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RESEARCH ARTICLE

Optimization of Direct Organogenesis and Callus Induction in *Curcuma sumatrana* Miq. for Conservation and Biotechnology

Rival Yuhendri[1], Wan Nurfatin Binti W Mohamad Baharudin[1], Nur Ain Syafiqa Binti Abdul Kadir[1], Nurainas[2], Tesri Maideliza[2], M. Idris[2], Ikarastika Rahayu Abdul Wahab[1*]

[1]Faculty of Agro-Based Industry, Universiti Malaysia Kelantan-Jeli Campus. 17600 Jeli, Kelantan, Malaysia.
[2]Faculty of Mathematics and Natural Sciences, Universitas Andalas. Limau Manis, Padang 25163, West Sumatra, Indonesia.

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☒ * CORRESPONDING AUTHOR
Dr. Ikarastika Rahayu Abdul Wahab
Faculty of Agro-Based Industry,
Universiti Malaysia Kelantan-Jeli
Campus. 17600 Jeli, Kelantan,
Malaysia.
Email: ikarastika@umk.edu.my

ABSTRACT

Curcuma sumatrana Miq., a ginger species classified as 'Vulnerable' (VU) by the IUCN, is threatened by habitat degradation and illegal harvesting. Explants from rhizome buds were cultured *in vitro* to develop a strategic approach for their *ex situ* conservation. For direct organogenesis, a combination of 5.0 mg/L 6-benzylaminopurine (BAP) and 0.5 mg/L 1-naphthaleneacetic acid (NAA) yielded the highest mean shoot number (2.33 ± 0.58) and length (4.47 ± 0.35 cm). However, a one-way ANOVA revealed no statistically significant differences among treatments for shoot proliferation ($p>0.05$). For root induction, the medium with 0.5 mg/L BAP and 1.0 mg/L NAA produced the highest average root number (3.33 ± 0.58 roots per shoot), though no significant differences were observed ($p>0.05$). The study also successfully induced compact callus for indirect regeneration using 6.75 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.2 mg/L BAP, with this treatment being significantly more effective than others ($p<0.05$). Additionally, Plant Preservative Mixture (PPM™) at 1.0 mL/L was effective in controlling contamination without phytotoxic effects. This protocol provides a crucial foundation for the sustainable conservation and mass propagation of *C. sumatrana*, supporting both species protection and the exploration of its pharmacological potential.

1. INTRODUCTION

Curcuma sumatrana Miq., locally known as *Koenih Bimbo* or *Koenih Rimbo*, is an endemic member of the Zingiberaceae family restricted to West Sumatra, Indonesia. This rare terrestrial herb, reaching up to 135 cm in height, produces aromatic rhizomes with pale-purple pigmentation and leaves with distinct anatomical features. Traditionally, decoctions of its leaves are used to treat skin ailments, indicating potential pharmacological properties (Ardiyani et al., 2011). The species was rediscovered in 2011 after more than a century of absence from scientific records and is now listed as “Vulnerable” (VU) under the IUCN Red List due to deforestation, overharvesting, and limited natural populations (IUCN, 2018; Nurainas and Ardiyani, 2019). Given its ethnomedicinal importance and conservation concern, biotechnological intervention through *in vitro* propagation has become a critical strategy for its preservation and sustainable utilization.

Conventional propagation methods such as rhizome division are inefficient, seasonal, and limited by slow growth, making them unsuitable for large-scale conservation. Moreover, explants derived from wild populations are prone to challenges such as microbial contamination, browning, and poor morphogenic response, which are commonly observed in *Curcuma* species (Hasnain et al., 2022). To overcome these biological and technical barriers, *in vitro* culture provides a powerful platform for clonal propagation, germplasm conservation, and the enhancement of bioactive compound production in threatened medicinal plants (Moyo et al., 2015). However, successful *in vitro* propagation requires optimization of plant growth regulators (PGRs), as their precise ratio determines whether explants will form shoots, roots, or callus.

