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Effects of Picloram–Kinetin and Light Conditions on Callus Induction of *Chrysanthemum morifolium* 'Puspita Nusantara'

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ABSTRACT

Callus induction is influenced by growth regulators and environmental factors. This study evaluated the effect of picloram–kinetin combination and light conditions on callus formation in internode explants of *Chrysanthemum morifolium* 'Puspita Nusantara'. A completely randomized block design was used with four concentrations of picloram (0–3 ppm), four concentrations of kinetin (0–3 ppm), and two light conditions (light and dark), resulting in 32 treatment combinations with three replications. Callus initiation time, percentage of callus formation, biomass, and callus morphology were observed in this study. The results showed that callus initiation time did not differ significantly among treatments. However, the interaction between picloram, kinetin, and light conditions significantly affected the percentage of callus formation, fresh weight, and dry weight. Most treatments containing picloram achieved 100% callus formation, while treatments without picloram showed a lower level of formation. The highest fresh weight was obtained in 2 ppm kinetin + 3 ppm picloram under light conditions (0.3510 ± 0.09 g), while the highest dry weight was recorded in 2 ppm kinetin + 2 ppm picloram under light conditions (0.0364 ± 0.015 g). Light conditions generally promoted greater biomass accumulation than dark conditions. All calli exhibited a compact texture, with green to yellow-green coloration under light and yellow to pale-yellow coloration under dark conditions. Overall, 2 ppm kinetin combined with 3 ppm picloram under light conditions was the most effective treatment for callus induction and biomass production. This study provides the first baseline data on callus induction in *C. morifolium* 'Puspita Nusantara', for which no previous reports are available. These findings support future callus-based propagation and secondary metabolite studies.

A. INTRODUCTION

Chrysanthemum morifolium is widely cultivated as an ornamental plant and is also known to contain various secondary metabolites, including flavonoids, alkaloids, and terpenoids (Xue et al., 2019). Such compounds have antioxidant, antimicrobial, and antifungal properties as well. These findings indicate that chrysanthemums have potential as a source of bioactive compounds. Plant tissue culture has been widely utilized to support biomass and secondary metabolite production under controlled conditions. Callus culture plays a crucial role because it can provide a sustainable source of

undifferentiated cells for metabolite production, plant propagation, and germplasm conservation (Efferth, 2019).

The type and concentration of plant growth regulators (PGRs) have an essential role in callus induction. Auxins promote cell dedifferentiation and callus initiation, while cytokinins stimulate cell division and therefore induce callus development (Asghar et al., 2023). For example, picloram is a synthetic auxin that has been proved to be highly efficient for callus induction and induced significantly higher levels of callusing and biomass production in *Gerbera jamesonii* and *Eleutherine palmifolia* compared to 2,4-D and NAA (Gantait & Mahanta, 2021; Habibah et al., 2023). Kinetin is a hormone which acts as a cytokinin that promotes cell division and tissue development by regulating the cell-cycle (Wang, 2021). *Eleutherine palmifolia* and *Pogostemon cablin* have also been dosed with picloram and kinetin to induce callus that can be induced successively (Habibah et al., 2023; Mazlan & Karim, 2018; Normasari et al., 2023).

Lighting conditions also influence callus induction and development. Light plays a role in regulating morphogenesis, cell division, callus development, and the production of primary and secondary metabolites through its effects on endogenous hormones and enzyme activity (Deng et al., 2020). Dark conditions can increase cell dedifferentiation and reduce auxin degradation, thus supporting better callus initiation and proliferation (Nurchayati et al., 2020). Conversely, light can stimulate the biosynthesis of certain secondary metabolites, such as flavonoids and phenolic compounds, through the activation of key biosynthetic enzymes (Zhang et al., 2021). Callus responses to light vary across plant species, requiring optimization for each genotype.

C. morifolium 'Puspita Nusantara' is an Indonesian spray chrysanthemum cultivar that is widely favored due to its attractive flower color, shape, type, as well as its resistance to rust disease, and has been exported to several countries, indicating its potential value in chrysanthemum cultivation (Sanjaya et al., 2015). However, data on in vitro callus induction in this cultivar are still scarce. Furthermore, studies examining the combination of picloram and kinetin under light and dark conditions for callus induction are still limited. This study was conducted to examine the effects of various concentrations of picloram and kinetin under different lighting conditions and to identify the best treatment combination for callus induction from internode explants of *C. morifolium* 'Puspita Nusantara'.

