

Effect of BAP And NAA on Callus Proliferation and Characteristics of Papaya (*Carica Papaya* L.) cv. Sekaki Explants

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Abstract

Efficient *in vitro* systems are essential for rapid multiplication and genetic improvement of papaya (*Carica papaya* L.), a commercially important tropical fruit crop. This study evaluated the effects of different concentrations and ratios of 6-benzylaminopurine (BAP) and α -naphthaleneacetic acid (NAA) on callus proliferation from stem and petiole explants of papaya cv. Sekaki. Explants from 45-day-old seedlings were cultured on Murashige and Skoog medium supplemented with BAP (0–2.0 mg L⁻¹) and NAA (0–4.0 mg L⁻¹), and the four best induction treatments for each explant were further assessed for callus fresh weight, dry weight, length, and morphology in a randomized complete block design. In stem explants, 2.0 mg L⁻¹ BAP + 2.0 mg L⁻¹ NAA produced the highest biomass (8.13 ± 3.64 g fresh weight; 0.33 ± 0.08 g dry weight) and longest callus (3.88 ± 0.61 cm), with light-yellow, soft, intact tissues, whereas lower NAA concentrations resulted in significantly reduced biomass and friable, browned callus. In petiole explants, 2.0 mg L⁻¹ BAP + 3.0 mg L⁻¹ NAA gave the greatest biomass (10.56 ± 2.07 g fresh weight; 0.37 ± 0.07 g dry weight) and length (4.12 ± 0.19 cm), while maintaining light-yellow, compact callus structure. Across optimized treatments, petiole explants consistently produced higher biomass and longer, better-structured callus than stem explants, indicating petiole as the preferred tissue for large-scale callus production in cv. Sekaki. These explant-specific, optimized BAP–NAA combinations provide a reproducible protocol for high-quality callus proliferation in papaya cv. Sekaki that can be directly applied as a starting point for micropropagation, somatic embryogenesis, genetic transformation, and *in vitro* production of bioactive compounds in horticultural breeding and propagation programmes.

Key Message

Optimal BAP: NAA combinations (2:2 mg/L for stem; 2:3 mg/L for petiole) maximized papaya callus biomass and quality, providing explant-specific protocols for micropropagation and genetic improvement.

INTRODUCTION

Papaya (*Carica papaya* L.) is a fast-growing, herbaceous fruit crop that can reach 3–10 m in height and is widely cultivated throughout tropical and subtropical regions. It originated in southern Mexico and Central America but is now a major commodity in Africa, Asia, and the Pacific, with India, Indonesia, Nigeria, Brazil, Mexico, and Thailand among the leading producers. The plant has a hollow, soft stem with prominent leaf scars, large palmate leaves, and separate male, female, or hermaphroditic flowers, with fruit set occurring on female and hermaphrodite plants. The fruit is oval to oblong with a thin, smooth skin that turns from green to yellow or orange at maturity, and the Sekaki variety

is characterized by 3 to 4 m plant height, deeply lobed leaves, and large, sweet, orange-fleshed fruits.

In addition to its agronomic and economic benefits, papaya is a nutritional product containing vitamin C, **provitamin A carotenoids**, lycopene and flavonoids like quercetin. These compounds play a role in potent antioxidant and immune-supportive effects and have also been linked to cardiometabolic advantages, but **clinical evidence for disease-specific effects remains limited**. Besides this, papaya leaf, papaya seeds and papaya latex are reported to have pharmacological and industrial uses such as anti-inflammatory effects, anti-cancer effects in vitro, papain as a meat tenderizer and papain in biopharmaceutical preparations. Together, these attributes make papaya an important target for intensive crop improvement, rapid multiplication of elite genotypes, and sustainable production.

Efficient in vitro systems are essential for micropropagation, germplasm conservation, genetic transformation, and secondary metabolite production in papaya, which is often considered recalcitrant in culture. Plant growth regulators (PGRs), especially the cytokinin 6-benzylaminopurine (BAP) and the auxin α -naphthaleneacetic acid (NAA), are central to controlling cell division, dedifferentiation, callus proliferation, and subsequent regeneration. However, published work on papaya tissue culture reports divergent outcomes regarding the optimal BAP and NAA concentrations and ratios, and these differences are further confounded by cultivar, explant type, and culture conditions. As a result, there is still no widely accepted, standardized BAP: NAA protocol for robust callus proliferation in *Carica papaya*, particularly for commercially relevant Southeast Asian cultivars such as Sekaki.