Plant growth regulators are pivotal in directing morphogenetic pathways by influencing cell division, elongation, and differentiation. Cytokinins such as 6-benzylaminopurine (BAP) stimulate shoot initiation by activating meristematic cell division, while auxins such as 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) promote rhizogenesis and callus induction, respectively (Šimášková et al., 2015; Cavallari et al., 2021). The interplay between auxins and cytokinins determines organogenic fate, and their species-specific responses necessitate independent optimization for each plant system. In *C. longa*, *C. caesia*, and *C. aeruginosa*, higher BAP concentrations have been reported to enhance shoot proliferation (Khumaida et al., 2019; Rahayu and Adil, 2012; Zuraida, 2013), whereas 2,4-D is widely recognized as an effective inducer of callus formation (Teoh et al., 2023).

Despite the extensive research on other *Curcuma* species, *C. sumatrana* remains poorly studied at the biotechnological level. Previous works have primarily focused on its taxonomy, anatomy, and phytochemistry (Ardiyani et al., 2011; Yuhendri et al., 2025), leaving a critical gap in the development of an *in vitro* propagation system. Addressing this gap is essential not only for *ex situ* conservation but also for enabling downstream applications such as secondary metabolite production and genetic improvement. Unlike earlier descriptive or phytochemical reports, the present study establishes the first comprehensive micropropagation and callus induction protocol for *C. sumatrana*, integrating hormonal optimization and contamination control using Plant Preservative Mixture (PPM™). This methodological advancement represents an innovative contribution to conservation biotechnology for vulnerable *Curcuma* species.

Therefore, the objectives of this study were: (1) to evaluate the effects of BAP and NAA on direct organogenesis for efficient shoot and root proliferation, (2) to determine an effective protocol for callus induction using 2,4-D as a potential source for indirect regeneration, and (3) to assess the effectiveness of PPM™ in controlling microbial contamination during *in vitro* culture. The findings of this work aim to provide a reproducible and scalable propagation framework for *C. sumatrana*, facilitating both conservation and future biotechnological exploration.

2. MATERIALS AND METHODS

2.1. Plant Material and Voucher Spescimen

Rhizome buds of *Curcuma sumatrana* Miq., were collected from their native habitat in Kambang, Pesisir Selatan Regency, West Sumatra, Indonesia (1°38'41.2" S, 100°49'36.7" E). The collection was authorised by the Ministry of Agriculture's Agency for Agricultural Quarantine of the Republic of Indonesia (Permit No: 19655/KR.020/K.3/09/2023). Dr. Nurainas taxonomically identified the species from the Department of Biology, Andalas University, Indonesia. A voucher specimen (accession number: ANDA 00049122) was deposited at the Herbarium of Andalas University (ANDA). All experiments were conducted at the Tissue Culture Laboratory, Universiti Malaysia Kelantan (UMK).

2.2. Explant Preparation and Surface Sterilization

Sprouted rhizome buds of approximately 3-4 cm in length were selected as the primary explants for in vitro culture initiation. These were chosen due to their high regenerative potential and low risk of microbial contamination. Before the sterilisation procedure, the explants underwent a preliminary cleaning process to remove surface debris and reduce the microbial load. This involved washing the buds thoroughly with a mild commercial detergent, followed by rinsing under running tap water for 30 minutes. This initial step is crucial for the elimination of soil particles and epiphytic microorganisms that could compromise the surface sterilisation process (Yu et al., 2022).

Surface sterilisation was performed using a sequential disinfection protocol to achieve optimal microbial decontamination while maintaining explant viability. Following the initial pre-washing, the explants were first immersed in 70% (v/v) ethanol for 1 minute. This served as a rapid surface disinfectant by denaturing microbial proteins (Kebede, 2021; Dickinson et al., 2021). Subsequently, the explants were treated with a sodium hypochlorite solution (1.5% v/v active chlorine) for 15 minutes. This solution was prepared by diluting commercial bleach (5.25% active chlorine) to the required final concentration. One to two drops of Tween 20 were added to the solution as a wetting agent to ensure uniform coverage of the explants, and the sterilisation was carried out under constant agitation at 120 rpm. This potent sterilant is effective against a broad spectrum of bacteria and fungi; however, its concentration and exposure time were carefully controlled to prevent phytotoxicity (Boonprasert et al., 2025).

