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The Effect of the Combination of 2,4-D and BAP and Lighting Conditions on Callus Induction of Sugarcane (*Saccharum officinarum L.*) Variety CMG Agribun

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ABSTRACT

This study aimed to evaluate the effect of different combinations of 2,4-D and BAP, as well as light conditions, on callus induction of sugarcane variety 'CMG Agribun'. A factorial Randomized Complete Block Design (RCBD) was applied with two factors: plant growth regulators (PGRs) combinations (2,4-D and BAP) and lighting conditions (light and dark). Observed parameters included callus initiation time, percentage of callus formation, fresh and dry weight, and callus morphology. The results showed that the treatments significantly affected callus initiation time but not the percentage of callus formation. The fastest callus induction (2.83 DAP) occurred under dark conditions with 1 ppm 2,4-D + 0 ppm BAP and 4 ppm 2,4-D + 1 ppm BAP. The fresh weight of the callus did not show any significant differences among the treatments, but the treatment under dark conditions tended to produce a higher fresh weight of the callus. The highest dry weight (0.092 g) was also obtained from the treatment 1 ppm 2,4-D + 0.5 ppm BAP under dark conditions. Callus morphology was generally compact with varying colors. Further research is recommended using a wider range of 2,4-D and BAP concentrations, as well as different light regimes, to optimize callus induction and development. This study supports further research on callus-based propagation and the production of secondary metabolites in sugarcane.

A. INTRODUCTION

Indonesia is a country that has abundant natural potential, especially in food self-sufficiency and great agrarian potential. One of the greatest natural resource wealth potentials in the national economy is sugarcane cultivation (Thoriq, 2021). The sugarcane variety used in this study is the 'CMG Agribun' variety of sugarcane. The 'CMG Agribun' variety is a sugarcane variety released in 2018. The 'CMG Agribun' sugarcane variety is one of the new superior varieties resulting from plant breeding by agricultural research institutes in Indonesia, which was developed to increase productivity on suboptimal land (Riajaya et al., 2022).

This variety is included in the group of Agribun varieties that are tested to have good agronomic characteristics such as plant height, number of seedlings, and stem diameter suitable for dry land. In addition, 'CMG Agribun' was also developed through mutation induction techniques using gamma ray radiation to increase genetic diversity and tolerance to environmental stressors (Karimuna et al., 2023).Sugarcane is primarily cult ivated for sugar production due to its high sucrose content, but it also possesses medicinal

properties and contains valuable secondary metabolites such as flavonoids, phenolics, saponins, alkaloids, terpenoids, and tannins (Ali et al., 2019; Sakadzo et al., 2024).

Callus culture is an important technique for plant propagation and secondary metabolite production. Successful callus induction is largely influenced by plant growth regulators (PGRs), particularly auxins and cytokinins, which regulate cell division and differentiation (Meshram et al., 2022). Previous studies have shown that combinations of 2,4-D and BAP effectively promote callus induction in sugarcane, with specific concentrations producing faster callus formation (Busaifi, 2018). In addition to PGRs, lighting conditions play a crucial role in callus induction and development by affecting endogenous hormone activity. Dark conditions have been reported to enhance callus induction frequency in sugarcane, reaching up to 100% callus formation (Phamontree et al., 2022).

However, information on the in vitro callus induction response of 'CMG Agribun' sugarcane remains limited. Several previous studies have demonstrated that the combination of plant growth regulators (PGRs) and lighting conditions influences callus formation and growth. Therefore, this study aimed to evaluate the effects of different combinations of 2,4-D and BAP, as well as various lighting conditions, on callus induction and development in 'CMG Agribun' sugarcane (*Saccharum officinarum* L.), providing a foundation for future regeneration, genetic improvement, and biotechnology-based breeding programs.