Most previous studies have either focused on a limited range of hormone combinations, emphasized callus induction over sustained proliferation, or used a single explant type, which restricts direct comparison and practical application. There is also limited quantitative information on how the same set of BAP: NAA treatments differentially affect biomass accumulation and callus quality traits (colour, texture, and degree of differentiation) in stem versus petiole explants of the same cultivar. This lack of explant-specific optimization hampers the development of reliable platforms for downstream micropropagation and genetic manipulation.

Therefore, the present study systematically evaluated a full factorial series of BAP (0–2.0 mg L⁻¹) and NAA (0–4.0 mg L⁻¹) combinations for callus proliferation from stem and petiole explants of *Carica papaya* L. cv. Sekaki. Specifically, we aimed to (i) identify the best BAP: NAA combinations for maximizing callus fresh weight, dry weight, and length in each explant type, and (ii) characterize associated morphological traits, including callus colour and texture, as indicators of tissue quality. By directly comparing explant-specific

responses under controlled conditions, this work provides a clear, experimentally validated set of media formulations that closes a key protocol gap for Sekaki

papaya and offers a practical platform for micropropagation, genetic transformation, and bioactive compound production.

MATERIALS AND METHODS

The study was conducted at the Plant Biotechnology Laboratory, Universiti Teknologi MARA, Malaysia, under controlled laboratory conditions.

2.1 Experimental conditions

Experiments were performed at the Plant Biotechnology Laboratory, Universiti Teknologi MARA, Malaysia. Aseptic conditions were ensured by working in a laminar flow hood and all media and instruments were autoclaved prior to use.

2.2 Plant material and explant preparation

Stem and petiole explants were excised from 45-day-old *Carica papaya* L. cv. Sekaki seedlings obtained from Haniz Nursery (Kampung Alai, Merlimau, Malacca). Explants were initially cut into 4–5 cm segments to allow free movement in 250 mL conical flasks during surface sterilization, then trimmed to 1–2 mm immediately before culturing.

Surface sterilization was optimized as follows: stem explants were immersed in 20% (v/v) Clorox® for 15 min, whereas petiole explants were treated with 10% (v/v) Clorox® for 10 min. The sterilizing agent was supplemented with Tween-20 (two drops per 100 mL). Explants were rinsed four times with sterile distilled water following disinfection.

2.3 Culture media and growth regulator treatments

Full-strength Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) supplemented with 30 g L⁻¹ sucrose was used as the basal medium. The pH was adjusted to 5.8 ± 0.1 prior to gelling

with 2.5 g L⁻¹ Gelrite, and the medium was autoclaved at 121°C for 20 min. All equipment and instruments were sterilized under identical conditions.

Treatments consisted of MS medium fortified with **6-benzylaminopurine (BAP)** at 0, 0.5, 1.0, 1.5, or 2.0 mg L⁻¹, alone or in combination with **1-naphthaleneacetic acid (NAA)** at 0, 1.0, 2.0, 3.0, or 4.0 mg L⁻¹. This full factorial arrangement yielded 25 unique treatment combinations (Supplementary Table 1), adapted from protocols described by Waidyaratne et al. (2022) and Radzuan et al. (2018).

2.4 Callus induction and maintenance

Sterilized explants were cultured in 90 mm disposable Petri dishes (three explants per dish) containing 25 mL of the respective induction media. Dishes were sealed with Parafilm® and maintained in a controlled growth room at $25 \pm 2^\circ\text{C}$ under continuous illumination (24 h photoperiod) provided by cool-white, fluorescent lamps.

Six weeks post-induction, callus derived from the optimized treatments was carefully excised from the parental explant tissue. Callus fresh weight was standardized to 0.5 g per inoculum and subcultured onto fresh medium of the corresponding composition at a density of two callus clumps per vessel. A second subculture was performed four weeks later, reducing the density to one callus per vessel to permit optimal expansion.

2.5 Experimental design

The experiment was arranged in a **Randomized Complete Block Design (RCBD)**. For each explant type (stem and petiole), four optimized treatments were selected from the initial induction screen. Each treatment was replicated five times, yielding 20 experimental units per explant category.

2.6 Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS version 27.0 (IBM Corp., Armonk, NY, USA) at a significance level of $\alpha = 0.05$. Where F-values indicated significant treatment effects, means were separated using **Duncan's Multiple Range Test (DMRT)** (Gomez & Gomez, 1984).