Following the sodium hypochlorite treatment, the explants were rinsed three times with sterile distilled water, with each rinse lasting 5 minutes, to completely remove any residual chlorine compounds, which are toxic to plant tissues. Once sterilised and rinsed, the explants were blotted dry under aseptic conditions and immediately transferred to the initiation medium. The efficacy of the sterilisation protocol was assessed based on the resulting contamination rate and explant viability.

2.3. Culture Media and Incubation Conditions

The basal medium for all experiments was the Murashige and Skoog (MS) basal salt mixture (Murashige and Skoog, 1962), which was purchased from Duchefa Biochemie, Netherlands. The medium was prepared at a concentration of 4.4 g/L and supplemented with 1.0 mL/L of MS vitamin solution (Sigma-Aldrich, St. Louis, MO, USA), consisting of thiamine-HCl (0.1 mg/L), pyridoxine-HCl (0.5 mg/L), nicotinic acid (0.5 mg/L), and glycine (2.0 mg/L). It was further enriched with 30 g/L of sucrose and solidified with 8 g/L of agar (Merck, Darmstadt, Germany).

The pH of the medium was adjusted to 5.8 using either 0.1 N HCl or 0.1 N NaOH before autoclaving. Sterilization was conducted at 121°C and 15 psi pressure for 20 minutes (Ahmed et al., 2021). To mitigate microbial contamination, all media were supplemented with 1.0 mL/L of Plant Preservative Mixture (PPM™) (Plant Cell Technology, Washington, DC, USA), which was added after the autoclaving process (Ledo et al., 2019). This concentration was found to be effective in controlling contamination without exhibiting any observable phytotoxic effects on explant morphogenesis. All cultures were incubated in a growth chamber at 25 ± 2°C under a 16-hour photoperiod with cool white fluorescent light (2000 lux) (Ahmed et al., 2021).

2.4. Direct Organogenesis: Shoot and Root Induction

For direct organogenesis, sterilized rhizome bud explants were inoculated onto MS basal medium. This medium was supplemented with a combination of the plant hormones 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA), which was chosen to investigate the role of cytokinin and auxin interaction in driving morphogenetic responses, as widely documented in other *Curcuma* species (Šimášková et al., 2015).

The experiment was based on a two-factor treatment system. The first group evaluated three levels of BAP (1.0, 3.0, and 5.0 mg/L) with a constant NAA concentration of 0.5 mg/L. Conversely, the second group varied NAA concentrations (1.0, 3.0, and 5.0 mg/L) while keeping BAP fixed at 0.5 mg/L.

Cultures were maintained under controlled environmental conditions (25 ± 2°C, 16/8 h photoperiod, and 35-45 µmol/m²/s light intensity) for 8 weeks. The parameters evaluated included shoot induction frequency (%), the mean number of shoots per explant, the average shoot length (cm), and the number of roots per explant. For the rooting experiment, shoots with an average length of 2-3 cm were excised and transferred to the rooting medium. The rooting percentage was also evaluated as a key parameter.

2.5. Callus Induction

Callus induction was carried out using sprouted rhizome bud explants cultured on MS basal medium. This medium was supplemented with a fixed concentration of 6.75 mg/L 2,4-D, a synthetic auxin commonly used to induce callus due to its ability to revert cells to a dedifferentiated state (Teoh et al., 2023).

To evaluate the synergistic effects of different PGRs, the medium was further supplemented with one of the following: 2.2 mg/L 6-benzylaminopurine (BAP), 4.7 mg/L kinetin (KIN), or 2.7 mg/L 1-naphthaleneacetic acid (NAA). These specific combinations were chosen based on previous studies that demonstrated their effectiveness in inducing callus and promoting cell proliferation in related Zingiberaceae species (Boonprasert et al., 2025).