B. METHODS

1. Plant materials

C. morifolium 'Puspita Nusantara' plants were collected from a commercial chrysanthemum grower located in Bandungan, Semarang Regency, Central Java, Indonesia (Figure 1A). Internodal segments approximately 2 cm long from the first to fifth nodes of young stems were used as explants in the callus induction process.

2. Research design

The present study was prepared using a Randomized Complete Block Design (RCBD) with two factors: plant growth regulator (PGR) combinations and light conditions. Factor one included 0, 1, 2 and 3 ppm of picloram and kinetin. The second factor consisted of two levels of light (L) and dark (D). These factors yielded a total of 32 treatment combinations (K0P0L–K3P3D) replicated three times for a total of 96 experimental units. Each experimental unit consisted of one culture bottle containing two explants. Therefore, each treatment combination consisted of three observational units (replicates) and a total of

six explants. The three replicate values were used as observations in the statistical analysis.

3. Explant sterilization

Young internodal segments were washed under running water for 45 min, immersed in 1% detergent solution for 40 min, and rinsed three times with sterile distilled water. Explants were then soaked in 1% bactericide–fungicide solution for 45 min, rinsed, and transferred into a laminar air flow cabinet. Inside the cabinet, surface sterilization was performed using 10% sodium hypochlorite for 10 min, followed by three rinses with sterile distilled water. This procedure was repeated once and followed by five final rinses with sterile distilled water.

Figure 1. (A) Source plants of *C. morifolium* 'Puspita Nusantara' used for explant collection, (B) internodal explants cultured on MS medium.

4. Callus induction

Sterilized explants were cut into approximately 1 cm segments, slightly wounded, and cultured on MS medium supplemented with picloram and kinetin according to the treatment combinations (Figure 1B). Cultures were maintained at 24°C under either continuous fluorescent light (approximately 2,000 lux) or complete darkness. Observations on the culture were carried out up to 30 days after inoculation (DAI) by measuring the time of callus initiation, percentage of callus formation, morphology, fresh weight, and dry weight of the callus.

Callus initiation time was observed and recorded daily from the time the culture was initiated until the first visible callus appeared on the explant surface with a diameter of approximately 1 mm. The percentage of callus formation was calculated at 30 DAI based on the ratio between the number of explants that formed callus and the total number of explants cultured. Fresh weight was measured at 30 DAI after the callus was separated from the culture medium and weighed using an analytical balance. Dry weight was determined after oven-drying the calli at 45°C for 4 days or until a constant weight was reached. Callus color was observed at 30 DAI using the Munsell Color Chart as a reference. Callus texture was evaluated at 30 DAI and classified as compact or friable.

5. Data analysis

Quantitative data, including callus initiation time, callus formation percentage, fresh weight, and dry weight, were analyzed using IBM SPSS Statistics 26 at a 5% significance level. Data normality and homogeneity were assessed using the Kolmogorov–Smirnov

and Levene's tests. Since the quantitative data did not meet the assumptions of normality and homogeneity, differences among treatments were analyzed using the Kruskal–Wallis test followed by Dunn's post hoc test. Morphological data, including callus color and texture, were considered qualitative variables and analyzed descriptively.

C. RESULT AND DISCUSSION

1. Callus initiation time and callus formation percentage

Callus initiation time did not differ significantly among treatments (Table 1). Numerically, the fastest callus initiation was observed in 2 ppm kinetin + 3 ppm picloram under light conditions (K2P3L), with callus formation occurring at 9.67 ± 0.52 DAI, whereas the latest initiation was recorded in 0 ppm kinetin + 0 ppm picloram under dark conditions (K0P0D) at 28.00 ± 3.10 DAI.

Table 1. Effects of picloram, kinetin, and light conditions on callus initiation time and callus formation percentage of chrysanthemum internode explants.