RESULTS

3.1 Callus proliferation response

Following induction, four optimized treatments were selected for each explant type for further proliferation analysis. The effects of BAP and NAA concentrations on callus fresh weight, length, dry weight, and morphological characteristics are presented in Tables 1–4 and Figs. 1–5, respectively.

Table 1

Effects of BAP and NAA on fresh weight, length, and dry weight of stem-derived callus in *Carica papaya* cv. Sekaki

Treatment	BAP (mg/l)	NAA (mg/l)	Callus Fresh Weight (gm)	Callus Length (cm)	Callus Dry Weight (gm)
T12	1	1	3.87 ± 0.59^{bc}	3.78 ± 0.31^a	0.26 ± 0.02^a
T22	2	1	1.60 ± 0.61^c	2.68 ± 0.44^b	0.13 ± 0.01^b
T23	2	2	8.13 ± 3.64^a	3.88 ± 0.61^a	0.33 ± 0.08^a
T25	2	4	6.17 ± 4.43^{ab}	3.68 ± 0.44^a	0.29 ± 0.09^a

Values represent mean $\pm$ standard deviation (n = 5). Means followed by different superscript letters within the same column are significantly different at $p < 0.05$ according to Duncan's multiple range test.

Table 2

Effects of BAP and NAA on fresh weight, length, and dry weight of petiole-derived callus in *Carica papaya* cv. Sekaki

Treatment	BAP (mg/l)	NAA (mg/l)	Callus Fresh Weight (gm)	Callus Length (cm)	Callus Dry Weight (gm)
T12	1	1	6.12 ± 3.25^b	3.58 ± 0.56^a	0.24 ± 0.09^b
T22	2	1	1.84 ± 2.00^c	2.06 ± 0.70^b	0.09 ± 0.09^c
T23	2	2	3.96 ± 1.19^{bc}	3.66 ± 0.87^a	0.21 ± 0.06^b
T24	2	3	10.56 ± 2.07^a	4.12 ± 0.19^a	0.37 ± 0.05^a

Values represent mean $\pm$ standard deviation (n = 5). Means followed by different superscript letters within the same column are significantly different at $p < 0.05$ according to Duncan's multiple range test.

3.2 Effect of BAP and NAA on callus fresh weight

3.2.1 Stem-derived callus

There was a large difference in the fresh weight of callus among the stem treatments (Table 1). T23 (2.0 mg L⁻¹ BAP + 2.0 mg L⁻¹ NAA) resulted in the greatest fresh weight (8.13 ± 3.64 g). In contrast, T22 (2.0 mg L⁻¹ BAP + 1.0 mg L⁻¹ NAA) yielded the lowest fresh weight (1.60 ± 0.61 g).

3.2.2 Petiole-derived callus

The range of response of petiole explants to the various BAP and NAA combinations was greater than that of stem explants (Table 2). The best treatment, T24 (2.0 mg L⁻¹ BAP + 3.0 mg L⁻¹ NAA), produced the highest callus fresh weight (10.56 ± 2.07 g), which was significantly greater than the other treatments. Fresh weight declined sharply in T25 (2.0 mg L⁻¹ BAP + 4.0 mg L⁻¹ NAA). T22 again produced the lowest fresh weight (1.84 ± 2.00 g).

3.3 Effect of BAP and NAA on callus length

3.3.1 Stem-derived callus

Callus length for stem explants followed a similar pattern to fresh weight (Table 1). T12, T23, and T25 were statistically similar ($p > 0.05$), with mean callus lengths ranging from 3.68 to 3.88 cm. T22 produced shorter callus than these treatments.

3.3.2 Petiole-derived callus

Callus length in petiole-derived callus reflected the fresh weight trends (Table 2). The highest elongation (4.12 ± 0.19 cm) was obtained in T24, and this was statistically similar to T12 and T23 (3.58 ± 0.56 cm and 3.66 ± 0.87 cm, respectively). T22 gave the shortest callus (2.06 ± 0.70 cm).

3.4 Effect of BAP and NAA on callus dry weight

3.4.1 Stem-derived callus

Dry weight accumulation in stem-derived callus paralleled the fresh weight trends (Table 1). T23 (0.33 ± 0.08 g) and T25 (0.29 ± 0.09 g) produced the highest dry weights, while T22 recorded the lowest (0.13 ± 0.01 g).

3.4.2 Petiole-derived callus

For petiole-derived callus, maximum dry weight was recorded in T24 (0.37 ± 0.05 g), which was significantly higher than all other treatments (Table 2). T12 and T23 did not differ significantly from each other but were significantly higher than T22, which had the lowest dry weight (0.09 ± 0.09 g).

