

Effect of NAA and BAP on Callus Induction of Binahong Internodes (*Anredera cordifolia* (Ten.) Steenis) under Different Light Conditions

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Abstract. Binahong is a medicinal plant containing secondary metabolites with antibacterial and anti-inflammatory activities. The callus culture method is one of the commonly used methods to increase the content of secondary metabolites. The effect of plant growth regulators and light conditions on binahong internodes callus induction remains limited. The aim of this study was to determine the optimal combination of concentrations of the growth regulators NAA and BAP to induce callus in binahong internode explants in vitro under different light conditions. This study employed a randomized block design (RBD) with two factors: light (light and dark) and NAA (0; 1; 2; 3) ppm and BAP (0; 0.5; 1; 1.5) ppm. The parameters observed included callus emergence time, percentage of callus-forming explants, fresh weight, dry weight, and callus morphology. The results showed a significant interaction between the plant growth regulators and lighting conditions for all parameters. The fastest callus formation time (6.8 DAP) occurred with BAP 0.5 ppm + NAA 2–3 ppm under light conditions, the highest percentage under dark conditions (95.25%) while under light conditions (92.94%), and the highest fresh and dry weights were observed with BAP 1.5 ppm + NAA 3 ppm (light) at 0.4128 g (fresh weight) and 0.0219 g (dry weight). The callus was predominantly compact in texture, ranging in color from greenish-white to brown under light conditions and from white to brown under dark conditions. These findings demonstrate that the interaction between NAA, BAP and light conditions is critical for efficient callus induction and biomass production from binahong internode explants in vitro.

Key words: binahong, callus induction, NAA, BAP, lighting

INTRODUCTION

As an archipelagic country, Indonesia possesses an exceptionally high diversity of flora and fauna (Al qamari *et al.*, 2020) there are approximately 30.000 plant species, about 7.000 of which have potential as medicinal ingredients (Adiyasa & Meiyanti, 2021). Among these medicinal plants, binahong (*Anredera cordifolia* (Ten.) Steenis) is one of the species widely utilized in traditional medicine due to its potential biological activities (Fitriyah *et al.*, 2013). All parts of the binahong plant, from the leaves and stems to the tubers, can be used as herbal medicine. This vine from the Basellaceae family contains secondary metabolites such as polyphenols, flavonoids, tannins, alkaloids, and saponins, which act as antibacterial, antimicrobial, and anti-inflammatory agents (Kumalasari & Sulistyani, 2011). The high potential of binahong as a medicinal plant highlights the need for biotechnology approaches, such as callus culture, to support controlled production and optimization of valuable plant compounds.

The valuable medicinal properties and secondary metabolite content of binahong highlight the need for sustainable approaches to improve biomass production and optimize the availability of bioactive compounds (Wonganu, 2007). Tissue culture techniques offer a number of strategic advantages, particularly in the propagation of rare and long-lived plants that have potential as sources of secondary metabolites. Compared with direct extraction from whole plants, callus culture allows continuous production of plant-derived compounds under controlled environmental conditions, making it a promising strategy for medicinal plant improvement (Sulichantini, 2015). The callus culture method is one of the most frequently used methods to increase secondary metabolite content, as changes in plant morphology particularly color changes resulting from secondary metabolite production can be clearly observed (Kadapi *et al.*, 2024).

The induction of callus is affected by several factors, particularly the type and concentration of plant growth regulators (PGRs) and lighting. PGRs regulate cellular processes including cell division, expansion, dedifferentiation, and differentiation, thereby determining the developmental response of explant tissues (Damanik *et al.*, 2017). In tissue culture techniques, the application of plant growth regulators (PGRs) plays a crucial role in stimulating explant growth. NAA, a synthetic auxin, plays a role in cell elongation and is essential for inducing the formation of compact, green callus (Prasetyo *et al.*, 2020). Meanwhile, cytokinins such as BAP can stimulate cell division and callus proliferation in explants (Yanti & Isda, 2021). Swandari & Setyorini, (2018) reported that a combination of 3.0 ppm NAA + 0.5 ppm BAP yielded the best results in callus induction in *Gerbera jamesonii*. Research Purnamaningsih & Ashrina, (2011) showed that a combination of BAP 0.5 mg/L + NAA 0.5 mg/L in MS medium effectively induced callus in *Artemisia annua* L.

Besides hormonal regulation, lighting conditions represent an important environmental factor affecting callus growth and physiological responses. Different light intensities can have a significant effect on the color and other responses produced by the callus (Setiawati *et al.*, 2020). In addition, light also influences the production of metabolites in tissue culture, including both primary metabolites such as amino acids, enzymes, fats, and carbohydrates, as well as secondary metabolites such as carotenoids, anthocyanins, and flavonols (Ariany *et al.*, 2013). Light parameters such as intensity and light source type play a crucial role in increasing fresh weight and the formation of secondary metabolites in various plant species (Chen *et al.*, 2019). In a study by Cahayani & Habibah (2025), treatment with dark conditions and 0 ppm kinetin + 1.5 ppm picloram resulted in the optimal time for callus formation on binahong stems. Although previous studies have demonstrated the importance of PGRs and lighting conditions in callus induction, the combined effects of NAA, BAP, and different lighting conditions on binahong internodes explants remain insufficiently explored. Therefore, this study was conducted to determine the optimal combination of NAA and BAP concentrations for inducing callus from binahong internodes explants under different lighting conditions in vitro.