B. METHODS

This research was carried out at the Plant Tissue Culture Laboratory, Biology Study Program, Faculty of Mathematics and Natural Sciences, Semarang State University, from September 2025 to May 2026. This study used a factorial Randomized Complete Block Design (RCBD) with two factors. The first factor is a combination of plant growth regulators (PGRs) (2,4-D at concentrations of 1, 2, 3, and 4 ppm and BAP at concentrations of 0, 0.5, 1, and 1.5 ppm), and the second factor is lighting conditions with dark treatment (without light for 24 hours) and light (with light for 24 hours). Blocks were defined by planting period, with each planting batch treated as one block to control potential variability due to differences in planting time. There were controls (A0B0) and 16 combinations of PGR treatments, each repeated 3 times, resulting in 102 experimental units. Each unit consisted of one culture bottle containing two explants. The combination of treatments consisted of: (a) A0B0; (b) A1B0; (c) A1B0.5; (d) A1B1; (e) A1B1.5; (f) A2B0; (g) A2B0.5; (h) A2B1; (i) A2B1,5; (j) A3B0; (k) A3B0.5; (l) A3B1; (m) A3B1.5; (n) A4B0; (o) A4B0.5; (p) A4B1; and (q) A4B1.5.

Observed parameters included callus initiation time, percentage of callus formation, fresh and dry weight, and callus morphology (texture and color). Callus color was evaluated using the Munsell Color Chart, while texture was classified as compact or friable. Callus initiation was observed daily for 30 days after planting. The study used young leaf roll explants (3–6 months old) of sugarcane variety 'CMG Agribun' obtained from plants cultivated at the UPTD Distanbun Plantation Plant Seed Center, Central Java Province, Indonesia.

Preparation of Murashige & Skoog (MS) medium

The culture medium was prepared by dissolving 4.43 g/L of Murashige and Skoog medium from Phygenera, 30 g/L of sugar, and 0.1 g/L of myo-inositol in 500 mL of distilled water. The pH of the medium was measured using a pH indicator until it reached a value between 5 and 6. After that, 7 grams of agar were added and distilled water was added until the solution volume reached 1 liter. The medium solution was heated in

boiling water for 20 minutes and poured into a sterile culture bottle. PGRs 2,4-D (A) and BAP (B) were added to the medium according to the treatment. The medium bottle is closed and sterilized for 20 minutes using an autoclave at a temperature of 121 °C and a pressure of 17.5 psi.

Explants Sterilization

The 'CMG Agribun' variety sugarcane explant is taken from leaves that are still rolling and washed with running water for 30 minutes. The explant is cleaned with a detergent solution for 10 minutes and rinsed with sterile distilled water until the detergent is gone. Next, the explants are soaked in 200 ml of a mixture containing 0.5 grams of fungicides and 1 gram of bactericides for 40 minutes, then rinsed with sterile distilled water. The explant is sterilized with a 20% NaOCl 5,25% solution for 10 minutes in the LAF, then rinsed again with sterile distilled water. Sugarcane leaf roll explants are soaked in a liquid MS solution 100 ml.

Callus Induction

Sterile sugarcane leaf rolls are taken using tweezers and peeled from the outside to the deepest part with a diameter of approximately 5-8 mm and a thickness of 3-5 mm using a scalpel. The explants are then grown in bottles containing media according to the treatment, each bottle containing two explants. The explants are placed in an incubation room with different lighting conditions for 24 hours, light conditions (L) with 2000 lux LED lights with a light distance of ±15 cm above the culture bottle and dark conditions (D) with translucent black plastic covers. The temperature of the incubation chamber ranges from 25-26 °C.

Statistical Analysis

Thirty-day-old calli were harvested for data collection on fresh weight, dry weight, callus initiation time, callus formation percentage, callus color and callus texture. Quantitative data in the form of callus initiation time, callus formation percentage, and callus weight were analyzed using IBM SPSS Statistics 27 with a significance level of 5% ($p = 0.05$). Data were further evaluated to determine the effects of PGR combinations, lighting conditions, and the interaction between both factors on callus induction responses. Morphological parameters such as the color and texture of the callus are analyzed descriptively.

C. RESULTS AND DISCUSSION

1. Callus initiation time and callus formation percentage

The formation of callus is the process by which plant cells undergo differentiation and reprogramming due to the influence of internal (cell competition) and external factors (hormones, stress), so that an unorganized mass of cells is formed which then develops into new organs or embryos (Kruglova et al., 2023). The sugarcane leaf roll explants undergo differentiation and actively divide in the culture medium, so that calluses are formed. The callus initiation time data showed an abnormal and non-homogeneous distribution based on the Kolmogorov-Smirnov test and the Levene test, each with a significance value $p < 0.05$. So the analysis was continued using the Kruskal-Wallis nonparametric test to test the differences between the treatments.