3.5 Effect of BAP and NAA on callus morphology

Table 3
Morphological characteristics of stem-derived callus under selected BAP and NAA treatments

Treatment	BAP (mg L ⁻¹)	NAA (mg L ⁻¹)	Callus colour	Callus texture
T12	1.0	1.0	Brown with green patches	Soft, intact
T22	2.0	1.0	Brown with green patches	Friable
T23	2.0	2.0	Light yellow	Soft, intact
T25	2.0	4.0	Yellowish brown	Slightly friable

Table 4
Morphological characteristics of petiole-derived callus under selected BAP and NAA treatments

Treatment	BAP (mg L ⁻¹)	NAA (mg L ⁻¹)	Callus colour	Callus texture
T12	1.0	1.0	Light yellow	Soft, intact
T22	2.0	1.0	Yellow	Soft, intact
T23	2.0	2.0	Light yellow with white spongy tissues	Soft, intact
T24	2.0	3.0	Light yellow	Soft, intact

3.5.1 Callus colour

There were clear differences in callus colour among treatments and explant types (Figs. 1–5). In both stem and petiole explants, T23 produced light-yellow callus (Fig. 1). Petiole-derived callus under T23 formed white,

spongy tissues (Fig. 2). Brown coloration was observed in stem-derived callus in T12 and T25. Green patches were visible in stem-derived callus in T12 and T22 (Figs. 3 and 5). Petiole-derived callus under T12 was pale yellow (Fig. 4).

3.5.2 Callus texture

Callus texture also varies among treatments (Tables 3 and 4). Both explants under T12 produced soft, intact callus despite the less favourable colour. Under T22, petiole-derived callus was compact, whereas stem-derived callus was

friable. Callus induced by T23 was soft and intact in both explants, and petiole-derived callus under T24 remained soft and intact despite high proliferation. In contrast, stem-derived callus in T25 was soft, loosely formed, and easily fragmented.

3.6 Explant-specific responses

Overall, stem explants were more sensitive to changes in BAP-NAA composition than petiole explants. At their respective optima, petiole explants produced higher callus fresh weight (10.56 ± 2.07 g vs. 8.13 ± 3.64 g), greater dry weight (0.37 ± 0.07 g vs. 0.33 ± 0.08 g), and slightly longer callus (4.12 ± 0.19 cm vs. 3.88 ± 0.61 cm) than stem explants, indicating a higher biomass potential for petiole tissues under optimized hormonal conditions. Petiole-derived callus also maintained good structural integrity over a wider range of BAP-NAA combinations, whereas stem-derived callus showed browning, phenolic accumulation, and loss of coherence outside a narrow hormonal window.

3.7 Optimized hormonal combinations

Optimum BAP-NAA combinations were established in each type of explant. In the case of stem derived callus, the most favourable results were obtained with 2.0 mg L⁻¹ BAP + 2.0 mg L⁻¹ NAA in all the parameters. In the case of petiole derived callus, 2.0mg L⁻¹ BAP + 3.0mg L⁻¹ NAA (T24) was the most effective in terms of biomass accumulation, elongation and morphology. These cultures offered high proliferation, favourable colour, and texture to every type of explant.

DISCUSSION

This study establishes explant-specific, optimized BAP: NAA combinations that support vigorous, high-quality callus proliferation in *Carica papaya* L. cv. Sekaki. For stem explants, 2.0 mg L⁻¹ BAP + 2.0 mg L⁻¹ NAA (T23) produced the highest fresh and dry biomass and longest callus while maintaining a light-yellow, non-browning, soft-intact morphology. For petiole explants, 2.0 mg L⁻¹ BAP + 3.0 mg L⁻¹ NAA (T24) was superior across the same parameters and generated similarly healthy, light-yellow tissues. These results not only confirm the importance of balanced cytokinin-auxin ratios for papaya callus growth, but also demonstrate, for the first time in Sekaki, that stem and petiole explants require distinct, narrow hormonal windows to achieve optimal biomass and tissue quality. By combining a full BAP × NAA factorial with parallel assessment of two explant types and multiple quantitative and qualitative traits, this work refines and extends earlier papaya protocols and delivers a directly usable set of media recipes for reliable callus production in this commercially important cultivar.