METHODS

The study was conducted at the Plant Tissue Culture Laboratory of the Biology Program, Faculty of Mathematics and Natural Sciences, Semarang State University, from October 2025 to February 2026. This study used a Randomized Block Design (RBD) with two treatment factors consisting of the type/concentration of plant growth regulators (NAA 0; 1; 2; 3 ppm and BAP 0; 0.5; 1; 1.5 ppm). There were 16 treatment levels for the PGR factor (**Table 1**). The second factor, lighting, has two levels: light (with 24-hour lighting) and dark (without 24-hour lighting). There is a total of 32 treatments with 4 replicates. Each treatment combination was randomly assigned within each replication, and each replication represented a different planting period.

Table 1. Combination of BAP and NAA treatments

Concentration BAP (B)	Concentration NAA (N)			
	0 ppm (N0)	1 ppm (N1)	2 ppm (N2)	3 ppm (N3)
0 ppm (B0)	B0N0	B0N1	B0N2	B0N3
0.5 ppm (B0.5)	B0.5N0	B0.5N1	B0.5N2	B0.5N3
1 ppm (B1)	B1N0	B1N1	B1N2	B1N3
1.5 ppm (B1.5)	B1.5N0	B1.5N1	B1.5N2	B1.5N3

Preparation and Sterilization of Media: The media used consists of 4.43 g of MS medium, 30 g of sugar, 0.1 g of myo-inositol, 7 g of agar, and 1 liter of distilled water. Adjust the medium's pH to 5–6 using 1N NaOH or 1N HCl solution. The medium is poured into culture bottles in 25-ml portions, and stock solutions of PGRs, NAA, and BAP are added to the MS medium according to the treatment. The medium is then sterilized in an autoclave at 121°C and 1.5 atm for 20 minutes (Vishal Gupta & Shukshith, 2016).

Explant Sterilization: Binahong internode explants were initially washed under running tap water for 30 minutes to remove surface contaminants. The explants were sterilized using 0.2 g of detergent for 10 minutes and rinsed three times with sterile distilled water. Next, the explants were soaked in a solution containing 0.5 g of bactericide and 0.5 g of fungicide for 30 minutes and rinsed with sterile distilled water three times. The sterilization process continued in a Laminar Air Flow (LAF) cabinet by soaking the explants in a 10% sodium hypochlorite solution for 10 minutes and then rinsed three additional times with sterile distilled water.

Callus Induction: Binahong internodes explants were cut into 1 cm pieces and planted in MS medium according to the treatment. Each culture flask was filled with two explants. The culture flasks were stored in an incubation chamber at a temperature of 20–24°C and a humidity of 52–58% (Hidayat *et al.*, 2021). The explants were incubated for 30 days under two treatments: either exposed to light for 24 hours (light conditions) or kept in the dark (dark conditions).

Observations: Observations were conducted daily and included the time of callus emergence, the percentage of callus formation, the fresh weight and dry weight of the callus, as well as the texture and color of the callus, with an incubation period of 30 days after planting (DAP). The fresh weight of the callus was measured immediately after harvesting, after the remaining medium had been removed. The dry weight of the callus was determined by weighing the callus after drying it in an oven at 60°C until a constant weight was achieved. Callus texture was assessed visually based on whether the callus was friable or compact, while callus color was identified using the Munsell Color Chart.

Data Analysis: Quantitative data, including callus induction time, the percentage of explants with callus, fresh weight, and dry weight, were analyzed using IBM SPSS Statistics version 27 at a 5% significance level. Data including callus induction time, the percentage of explants with callus, fresh weight, and dry weight were tested for normality using the Kolmogorov-Smirnov test (Ghasemi & Zahediasl, 2012) and homogeneity using Levene's test. Since the data did not meet the assumptions of normality and homogeneity, a non-parametric analysis was performed using the *Kruskal-Wallis* test, followed by a *Dunn* post-hoc test to identify treatments with significant differences.

RESULTS

Callus Induction Time and Callus Formation Percentage

The data time of callus formation and percentage of callus formation were not normally distributed and were not homogeneous, they were analyzed using the *Kruskal-Wallis* test. The results showed that the interaction between PGR and light conditions had a significant effect ($p < 0.05$). Further analysis using *Dunn's* test showed that the fastest average callus formation time was observed in the BAP 0.5 + NAA 2–3 ppm treatment under light conditions. Overall, variations in NAA and BAP concentrations resulted in different callus induction responses, with several combinations showing faster formation under light conditions, while higher callus formation percentages were generally observed under dark conditions. The highest percentage of callus-forming explants under dark conditions was 95.25% (**Table 2**).

Table 2. Results of Dunn's multiple range test for the combination of BAP and NAA on the parameters of callus induction time and callus formation percentage under light and dark conditions.

