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Research article

Development of efficient micropropagation protocol of *Dendrocalamus* sp. using temporary immersion bioreactor system for commercial large-scale production

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Abstract

Importance of the work: *Dendrocalamus* sp. is a valuable Thai bamboo with high demand. Micropropagation ensures rapid, efficient plantlet production to fulfill supply requirements.

Objectives: To optimize *Dendrocalamus* sp. micropropagation using temporary immersion bioreactor (TIB) technology and plantlet acclimatization.

Materials and Methods: *In vitro* techniques were used to develop a micropropagation protocol. Various Murashige and Skoog (MS) medium treatments with different plant growth regulator combinations were tested to determine the optimal concentrations for explant growth.

Results: Semi-solid MS medium with 3 mg/L 6-benzylaminopurine (BAP) and 2 mg/L α-naphthalene acetic acid (NAA) produced the highest shoot induction, averaging 24.66 shoots per explant. In addition, the TIB was used for increasing production. The method showed that a feeding frequency of 2 hr in the same medium containing 500 mL to initiate 10 explants produced the significantly highest number of shoots (72.33 shoots per bottle) of all the tested conditions. The survival rate was 99% across all substrates for both the greenhouse and field conditions. Based on morphological studies, there were no differences between the donor mother plants and the regenerated plants. These findings highlighted the effectiveness of this micropropagation protocol for large-scale bamboo production, making it a reliable method for commercial propagation of *Dendrocalamus* sp.

Main finding: MS medium with 3 mg/L BAP and 2 mg/L NAA optimized *Dendrocalamus* sp. growth, ensuring 99% survival and no morphological changes. This procedure enhanced propagation efficiency, producing 1,382 times more plants than stem cutting for large-scale cultivation.

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Introduction

Bamboo is a highly prized resource in Asian, African and South American civilizations, having been utilized traditionally for food, building materials, firewood and small cottage industries, with its uses being expanded to include textiles production, engineered products, pulp and paper (Sood et al., 2002). The increasing global demand for bamboo has rendered traditional propagation techniques inadequate due to their slowness and low yield, resulting in scientific propagation methods gaining prominence (Nadgauda et al., 1997; United Nations Conference on Trade and Development, 2022). *Dendrocalamus* sp., or economic bamboo, is in the family Gramineae and it is endemic to northern Thai provinces (Chiang Mai, Phrae, Lampang and Lamphun), as well as other places. In Thailand, these economic bamboo species are referred to locally as 'sang mon bamboo' due to their strong, dense culms. Thus, it is highly sought after by industry, particularly for the manufacture of furniture, parquet flooring, chopsticks and skewers (Kumar and Mandel, 2022).

Nevertheless, in industrial economies, the rising dependence on bamboo has brought about severe over-harvesting and rapid natural bamboo resource depletion. For example, it is projected that a severe lack of planting material in the future would be brought about by the current trends unless an alternate source is pursued (Nadgauda et al., 1990; Mustafa et al., 2023). Micropropagation via tissue culture has become extremely popular as a sustainable approach for solving this problem. This method is commonly termed as overcoming most of the deficits in traditional bamboo propagation. Large-scale propagation and field establishment of several species of bamboo have been proven by tissue culture techniques and some research, as is clear from commercial exports and international practice (Gielis and Oprins, 2002; Ramanayake et al., 2006; Cardoso et al., 2018).

Tissue culture technology is a notable plant propagation method, which has a considerable advantage in that it produces a large number of plants within a limited period of time compared to other conventional techniques such as bud or stem cutting (Preeti and Nikita, 2018; Waraporn et al., 2022). In recent years, it has also become a new hope for the mass production of different plant species (Klaocheed et al., 2024a; Klaocheed et al., 2024b; Wongchaya et al., 2024). Temporary immersion bioreactor (TIB) technology is one of the main advancements in this area. Through facilitating liquid culture media to circulate effectively, TIB provides an even contact between plant tissues and nutrients, leading to rapid and even development (Zhang et al., 2018). With the aim of boosting propagation efficiency, the TIB system is suitable for commercial scale and can be tailored to be used in commercial production (Pérez et al., 2013).

Although there are benefits with liquid culture systems, there have been several limitations observed, including asphyxia, hyperhydricity and other physiological disorders caused by the permanent submersion of plant tissues (Gao et al., 2018; Schuchovski et al., 2020). Of these, the most common physiological disorder of *in vitro* plant culture is hyperhydricity, or vitrification, commonly referred to as glassiness (Kevers et al., 2004). Hyperhydricity manifests in the form of waterlogged, pale leaves by lowering chlorophyll content and an intracellular overabundance of water (Cassells and Curry, 2001). Hyperhydricity interferes with how plants normally function by reducing the activity of stomata and decreasing the pressure inside their cells. Hyperhydric plantlets thereby have catastrophic survival rates when shifted from *in vitro* to *ex vitro* conditions and their survival may even drop to zero in a few cases (Kei-ichiro et al., 1998).

