

Table 4. Number, percentage, intensity of roots, shoots, and calluses of tomato in six weeks

Treatment	Total Explant	Root		Shoot		Callus	
		Total	%	Total	%	Total	%
Control	8	8	100	1	12.5	0	0
K10	8	6	75	6	75	4	50
K20	8	7	87.5	7	87.5	5	62.5
K30	8	7	87.5	1	12.5	6	75
B0.5+I0.1	8	8	100	4	50	0	0
B1+I0.3	8	7	87.5	4	50	4	50
B1.5+I0.5	8	6	75	5	62.5	3	37.5
B0.5+I0.1 K10	8	7	87.5	3	37.5	3	37.5
B0.5+I0.1 K20	8	5	62.5	1	12.5	3	37.5
B0.5+I0.1 K30	8	4	50	0	0	0	0
B1+I0.3 K10	8	3	37.5	0	0	0	0
B1+I0.3 K20	8	4	50	0	0	1	12.5
B1+I0.3 K30	8	3	37.5	0	0	0	0
B1.5+I0.5 K10	8	3	37.5	0	0	3	37.5
B1.5+I0.5 K20	8	4	50	1	12.5	4	50
B1.5+I0.5 K30	8	5	62.5	0	0	3	37.5

along with vitamins, sugars, amino acids, and minerals, works synergistically to stimulate cell division.

Table 5. Intensity of calluses *Solanum lycopersicum* L.

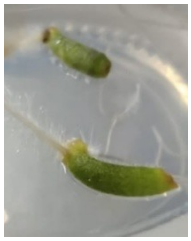
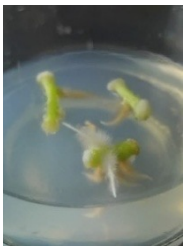
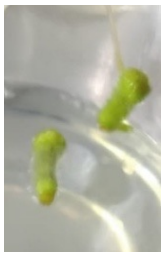
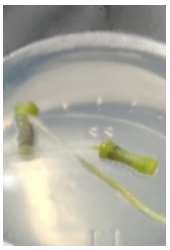
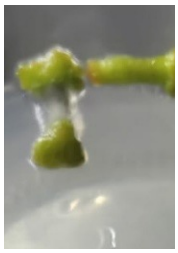
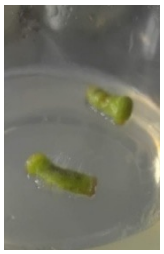
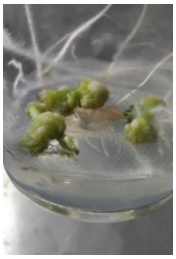
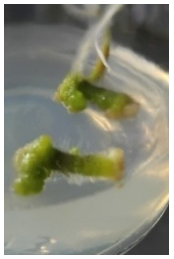
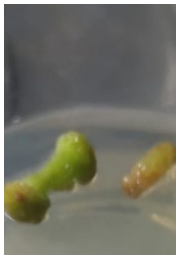
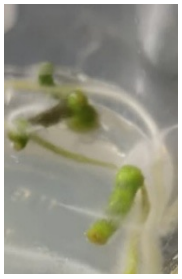
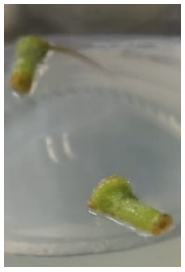
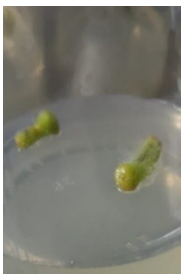
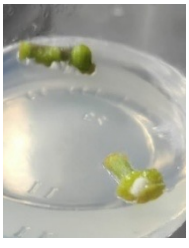
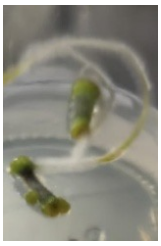
BAP & IAA Concentration (ppm)	Coconut water concentration (%)			
	0	**K10**	**K20**	**K30**
0	 -	 +++	 +++	 +++
B0.5+I0.1	 -	 ++	 +	 -
B1+I0.3	 ++	 -	 +	 -
B1.5+I0.5	 +	 +	 ++	 +