Treatment	Callus Induction Time (Days)	Callus Formation Percentage (%)
B0N0L	7.00 ± 5.52 ^a	50.00 ± 40.82 ^c
B0N1L	9.25 ± 2.59 ^{ab}	100.00 ± 00.00 ^a
B0N2L	7.87 ± 0.85 ^{ab}	100.00 ± 00.00 ^a
B0N3L	8.37 ± 2.86 ^{ab}	100.00 ± 00.00 ^a
B0.5N0L	15.87 ± 4.44 ^{cd}	100.00 ± 00.00 ^a
B0.5N1L	11.25 ± 1.32 ^{abc}	100.00 ± 00.00 ^a
B0.5N2L	6.87 ± 0.62^a	100.00 ± 00.00 ^a
B0.5N3L	6.87 ± 0.62^a	100.00 ± 00.00 ^a
B1N0L	16.75 ± 3.52 ^d	100.00 ± 00.00 ^a
B1N1L	8.50 ± 1.9 ^{ab}	100.00 ± 00.00 ^a
B1N2L	8.25 ± 1.19 ^{ab}	100.00 ± 00.00 ^a
B1N3L	9.87 ± 2.25 ^{ab}	100.00 ± 00.00 ^a
B1.5N0L	6.87 ± 2.25 ^a	62.50 ± 47.81 ^{bc}
B1.5N1L	7.25 ± 1.84 ^a	100.00 ± 00.00 ^a
B1.5N2L	7.87 ± 1.84 ^{ab}	100.00 ± 00.00 ^a
B1.5N3L	7.00 ± 1.65 ^a	100.00 ± 00.00 ^a
B0N0D	11.50 ± 7.93 ^{abcd}	75.00 ± 50.00 ^{abc}
B0N1D	10.12 ± 1.70 ^{ab}	100.00 ± 00.00 ^a
B0N2D	10.37 ± 0.75 ^{abc}	100.00 ± 00.00 ^a
B0N3D	11.00 ± 2.85 ^{abc}	100.00 ± 00.00 ^a
B0.5N0D	8.00 ± 5.94 ^{ab}	62.50 ± 47.87 ^{bc}
B0.5N1D	12.12 ± 2.65 ^{abcd}	100.00 ± 00.00 ^a
B0.5N2D	11.37 ± 3.25 ^{abcd}	100.00 ± 00.00 ^a
B0.5N3D	10.00 ± 3.55 ^{ab}	100.00 ± 00.00 ^a
B1N0D	13.25 ± 0.95 ^{bcd}	100.00 ± 00.00 ^a
B1N1D	11.75 ± 1.89 ^{abcd}	100.00 ± 00.00 ^a
B1N2D	9.00 ± 2.04 ^{ab}	100.00 ± 00.00 ^a
B1N3D	8.62 ± 2.28 ^{ab}	100.00 ± 00.00 ^a
B1.5N0D	12.12 ± 3.70 ^{abcd}	87.50 ± 25.00 ^{ab}
B1.5N1D	8.75 ± 1.70 ^{ab}	100.00 ± 00.00 ^a
B1.5N2D	10.12 ± 2.46 ^{ab}	100.00 ± 00.00 ^a
B1.5N3D	8.50 ± 2.38 ^{ab}	100.00 ± 00.00 ^a

Note: B: BAP (0; 0.5; 1; 1.5 ppm); N: NAA (0; 1; 2; 3 ppm); L: light conditions; D: dark conditions. Values are presented as mean ± SD of four replicates. Each treatment consisted of two explants. Bold values indicate the fastest average time to callus formation.

*Identical letters indicate no significant difference; different letters indicate a significant difference.

Fresh Weight of Callus

The fresh weight data were analyzed using the nonparametric *Kruskal-Wallis* test to assess the effects of adding the plant growth regulators BAP and NAA. The results showed that there was an interaction effect between the plant growth regulators and lighting conditions on callus wet weight, with $p < 0.05$. The highest average fresh weight of callus was found in the treatment of 1.5 ppm BAP + 3 ppm NAA under light conditions, which was 0.4128 g (**Figure 1**).

