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The Effect of Salicylic Acid Concentration on Callus Induction and Morphology in Young Stem Explants of *Momordica cochinchinensis* (Lour.) Spreng under In Vitro Conditions

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Abstract. *Dumbaya (Momordica cochinchinensis (Lour.) Spreng.) is a tropical plant with potential as a functional food and natural antioxidant source due to its high bioactive compound content. However, its propagation is constrained by low seed germination caused by a hard seed coat, necessitating alternative methods such as plant tissue culture. This study aimed to evaluate the effect of salicylic acid (SA) concentration on callus induction and morphology in young stem explants of Dumbaya under in vitro conditions. A completely randomized design with five treatments was used: MS medium without plant growth regulators (ID0), MS + 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP (ID1), and MS supplemented with 88.44 mg L^{-1} (ID2), 162.14 mg L^{-1} (ID3), and 235.84 mg L^{-1} (ID4) SA, each with three replicates. Observed parameters included callus formation percentage, initiation time, color, and texture. All treatments resulted in 100% callus formation at 14 days after inoculation. Analysis of variance indicated a highly significant effect on callus initiation time ($F = 156.339$; $p < 0.001$), with the fastest initiation observed in ID2 (3.33 ± 0.00 days) and the slowest in ID0 (8.67 ± 0.33 days). Callus morphology varied in color and was predominantly friable. The ID2 treatment produced the most desirable characteristics, including bright color and friable texture. These findings indicate that 88.44 mg L^{-1} SA was the most effective concentration for early callus induction and favorable callus morphology in Dumbaya.*

Keywords: *callus; dumbaya; in vitro; m. cochinchinensis; plan.*

1. Introduction

Dumbaya (*Momordica cochinchinensis* (Lour.) Spreng.), also known as gac fruit, is a tropical plant belonging to the Cucurbitaceae family with considerable potential as a source of functional food and nutraceutical compounds. This species contains important bioactive compounds, particularly carotenoids such as lycopene and β-carotene, which act as natural antioxidants and may contribute to human health benefits (Al-Amery *et al*., 2023). In addition, *M. cochinchinensis* has been reported to possess pharmacological value and potential for development as a local economic commodity, especially in regions where this plant genetic resource is naturally

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available (B *et al*., 2023; Dunggio *et al*., 2025). However, the existence of Dumbaya in its natural habitat faces several challenges, including limited land availability, insufficient conservation efforts, and the lack of an effective propagation system for sustainable plant production (Pagalla *et al*., 2023).

Generative propagation of Dumbaya remains a major limitation in seedling production. The hard seed coat of Dumbaya may restrict water imbibition, delay germination, and reduce germination percentage coat that may restrict water imbibition, delay germination, and reduce germination percentage (Nidaulhasanah *et al*., 2025). This condition limits the availability of planting materials, particularly when the species is intended for conservation, research, or development as a functional food crop. Therefore, a more efficient and controlled propagation method capable of producing large numbers of uniform plant materials is required.

Plant tissue culture is one of the biotechnological approaches that can support the propagation, conservation, and development of economically valuable plant species. This technique enables the rapid production of uniform and relatively pathogen-free plant materials under aseptic and controlled conditions (Khan *et al*., 2021). In in vitro culture, callus induction was an important stage because callus tissue served as a source for plant regeneration through organogenesis or somatic embryogenesis. Moreover, callus culture can also be utilized as a system for producing bioactive secondary metabolites through medium manipulation and the application of specific elicitors (Bapat *et al*., 2023).

Effective early callus initiation is strongly influenced by explant type, physiological condition of the tissue, medium composition, and the type and concentration of plant growth regulators or physiological modulators added to the culture medium. Explants derived from young tissues generally have a higher dedifferentiation capacity because their cells are actively dividing and more responsive to stimuli from the culture medium. Young stems are therefore considered a promising source of explants because they are physiologically active and have the potential to form callus under in vitro conditions. Plant regeneration through tissue culture is closely associated with the ability of cells to undergo dedifferentiation, reprogramming, and renewed cell division toward a meristematic state (Long *et al*., 2022).