The cultures were incubated in complete darkness at 25 ± 2°C for 56 days. These dark conditions were chosen because they promote undifferentiated cell growth and prevent premature organogenesis (Zhao et al., 2013). After this period, the callus induction frequency (%), callus diameter (mm), colour (e.g., creamy white, yellowish, or brown), and texture (compact or friable) were recorded to evaluate callus quality.

2.6. Data and Statistical Analysis

The experimental design was completely randomized with three replicates per treatment. To minimize the impact on the minimal and endangered plant population, each replicate was restricted to a single explant per bottle. Quantitative data are expressed as the mean ± standard deviation (SD) and were analyzed using a one-way analysis of variance (ANOVA) to determine significant differences between treatment means. A post-hoc Tukey's Honestly Significant Difference (HSD) test was applied when the ANOVA result was significant ($p<0.05$). All statistical analyses were performed with SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA).

2.7. Limitations and Future Perspectives

This study was limited to the assessment of morphogenetic and physical characteristics of *C. sumatrana* under in vitro conditions. Although the findings successfully established a baseline for shoot and callus induction, the biochemical and molecular dimensions were not explored. Future research should therefore integrate metabolic profiling and gene-expression analyses to clarify the physiological mechanisms underlying morphogenesis, secondary metabolite synthesis, and stress responses. In addition, assessment of genetic fidelity using molecular markers such as RAPD or ISSR is recommended to verify clonal uniformity and confirm the genetic stability of regenerated plantlets, thereby strengthening the reliability of this micropropagation protocol for long-term conservation and biotechnological applications.

3. RESULT AND DISCUSSION

The *in vitro* propagation of *C. sumatrana*, a vulnerable and endemic member of the Zingiberaceae family, was successfully initiated in this study through two distinct morphogenetic pathways: direct organogenesis and indirect organogenesis via callus induction. This pioneering work not only provides a critical tool for the conservation of this rare species but also establishes a foundational protocol for future biotechnological applications. This section details the results, discusses their biological and practical implications, and addresses the broader challenges and opportunities in the field.

3.1 Effect of BAP and NAA on Direct Organogenesis from Rhizome Bud Explants

Following an eight-week culture period, the rhizome bud explants of *C. sumatrana* demonstrated a range of morphogenetic responses. A one-way ANOVA revealed no statistically significant differences among treatments for shoot proliferation ($p>0.05$). However, the medium containing 5.0 mg/L BAP and 0.5 mg/L NAA consistently produced the highest mean shoot number (2.33 ± 0.58) and length (4.47 ± 0.35 cm) numerically. This finding aligns with the fundamental principles of plant hormone physiology, which posit that the cytokinin-to-auxin ratio is a critical determinant of cellular fate (Su and Zhang, 2014).

This outcome is not only consistent with basic principles of plant hormone action but also aligns with recent reports on related monocotyledonous species, particularly within the Zingiberaceae family. For instance, optimal shoot multiplication in *C. aeruginosa* was also achieved with high BAP concentrations (Khumaida et al., 2019), and other *Curcuma* species, such as *C. xanthorrhiza* (Rahayu and Adil, 2012) and *C. caesia* (Zuraida, 2013), responded optimally to approximately 5.0 mg/L BAP. The consistency of these results across *Curcuma* species suggests a high degree of evolutionary conservation in cytokinin signalling pathways that regulate shoot development, likely reflecting shared evolutionary traits among rhizomatous geophytes.