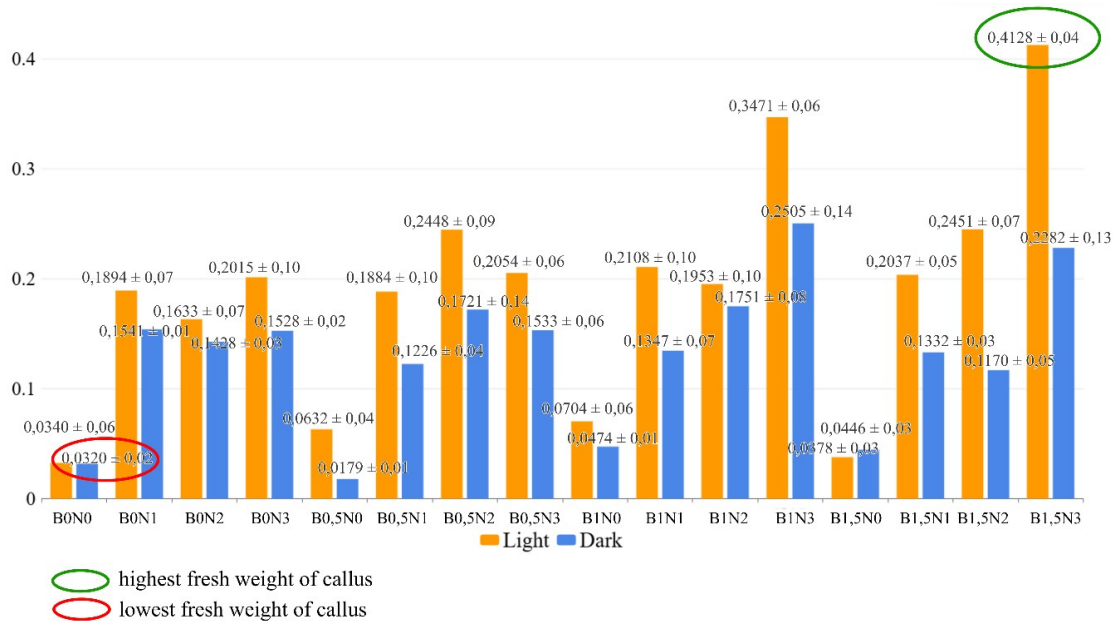


Figure 1. Fresh weight of binahong callus internodes under light and dark conditions

Dry Weight of Callus

The test showed that the interaction between the PGR and light conditions had a significant effect ($p < 0.05$) on the callus dry weight parameter. The highest average callus dry weight was obtained in the BAP 1.5 ppm + NAA 3 ppm treatment under light conditions, namely 0.0219 g, while the lowest was observed in the control under dark conditions, at 0.0022 g. (Figure 2).

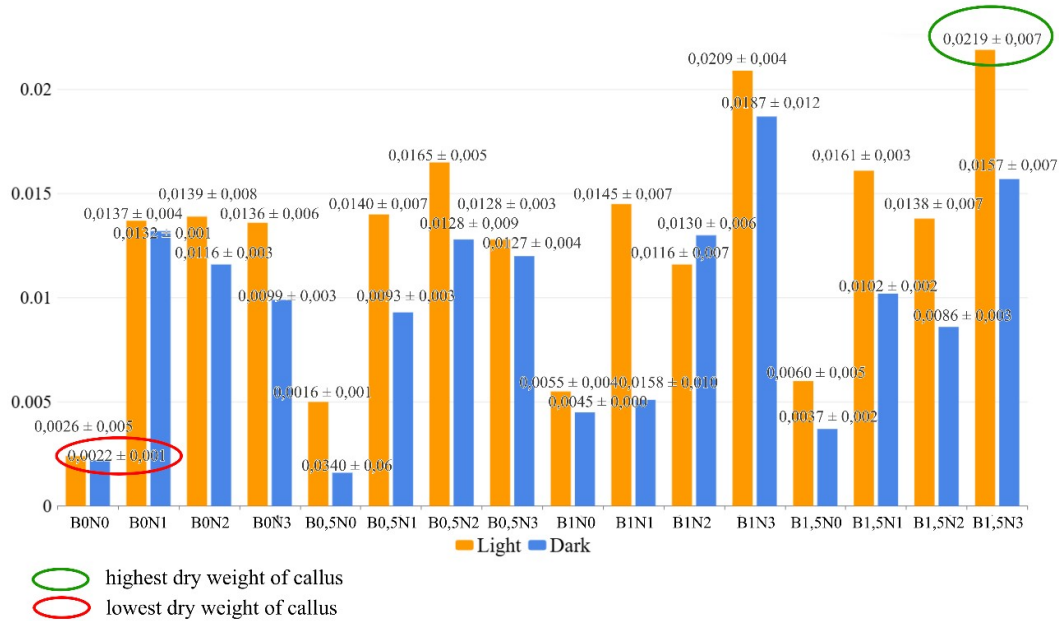


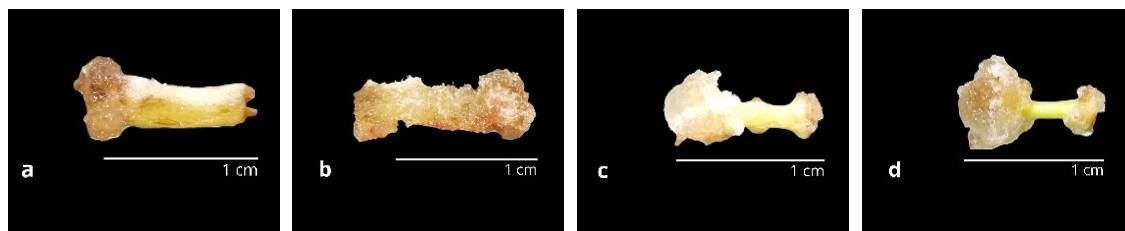
Figure 2. Dry weight of binahong callus internodes under light and dark conditions

Callus Morphology

The morphological observations of binahong internode callus under light and dark conditions are presented in **Figure 3** and **Figure 4**. In general, callus development showed variations in color, texture, and growth pattern among treatments.



Figure 3. Morphology of binahong callus internodes under light conditions
(a) B0N0; (b) B0N1; (c) B0N2; (d) B0N3; (e) B0.5N0; (f) B0.5N1; (g) B0.5N2; (h) B0.5N3;
(i) B1N0; (j) B1N1; (k) B1N2; (l) B1N3; (m) B1.5N0; (n) B1.5N1; (o) B1.5N2;
(p) B1.5N3; L: light conditions; D: dark conditions.