The Kruskal-Wallis test showed that the combination of 2,4-D, BAP, and lighting conditions significantly affected callus initiation time ($p = 0.001$). The fastest callus induction was observed in A1B0D (1 ppm 2,4-D + 0 ppm BAP) and A4B1D (4 ppm 2,4-D + 1 ppm BAP) treatments, with a mean of 2.83 DAP (Days After Planting), whereas the control treatment (A0B0D) showed the longest initiation time. Dunn's test further indicated that treatments containing PGRs induced callus significantly faster than the

control, and dark conditions generally promoted earlier callus formation than light conditions.

The addition of 2,4-D promotes cell division and enlargement, thereby accelerating callus initiation and growth. Abdullah et al. (2023) reported that 2 ppm 2,4-D without BAP was the optimal treatment for inducing callus in ASA Agribun sugarcane. Moreover, the combination of auxins and cytokinins enhances callus development, with auxins inducing callus formation and cytokinins stimulating its proliferation. Similarly, Manurung et al. (2018) found that medium supplemented with 15 mg/L 2,4-D and 2 mg/L BAP induced the fastest callus formation.

The optimal response of A1B0D and A4B1D suggests that 'CMG Agribun' tissues are highly responsive to auxin-mediated dedifferentiation. In this variety, low to moderate concentrations of 2,4-D appear sufficient to induce rapid cell division, while the addition of BAP at appropriate levels supports callus initiation without disrupting auxin dominance required during the early induction stage.

Table 1. Average time of callus emergence (DAP) and percentage of callus explants (%)

Treatment	Callus induction time (DAP)	Percentage of callus (%)	Treatment	Callus induction time (DAP)	Percentage of callus (%)
A0B0L	12.00 ± 10.58^{fg}	66.66 ± 57.73	A0B0D	14.00 ± 1.00^{g}	66.67 ± 28.86
A1B0L	5.33 ± 0.57^{abcd}	66.66 ± 28.86	A1B0D	2.83 ± 2.46^{a}	50.00 ± 50.00
A1B0,5L	5.83 ± 1.75^{abcd}	83.33 ± 28.86	A1B0,5D	4.16 ± 1.04^{abc}	83.33 ± 28.86
A1B1L	6.83 ± 0.76^{abcde}	100.00 ± 0.00	A1B1D	7.83 ± 1.04^{bcdef}	83.33 ± 28.86
A1B1,5L	5.00 ± 0.00^{abcd}	83.33 ± 28.86	A1B1,5D	5.16 ± 1.04^{abcd}	100.00 ± 0.00
A2B0L	7.50 ± 0.86^{abcde}	83.33 ± 28.86	A2B0D	6.33 ± 0.76^{abcde}	100.00 ± 0.00
A2B0,5L	8.16 ± 1.75^{bcdef}	100.00 ± 0.00	A2B0,5D	6.00 ± 1.00^{abcd}	100.00 ± 0.00
A2B1L	5.83 ± 1.04^{abcd}	100.00 ± 0.00	A2B1D	5.16 ± 0.28^{abcd}	83.33 ± 28.86
A2B1,5L	5.66 ± 1.15^{abcd}	83.33 ± 28.86	A2B1,5D	4.00 ± 1.00^{abc}	83.33 ± 28.86
A3B0L	6.00 ± 1.32^{abcd}	83.33 ± 28.86	A3B0D	6.50 ± 0.86^{abcde}	83.33 ± 28.86
A3B0,5L	6.33 ± 1.52^{abcde}	100.00 ± 0.00	A3B0,5D	4.33 ± 0.57^{abcd}	83.33 ± 28.86
A3B1L	11.00 ± 2.17^{efg}	100.00 ± 0.00	A3B1D	6.83 ± 1.75^{abcde}	83.33 ± 28.86
A3B1,5L	5.33 ± 0.57^{abcd}	83.33 ± 28.86	A3B1,5D	9.16 ± 2.92^{def}	100.00 ± 0.00
A4B0L	6.33 ± 0.57^{abcde}	83.33 ± 28.86	A4B0D	5.83 ± 0.76^{abcd}	100.00 ± 0.00
A4B0,5L	3.83 ± 3.32^{abc}	66.66 ± 57.75	A4B0,5D	3.33 ± 3.05^{ab}	50.00 ± 50.00
A4B1L	5.16 ± 1.25^{abcd}	83.33 ± 28.86	A4B1D	2.83 ± 2.46^{a}	66.66 ± 57.73
A4B1,5L	5.00 ± 1.00^{abcd}	50.00 ± 0.00	A4B1,5D	8.33 ± 2.88^{cdef}	66.66 ± 28.86

Note: A: 2,4-D; B: BAP; L: light conditions; D: dark conditions. Values are presented as mean ± SD of three replicates. Each treatment consisted of three culture bottles, with two explants per bottle.