Physiologically, shoot induction by cytokinins like BAP is based on their role in promoting cell division within meristems. BAP binds to receptors on the cell membrane, which initiates a signalling cascade that ultimately activates transcription factors and promotes shoot formation (Hutchison and Kieber, 2002; Yang et al., 2021). The addition of 0.5 mg/L NAA complements this process by supporting shoot elongation and structural integrity through the promotion of cell expansion and vascular differentiation (Wu et al., 2021). Thus, the synergistic interaction between 5.0 mg/L BAP and 0.5 mg/L NAA appears to have established an ideal hormonal balance that maximised both proliferation and cell elongation, resulting in robust and well-developed shoots. The shoots produced under this regimen were also healthy, green, and free from hyperhydricity (vitrification). This common physiological disorder can reduce plantlet survival by up to 70% during acclimatisation (Shaheen et al., 2023). This observation underscores that the chosen hormone combination not only encouraged rapid proliferation but also ensured normal morphological development.

Conversely, treatments with a lower cytokinin-to-auxin ratio favoured root induction. The highest average root count was recorded on the medium containing 0.5 mg/L BAP and 1.0 mg/L NAA (Figure 1A), which is consistent with the well-documented role of auxins in promoting rhizogenesis via the activation of auxin-responsive genes (Cavallari et al., 2021). As detailed in Table 1, this treatment also achieved a 100% rooting frequency, confirming its high efficacy for root induction. The resulting well-developed roots are visible in Figure 1B.

Table 1. Effect of different BAP and NAA concentrations on root number, rooting percentage, shoot number, and shoot length after eight weeks of culture.

BAP (mg/L)	NAA (mg/L)	Mean Root Number	Rooting Percentage (%)	Mean number of shoots	Mean Length of Shoot (cm)
0.0	0.0	0.0	0.0	0.67 ± 0.58^{a}	0.43 ± 0.06^{a}
1.0	0.5	0.0	0.0	1.00 ± 0.00^{a}	2.30 ± 0.36^{a}
3.0	0.5	1.00 ± 0.58^{a}	33^{a}	1.67 ± 0.58^{a}	3.17 ± 0.15^{a}
5.0	0.5	1.00 ± 0.58^{a}	33^{a}	2.33 ± 0.58^{a}	4.47 ± 0.35^{a}
0.5	1.0	3.33 ± 0.58^{a}	100^{a}	1.00 ± 0.00^{a}	1.97 ± 0.20^{a}
0.5	3.0	1.67 ± 0.58^{a}	67^{a}	1.33 ± 0.58^{a}	1.90 ± 0.36^{a}
0.5	5.0	1.00 ± 0.58^{a}	33^{a}	0.0	0.0

Values are expressed as mean ± standard deviation (n=3). Means within columns followed by the same letter are not significantly different (*p*>0.05).

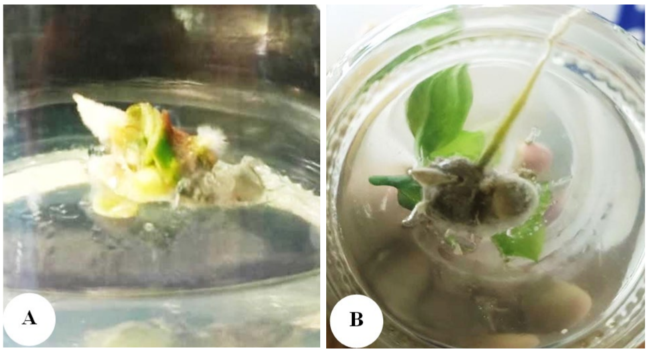

Figure 1: Root initiation of *C. sumatrana*. (A) Root initiation after week-1 of culture, showing the effect of 0.5 mg/L BAP and 1.0 mg/L NAA. (B) Root development after 8 weeks of culture. Scale bars = 1 cm.

While the numerical trends in our data were evident, a one-way ANOVA indicated no statistically significant differences between treatments ($p > 0.05$). This does not imply the treatments were ineffective; rather, it highlights several biological and experimental limitations. As a wild-sourced species, *C. sumatrana* likely possesses inherent genetic heterogeneity, which could lead to varied responses from individual explants. Furthermore, the use of rhizome bud explants, which already contain meristematic tissue, possesses a high inherent regenerative capacity. This capacity may diminish the observable impact of exogenous hormones, a common phenomenon in micropropagation using differentiated explants. The limited number of replicates (n=3) may also have prevented the statistical analysis from detecting significant differences, even when clear biological trends were present. Nevertheless, the consistent numerical superiority of the 5.0 mg/L BAP treatment provides a biologically meaningful trend that is highly relevant for practical micropropagation protocols.