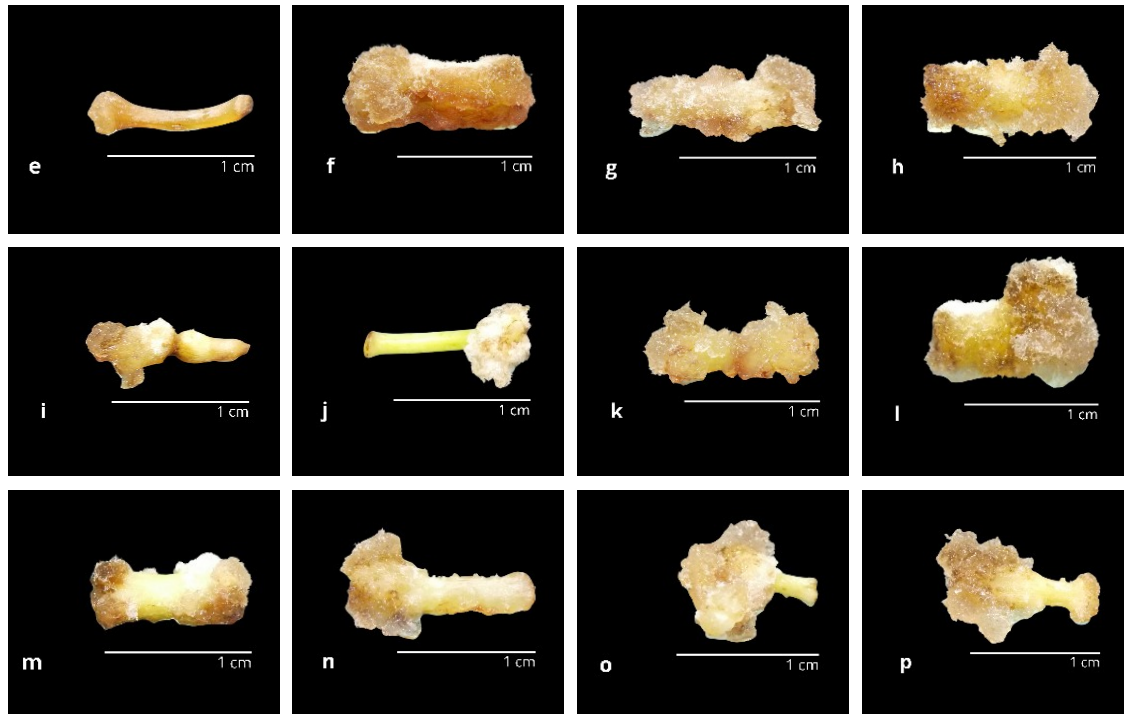
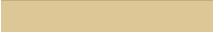
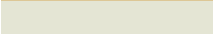


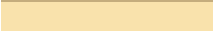
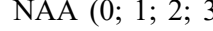
Figure 4. Morphology of binahong callus internodes under dark conditions (a) B0N0; (b) B0N1; (c) B0N2; (d) B0N3; (e) B0.5N0; (f) B0.5N1; (g) B0.5N2; (h) B0.5N3; (i) B1N0; (j) B1N1; (k) B1N2; (l) B1N3; (m) B1.5N0; (n) B1.5N1; (o) B1.5N2; (p) B1.5N3; L: light conditions; D: dark conditions.

Texture and Color of Callus

The results of the texture and color observations of binahong internodes callus using the Munsell Color Chart application are presented in **Table 3**. Almost all treatments produced compact callus, except the BAP 1.5 ppm + NAA 0 ppm treatment under light conditions, which produced friable callus.

Table 3. Morphological observations of binahong internodes explants under light and dark conditions

Treatment	Texture	Color and Code (<i>Munsell Color Chart</i>)	
B0N0L	Compact		Brown - 2.5Y 7/4
B0N1L	Compact		Brown - 2.5Y 8/4
B0N2L	Compact		Brownish white - 2.5Y 9/2
B0N3L	Compact		Brown - 2.5Y 8/4
B0.5N0L	Compact		White - 2.5GY 9/1
B0.5N1L	Compact		Brown - 2.5Y 7/6
B0.5N2L	Compact		Brownish white - 10YR 9/4
B0.5N3L	Compact		Brown - 7.5YR 5/4
B1N0L	Compact		Greenish white - 2.5GY 9/4
B1N1L	Compact		Brown - 2.5Y 8/4
B1N2L	Compact		Brown - 10YR 7/4
B1N3L	Compact		Brown - 7.5YR 5/4
B1.5N0L	Friable		Greenish white - 7.5Y 8/4
B1.5N1L	Compact		Brown - 2.5Y 7/4
B1.5N2L	Compact		Greenish white - 5Y 8/4