Table 6. Intensity of *Solanum lycopersicum* L. shoots

BAP & IAA Concentration (ppm)	Coconut water concentration (%)			
	0	**K10**	**K20**	**K30**
0	 +	 +++	 +++	 +
B0.5+I0.1	 ++	 ++	 +	 -
B1+I0.3	 +++	 -	 -	 -
B1.5+I0.5	 +	 -	 +	 -

The intensity of shoot formation, as shown in Table 6, was highest in the K10 and K20 treatments, consistent with their higher shoot growth percentages in Table 4. Coconut water contains bioactive compounds that activate endogenous hormonal pathways, especially cytokinins, which stimulate the transition of cells into active division and increase the expression of genes regulating shoot morphogenesis and apical meristem formation. In addition to coconut water treatments, the single treatment of B1 + I0.3 also showed high shoot formation intensity.

Table 7. Root intensity of *Solanum lycopersicum* L.

BAP & IAA Concentration (ppm)	Coconut water concentration (%)			
	0	**K10**	**K20**	**K30**
0	 ++	 ++	 ++	 +++
B0.5+I0.1	 +++	 +++	 ++	 +
B1+I0.3	 +++	 +	 +	 +
B1.5+I0.5	 +++	 +	 +	 ++

Root intensity (Table 7) showed that although the control treatment produced 100% root growth, its root intensity was lower than the BAP + IAA treatments without coconut water. Higher root intensities were observed in K30, B0.5 + I0.1, B0.5 + I0.1 K10, B1+ I0.3, and B1.5 + I0.5. This enhancement is due to the synergistic effects of IAA, which promotes root initiation and elongation, and low concentrations of BAP, which support cell division without suppressing root formation. The balance between auxin and cytokinin ultimately determines whether explants form roots, shoots, or callus, depending on hormone ratios and tissue sensitivity.

CONCLUSION

The application of 0.5 ppm, 1 ppm, 1.5 ppm BAP, 0.1 ppm, 0.3 ppm, 0.5 ppm IAA, and 10%, 20%, 30% coconut water at specific concentrations in tomato hypocotyl in vitro cultures successfully induced the formation of roots, shoots, and callus with varying responses. The highest response was root formation, followed by shoot, and the lowest was callus, indicating a tendency toward direct organogenesis. Based on the statistical analysis using Dunn's test, the K20 treatment (20% coconut water) showed a significant difference (P = 0.05) compared to the treatments B1.5+I0.5 K10, B1.5+I0.5 K30, B1+I0.3 K10, B1+I0.3 K20, and B1+I0.3 K30. This indicates that the single application of 20% coconut water resulted in a significantly higher mean shoot height compared to the combinations of coconut water with BAP and IAA. These results indicate that the 20% coconut water treatment optimizes the organogenesis process in *in vitro* tomato culture, primarily by enhancing the efficiency of shoot initiation and elongation compared to its combinations with BAP and IAA.

AUTHOR CONTRIBUTION

A.W.M. designed the research, conducted the experiments, collected and analyzed the data, and wrote the manuscript. **R.R.** supervised the research, provided funding support, and assisted in the manuscript submission, **A.P.** supervised the research and assisted in the manuscript submission.

ACKNOWLEDGMENTS

The authors would like to thank the laboratory staff of the Faculty of Biotechnology, Universitas Kristen Duta Wacana, for technical assistance during the experiments. The authors also express gratitude to the supervising lecturer for valuable guidance and supervision throughout the research. This study was financially supported through a research grant provided by the Faculty of Biotechnology, Universitas Kristen Duta Wacana.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Agustina, M., Maisura, M., & Handayani, Rd. S. (2020). The Effect of Different Seed Cutting Treatments and Concentrations of BAP for the Successful In vitro Micrografting of Mangosteen (*Garcinia mangostana* L.). *Journal of Tropical Horticulture*, 3(1), 1. DOI: 10.33089/jthort.v3i1.37

Ahmad, I., Ahmad, S., Rao, H., Shaukat, U., Shahzad, M. N., Rehman, M. S.-, Ba-

sit, A., Arshad, M. A., & Ahmad, B. (2020). Multi-Method Determination of Antioxidant Capacity, Phytochemical and Biological Investigation of Four Different Solvent Extractives of *Leucophyllum frutescens* (cenizo). DOI: 10.20944/preprints202011.0305.v1