*Different superscript letters in the same column indicate significant differences

Light conditions are another important factor affecting callus induction. Dark conditions promote auxin accumulation in explant tissues, which enhances cell division and accelerates callus formation (Basri, 2016). The photoperiod used in tissue culture generally ranges from 8–16 h light and 16–8 h dark, depending on the variety and explant type. Syabana et al. (2017) reported that callus appeared significantly earlier under dark conditions, with an average of 13.58 DAP, compared to 16.33 DAP under light conditions.

The next parameter is the percentage of callus formation (%). The percentage of callus formation is an important indicator of successful callus induction. Normality and homogeneity tests showed that the data were non-normal and heterogeneous ($p = 0.001 < 0.05$), therefore a Kruskal-Wallis test was performed. The results indicated no significant differences among treatments ($p = 0.679 > 0.05$). As shown in Table 1, ten treatments achieved 100% callus formation, while three treatments showed the lowest percentage (50%). This suggests that CMG Agribun leaf-roll explants have a high intrinsic

capacity for callus induction, allowing most PGR combinations to effectively initiate callogenesis.

Callus formation is characterized by swelling and the appearance of clear white tissue on explant wounds, which subsequently develops into callus aggregates. Early callus induction within the first two weeks can increase both the percentage and surface area of callus formation. Fatahillah et al. (2024) reported that 3 ppm 2,4-D without BAP resulted in the highest explant survival rate (100%) and the fastest callus initiation, occurring at 15.67 DAP.

2. Fresh and Dry Weight

Table 2. Fresh weight and dry weight sugarcane callus of CMG Agribun variety

Treatment	Fresh weight (g)	Dry weight (g)	Treatment	Fresh weight (g)	Dry weight (g)
A0B0L	0.45 ± 0.10	0.048 ± 0.01^{abc}	A0B0D	0.32 ± 0.07	0.036 ± 0.00^{ab}
A1B0L	0.38 ± 0.05	0.055 ± 0.01^{abcd}	A1B0D	0.52 ± 0.11	0.052 ± 0.00^{abcd}
A1B0,5L	0.60 ± 0.16	0.070 ± 0.03^{bcd}	A1B0,5D	0.66 ± 0.07	0.092 ± 0.02^{a}
A1B1L	0.65 ± 0.16	0.074 ± 0.01^{bcd}	A1B1D	0.56 ± 0.24	0.063 ± 0.02^{abcd}
A1B1,5L	0.57 ± 0.18	0.057 ± 0.01^{abcd}	A1B1,5D	0.46 ± 0.08	0.062 ± 0.00^{abcd}
A2B0L	0.51 ± 0.20	0.061 ± 0.02^{abcd}	A2B0D	0.59 ± 0.03	0.065 ± 0.00^{abcd}
A2B0,5L	0.48 ± 0.08	0.077 ± 0.03^{bcd}	A2B0,5D	0.64 ± 0.32	0.074 ± 0.02^{bcd}
A2B1L	0.61 ± 0.09	0.069 ± 0.01^{bcd}	A2B1D	0.50 ± 0.13	0.050 ± 0.01^{abcd}
A2B1,5L	0.53 ± 0.04	0.069 ± 0.01^{bcd}	A2B1,5D	0.47 ± 0.06	0.055 ± 0.01^{abcd}
A3B0L	0.59 ± 0.20	0.079 ± 0.01^{cd}	A3B0D	0.53 ± 0.11	0.050 ± 0.01^{abcd}
A3B0,5L	0.56 ± 0.21	0.073 ± 0.03^{bcd}	A3B0,5D	0.63 ± 0.23	0.062 ± 0.00^{abcd}
A3B1L	0.54 ± 0.02	0.074 ± 0.02^{bcd}	A3B1D	0.56 ± 0.06	0.060 ± 0.00^{abcd}
A3B1,5L	0.64 ± 0.31	0.074 ± 0.02^{bcd}	A3B1,5D	0.69 ± 0.10	0.077 ± 0.00^{bcd}
A4B0L	0.44 ± 0.19	0.068 ± 0.03^{abcd}	A4B0D	0.60 ± 0.14	0.058 ± 0.01^{abcd}
A4B0,5L	0.54 ± 0.35	0.072 ± 0.04^{bcd}	A4B0,5D	0.31 ± 0.16	0.027 ± 0.00^{d}
A4B1L	0.48 ± 0.15	0.057 ± 0.00^{abcd}	A4B1D	0.58 ± 0.20	0.063 ± 0.01^{abcd}
A4B1,5L	0.53 ± 0.08	0.059 ± 0.02^{abcd}	A4B1,5D	0.55 ± 0.10	0.060 ± 0.01^{abcd}