The present study successfully identified an effective hormonal regimen for the direct organogenesis of *C. sumatrana*. A key advantage of this approach is the minimisation of the risk of somaclonal variation, genetic or epigenetic changes that can arise during prolonged *in vitro* culture (Krishna et al., 2016; Thorat et al., 2018). This is particularly crucial for conservation purposes, where genetic fidelity is paramount. However, certain limitations must be acknowledged. First, rooting did not occur during the multiplication phase, necessitating a separate rooting stage, which adds to the duration and complexity of the protocol. Second, while this method is effective in a laboratory setting, its scalability for commercial or large-scale conservation efforts will present a significant challenge.

Although micropropagation is often considered a costly method of propagation, its benefits for *C. sumatrana*, a rare and endangered species, can justify the high cost-benefit ratio. This technique allows for the rapid mass propagation of uniform and disease-free plant material, which would be difficult to achieve using conventional propagation via rhizomes. However, a major challenge lies in the scale-up process. The transition from a culture vessel to an external environment (acclimatisation) can lead to considerable losses if not properly optimised. Future research should therefore focus on (1) optimising the rooting and acclimatisation stages, (2) conducting a more detailed economic feasibility analysis, and (3) exploring alternative basal media and organic additives to enhance efficiency (Mishra et al., 2019).

3.2. Callus Induction

Beyond direct organogenesis, this study also explored the potential for indirect organogenesis through callus induction, a vital step for advanced biotechnological applications. Callus formation was successfully achieved using a combination of 6.75 mg/L 2,4-D and 2.2 mg/L BAP, which resulted in a compact, brown callus with a mean diameter of 0.95 ± 0.05 cm after 56 days (Figure 2B). As shown in Table 2, this treatment was statistically more effective than others ($p < 0.05$), demonstrating a clear synergistic interaction between the synthetic auxin 2,4-D and the cytokinin BAP.

Table 2: Effect of different combinations of auxins and cytokinins on callus induction and growth in *C. sumatrana* after 56 days of culture on MS medium.

Treatment (PGR + 6.75 mg/L 2,4-D)	BAP (mg/L)	KIN (mg/L)	NAA (mg/L)	Induction Response	Measurement of Callus Growth (cm)	Color of Callus	Texture of Callus
Control	0.0	0.0	0.0	–	–	–	–
MS + 2,4-D + BAP	2.2	0.0	0.0	+++	0.95 ± 0.05^a	Brown	Compact
MS + 2,4-D + KIN	0.0	4.7	0.0	+	0.25 ± 0.05^b	Brown	Compact
MS + 2,4-D + NAA	0.0	0.0	2.7	–	–	–	–

Note: + = low response, +++ = well-developed, - = no response. Values are mean ± standard deviation (n=3). Within the same column, means followed by different letters are significantly different at $p < 0.05$ (Tukey's test).

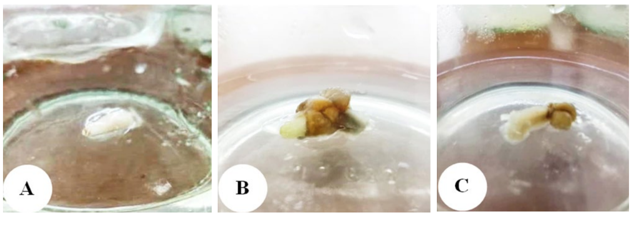

Figure 2: *In vitro* callus induction in *C. sumatrana* shoot bud explants after 56 days of culture. (A) No callus formation on hormone-free MS medium (control). (B) Compact, brown callus on MS medium supplemented with 6.75 mg/L 2,4-D and 2.2 mg/L BAP. (C) Sparse and underdeveloped callus on MS medium with 6.75 mg/L 2,4-D and 4.7 mg/L KIN. Scale bars = 1 cm.