Previous studies on the tissue culture of Dumbaya are still limited and have mostly focused on the use of conventional plant growth regulators, such as 2,4-D, BAP, and IAA, in specific types of explants. Several studies have shown that combinations of plant growth regulators play an important role in callus induction of Dumbaya using leaf, petiole, and young stem explants (Dunggio *et al*., 2025; Nidaulhasanah *et al*., 2025). These studies demonstrated that combinations of auxins and cytokinins can successfully induce callus formation in M. cochinchinensis.

However, information regarding the use of salicylic acid (SA) as a physiological modulator for callus induction in this species remains unavailable.

Salicylic acid is an endogenous signaling molecule involved in plant growth regulation, stress responses, wound signaling, and secondary metabolite biosynthesis (Li *et al*., 2022; Li *et al*., 2025). In plant tissue culture, SA may function as an elicitor and physiological regulator capable of influencing cellular dedifferentiation and callus development. Previous studies in various plant species have reported that the effects of SA are concentration-dependent, with low concentrations generally stimulating cellular activity, whereas excessive concentrations may induce oxidative stress and inhibit tissue growth (Amoanimaa-Dede *et al*., 2022; Fatma *et al*., 2022). Despite these findings, the role of SA in callus induction and morphology of *M. cochinchinensis* has not yet been investigated.

Therefore, this study addresses an important research gap concerning the application of salicylic acid as a non-conventional regulator for callus induction in Dumbaya. The novelty of this study lies in evaluating salicylic acid as the sole supplement for inducing callus formation in young stem explants of M. cochinchinensis and assessing its effects on callus initiation time and callus morphological characteristics. Unlike previous studies that primarily employed auxin-cytokinin combinations, this research explores the potential of SA as an alternative physiological regulator for improving early callus development.

The concentration range evaluated in this study was selected to represent different physiological response levels and was based on previous reports demonstrating that salicylic acid can influence plant growth, stress responses, and tissue development in in vitro culture systems (Amoanimaa-Dede *et al*., 2022; Fatma *et al*., 2022; Li *et al*., 2022).

The salicylic acid concentrations used in this study (88.44, 162.14, and 235.84 mg L^{-1}) were selected to represent low, medium, and high concentration levels to evaluate the concentration-dependent response of Dumbaya explants under in vitro conditions. These concentration levels were chosen based on reports indicating that SA may promote cellular activity and tissue development at lower concentrations while potentially causing physiological stress at higher concentrations (Amoanimaa-Dede *et al*., 2022; Fatma *et al*., 2022; Li *et al*., 2022). Therefore, the selected concentrations were expected to provide a suitable range for identifying the most effective SA concentration for early callus induction and favorable callus morphology.

Based on these considerations, this study aimed to evaluate the effect of salicylic acid concentration on callus induction and morphology in young stem explants of Dumbaya under in vitro conditions.

However, information on the use of salicylic acid as an additional compound in the culture medium to support callus induction of Dumbaya, particularly in young stem explants, remains scarce. This indicates a research gap regarding the optimization of nonconventional physiological modulators that may enhance the early response of callus formation.

Salicylic acid (SA) is an endogenous signaling compound in plants that plays important roles in growth regulation, stress responses, plant defense, and cellular metabolic activity. In in vitro culture, SA may function as both an elicitor and a physiological modulator that influences tissue responses to wounding, cellular dedifferentiation, and secondary metabolite formation (Li *et al*., 2022; Li *et al*., 2025). The effect of SA on plant tissue growth is concentration dependent. At certain concentrations, SA may stimulate cellular physiological activity, whereas higher concentrations may induce oxidative stress and inhibit tissue growth (Amoanimaa-Dede *et al*., 2022; Fatma *et al*., 2022)

Based on these considerations, this study aimed to evaluate the effect of salicylic acid concentration on callus induction and morphology in young stem explants of Dumbaya under in vitro conditions. The SA concentrations used in this study were 88.44 mg L^{-1}, 162.14 mg L^{-1}, and 235.84 mg L^{-1}, which were compared with MS medium without plant growth regulators and MS medium supplemented with 2 mg L^{-1} 2,4-D and 2 mg L^{-1} BAP as a positive control. This study is expected to provide basic information on the effective concentration of SA for supporting early callus induction in Dumbaya as an initial step toward developing tissue culture techniques for propagation, conservation, and utilization of this bioactive local plant.