The fresh and dry weight responses of stem-derived callus confirmed that an equal ratio of BAP:NAA at higher absolute concentrations (2.0:2.0 mg L⁻¹) is particularly favourable for biomass accumulation, in agreement with earlier reports that equal cytokinin-auxin ratios

can promote optimum callus growth in papaya. At the same time, the poor performance of T22, with adequate BAP but insufficient NAA, shows that auxin levels must reach a critical threshold before vigorous proliferation can occur, despite the presence of an effective cytokinin dose. In petiole explants, the optimum at 2.0 mg L⁻¹ BAP + 3.0 mg L⁻¹ NAA reflects a higher auxin requirement, consistent with their broader response range and greater biomass. The parallel trends in fresh weight, callus length, and dry weight indicate that these hormonal combinations increased true biomass rather than merely changing water content. These are optimized hormonal ratios that focus on the importance of moderate to high levels of NAA in maintaining cell division and cell growth. In comparison, the lower biomass in T22, in both explants, shows that low levels of NAA inhibit growth and elongation.

The morphological features are also a further indication of the ideal hormonal balance. The yellowish colour, intact and soft callus texture in T23 and T24 are characteristic of the healthy and actively growing cultures with restricted oxidative browning. The brown coloration and green patches seen in T12, T22, and T25 for stem-derived callus point to phenolic accumulation and partial differentiation under suboptimal BAP: NAA ratios, whereas the pale-yellow callus in petiole-derived T12 suggests a relatively greater tolerance to these conditions. The friable texture of stem-derived callus in T22 and T25 further indicates that inappropriate auxin levels can compromise tissue integrity even when proliferation occurs.

These findings show that stem and petiole explants of *C. papaya* cv. Sekaki, differ in their sensitivity to BAP-NAA combinations, with the stem tissues requiring a narrower window of the hormonal ratios to avoid browning and structural weakness, and petiole tissues tolerating a wider range while still achieving high biomass. Explant-specific differences of this type have also been reported in other dicot species and emphasize the need to tailor hormone regimes to tissue type rather than applying a single protocol across all explants.

The explant-specific hormonal windows identified here have direct horticultural implications for papaya propagation and improvement. For cv. Sekaki, the media combinations of 2.0 mg L⁻¹ BAP + 2.0 mg L⁻¹ NAA for stem explants and 2.0 mg L⁻¹ BAP + 3.0 mg L⁻¹ NAA for petiole explants consistently produced high callus biomass with favourable, non-browning morphology, offering robust starting material for downstream regeneration systems. Importantly, petiole explants outperformed stem explants at their respective optima in terms of fresh and dry biomass and callus elongation, and they tolerated a broader range of BAP-NAA ratios while maintaining tissue quality. This finding suggests that petiole tissue, which has traditionally received less emphasis than stem or nodal segments in papaya tissue-culture protocols, should be considered a preferred explant for large-scale callus production in this cultivar. Given the importance of stable, high-quality callus for micropropagation, somatic embryogenesis, and genetic

transformation in papaya, these conditions can be readily integrated into breeding pipelines aimed at disseminating elite hermaphroditic lines and developing papaya ringspot virus-resistant cultivars. Future horticultural work can build on this protocol by coupling it with shoot or embryo regeneration steps, testing responsiveness across diverse papaya germplasm, and assessing field performance of regenerated plants.

CONCLUSION

This study establishes explant-specific, optimized BAP–NAA combinations for high-quality callus proliferation in papaya (*Carica papaya* L.) cv. Sekaki. For stem explants, 2.0 mg L⁻¹ BAP + 2.0 mg L⁻¹ NAA, and for petiole explants, 2.0 mg L⁻¹ BAP + 3.0 mg L⁻¹ NAA, maximized callus biomass and elongation while producing light-yellow, soft, intact tissues that were less prone to browning and friability. At their respective optima, petiole explants generated higher biomass and slightly longer callus than stem explants, and they maintained good morphology across a wider range of hormonal combinations, identifying petiole as the preferred explant for routine, high-throughput callus production in this cultivar. These reproducible protocols provide practical media formulations for horticultural laboratories and can serve as reliable starting points for micropropagation, somatic embryogenesis, genetic transformation, and in vitro production of papaya bioactives within commercial and breeding programmes.

Declarations

Funding

No external funding was received for this research.

Data Availability

Data used in this article is generated by the first author from the experimental setup conducted at the Plant Tissue Culture Laboratory, Universiti Teknologi MARA, Malaysia.