Treatment	Callus initiation time (DAI)	Percentage of callus (%)
K0P0L	0.00 ± 0.00	0.00 ± 0.00^{c}
K0P1L	11.00 ± 1.55	100.00 ± 0.00^{a}
K0P2L	13.67 ± 4.23	100.00 ± 0.00^{a}
K0P3L	15.00 ± 7.46	83.33 ± 20.00^{ab}
K1P0L	19.67 ± 8.96	66.66 ± 25.00^{ab}
K1P1L	14.17 ± 1.83	100.00 ± 0.00^{a}
K1P2L	13.00 ± 2.37	100.00 ± 0.00^{a}
K1P3L	9.83 ± 0.41	100.00 ± 0.00^{a}
K2P0L	26.33 ± 5.68	33.33 ± 25.00^{bc}
K2P1L	12.33 ± 2.25	100.00 ± 0.00^{a}
K2P2L	17.33 ± 8.04	83.33 ± 20.00^{ab}
K2P3L	9.67 ± 0.52	100.00 ± 0.00^{a}
K3P0L	25.00 ± 7.75	33.33 ± 25.00^{bc}
K3P1L	14.67 ± 1.03	100.00 ± 0.00^{a}
K3P2L	20.00 ± 8.56	66.66 ± 25.00^{ab}
K3P3L	12.00 ± 3.85	100.00 ± 0.00^{a}
K0P0D	28.00 ± 3.10	33.33 ± 25.00^{bc}
K0P1D	18.00 ± 3.22	100.00 ± 0.00^{a}
K0P2D	17.17 ± 2.14	100.00 ± 0.00^{a}
K0P3D	14.67 ± 4.50	100.00 ± 0.00^{a}
K1P0D	27.33 ± 3.01	50.00 ± 25.00^{abc}
K1P1D	18.67 ± 3.01	100.00 ± 0.00^{a}
K1P2D	17.67 ± 6.35	83.33 ± 20.00^{ab}
K1P3D	21.00 ± 7.32	66.66 ± 25.00^{ab}
K2P0D	27.67 ± 3.61	33.33 ± 25.00^{bc}
K2P1D	21.17 ± 0.98	100.00 ± 0.00^{a}
K2P2D	16.67 ± 2.34	100.00 ± 0.00^{a}
K2P3D	16.50 ± 2.07	100.00 ± 0.00^{a}
K3P0D	24.00 ± 7.35	50.00 ± 25.00^{abc}
K3P1D	20.67 ± 6.41	83.33 ± 20.00^{ab}
K3P2D	18.00 ± 4.05	100.00 ± 0.00^{a}
K3P3D	18.67 ± 3.14	100.00 ± 0.00^{a}

*Different superscript letters in the same column indicate significant differences.
K: kinetin (0, 1, 2, 3 ppm); P: picloram (0, 1, 2, 3 ppm); L: light conditions; D: dark conditions.
Values are presented as mean ± SD of three replicates. Each treatment consisted of six explants.

The relatively rapid callus initiation observed in K2P3L may be associated with the synergistic effect of auxin and cytokinin, as a balanced hormonal ratio supports the early stages of callogenesis (Sosnowski et al., 2023). A review of propagation methods in chrysanthemum established picloram, 2,4-D, and TDZ as three of the best auxins for inducing callus production in chrysanthemum explants. It also established that balanced auxin–cytokinin ratios always reduced callus induction time (Eisa et al., 2022). Similarly, picloram-treated explants of *Zingiber officinale* var. *rubrum* showed faster callus initiation than those treated with 2,4-D and NAA, supporting the role of picloram in early callogenesis (Gnasekaran et al., 2023).

The treatments where no exogenous PGR were added under light conditions (K0P0L) resulted in a complete inhibition of callus production (0.00%). However, there was still a production of callus (%) under the dark control treatment (K0P0D) at 33.33%. This response suggests that dark conditions promote cellular dedifferentiation even without exogenous hormones. Similar results were also reported in *Rosa hybrida, Nelumbo nucifera, Datura innoxia,* and *Paeonia lactiflora*, where dark conditions proved to be more effective in inducing callus than light conditions (Deng et al., 2020; Mahmoud & Hassanein, 2018; Twaij et al., 2019; Song et al., 2023). Exposure to light could lower the level of activated auxin, while dark might keep the proper functioning of winning auxins with earlier cell dedifferentiation (Nurchayati et al., 2020). In addition, it also promotes RNA synthesis and DNA replication, which is a necessary step for callus initiation and proliferation (Arief et al., 2025).

The results showed that the percentage of callus formation was significantly influenced by the interaction between picloram, kinetin, and lighting conditions (Table 1). Treatments containing picloram resulted in higher callus formation than treatments without picloram. Most treatment combinations containing picloram achieved 100% callus formation, while treatments without picloram showed a lower percentage. Treatments K2P0L, K3P0L, K0P0D, and K2P0D only resulted in 33.33% callus formation, while K0P0L showed no callus formation at all. These results suggest that picloram played an important role in callus induction, although its effect was influenced by kinetin and lighting conditions.

The effectiveness of picloram in stimulating callus formation supports its role as a highly potent synthetic auxin. This auxin is generally considered more effective than 2,4-D and NAA in inducing callus due to its greater affinity for auxin receptors and its resistance to metabolic degradation processes in plant tissues (Gantait & Mahanta, 2021; Habibah et al., 2023). The ability of picloram at a low concentration, namely 1 ppm, to produce up to 100% callus formation in several treatments indicates the high sensitivity of this cultivar to picloram-induced auxin signals. Similar responses have also been reported in *Pogostemon cablin* and *Manihot esculenta* where picloram application increased the frequency of callus formation while supporting its development. (Latif et al., 2019; Azizi et al., 2023).