Alavijeh, M.K., Bayat, H., Kianpour, D. et al. (2025) Optimization of in vitro propagation and virus eradication using meristem culture and thermotherapy in two geranium species *Pelargonium X hortorum* ('Zonal') and *Pelargonium* × *domesticum* ('Regal'). *BMC Plant Biol* 25, 9. DOI: 10.1186/s12870-024-06027-y

Andriani, C., Kadapi, M., Suminar, E., & Nuraini, A. (2023). Comparison of the Effects of BAP and Kinetin on the Multiplication Rate of Sweet Charlie Strawberry Cultivar. *Jurnal Agroteknologi*, 14, 13. DOI: 10.24014/ja.v14i1.18824

Ario, A., & Setiawan, S. (2020). The Effect of Benzyl Amino Purine (BAP) Concentration on the Growth Amount of the Explant of Dendrobium spectabile Orchid by In-Vitro. *International Journal of Multi Discipline Science* (IJ-MDS), 3(2), 33-38. DOI:10.26737/ij-mds.v3i2.2397.

Cavallaro, V., Pellegrino, A., Muleo, R., & Forgione, I. (2022). Light and Plant Growth Regulators on In Vitro Proliferation. *Plants*, 11(7), 844. DOI: 10.3390/plants11070844

Chutimanukul, P., Sukdee, S., Prajuabjinda, O., Thepsilvisut, O., Panthong, S., Ehara, H., & Chutimanukul, P. (2023). Exogenous Application of Coconut Water to Promote Growth and Increase the Yield, Bioactive Compounds, and Antioxidant Activity for *Hericium erinaceus* Cultivation. *Horticulturae*, 9(10), 1131. DOI: 10.3390/horticulturae9101131

Collins, E. J., Bowyer, C., Tsouza, A., & Chopra, M. (2022). Tomatoes: An Extensive Review of the Associated Health Impacts of Tomatoes and Factors That Can Affect Their Cultivation. *Biology*, 11(2), 239. DOI: 10.3390/biology11020239.

Conner, A. & Jacobs, Jeanne. (2025). Defining the Use of MS Medium In Plant Science Research. *Plant Cell, Tissue and Organ Culture (PCTOC)*. 162. 10.1007/s11240-025-03210-3.

Cosic, T., Savic, J., Raspor, M., Cingel, A., Ghalawnji, N., Vinterhalter, B., & Ninkovic, S. (2020). Effects of different types of sugars and plant growth regulators on kohlrabi seedling growth and development in vitro. *Archives of Biological Sciences*, 72(3), 349–357. DOI: 10.2298/abs200622029c

Debnath, A. J., Gangopadhyay, G., Basu, D., & Sikdar, S. R. (2018). An efficient protocol for *in vitro* direct shoot organogenesis of *Sesamum indicum* L. using cotyledon as explant. *3 Biotech*, 8(3), 146. DOI: 10.1007/s13205-018-1173-7

Desi Melianawati, Prihastanti, E., Haryanti, S., Hastuti, E. D., & Irawan, F. P (2025). The Utilization *Vigna radiata* Extract, Coconut Water and Root-Up on The Growth of *Bruguiera gymnorrhiza* Seedlings. *Jurnal Biodjati*, 10(1), 201–216. DOI: 10.15575/biodjati.v10i1.39354

Hartmann, H. T., Kester, D. E., Davies, F. T., & Geneve, R. L. (2011). *Plant propagation: Principles and practices* (8th ed.). Prentice Hall.

Kumar, P., & Srivastava, D. K. (2015). High

frequency organogenesis in hypocotyl, cotyledon, leaf and petiole explants of broccoli (*Brassica oleracea* L. var. Italica), an important vegetable crop. *Physiology and Molecular Biology of Plants*, 21(2), 279–285. DOI: 10.1007/s12298-015-0282-6.

Mardhikasari, S., Yunus, A., & Samanhudi, S. (2019). Modification of Media for Banana *In Vitro* Propagation with Foliar Fertilizer and Coconut Water in cv. Rajabulu. *Caraka Tani: Journal of Sustainable Agriculture*. 35. 23. DOI: 10.20961/carakatani.v35i1.27756.