Note: A: 2,4-D; B: BAP; L: light conditions; D: dark conditions. Values are presented as mean ± SD of three replicates. Each treatment consisted of three culture bottles, with two explants per bottle.

*Different superscript letters in the same column indicate significant differences.

Sugarcane calli were harvested to determine fresh and dry weights. Fresh weight data were normally distributed but non-homogeneous; therefore, they were analyzed using Welch's ANOVA. The results showed no significant differences among treatments ($p = 0.147$). Nevertheless, the highest fresh weight under dark conditions was observed in A3B1.5D (0.69 g), while under light conditions it was observed in A1B1L (0.65 g). The relatively high fresh weight obtained in A3B1.5D suggests that this combination promoted greater water accumulation and cell expansion. The presence of both auxin and cytokinin at higher concentrations may have enhanced tissue proliferation, resulting in larger callus biomass. Dark conditions tended to promote higher callus biomass because auxin activity is more favorable for cell division and proliferation under limited light (Suhartanto et al., 2022). This is in accordance with the results of the research by Suhartanto et al. (2022), which shows that callus weight in dark conditions is higher (115.1 mg) compared to light conditions (96.3 mg).

Dry weight data were normally distributed but non-homogeneous and were therefore analyzed using Welch's ANOVA, which revealed significant differences among treatments ($p = 0.043$). The highest dry weight was obtained in A1B0.5D (0.092 g), while the lowest was recorded in A4B0.5D (0.027 g). Under light conditions, A3B0L produced the highest dry weight (0.079 g). The superior response of A1B0.5D suggests that the combination of

1 ppm 2,4-D and 0.5 ppm BAP provided a balanced hormonal condition for biomass accumulation in CMG Agribun callus. Dry weight reflects actual biomass because the drying process removes most of the water content, which constitutes the major component of fresh callus weight (Setiawati et al., 2021).

The highest dry weight observed in A1B0.5D indicates that this combination was more effective in increasing actual biomass accumulation rather than merely promoting water uptake. A low concentration of BAP combined with 1 ppm 2,4-D may provide a balanced hormonal environment that supports sustained cell division and biomass production in CMG Agribun callus.

3. Callus Morphology

The results of morphological observations of callus on sugarcane leaf rolls included callus texture and color. Most treatments produced compact callus under both light and dark conditions, whereas friable callus was observed in A2B0L, A3B1L, and A3B1.5 (light and dark). Callus texture reflects the explant response to culture media and PGRs. Compact callus is characterized by densely arranged cells and a firm structure, while friable callus has a looser cellular arrangement (Fu et al., 2025).