B1.5N3L	Compact		Brown - 2.5Y 8/4
B0N0D	Compact		Brown - 10YR 7/6
B0N1D	Compact		Brown - 10YR 8/4
B0N2D	Compact		White - 2.5Y 9/2
B0N3D	Compact		Yellowish white - 10YR 8/4
B0.5N0D	Compact		Brown - 10YR 7/6
B0.5N1D	Compact		Brown - 10YR 7/6
B0.5N2D	Compact		Brown - 10YR 8/4
B0.5N3D	Compact		Brown - 2.5Y 8/6
B1N0D	Compact		Brown - 10YR 7/6
B1N1D	Compact		White - 2.5Y 9/2
B1N2D	Compact		Brown - 2.5Y 8/4
B1N3D	Compact		Brown - 2.5Y 7/4
B1.5N0D	Compact		Brownish white - 2.5Y 9/4
B1.5N1D	Compact		Brown - 2.5Y 8/4
B1.5N2D	Compact		Brown - 10YR 8/4
B1.5N3D	Compact		Brown - 10YR 8/4

Note: B: BAP (0; 0.5; 1; 1.5 ppm); N: NAA (0; 1; 2; 3 ppm); L: light conditions; D: dark conditions.

DISCUSSION

Callus Induction Time

Based on the results showed that the fastest average time to callus formation was observed in the BAP 0.5 + NAA 2–3 ppm treatment under light conditions, which stimulated callus formation on binahong internodes segments at 6.87 DAP, while the treatment with the longest time to callus formation was BAP 1 + NAA 0 under light conditions, as shown in Table 2. This is consistent with the study by Swandari & Setyorini (2018), who found that the application of 0.5 ppm BAP was the best combination for callus induction in *Gerbera jamesonii*. The BAP 1 + NAA 0 treatment resulted in the longest time to callus formation, which is thought to be related to an imbalance in the ratio of auxin to cytokinin in the culture medium. The balance between these two types of plant growth regulators plays a crucial role in regulating cell growth and development during the callus induction process. According to Walangadi *et al.*, (2025), effective stimulation of callus development can occur if the ratio of auxin to cytokinin in the culture medium is appropriate, as these two hormones have interrelated functions in controlling cell division, differentiation, and development.

The success of callus induction depends not only on the explants and in vitro culture conditions but is also influenced by the exogenous application of plant growth regulators (PGRs) including auxins, cytokinins, or their combinations which play a role in the callus formation process in various plant species (Abou El-Dis *et al.*, 2021). The rate of callus induction varies among explants, as the rate of cell division differs in each explant (Khoiriyah *et al.*, 2023). Generally, callus growth can be stimulated by the addition of auxin and cytokinin hormones at relatively high concentrations (Kristianto & Setyorini, 2021). The application of an appropriate combination of auxin and cytokinin influences callus formation and indicates that cells in the binahong internodes explants can respond to plant growth regulators. This callus induction process begins with changes in the explant's shape, appearing swollen or curved, followed by the emergence of white protrusions at the cut wound site, which subsequently develop into callus (**Figure 5**) (Puteri *et al.*, 2014).

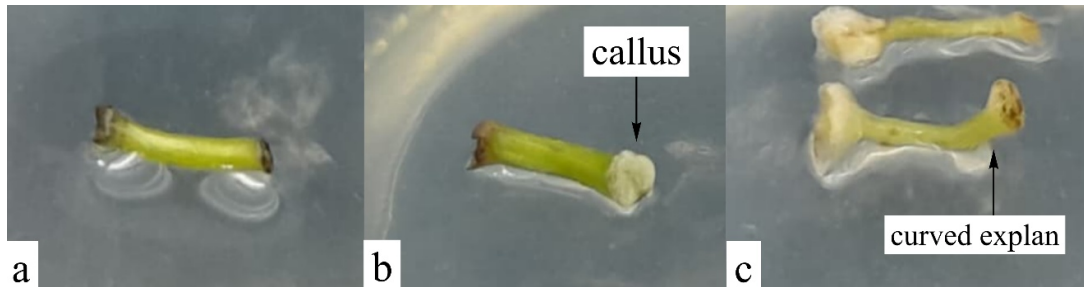


Figure 5. (a) Binahong internode explants after planting (0 days after planting); (b) Callus begins to form white protrusions (7 days after planting); (c) a curved explant after callus formation

The observed response of callus induction in this study may be associated with molecular changes triggered by plant growth regulators during cell reprogramming. The basic mechanism of callus induction involves complex processes, including histone modifications and transcription factor activity, in response to plant growth regulators (Mulyono *et al.*, 2025). Changes in active histone markers H3ac and H3K4me3 during callus formation can promote chromatin reprogramming and regulate gene expression related to cell division, sugar metabolism, and hormonal signaling. These molecular processes may support the dedifferentiation and proliferation of explant cells during callus development. Media formulated with a combination of NAA and BAP at specific concentrations tend to result in faster callus formation compared to using only one of the hormones. The concentration required for callus formation in tissue culture is achieved when the ratio of NAA (an auxin) to BAP (a cytokinin) is higher. The addition of NAA, as an auxin, can stimulate cell elongation by loosening the cell walls due to a decrease in pH. This low pH activates enzymes involved in breaking down cell wall components, such as cellulose. These enzymes are thought to break the bonds in the polysaccharides that make up the cell wall, making the wall more elastic and easier to stretch (Puteri *et al.*, 2014), while cytokinin supports the acceleration of division and growth of redifferentiated cells (Walangadi *et al.*, 2025).