Mehbub, H., Akter, A., Akter, Mst. A., Mandal, M. S. H., Hoque, Md. A., Tuleja, M., & Mehraj, H. (2022). Tissue Culture in Ornamentals: Cultivation Factors, Propagation Techniques, and Its Application. *Plants*, 11(23), 3208. DOI: 10.3390/plants11233208

Michael, P. S. (2011). Effects of Coconut Water on Callus Initiation and Plant Regeneration Potentials of Sweetpotato. *Journal and Proceedings of the Royal Society of New South Wales*, 144(3–4), 91–101. DOI: 10.5962/p.361652

Mok, M. C., Martin, R. C., & Mok, D. W. S. (2000). Cytokinins: Biosynthesis metabolism and perception. *In vitro Cellular & Developmental Biology - Plant*, 36(2), 102–107. DOI: 10.1007/s11627-000-0021-7.

Motlhalamme, T., Kaningini, A. G., Maropeng, R., Thema, F. T., Mohale, K. C., & Maaza, M. (2025). Tomato lycopene, total flavonoids, vitamin C content and mineral composition as affected by biosynthesized calcium carbonate nanoparticles foliar application. *Journal of Food Composition and Analysis*, 139, 107173. DOI: 10.1016/j.jfca.2024.107173

Mu Z, Tran BM, Xu H, Yang Z, Qamar UZ, et al. 2024. Exploring the potential application of coconut water in healthcare and biotechnology: a review. *Beverage Plant Research* 4: DOI: 10.48130/bpr-0024-0009

Ng, Z. C., Tan, S. H., Shiekh Mahmud, S. H. R., & Ma, N. L. (2020). Preliminary Study on Micropropagation of *Hylocereus polyrhizus* with Waste Coconut Water and Sucrose. *Materials Science Forum*, 981, 316–321. DOI: 10.4028/www.scientific.net/msf.981.316

Norouzi, O., Hesami, M., Pepe, M., Dutta, A., & Jones, A. M. P. (2022). *In vitro* plant tissue culture as the fifth generation of bioenergy. *Scientific Reports*, 12(1), 5038. DOI: 10.1038/s41598-022-09066-3

Nyamah, E.Y., Brenya, E., & Yeboah, S. Resurgence of culture tissue of three cultivars of tomatoes.

Olatunji, D., Geelen, D., & Verstraeten, I. (2017). Control of Endogenous Auxin Levels in Plant Root Development. *International Journal of Molecular Sciences*, 18(12), 2587. DOI: 10.3390/ijms18122587

Oluwakemi, A. B., Adedeji, A. D., & Obembe, O. O. (2024). Potential utilization of coconut water as a natural substitute to plant growth regulators for *in vitro* propagation of *Hibiscus sabdariffa*. *International Journal of Agriculture and Biology*, 33, Article 330215. DOI: 10.17957/IJAB/15.2275.

Pasternak, T. P., & Steinmacher, D. (2024). Plant Growth Regulation in Cell and Tissue Culture *In vitro*. *Plants*, 13(2), 327. DOI: 10.3390/plants13020327

Prando, M. A. S., Chiavazza, P., Faggio, A., & Contessa, C. (2014). Effect of coconut water and growth regulator supple-

ments on in vitro propagation of *Corylus avellana* L. *Scientia Horticulturae*, 171, 91–94. DOI: 10.1016/j.scienta.2014.03.052

Pratiwi, H. G., Wardana, R., & Jumiatun, J. (2024). The Effect of IAA and BAP Plant Growth Regulators on the *In Vitro* Growth of Purple Sweet Potato (*Ipomoea batatas* L.): The Effect of IAA and BAP Plant Growth Regulators on the *In Vitro* Growth of Purple Sweet Potato (*Ipomoea batatas* L.). *Jurnal Ilmiah Inovasi*. 24(1), 1–7. DOI: 10.25047/jii.v24i1.4092

Pudjiwati, E. H., & Rindiani, R. (2022). Prospects of Indole Acetic Acid (IAA)-Producing and Nitrate-Supplying Rhizobacteria as Plant Growth-Promoting Rhizobacteria (PGPR).