The success of 2,4-D in inducing callus, in stark contrast to the failure of NAA, highlights a key difference in auxin potency and stability. 2,4-D is a potent synthetic auxin known for its ability to trigger cellular dedifferentiation and initiate uncontrolled cell division, making it the auxin of choice for callus induction in many plant species (Karami et al., 2023). Its resistance to degradation by endogenous enzymes allows it to accumulate to effective concentrations within the tissue (Goggin et al., 2016), whereas NAA exhibits higher metabolic lability. The morphology of the obtained callus, being both compact and friable, is particularly promising as it indicates a high degree of cellular organisation and mitotic activity, which are crucial for subsequent subculturing and regeneration. This condition also mitigated common issues such as excessive browning often encountered in Zingiberaceae cultures, a challenge successfully managed through the use of PPM™ (Plant Preservative Mixture).

The successful induction of callus in *C. sumatrana* is a significant achievement, given the known difficulties of *in vitro* culture in many Zingiberaceae species, which are often sensitive to hormones, produce toxic secondary metabolites, or experience severe browning. This success not only validates the effectiveness of the tested treatment but also establishes a valuable foundational protocol for conserving and bioprospecting other endangered or bioeconomically important Zingiberaceae species. However, it is essential to acknowledge some inherent limitations and challenges. Firstly, the use of indirect organogenesis through callus induction carries an inherent risk of somaclonal variation. The process of cellular dedifferentiation and redifferentiation can lead to genetic or epigenetic changes. While these changes can sometimes be beneficial for breeding programmes, they pose a serious problem in conservation efforts where genetic fidelity is paramount.

4. CONCLUSION

This study successfully established the first *in vitro* micropropagation protocol for *C. sumatrana*, a vulnerable endemic species. Our findings demonstrate that a specific hormonal balance is crucial for directing its morphogenesis. For direct organogenesis, the combination of 5.0 mg/L BAP and 0.5 mg/L NAA proved most effective for shoot proliferation, while 0.5 mg/L BAP and 1.0 mg/L NAA were optimal for root induction. Furthermore, the successful induction of a compact, viable callus using 6.75 mg/L 2,4-D and 2.2 mg/L BAP provides a critical foundation for advanced biotechnological applications.

Our work has significant implications for the conservation and sustainable production of *C. sumatrana*. By providing a reliable method for rapid clonal propagation, this protocol serves as a vital tool for *ex situ* conservation, helping to preserve the species' genetic material outside of its natural habitat. On a broader scale, the ability to propagate this plant efficiently and reliably offers a path toward sustainable bioproduction. Instead of relying on the unsustainable harvesting of wild populations, this technology enables the mass production of uniform, high-quality plantlets. This, in turn, can secure a consistent supply of valuable curcuminoids and other bioactive compounds for the medicinal and industrial sectors. The established callus line can also be further exploited for metabolic engineering and bioreactor-based production, transforming an academic finding into a viable, long-term commercial strategy.

While our findings lay a strong foundation, we recognize several inherent limitations and challenges that require further research. A critical practical consideration is the economic feasibility, as the high costs of *in vitro* production and the technical challenges of scaling up to commercial bioreactors need to be addressed. Furthermore, although direct organogenesis is generally associated with high genetic fidelity, the genetic stability of the regenerated plants has not yet been assessed, necessitating the use of molecular markers in future studies. Finally, the post-transplantation acclimatization survival rate of *in vitro*-derived plantlets was not evaluated. Optimizing hardening procedures is crucial for improving plantlet survival rates and ensuring the long-term success of this protocol. In essence, this research not only addresses an urgent conservation need but also provides a clear technological solution for the sustainable utilization of this rare and valuable species.

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