2. Materials and Methods

2.1 Research Subject

This study was conducted from December 2025 to March 2026 at the Plant Tissue Culture Laboratory, Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo. Young stem explants of Dumbaya (*Momordica cochinchinensis* (Lour.) Spreng.) were collected from healthy plants grown in Wongkaditi Barat, Kota Utara District, Gorontalo City, Indonesia (0°33′42.0″ N, 123°04′42.2″ E). Young stem tissues were selected as explants because juvenile tissues generally exhibit high physiological activity and are more responsive to dedifferentiation under in vitro culture conditions (Long *et al*., 2022).

2.2.Tools and Materials Used

The equipment used in this study included a laminar air flow cabinet, autoclave, analytical balance, magnetic stirrer, hot plate, pH meter or universal pH indicator, micropipettes, sterile scalpel, sterile forceps, Erlenmeyer flasks, measuring cylinders, beakers, and culture bottles. The laminar air flow cabinet was used to maintain aseptic conditions during explant inoculation, while

the autoclave was used to sterilize culture media and instruments. Sterilization of the working area using alcohol and ultraviolet irradiation was carried out to reduce the risk of microbial contamination during tissue culture procedures (Sulichantini *et al*., 2024).

The materials used included Murashige and Skoog (MS) basal medium at 4.43 g L^{-1}, sucrose at 30 g L^{-1}, agar at 8 g L^{-1}, salicylic acid (SA), 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP), 5.25% NaOCl, 70% ethanol, 96% or absolute ethanol, sterile distilled water, NaOH, and HCl. MS medium was used because it provides macro and micronutrients required to support plant tissue growth under in vitro conditions (Adugna *et al*., 2020; Tarigan *et al*., 2023).

2.3. Experimental Design

The experiment was arranged in a completely randomized design with one factor, namely medium formulation for callus induction. The experiment consisted of five treatments as follows Table 1.

Table 1. Treatment codes and medium formulations used for callus induction.

Treatment code	Medium formulation
ID0	MS medium without plant growth regulators
ID1	MS + 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP
ID2	MS + 88.44 mg L^{-1} SA
ID3	MS + 162.14 mg L^{-1} SA
ID4	MS + 235.84 mg L^{-1} SA

Each treatment was replicated three times. Each replicate consisted of three culture bottles, with three explants cultured in each bottle.Thus, a total of 27 explants were used for each treatment. Each culture bottle contained 25 mL of medium. For statistical analysis, the value of each replication was obtained from the mean response of explants within the corresponding treatment replication.

2.4. Preparation of Stock Solutions and Concentration Conversion

The salicylic acid stock solution was prepared at a concentration of 14,740 ppm, equivalent to 14,740 mg L^{-1}. The stock solution was added to MS medium at 6, 11, and 16 mL per liter of medium. Based on dilution calculations, the final SA concentrations in the medium were equivalent to 88.44, 162.14, and 235.84 mg L^{-1}, respectively. The stock solutions of 2,4-D and BAP were each prepared at a concentration of 1000 ppm, equivalent to 1000 mg L^{-1}. Each stock solution was added to MS medium at 2 mL per liter of medium, resulting in final concentrations of 2 mg L^{-1} 2,4-D and 2 mg L^{-1} BAP.

The final concentrations were calculated using the dilution Equation (1).

$$C_2 = \frac{C_1 \times V_1}{V_2} \quad (1)$$

where C_1 is the stock solution concentration, V_1 is the volume of stock solution added, V_2 is the final volume of the medium, and C_2 is the final concentration of the compound in the medium.