The lower callus induction percentage in the treatment without picloram indicates that kinetin is not effective enough to initiate callus formation when used alone. Auxin controls callus induction by promoting cell cycle reactivation and the dedifferentiation of somatic cells into actively dividing tissues (Yu et al., 2017). During this process, the re-differentiated cells become meristematic and capable of continuous division. Picloram also increases RNA synthesis and DNA replication, which support cell growth and proliferation. Furthermore, picloram stimulates cell elongation and enlargement by modifying the activity of enzymes involved in cell wall formation and breakdown, thereby

increasing water uptake and cell expansion (Yu et al., 2017). On the other hand, cytokinins play a primary role in supporting cell division and proliferation, with their activity significantly influenced by the presence of auxin (Sosnowski et al., 2023).

2. Fresh and dry weight

The combination of picloram–kinetin concentration and lighting conditions significantly affected the fresh weight of callus (Table 2). Under continuous light conditions, the highest fresh weight was obtained in the treatment of 2 ppm kinetin + 3 ppm picloram (K2P3L), which was 0.3510 ± 0.09 g. Meanwhile, under dark conditions, the highest fresh weight was achieved by the treatment of 1 ppm kinetin + 1 ppm picloram (K1P1D), which was 0.1668 ± 0.01 g. In general, callus cultured under light conditions produced a higher fresh weight than callus grown under dark conditions, indicating greater biomass accumulation.

Table 2. Effects of picloram, kinetin, and light conditions on fresh and dry weight of chrysanthemum internode explants.

Treatment	Fresh weight (g)	Dry weight (g)
K0P0L	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}
K0P1L	0.1072 ± 0.03^{bcd}	0.0160 ± 0.007^{ab}
K0P2L	0.1786 ± 0.07^{bc}	0.0166 ± 0.008^{ab}
K0P3L	0.0999 ± 0.03^{bcd}	0.0350 ± 0.015^{a}
K1P0L	0.0937 ± 0.04^{bcd}	0.0135 ± 0.006^{ab}
K1P1L	0.1024 ± 0.03^{bcd}	0.0189 ± 0.011^{ab}
K1P2L	0.1663 ± 0.06^{bcd}	0.0235 ± 0.004^{ab}
K1P3L	0.2039 ± 0.08^{ab}	0.0307 ± 0.012^{a}
K2P0L	0.0146 ± 0.01^{cd}	0.0023 ± 0.001^{b}
K2P1L	0.2107 ± 0.08^{ab}	0.0319 ± 0.009^{a}
K2P2L	0.2310 ± 0.09^{ab}	0.0364 ± 0.015^{a}
K2P3L	0.3510 ± 0.09^{a}	0.0317 ± 0.005^{a}
K3P0L	0.0704 ± 0.03^{bcd}	0.0021 ± 0.001^{b}
K3P1L	0.1155 ± 0.04^{bcd}	0.0242 ± 0.010^{ab}
K3P2L	0.0926 ± 0.04^{bcd}	0.0108 ± 0.005^{ab}
K3P3L	0.1338 ± 0.05^{bcd}	0.0218 ± 0.010^{ab}
K0P0D	0.0130 ± 0.01^{cd}	0.0018 ± 0.001^{b}
K0P1D	0.1220 ± 0.03^{bcd}	0.0139 ± 0.001^{ab}
K0P2D	0.0807 ± 0.03^{bcd}	0.0171 ± 0.008^{ab}
K0P3D	0.1553 ± 0.05^{bcd}	0.0137 ± 0.006^{ab}
K1P0D	0.0231 ± 0.01^{cd}	0.0029 ± 0.002^{b}
K1P1D	0.1668 ± 0.01^{bcd}	0.0185 ± 0.002^{ab}
K1P2D	0.1108 ± 0.02^{bcd}	0.0130 ± 0.004^{ab}
K1P3D	0.1314 ± 0.05^{bcd}	0.0191 ± 0.008^{ab}
K2P0D	0.0137 ± 0.01^{cd}	0.0020 ± 0.001^{b}
K2P1D	0.1394 ± 0.05^{bcd}	0.0131 ± 0.005^{ab}
K2P2D	0.1056 ± 0.04^{bcd}	0.0116 ± 0.003^{ab}
K2P3D	0.1536 ± 0.06^{bcd}	0.0161 ± 0.007^{ab}
K3P0D	0.0188 ± 0.01^{cd}	0.0020 ± 0.002^{b}
K3P1D	0.1508 ± 0.05^{bcd}	0.0139 ± 0.005^{ab}
K3P2D	0.1292 ± 0.04^{bcd}	0.0146 ± 0.005^{ab}
K3P3D	0.1051 ± 0.04^{bcd}	0.0101 ± 0.002^{ab}