Table 3. Texture and color of sugarcane callus of CMG Agribun variety

Treatment	Texture	Color	Munsell Code	Treatment	Texture	Color	Munsell Code
A0B0L	Compact		2,5GY 7/4	A0B0D	Compact		10YR 8/6
A1B0L	Compact		2,5GY 6/6	A1B0D	Compact		2,5Y 8/4
A1B0,5L	Compact		10Y 5/4	A1B0,5D	Compact		2,5Y 7/4
A1B1L	Compact		7,5Y 6/6	A1B1D	Compact		2,5Y 8/4
A1B1,5L	Compact		10Y 5/6	A1B1,5D	Compact		2,5Y 7/6
A2B0L	Friable		10Y 6/6	A2B0D	Compact		2,5Y 7/4
A2B0,5L	Compact		10Y 6/4	A2B0,5D	Compact		10YR 7/4
A2B1L	Compact		10Y 7/4	A2B1D	Compact		2,5Y 8/4
A2B1,5L	Compact		10Y 7/8	A2B1,5D	Compact		2,5Y 7/4
A3B0L	Compact		10Y 5/4	A3B0D	Compact		2,5Y 7/6
A3B0,5L	Compact		7,5Y 5/4	A3B0,5D	Compact		2,5Y 7/2
A3B1L	Friable		10Y 6/6	A3B1D	Compact		5Y 7/4
A3B1,5L	Friable		10Y 7/6	A3B1,5D	Friable		5Y 8/4
A4B0L	Compact		2,5GY 6/4	A4B0D	Compact		2,5Y 7/4
A4B0,5L	Compact		2,5GY 5/6	A4B0,5D	Compact		2,5Y 7/4
A4B1L	Compact		10Y 7/6	A4B1D	Compact		5Y 8/6
A4B1,5L	Compact		2,5GY 6/6	A4B1,5D	Compact		5Y 9/6

Note: A: 2,4-D; B: BAP; L: light conditions; D: dark conditions.

The compact texture observed in most treatments may be associated with lignification and cytokinin activity, which contribute to denser tissue formation. Similarly, Haring et al. (2024) reported that combinations of 2,4-D and BAP produced predominantly compact callus, suggesting that these PGRs influence not only callus induction but also callus characteristics. Although compact callus is suitable for plant regeneration, friable callus is generally preferred for cell suspension cultures and secondary metabolite production (Rahayu et al., 2023). The predominance of compact callus across treatments suggests that CMG Agribun tends to exhibit organized cell proliferation during callus development. This response may indicate that the PGR combinations used were more favorable for structural callus formation than for producing highly friable callus.

Visual observations using the Munsell Color Chart showed that callus color varied with lighting conditions. Calli cultured under light conditions were generally bright yellow to greenish, whereas those grown in dark conditions ranged from pale yellow to brown. These differences indicate the influence of light on physiological and biochemical processes during in vitro tissue development (Munira et al., 2022).

Calluses induced with bright lighting conditions are green in color due to increased light intensity favoring the development of chlorophyll and tissue differentiation structures, resulting in greener calluses color (Ruíz-Rivas et al., 2022). In in vitro culture, the quality and intensity of light have been shown to have a significant effect on increasing chlorophyll content and plant tissue photosynthesis activity. Based on the results of the study Setiawati et al. (2020), at the end of the observation period of 45 days after planting (DAP), calluses cultured in dark conditions showed a lighter color and appeared pale compared to calluses grown under lighting. In dark conditions, calluses are generally white, yellowish, due to the absence of chlorophyll. In addition, calluses that grow in dark conditions show a low degree of differentiation and very minimal photosynthetic activity (Han et al., 2024).