2.5. Culture Incubation and Observation

The culture medium was prepared by dissolving MS basal medium at 4.43 g L^{-1} and sucrose at 30 g L^{-1} in distilled water. The solution was homogenized using a magnetic stirrer. The stock solutions of 2,4-D, BAP, and SA were added to the medium according to the treatment formulations. The pH of the medium was adjusted to 5.8–6.0 using NaOH or HCl. After pH adjustment, agar at 8 g L^{-1} was added as a gelling agent, and the medium was heated until the agar was completely dissolved. A total of 25 mL of medium was dispensed into each culture bottle. The complete medium containing all treatment components, including SA, was then sterilized by autoclaving at 121°C for 20 min. Autoclaving at 121°C is commonly used in plant tissue culture to ensure that the culture medium is free from microbial contaminants (Adugna *et al*., 2020).

2.6 Explant Sterilization and Inoculation

Young stem explants of Dumbaya were excised to approximately 5 cm in length from healthy mother plants. The explants were washed under running tap water to remove surface debris and then rinsed with sterile distilled water. Initial surface sterilization was performed by immersing the explants in 5.25% NaOCl for approximately 10 min, followed by rinsing three times with sterile distilled water.

Further sterilization was carried out inside the laminar air flow cabinet by immersing the explants in 70% ethanol for five min, followed by rinsing three times with sterile distilled water. The explants were then cut into approximately 0.5 cm segments using a sterile scalpel and aseptically inoculated onto the treatment media. Surface sterilization is essential to reduce external contaminants while maintaining the viability of the explant tissues used for culture (Manuhara *et al*., 2024).

2.7. Culture Incubation and Observation

The cultures were incubated at a temperature of ±25°C for 14 days under a photoperiod of 12 h light and 12 h dark. The observed parameters included callus formation percentage, callus initiation time, callus color, and callus texture. Callus formation percentage was calculated based on the number of explants that formed callus relative to the total number of cultured explants using the following Equation (2).

$$\text{Callus formation percentage} = \frac{\text{Number of explants forming callus}}{\text{Total number of explants}} \times 100\% \quad (2)$$

Callus initiation time was recorded as the number of days from inoculation until the first visible appearance of callus on the explant surface. Callus was defined as a newly formed tissue mass, commonly emerging from the wounded region of the explant and appearing white or

translucent during the early stage of formation (Prashariska *et al*., 2021; Tarigan *et al*., 2023). Callus morphology was visually observed based on callus color and texture. Callus color was categorized according to the dominant color observed, while callus texture was classified as friable or compact. Morphological observation is important because callus color and texture may reflect the physiological condition of callus tissue during in vitro culture (Prashariska *et al*., 2021).

2.8. Data Analysis and Interpretation

Quantitative data, namely callus initiation time, were analyzed using one-way analysis of variance (ANOVA). Prior to ANOVA, data normality was assessed using the Shapiro-Wilk test. When ANOVA indicated significant differences among treatments, the analysis was followed by the Waller-Duncan post hoc test at a significance level of $\alpha = 0.05$. Qualitative data, including callus color and texture, were analyzed descriptively. All statistical analyses were performed using IBM SPSS Statistics version 30.

3. Results and Discussion

3.1. Morphology of the Donor Plant

The Dumbaya plant (*Momordica cochinchinensis* (Lour.) Spreng.) used as the explant source exhibited typical morphological characteristics of a climbing species belonging to the Cucurbitaceae family. The plant had young green stems with a relatively rough surface and tendrils. The leaves were cordate with serrated margins and pointed tips, while the flowers were white to yellowish. The fruits were round to oval with a rough or spiny surface and changed from green to orange when mature. The seeds had a hard coat, which is one of the main constraints in generative propagation of this species. Young stems were selected as explants because juvenile tissues generally have high physiological activity and greater dedifferentiation capacity than older tissues. In plant tissue culture, the ability of cells to undergo dedifferentiation, reprogramming, and renewed cell division is a key factor determining successful callus induction (Long *et al*., 2022). Therefore, young stem explants of Dumbaya have potential as explant sources for in vitro callus induction.