*Different superscript letters in the same column indicate significant differences.
K: kinetin (0, 1, 2, 3 ppm); P: picloram (0, 1, 2, 3 ppm); L: light conditions; D: dark conditions.
Values are presented as mean ± SD of three replicates. Each treatment consisted of six explants.

The higher fresh weight under light conditions can be explained by the ability of light to support chloroplast development in callus cells. When callus contains functional chloroplasts, it can carry out photosynthesis and produce its own carbohydrates, adding to the sucrose already provided in the culture medium. This gives the cells more energy and building materials to grow and divide (Deng et al., 2020). Higher light intensity during callus culture increased chloroplast formation and biomass have been reported in *Camellia sinensis* (Zubova et al., 2024). In contrast, callus grown in the dark relies entirely on exogenous sucrose from the medium, which limits dry matter accumulation (Martinez et al., 2021).

The highest fresh weight obtained from the combination of 2 ppm kinetin + 3 ppm picloram suggests that exogenous supplementation of auxin and cytokinin plays an important role in promoting callus biomass accumulation. Research comparing auxins and cytokinins in callus culture of *Orthosiphon aristatus* showed that auxin-dominant treatments consistently produce higher biomass than cytokinin-dominant treatments (Tho et al., 2026). Similar results were found in *Gerbera jamesonii*, where picloram produced the highest fresh and dry weight among all auxins tested (Gantait & Mahanta, 2021). However, because all calli were compact, high fresh weight values may partly reflect water content rather than actual biomass accumulation.

The interaction between picloram–kinetin levels and the effects of light had a significant influence on callus dry weight (Table 2). In the presence of light, maximum dry weight was observed in K2P2L at 0.0364 ± 0.015 g, which is statistically comparable to K0P3L, K1P3L, K2P1L, and K2P3L. In the absence of light, maximum dry weight was observed in K1P3D at 0.0191 ± 0.008 g, although no significant difference was observed between the treatments in group ab (Table 2).

Dry weight is said to be a better biomass accumulation indicator than fresh weight since it does not include water content in the tissue. Even though the best fresh weight was achieved at 2 ppm kinetin and 3 ppm picloram under light treatment, the best dry weight was noted using 2 ppm kinetin and 2 ppm picloram, but no significant differences were observed. The inconsistency in ranking values indicates that water content and organic matter accumulation may be disproportionate since there could be variations in tissue density and ability to retain water without much impact on dry biomass accumulation (Isalar, 2020)

3. Callus morphology

Callus morphology was observed based on its color and texture. Representative callus morphology under light and dark conditions is shown in Figures 2 and 3, while callus color characteristics based on the Munsell Color Chart are presented in Table 3. Under light conditions, most callus was green to yellowish green, with some treatments producing bright yellowish green callus. In contrast, under dark conditions, callus was generally yellow, pale yellow, or yellowish green. Callus formed without the addition of picloram tended to be darker green under light conditions and yellow under dark conditions, while the addition of picloram caused the callus color to shift to a brighter yellowish green.

The variation in callus color that appears in light and dark conditions is related to the development of chloroplasts in the callus tissue. Light promotes the conversion of plastid precursors into functional chloroplasts containing chlorophyll, resulting in greener callus tissues (Cackett et al., 2022). The greener coloration observed under light conditions may reflect greater photosynthetic activity, which may have contributed to the higher fresh and dry weights observed under light conditions in this study. In contrast, callus cultured

in darkness lacked fully developed chloroplasts and therefore exhibited yellow or pale coloration, a phenomenon associated with etiolation (Armarego-marriott & Sandoval-ibañez, 2020). Similar light-dependent color changes have been reported in *Tussilago farfara*, where callus color changed from yellow-white to green under light exposure (Bojko et al., 2024)

Table 3. Callus color of chrysanthemum 'Puspita Nusantara' internode explants cultured under different picloram–kinetin combinations and light conditions.