Septiawati, N., Hasibuan, S., & Aziz, R. (2021). The use of coconut water and indole-3-butyric acid (IBA) for shoot multiplication induction of potato (*Solanum tuberosum* L.) explants *in vitro*. *Jurnal Ilmiah Pertanian*, 3, 76–85. DOI: 10.31289/jiperta.v3i1.432

Setiaji, Arkan & Annisa, Rr & Rumiyati, Rumiyati & Semiarti, Endang. (2020). Induction and Growth Kinetics Callus of Tomato (*Solanum lycopersicum*). *Biosaintifika: Journal of Biology & Biology Education*. 12. 35-41. 10.15294/biosaintifika.v12i1.21704.

Setyowidianto, E. P., Basuki, N., & Damanhuri, D. (2017). Combining Ability and Heterosis of Maize (*Zea mays* L.) Lines on Yield and Yield Component Traits. *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*. 45(2), 124. DOI: 10.24831/jai.v45i2.11650

Shayanthavi, S., Kapilan, R., & Wickramasinghe, I. (2024). Comprehensive analysis of physicochemical, nutritional, and antioxidant properties of various forms and varieties of tender coconut (*Cocos nucifera* L.) water. *Food Chemistry Advances*, 4, 100645. DOI: 10.1016/j.focha.2024.100645.

Sunaryanti, D. P., & Dwiyana, M. (2020). Tomato (*Solanum lycopersicum* L.) Cultivation Techniques Using a Drip Irrigation Hydroponic System at PT Hidroponik Agrofarm Bandungan. *Jurnal Inovasi Penelitian*. , 5, DOI: 1059–1066.

Singh, V., Chauhan, N. S., Singh, M., Idris, A., Madanala, R., Pande, V., & Mohanty, C. S. (2014). Establishment of an efficient and rapid method of multiple shoot regeneration and a comparative phenolics profile in *in vitro* and greenhouse-grown plants of *Psophocarpus tetragonolobus* (L.) DC. *Plant Signaling & Behavior*, 9(10), e970443. DOI: 10.4161/15592316.2014.970443

Sivankalyani, V., Takumi, S., Thangasamy, S., Ashakiran, K., & Girija, S. (2014). Punctured-hypocotyl method for high-efficient transformation and adventitious shoot regeneration of tomato. *Scientia Horticulturae*, 165, 357–364. DOI: 10.1016/j.scienta.2013.11.034

Sumberg, J., & Giller, K. E. (2022). What is 'conventional' agriculture? *Global Food Security*, 32, 100617. DOI: 10.1016/j.gfs.2022.100617

Thakur, S., Shruti, Hashmi, S., Mishra, S., Ekka, S. K., Kushwaha, A., & Kujur, R. (2024). A Review on Plant Tissue Culture. *Asian Journal of Biology*, 20(2), 14–18. DOI: 10.9734/ajob/2024/v20i2387

Vidyagina, E. O., Kharchenko, N. N., & Shestibratov, K. A. (2021). Efficient Cryopreservation of *Populus tremula* by In vitro-Grown Axillary Buds and Genetic Stability of Recovered

Plants. *Plants*, 10(1), 77. DOI: 10.3390/plants10010077

Wulandari, C. (2018). Effect of Cytokinin and Auxin Concentrations on the Growth of *Anthurium plowmanii* Croat Plantlets.

Yaroshko, O., Pasternak, T., Larriba, E., & Pérez-Pérez, J. M. (2023). Optimization of Callus Induction and Shoot Regeneration from Tomato Cotyledon Explants. *Plants (Basel, Switzerland)*, 12(16), 2942. DOI: 10.3390/plants12162942

Zhang, Q., Gong, M., Xu, X., Li, H., & Deng, W. (2022). Roles of Auxin in the Growth, Development, and Stress Tolerance of Horticultural Plants. *Cells*, 11(17), 2761. DOI: 10.3390/cells11172761

Zhang, Y., Kan, J., Liu, X., Song, F., Zhu, K., Li, N., & Zhang, Y. (2024). Chemical Components, Nutritional Value, Volatile Organic Compounds and Biological Activities *In Vitro* of Coconut (*Cocos nucifera* L.) Water with Different Maturities. *Foods*, 13(6), 863. DOI: 10.3390/foods13060863