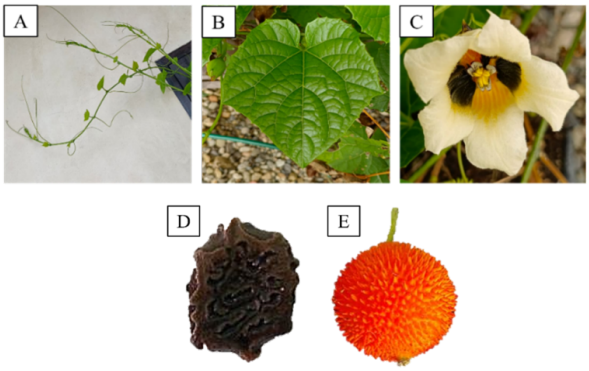


Figure 1. Morphology of *Momordica cochinchinensis* (Lour.) Spreng.: Figure 1A) young stem; Figure 1B) leaf; Figure 1C) flower; Figure 1D) seed; Figure 1E) fruit.

Figure 1 shows the morphology of *Momordica cochinchinensis* (Lour.) Spreng, including the young stem (Figure 1A), leaf (Figure 1B), flower (Figure 1C), seed (Figure 1D), and fruit (Figure 1E).

3.2 Percentage of Explants Forming Callus

Callus formation percentage is an early indicator of explant response to culture medium. Based on observations at 14 days after inoculation (Table 2), all treatments produced 100% callus formation. This result indicates that young stem explants of Dumbaya had a high dedifferentiation response on MS medium, either without plant growth regulators, with a combination of 2,4-D and BAP, or with the addition of salicylic acid.

Table 2. Callus formation percentage under different medium formulations.

Treatment code	Medium formulation	Callus formation (%)
ID0	MS medium without PGRs	100
ID1	MS + 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP	100
ID2	MS + 88.44 mg L^{-1} SA	100
ID3	MS + 162.14 mg L^{-1} SA	100
ID4	MS + 235.84 mg L^{-1} SA	100

The high percentage of callus formation may be associated with the physiological condition of young stem explants, which still actively divide. Juvenile tissues tend to be more responsive to stimuli from the culture medium because they have higher regenerative potential and metabolic activity. In addition, Murashige and Skoog (MS) medium provides macro- and micronutrients required to support metabolic activity, cell division, and new tissue formation under in vitro conditions (Adugna *et al*., 2020; Tarigan *et al*., 2023). In ID0, MS medium without plant growth regulators, the explants still formed callus at 100%. This indicates that endogenous hormones in young stem tissues may be sufficient to initiate callus formation. Such a response may occur because wounding during explant excision triggers physiological changes in the tissue and induces cells around the wounded region to undergo dedifferentiation, thereby regaining the ability to divide and form callus tissue (Zulkarnain, 2009; Long *et al*., 2022). However, because all treatments produced the same callus formation percentage, this parameter could not distinguish the effectiveness of the treatments. Therefore, further evaluation was based on callus initiation time and callus morphological characteristics.

3.2.Callus Initiation Time

Callus initiation time is an important parameter for evaluating the speed of explant response to medium treatment. Statistical analysis showed that medium formulation had a highly significant effect on callus initiation time. One-way ANOVA showed an F value of 156.339 with $p < 0.001$, indicating highly significant differences among treatments (Table 3).

Table 3. ANOVA results for callus initiation time.