Callus Formation Percentage

Percentage of callus-forming explants was used as an indicator of callus growth success. In **Table 2**, all treatments showed the best results in callus formation except for the 0 ppm BAP + 0 ppm NAA under light treatment (50%), 0.5 ppm BAP + 0 ppm NAA under dark treatment (62.5%), and 1.5 ppm BAP + 0 ppm NAA under light (62.5%), which showed lower percentages. Treatment with NAA resulted in a 100% callus formation rate, whereas treatment without NAA showed a lower callus formation rate. This indicates that the addition of NAA as an auxin enhances the ability of explants to form callus, as NAA plays a role in triggering cell dedifferentiation and cell division (Andany & Ratnasari, 2023).

Under light conditions, the average callus formation rate in binahong reached 92.94%, while under dark conditions, the average was higher at 95.25%. This result indicates that lighting can influence the callus formation process in binahong internodes. Light plays a crucial role in influencing morphogenesis, biochemical activity, and the biosynthesis of secondary metabolites (Ali & Abbasi, 2014). In line with the results of a study by Syabana *et al.*, (2017) dark conditions are optimal for inducing callus formation in stevia (*Stevia rebaudiana* Bertoni M.), with an average callus formation rate of 100%. According to Safitri *et al.*, (2017), cell division rate is influenced by the combination of auxin and cytokinin at specific concentrations depending on the plant species, as well as environmental factors such as light intensity. Light is known to reduce auxin activity, even causing its distribution to

move away from the direction of the light source. Therefore, tissue culture under dark conditions can be one approach to optimize auxin function, thereby accelerating the callus formation process (Syabana *et al.*, 2017). Additionally, several studies have reported that media supplemented with the plant growth regulators BAP and NAA constitute an optimal combination for callus induction in certain medicinal plants, such as *Withania somnifera* (Adhikari & Pant, 2013) and *Centella asiatica* (Joshi *et al.*, 2013).

Fresh Weight of Callus

Callus biomass is an important factor in the *in vitro* production of secondary metabolites because secondary metabolites are produced by the cells that make up the callus. Therefore, a high fresh weight indicates that the cells are actively dividing (Suhartanto *et al.*, 2022). The interaction effect between the plant growth regulators and lighting conditions on callus wet weight, with $p < 0.05$. Based on the results in **Figure 1**, the highest average fresh weight of callus was found in the treatment of 1.5 ppm BAP + 3 ppm NAA under light conditions, which was 0.4128 g. This is consistent with the study by Setiawati *et al.*, (2019) which found that chrysanthemum (*C. morifolium*) callus under light conditions with a concentration of 4 ppm 2,4-D + 0.5 ppm BAP had a higher fresh weight compared to that under dark conditions.

Callus weight is influenced by the morphological condition of the callus, the rate of cell division, the cells' ability to multiply, and the growth of the callus itself (Kristianto & Setyorini, 2021). In tissue culture, callus formation is generally influenced by the concentration ratio of plant growth regulators (PGRs), where NAA, as an auxin, is typically required in higher amounts compared to BAP or cytokinin. The addition of the auxin NAA plays a role in promoting cell wall stretching and relaxation through the secretion of acids that activate specific enzymes. These enzymes then break the bonds between cellulose molecules in the cell wall, making the cells more elastic and easier to stretch (Paryatin *et al.*, 2024). Meanwhile, BAP functions in cell division and protein synthesis, which leads to cell proliferation, causing an increase in cell volume and resulting in an increase in the weight of the callus produced (Latifah *et al.*, 2017).

In addition to being influenced by the application of plant growth regulators (PGRs), callus fresh weight is also related to cell proliferation activity. The active proliferation process leads to an increase in cell number, causing the explant tissue to thicken, enlarge, or swell. This proliferation activity is generally the tissue's initial response to the injury caused by the induction process. The availability of endogenous and exogenous hormones in appropriate concentrations can stimulate continuous cell division and regulate the formation of disorganized (amorphous) callus mass (Mastuti *et al.*, 2020). The increase in callus fresh weight is not only related to cell proliferation but is also influenced by cell expansion due to water accumulation in the vacuoles. The influx of water into the vacuoles increases turgor pressure, which exerts a mechanical force on the plasma membrane and causes the cell wall to stretch through changes in the structure of polysaccharides. These conditions allow the cells to enlarge, thereby increasing the volume of the callus tissue and resulting in an increase in the fresh weight of the callus (Majda & Robert, 2018).

Dry Weight of Callus

The dry weight of callus is influenced by cell growth activity, primarily through the rate of cell division and proliferation, which is then followed by cell enlargement. This process contributes to an increase in the accumulation of dry biomass in callus tissue (Setiawati *et al.*, 2021). Statistical analysis confirmed that the interaction between PGR and light conditions significantly affected callus dry weight ($p < 0.05$). The highest average callus

dry weight was obtained in the BAP 1.5 ppm + NAA 3 ppm treatment under light conditions, namely 0.0219 g (**Figure 2**). The lowest average dry weight was obtained from callus not treated with growth regulators, namely 0.0022 g under dark conditions and 0.0024 g under light conditions.