Treatment	Callus color		
	Munsell code	Color	Color description
K0P0L	-	-	-
K0P1L	5GY 6/8		Bright yellow-green
K0P2L	5GY 7/8		Yellow-green
K0P3L	5GY 7/8		Yellow-green
K1P0L	5GY 4/6		Dark green
K1P1L	2.5GY 6/8		Bright yellow-green
K1P2L	2.5GY 6/6		Bright yellow-green
K1P3L	5GY 4/4		Dark green
K2P0L	10Y 6/8		Bright yellow
K2P1L	2.5GY 7/8		Bright yellow-green
K2P2L	2.5GY 7/6		Bright yellow-green
K2P3L	5GY 7/2		Grayish green
K3P0L	2.5GY 6/4		Moderate yellow-green
K3P1L	10Y 5/6		Moderate yellow
K3P2L	7.5GY 5/10		Bright green
K3P3L	5GY 5/6		Bright yellow-green
K0P0D	5Y 6/8		Bright yellow
K0P1D	7.5YR 6/2		Pale yellow
K0P2D	5Y 6/6		Bright yellow
K0P3D	5Y 3/4		Dark yellow
K1P0D	2.5GY 7/6		Bright yellow-green
K1P1D	2.5GY 6/4		Moderate yellow-green
K1P2D	2.5GY 9/2		Very pale green
K1P3D	2.5GY 5/4		Moderate yellow-green
K2P0D	5Y 5/6		Moderate bright yellow
K2P1D	5GY 7/4		Bright green
K2P2D	2.5GY 6/4		Moderate yellow-green
K2P3D	2.5GY 5/4		Moderate yellow-green
K3P0D	7.5Y 6/8		Bright yellow
K3P1D	2.5GY 5/4		Moderate yellow-green
K3P2D	2.5GY 5/4		Moderate yellow-green
K3P3D	2.5GY 6/4		Moderate yellow-green

K: kinetin (0, 1, 2, 3 ppm); P: picloram (0, 1, 2, 3 ppm); L: light conditions; D: dark conditions.

Picloram also influenced callus colour. Treatments supplemented with picloram consistently produced brighter yellow-green callus than treatments without picloram under both light and dark conditions. This response may be associated with the ability of picloram to stimulate more active cell division (Sosnowski et al., 2023), which leads to more vigorous and healthier-looking callus tissue (Siddique & Islam, 2018). The greener coloration may indicate the development of chlorophyll-containing plastids, as green callus tissues have been shown to contain chloroplasts with developed grana structures and photosynthetic activity (Lysenko et al., 2023). Chlorophyll development may also be

influenced by the balance between auxin and cytokinin, with the relative concentrations of these growth regulators affecting chlorophyll accumulation in callus tissues (Kulus & Tymoszuk, 2020). The brighter coloration observed in treatments containing moderate concentrations of picloram and kinetin is also consistent with findings in *Allium cepa*, where auxin–cytokinin combinations influenced callus colour and texture characteristics (Habibah et al., 2024).

No variation in callus texture was observed among treatments, as all calli exhibited a compact texture throughout the culture period (Figures 2 and 3). Compact callus consists of tightly packed cells and is often associated with organized meristematic regions that support somatic embryogenesis or shoot regeneration (Asmono et al., 2026).