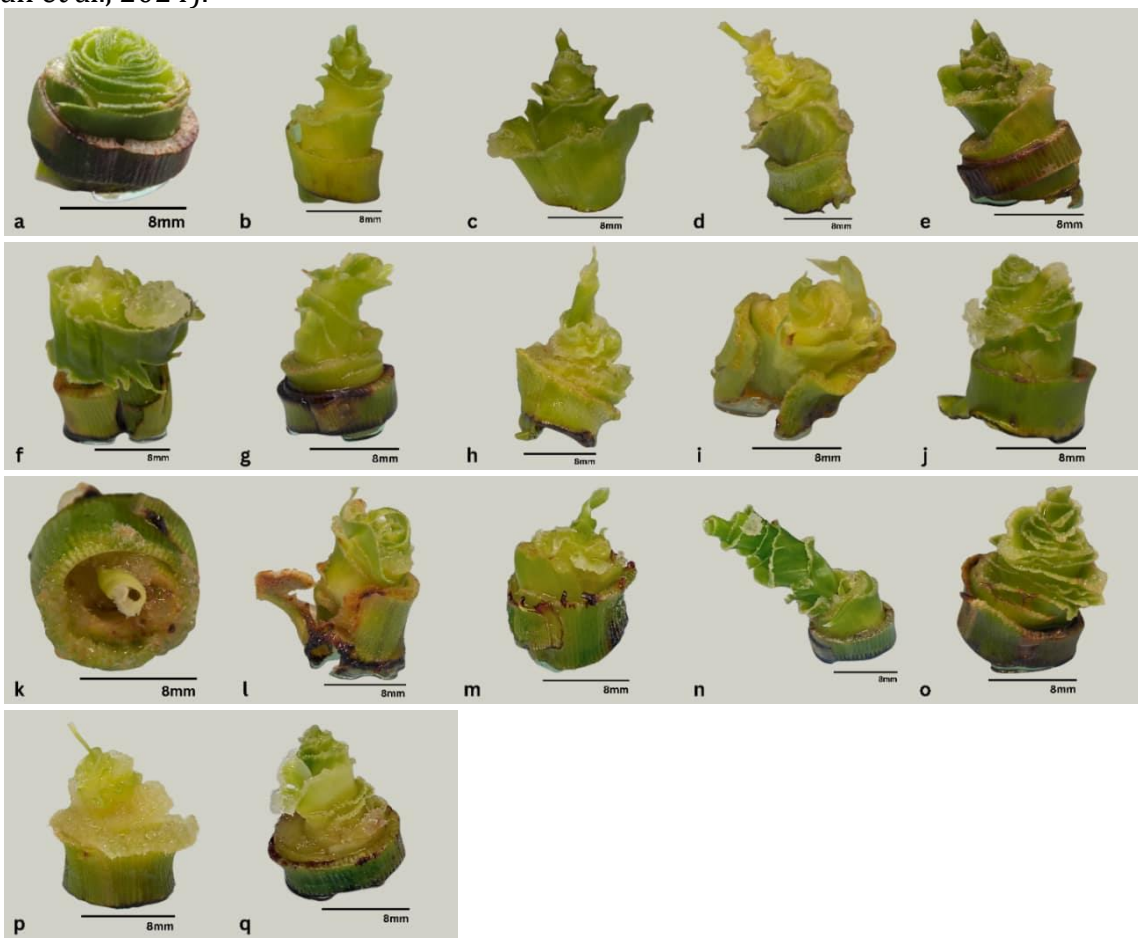

Figure 1. Callus morphology of sugarcane 'CMG Agribun' under light conditions. (a) A0B0; (b) A1B0; (c) A1B0.5; (d) A1B1; (e) A1B1.5; (f) A2B0; (g) A2B0.5; (h) A2B1; (i) A2B1,5; (j) A3B0; (k) A3B0.5; (l) A3B1; (m) A3B1.5; (n) A4B0; (o) A4B0.5; (p) A4B1; and (q) A4B1.5.
Note: scale bar (-): 8 mm; A: 2,4-D; B: BAP; L: light conditions; D: dark conditions.

Callus color is influenced by the PGR composition in the culture medium. Variations in 2,4-D and BAP concentrations under different lighting conditions significantly affect callus color and growth responses. The interaction between these PGRs can produce calluses ranging from green to brown, reflecting differences in metabolic activity and tissue physiological status. Green callus formation is often associated with cytokinin (BAP), which promotes chlorophyll synthesis, resulting in a brighter green appearance (Arsyam & Said, 2020). The yellow to pale-yellow coloration observed in most calli indicates actively growing tissues with limited chlorophyll development, particularly

under dark conditions. This characteristic is consistent with the treatments that promoted rapid callus induction and growth in CMG Agribun.