In addition to being influenced by fresh weight, callus growth is also influenced by dry weight. A high fresh weight is not always accompanied by a high dry weight, as each cell has a different water absorption capacity. According to Rahayu *et al.*, (2003) the fresh weight of callus is generally due to the high water content within the tissue. The difference between the fresh weight and dry weight of callus is also influenced by the medium to which growth regulators such as auxin and cytokinin are added at various concentrations, resulting in different responses in the cultured explants (Junairiah *et al.*, 2018).

Callus Morphology

Callus Texture

Callus morphology refers to the physical characteristics of the callus produced by each treatment, as observed based on its color and texture. Callus color and texture can serve as indicators for assessing callus growth in in vitro culture (Salsabila & Isda, 2022). Callus texture is one of the indicators used to assess the quality of the callus formed. Generally, callus texture is classified into two types compact (non-friable) and crumbly (friable) (Mahadi *et al.*, 2016). Based on the observations in **Table 3**, nearly all treatment combinations of the plant growth regulators NAA and BAP under both light and dark conditions produced callus with a compact texture, only the 3 ppm NAA + 0 ppm BAP treatment under light conditions produced friable callus.

Compact callus has a dense cellular structure with thicker polysaccharide walls, making it difficult to separate (Anwar & Isda, 2020). The compact texture of the callus is thought to be due to the influence of cytokinins, which play a role in nutrient transport. Cytokinins, which are translocated from the basal region to the apex via the vascular tissue, help transport water and nutrients and affect the osmotic potential of cells. In addition, the presence of sucrose supports an increase in turgor pressure because this compound is transported through the phloem vessels. Turgor pressure results from differences in solution concentration between the environment and the cell, causing water and nutrients including sucrose from the culture medium to enter the cell via osmosis. This causes the cell walls to become stiffer, resulting in compact callus cells (Wulandari *et al.*, 2021). Meanwhile, crumbly-textured callus forms from small cells with looser intercellular bonds, a process generally influenced by the presence of auxin (Sari *et al.*, 2018). Auxin plays a role in stimulating cell elongation by increasing cell wall plasticity, thereby loosening the cell walls. Consequently, water enters the cells more easily through osmosis, which then promotes cell elongation. According to Arieswari *et al.*, (2018) compact callus is capable of accumulating higher amounts of secondary metabolites than loose callus. This is due to the higher water content in loose callus, which results in lower accumulation of secondary metabolites.

Callus Color

The results of the color observations of binahong internodes callus using the Munsell Color Chart application are presented in **Table 3**. The addition of NAA+BAP combinations to the medium under different lighting conditions affected the resulting callus color. Under light conditions, the color of binahong internodes callus ranged from greenish white to brown, while under dark conditions, it was white, brownish white, and brown. This is consistent with the findings of Wahyuni *et al.*, (2020) who stated that callus color under dark conditions appeared brighter compared to light conditions. Variations in callus color may also be caused

by light-induced pigmentation, which can be distributed either uniformly across the callus surface or localized in specific regions (Setiawati *et al.*, 2020). The development of chlorophyll in explants incubated under light conditions occurs due to light stimulation that initiates photosynthetic processes. Chlorophyll biosynthesis occurs through the photoreduction of protochlorophyllide into chlorophyllide a, which is subsequently esterified with phytol to produce chlorophyll a, with chlorophyllase enzymes facilitating this process.

The greenish-white color of the callus indicates the formation of chlorophyll within the callus tissue in response to light stimulation. Jannah & Nurhidayah, (2024) state that the application of BAP, either alone or in combination with NAA, can stimulate chlorophyll formation, causing the callus to turn greener. The white color of the callus occurs because plastids have formed but have not yet developed into chloroplasts, so the tissue is not yet capable of performing photosynthesis optimally even under light conditions (Puteri *et al.*, 2014). Meanwhile, a brownish color change indicates the presence of polyphenolic compounds in the part used for callus induction, while a darker brown color indicates the occurrence of the browning process (Paryatin *et al.*, 2024). Purnamaningsih & Ashrina, (2011) explain that browning can inhibit cell growth and differentiation, as the tissue begins to enter a stationary phase or undergoes aging, and the production of secondary metabolites decreases significantly due to the oxidation of phenolic compounds catalyzed by the enzyme polyphenol oxidase (PPO) (Patel & Avhad, 2025).

CONCLUSION

Based on the research results, there was a significant interaction between plant growth regulator treatments and light conditions on all observed parameters. The fastest callus induction (6.8 DAP) was observed in the 0.5 ppm BAP + 2–3 ppm NAA treatment under light conditions. The highest callus growth percentage under dark conditions was 95.25%, while the highest fresh and dry weights were obtained with the 1.5 ppm BAP + 3 ppm NAA treatment under light conditions, reaching 0.4128 g and 0.0219 g, respectively. Treatments containing NAA were effective in accelerating callus formation, achieving nearly 100% callus induction and yielding the highest fresh and dry callus weights. The resulting callus mostly had a firm texture. Under light conditions, callus color ranged from greenish-white to brown, whereas under dark conditions, the color ranged from white and brownish-white to brown. Overall, the combined application of NAA and BAP, along with appropriate lighting conditions, enhanced callus induction and growth from binahong internode explants.

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