Ethics and consent to participate

No ethics approval was required for this study, as it involved *in-vitro* callus induction and proliferation from *Carica papaya* explants, with no use of humans, animals, or environmentally sensitive materials. The papaya (*Carica papaya* L.) cv. Sekaki explants used in this study were obtained from cultivated seedlings purchased from Haniz Nursery Kampung Alai, Merlimau, Malacca. No wild plant material was collected, and the collection and use of the plant material complied with all relevant local and national guidelines of Malaysia. No specific permits or licences were required for this study.

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Figures



Figure 1

Stem-derived callus exhibiting bright yellow coloration and compact, intact texture on MS medium supplemented with 2.0 mg L⁻¹ BAP and 2.0 mg L⁻¹ NAA (T23)

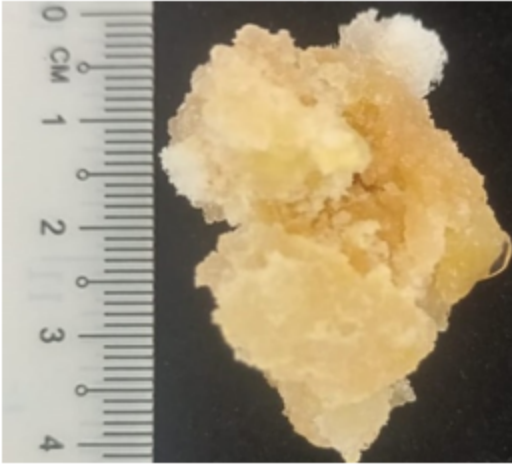


Figure 2

Petiole-derived callus showing characteristic white, spongy tissue formation indicative of rapid cellular expansion on MS medium supplemented with 2.0 mg L⁻¹ BAP and 2.0 mg L⁻¹ NAA (T23)



Figure 3

Stem-derived callus exhibiting green pigmentation patches indicative of partial differentiation on MS medium supplemented with 1.0 mg L⁻¹ BAP and 1.0 mg L⁻¹ NAA (T12)

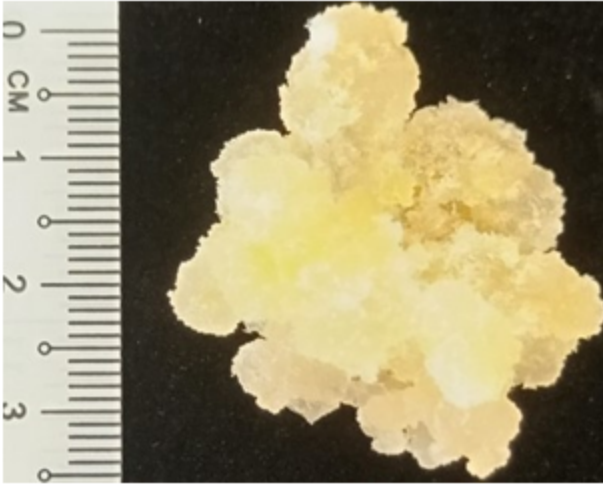


Figure 4

Petiole-derived callus displaying pale yellow coloration and intact texture on MS medium supplemented with 1.0 mg L⁻¹ BAP and 1.0 mg L⁻¹ NAA (T12)

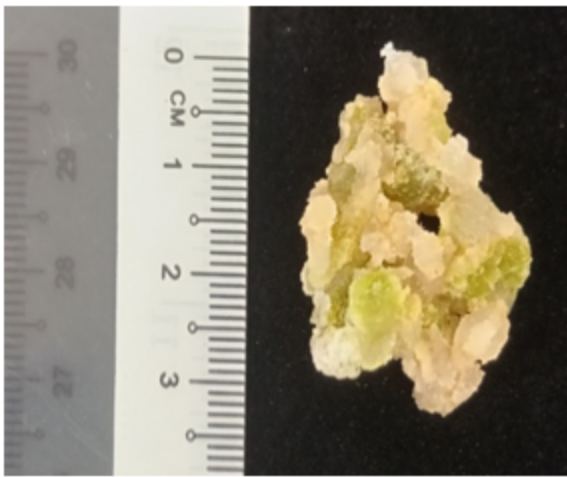


Figure 5

Stem-derived callus of *Carica papaya* cv. Sekaki induced on MS medium supplemented with 2.0 mg L⁻¹ BAP and 1.0 mg L⁻¹ NAA (T22), showing green colouration patches and friable texture resulting from suboptimal auxin concentration. Scale bar = 1 cm.

Supplementary Files

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