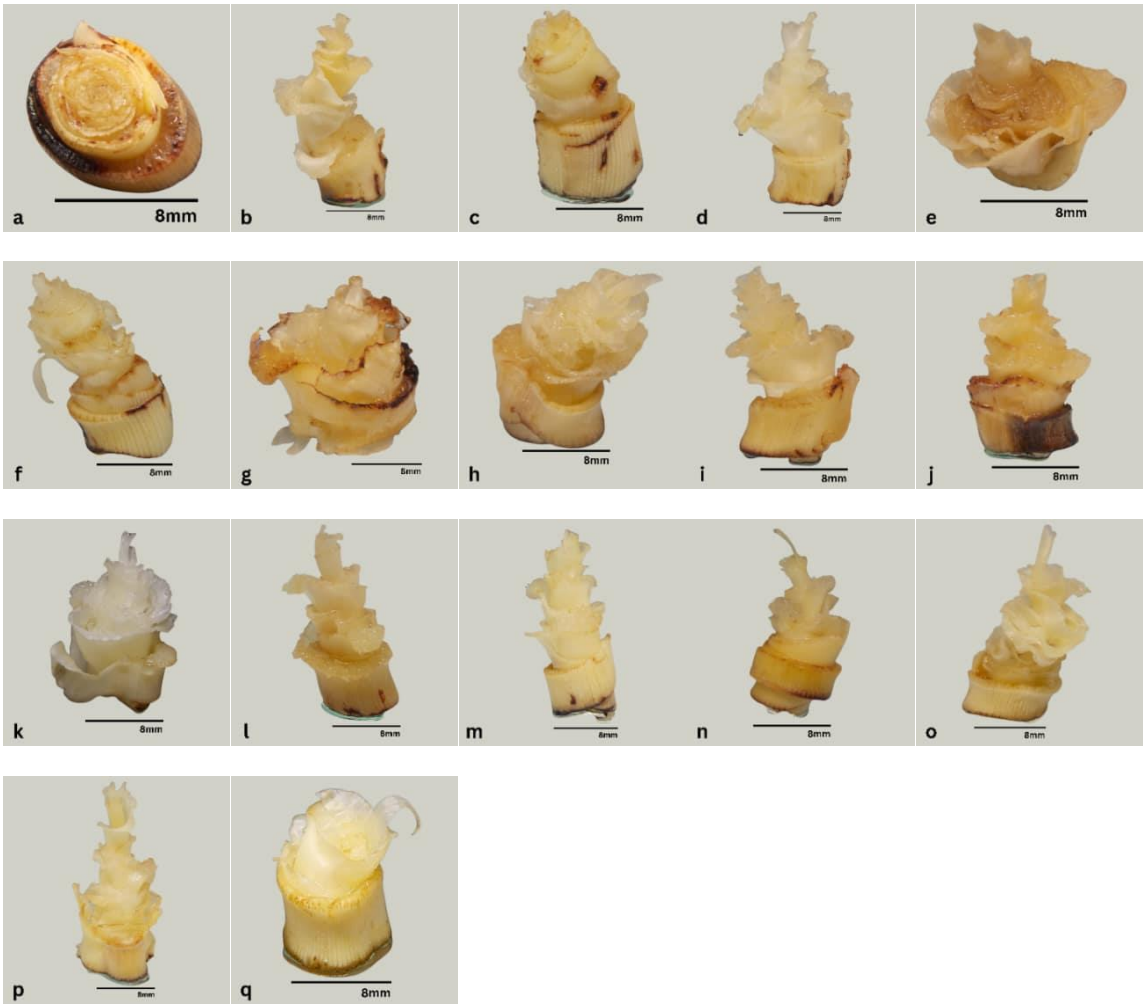

Figure 2. Callus morphology of sugarcane 'CMG Agribun' under dark conditions. (a) A0B0; (b) A1B0; (c) A1B0.5; (d) A1B1; (e) A1B1.5; (f) A2B0; (g) A2B0.5; (h) A2B1; (i) A2B1,5; (j) A3B0; (k) A3B0.5; (l) A3B1; (m) A3B1.5; (n) A4B0; (o) A4B0.5; (p) A4B1; and (q) A4B1.5.
Note: scale bar (-): 8 mm; A: 2,4-D; B: BAP; L: light conditions; D: dark conditions.

D. CONCLUSION AND SUGGESTIONS

Based on the results, the combination of 2,4-D and BAP, together with light conditions, significantly affected the time of callus initiation and callus dry weight, but not the percentage of callus formation or fresh weight. The fastest callus formation was observed in 1 ppm 2,4-D + 0 ppm BAP and 4 ppm 2,4-D + 1 ppm BAP under dark conditions (2.83 DAP). All treatments successfully induced callus, with formation rates ranging from 50% to 100%. Dark conditions generally produced higher callus fresh weights, while the highest dry weight (0.092 g) was obtained with 1 ppm 2,4-D + 0.5 ppm BAP under dark conditions. Callus morphology was characterized by a compact texture and varying colors influenced by PGR combinations and light regimes. These findings provide a basis for further optimization of sugarcane callus induction and its application in propagation and secondary metabolite production.

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