Source of variation	Sum of squares	df	Mean square	F value	Sig.
Between treatments	46.341	4	11.585	156.339	<0.001
Error	0.741	10	0.074	-	-
Total	47.082	14	-	-	-

The Waller-Duncan post hoc test showed (Table 4) that all treatments were significantly different from one another at $\alpha = 0.05$. The fastest callus initiation was observed in ID2, consisting of MS medium supplemented with 88.44 mg L^{-1} SA, with callus appearing at 3.33 ± 0.00 days after inoculation. ID3 showed callus initiation at 4.78 ± 0.19 days, followed by ID4 at 5.33 ± 0.33 days and ID1 at 6.00 ± 0.33 days. In contrast, ID0, the MS medium without plant growth regulators, showed the slowest callus initiation time at 8.67 ± 0.33 days after inoculation.

Table 4. Callus initiation time under different medium formulations.

Treatment code	Medium formulation	Callus initiation time (DAI)
ID0	MS medium without PGRs	8.67 ± 0.33^{e}
ID1	MS + 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP	6.00 ± 0.33^{d}
ID2	MS + 88.44 mg L^{-1} SA	3.33 ± 0.00^{a}
ID3	MS + 162.14 mg L^{-1} SA	4.78 ± 0.19^{b}
ID4	MS + 235.84 mg L^{-1} SA	5.33 ± 0.33^{c}

Note: Values are presented as mean ± standard deviation. Different superscript letters within the same column indicate significant differences among treatments based on the Waller-Duncan post hoc test at $\alpha = 0.05$.

The application of 88.44 mg L^{-1} SA accelerated callus initiation compared with the other treatments. This may be related to the role of salicylic acid as a signaling molecule that modulates physiological responses to wounding, early stress, and cellular metabolic activation. During the early stage of culture, wounding caused by explant excision may trigger stress responses that stimulate cells around the wounded region to dedifferentiate and resume cell division. At an appropriate concentration, salicylic acid may regulate these responses, thereby accelerating callus initiation (Fatma *et al*., 2022; Li *et al*., 2022).

However, increasing SA concentrations to 162.14 and 235.84 mg L^{-1} did not further accelerate callus initiation compared with ID2. Instead, callus initiation was delayed. This result suggests that the response of young stem explants of Dumbaya to SA was concentration-dependent. At a lower concentration, SA may exert a stimulatory effect, whereas higher concentrations may impose physiological stress that slows tissue dedifferentiation. Plant responses to growth regulators or signaling compounds are often concentration-dependent, meaning that increasing concentration does not always result in a better growth response (Amoanimaa-Dede *et al*., 2022).

The ID1 treatment containing 2,4-D and BAP also accelerated callus initiation compared with the control ID0, but it was still slower than the SA treatments. A combination of auxin and cytokinin such as 2,4-D and BAP is commonly used in plant tissue culture to stimulate cell division and callus formation. However, the final response depends on explant type, medium composition,

and the physiological balance of the tissue. In this study, 88.44 mg L^{-1} SA produced the fastest callus initiation response in young stem explants of Dumbaya.