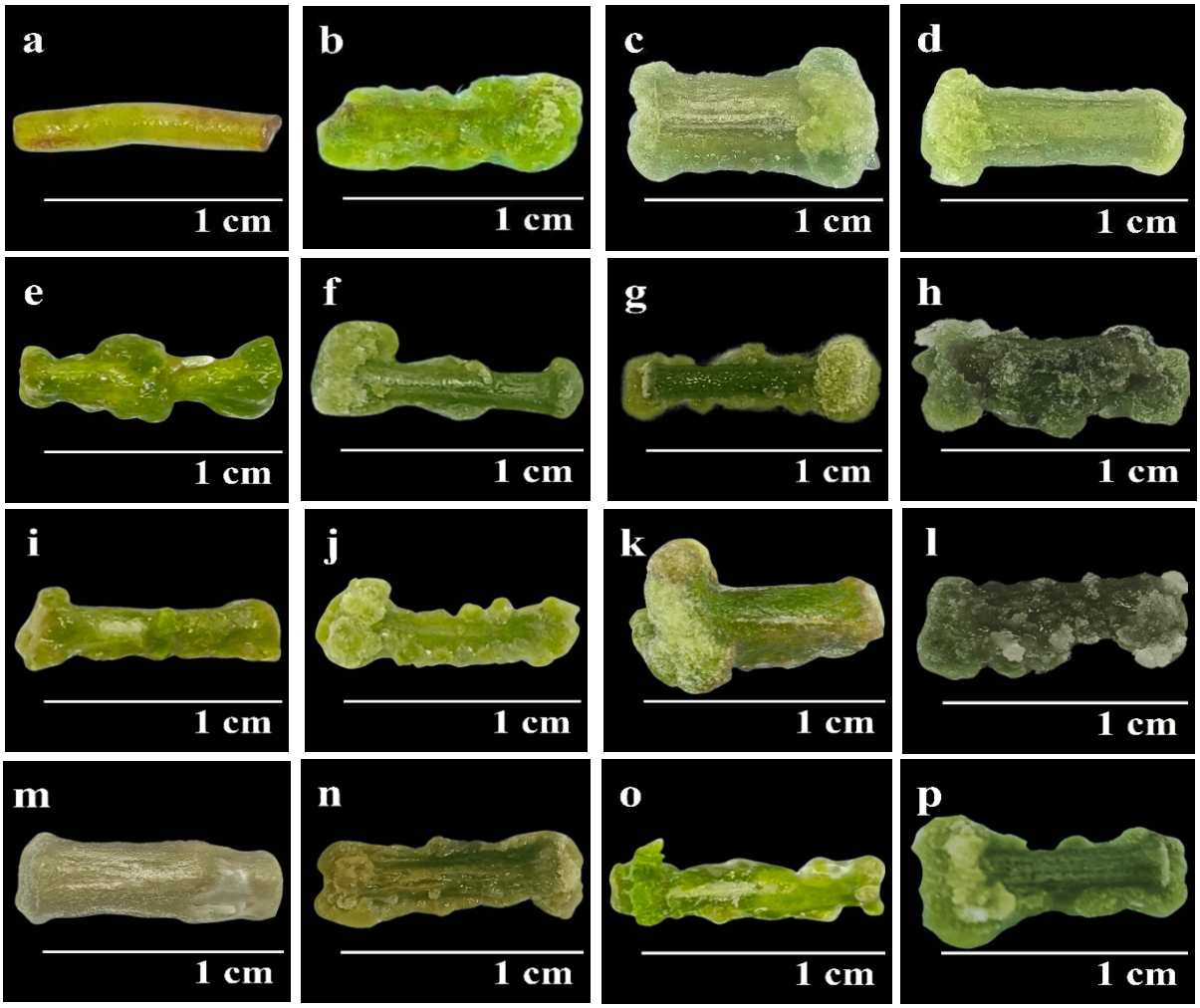

Figure 2. Callus morphology of chrysanthemum 'Puspita Nusantara' under light conditions. (a) K0P0L; (b) K0P1L; (c) K0P2L; (d) K0P3L; (e) K1P0L; (f) K1P1L; (g) K1P2L; (h) K1P3L; (i) K2P0L; (j) K2P1L; (k) K2P2L; (l) K2P3L; (m) K3P0L; (n) K3P1L; (o) K3P2L; (p) K3P3L. K: kinetin (0, 1, 2, 3 ppm); P: picloram (0, 1, 2, 3 ppm); L: light conditions; D: dark conditions