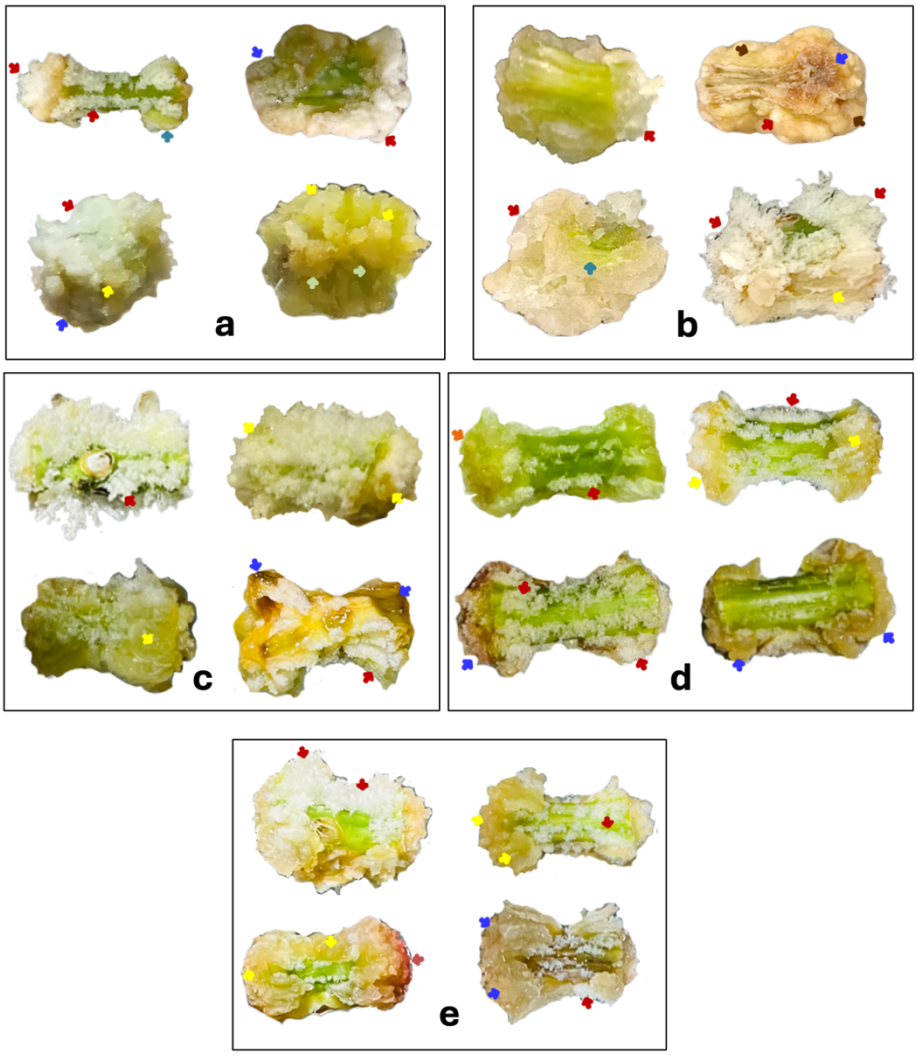
3.3. Callus Morphology

The callus formed from young stem explants of Dumbaya showed morphological variation, particularly in color and texture (Table 5). The observed callus colors included white, yellowish-white, greenish-white, yellowish cream, brown, and pink. In general, white to yellowish-white callus indicates actively dividing tissue that has not undergone advanced differentiation. Greenish-white Callus may indicate chlorophyll formation due to light exposure during incubation, whereas brown Callus indicates browning associated with phenolic oxidation (Phillips & Garda, 2019; Twaij *et al*., 2020).

Table 5. Callus morphological characteristics under different medium formulations.

Treatment code	Medium formulation	Callus color	Callus texture	General interpretation
ID0	MS medium without PGRs	White, yellowish-white, greenish-white, brown	Friable	Callus formed well, but color variation and browning were observed
ID1	MS + 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP	White, greenish-white, yellowish-white, brown	Friable and partly compact	Callus formed well, but some Callus showed compact texture
ID2	MS + 88.44 mg L^{-1} SA	White, yellowish-white, slightly brown	Friable	Best callus characteristics, dominated by bright color and friable texture
ID3	MS + 162.14 mg L^{-1} SA	White, greenish-white, yellowish-white, brown	Friable	Callus formed, but browning symptoms began to appear
ID4	MS + 235.84 mg L^{-1} SA	Yellowish-white, white, brown, pink	Friable	Callus formed, but higher SA concentration tended to increase color variation

Callus color was an important indicator of the physiological condition of the cultured tissue. White or yellowish-white callus was generally associated with physiologically active tissue, whereas brown callus indicated oxidation of phenolic compounds caused by tissue stress. Browning is a common problem in plant tissue culture because oxidized phenolic compounds can be toxic to cells and inhibit callus growth (Twaij *et al*., 2020). In this study, browning was observed in several treatments, particularly in the callus showing brown discoloration. In terms of texture, most Callus exhibited a friable texture. Friable Callus has a relatively loose cellular arrangement, is easily separated, and generally has a higher potential for further proliferation than compact Callus. Friable callus is also more suitable for developing cell suspension cultures or further regeneration stages because they tend to have better proliferative capacity (Arora *et al*., 2022).



Note: red arrows indicate white callus; yellow arrows indicate yellowish-white callus; green arrows indicate greenish-white callus; blue arrows indicate brown callus; brown arrows indicate yellowish-cream callus; and orange arrows indicate pink callus.

Figure 2. Callus morphology of young stem explants of *Momordica cochinchinensis* under different medium formulations after 14 days of incubation. Figur 1a) ID0 = MS medium without plant growth regulators; Figur 2b) ID1 = MS + 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP; Figur 1c) ID2 = MS + 88.44 mg L^{-1} SA; Figur 1d) ID3 = MS + 162.14 mg L^{-1} SA; and Figur 1e) ID4 = MS + 235.84 mg L^{-1} SA.

Figure 2 shows the callus morphology of young stem explants of *M. cochinchinensis* cultured on different medium formulations after 14 days of incubation, including ID0 on MS medium without plant growth regulators (Figure 2A), ID1 on MS + 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP (Figure 2B), ID2 on MS + 88.44 mg L^{-1} SA (Figure 2C), ID3 on MS + 162.14 mg L^{-1} SA (Figure 2D), and ID4 on MS + 235.84 mg L^{-1} SA (Figure 2E).

The ID2 treatment produced the best callus morphological characteristics, indicated by a predominance of bright color and friable texture. This finding is consistent with the callus initiation time data, which showed that ID2 had the fastest response. Thus, SA at 88.44 mg L^{-1} not only accelerated callus initiation but also supported the formation of Callus with better morphological characteristics. In contrast, increasing SA concentrations in ID3 and ID4 tended to delay callus

initiation and increase color variation, including browning, which may indicate an enhanced tissue stress response.

Physiologically, SA may act as a signaling compound that affects metabolic activity, stress responses, and secondary metabolite formation in plants (Li *et al*., 2022; Li *et al*., 2025). However, at high concentrations, SA may induce excessive accumulation of reactive oxygen species (ROS), leading to oxidative stress. This condition may accelerate phenolic oxidation and cause callus browning (Khan *et al*., 2021; Twaij *et al*., 2020). Therefore, selecting an appropriate SA concentration is essential to support callus induction and maintain callus quality in Dumbaya under in vitro conditions.

4. Conclusion

Salicylic acid affected callus initiation time and callus morphology in young stem explants of Momordica cochinchinensis (Lour.) Spreng. cultured under in vitro conditions. All treatments resulted in 100% callus formation at 14 days after inoculation. The application of 88.44 mg L^{-1} SA produced the fastest callus initiation (3.33 ± 0.00 days after inoculation) and generated callus with desirable morphological characteristics, including bright coloration and a friable texture. In contrast, higher SA concentrations tended to delay callus initiation and increase callus browning. This study was limited to observations of callus induction and morphological characteristics during the early culture stage. Therefore, further studies are required to evaluate callus biomass production, viability, secondary metabolite accumulation, and regeneration potential to better understand the long-term effects of salicylic acid on Dumbaya tissue culture.

Abbreviations

ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
DAI	Days After Inoculation
IAA	Indole-3-Acetic Acid
MS	Murashige and Skoog
PGRs	Plant Growth Regulators
ROS	Reactive Oxygen Species
SA	Salicylic Acid

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

CRediT Authorship Contribution Statement

Indayani Tandililing: drafting, conceptualization, investigation, data curation, formal analysis, visualization, and initial draft writing. **Jusna Ahmad**: supervision, methodology, validation, review and editing. **Devi Bunga Pagalla**: supervision, conceptualization,

methodology, review and editing. **Novri Youla Kandowangko**: investigation, resources, and validation. **Indriati Husain**: methodology, resources, validation, review and editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest or competing interests.

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