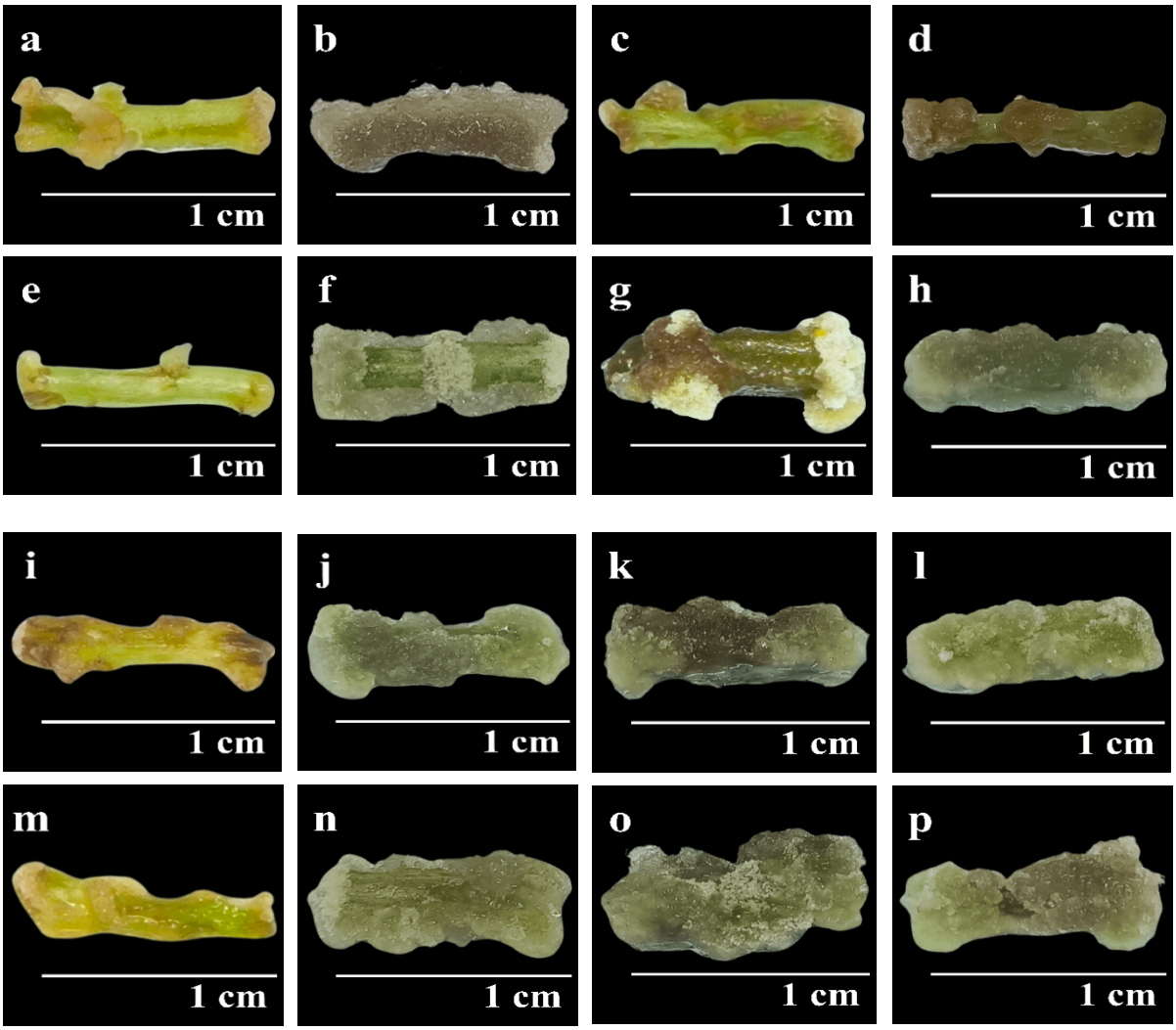

Figure 3. Callus morphology of chrysanthemum 'Puspita Nusantara' under dark conditions. (a) K0P0D; (b) K0P1D; (c) K0P2D; (d) K0P3D; (e) K1P0D; (f) K1P1D; (g) K1P2D; (h) K1P3D; (i) K2P0D; (j) K2P1D; (k) K2P2D; (l) K2P3D; (m) K3P0D; (n) K3P1D; (o) K3P2D; (p) K3P3D. K: kinetin (0, 1, 2, 3 ppm); P: picloram (0, 1, 2, 3 ppm); L: light conditions; D: dark conditions.

Similar characteristics have been reported in chrysanthemum callus cultures, where compact callus is linked to embryogenic potential (Eisa et al., 2022). Since the use of friable callus in suspension culture and secondary metabolites production is favored due to its easy dispersion characteristics, compact callus is thought to be the preferable choice for regeneration purposes and germplasm preservation (Kocot et al., 2024). The consistent emergence of compact callus among all experimental treatments may suggest that PGRs concentration and light had more impact on callus color rather than its texture.

D. CONCLUSION AND SUGGESTIONS

The combination of picloram, kinetin, and lighting conditions significantly affected the percentage of callus formation, biomass accumulation, and callus morphology in internode explants of *C. morifolium* 'Puspita Nusantara' but did not affect the time of callus initiation. Picloram was the main factor in stimulating callus formation, with most treatments containing picloram resulting in callus induction of up to 100%. In general, light conditions increased the fresh and dry weight of callus. All treatments produced compact callus, while callus colour varied with light exposure and PGR composition. 2 ppm kinetin + 3 ppm picloram under light conditions treatment was the most effective,

producing 100% callus formation and the highest fresh weight. This study provides baseline information on callus induction in *C. morifolium* 'Puspita Nusantara', particularly regarding the effects of picloram-kinetin combinations and lighting conditions on callus formation, biomass accumulation, and morphology.

Further studies are recommended to evaluate the potential of the induced callus for subsequent regeneration and secondary metabolite production. The effectiveness of the optimal treatment identified in this study should be further investigated using other explant types and culture conditions to support the development of an efficient in vitro culture system for *C. morifolium* 'Puspita Nusantara'.

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