TIB technology provides an advanced alternative to the limitations of continuous liquid culture, specifically in the prevention of hyperhydricity. With this technology, plantlets are only immersed in the liquid medium for brief, scheduled periods to take in nutrients and experience subsequent times of aeration or drying, greatly limiting physiological disorders such as hyperhydricity (Frometa et al., 2017). At the immersion stage, the plants absorb necessary nutrients and later aeration allows for the uptake of oxygen and carbon dioxide—gases essential in respiration and photosynthesis (Etienne and Berthouly, 2002). Contemporary bioreactor systems with pressure and flow controllers can also further improve gas exchange to maximize plant growth and shoot multiplication. Proper regulation of concentrations of oxygen and carbon dioxide are essential, since these gases directly influence photosynthetic rates and metabolic processes, thereby promoting overall plantlet growth (Mirzabe et al., 2022).

Research into propagation methods needs ongoing urgent attention to unlock the true potential of bamboo as a renewable resource. For many years now, tissue culture propagation has been known to be central to meeting the world's increasing demand for bamboo plantlets (Mudoi et al., 2013). Hence, background studies on bamboo tissue culture

are necessary to provide consistent yields of quality plantlets. Of the propagation methods available, micropropagation continues to be the most scalable and sustainable mass production method. It is still widely used because it is efficient and reliable in producing multiple uniform, healthy plants (Dar et al., 2021; Marueng et al., 2021; Wongchaya et al., 2024).

Thus, the current research aimed to establish a method of *in vitro* micropropagation of *Dendrocalamus* sp. Involving the exploration of the role of different concentrations and combinations of plant growth regulators (PGRs) on explant growth based on greenhouse and *ex vitro* survival of the plantlets formed in different substrate materials. The results might lead to a suggested micropropagation method that provides a rapid, efficient and affordable way to produce *Dendrocalamus* sp. on a large scale, meeting the growing need for this important bamboo species.

Materials and Methods

The *Dendrocalamus* species (Sang Mon bamboo) used was obtained from the Scientific Instrument and Product Standard Quality Inspection Center at the University of Phayao, Thailand and served as the donor plant material. The selected plant was confirmed to be disease-free and was maintained in a controlled environment room, with the temperature regulated at 25 ± 2°C.

Plant material and explant preparation

Nodal segments from the donor *Dendrocalamus* sp. were used to initiate tissue culture. The culture medium was prepared using Murashige and Skoog (MS) semi-solid medium supplemented with 30 g/L sucrose and 8 g/L agar powder, with the pH adjusted to 5.6–5.8. The medium was sterilized using autoclaving at 121°C for 30 min (Lee et al., 2019). Explants were surface disinfected using 0.1% mercuric chloride ($HgCl_2$) for 10 min, followed by three thorough rinses with sterile distilled water. Then, the disinfected nodal segments were cultured on MS medium for 1 wk. Only explants that remained free of contamination or disease symptoms were selected for subsequent experiments.

Effect of plant growth regulators on shoot and root formation

The effects were investigated of different concentrations and combinations of plant growth regulators (PGRs) on *in vitro* shoot multiplication. Specifically, MS medium was supplemented with 1–3 mg/L of 6-benzylaminopurine (BAP) and 0.5–2 mg/L α-naphthalene acetic acid (NAA). In total, 20 replicates were used, with one sterile nodal segment inoculated per culture bottle containing the prepared medium. All cultures were maintained at 25 ± 2°C under a 16 hr/8 hr light/darkness photoperiod, with a photosynthetic photon flux density of 40 $\mu mol/m^2/s^1$. After 8 wk of cultivation, data were recorded on the number of explants forming multiple shoots and roots, as well as shoot and root lengths.

Study using temporary immersion bioreactor conditions

In a separate experiment, the effects were evaluated of different medium volumes and feeding frequencies on durations in the TIB system on the growth of the *Dendrocalamus* sp. plantlets (Table 1). The explants were exposed to MS medium supplemented with 3 mg/L BAP and 2 mg/L NAA, using feed volumes in the range 250–1,000 mL and feeding frequencies of the medium for 5 min every 2 hr, 3 hr or 4 hr. After cultivation for 8 wk, growth parameters were measured (number of shoots, shoot height, number of leaves, leaf length and leaf width). Data were analyzed using the least significant difference test at a 95% confidence level, with statistical significance set at $p < 0.05$.

Table 1 Experimental design to evaluate the effects of volume of culture medium and feeding frequencies on duration in temporary immersion bioreactor.

Treatment	Volume of culture medium (mL)	Feeding frequency
T1	250	Every 2 hr
T2	250	Every 3 hr
T3	250	Every 4 hr
T4	500	Every 2 hr
T5	500	Every 3 hr
T6	500	Every 4 hr
T7	1,000	Every 2 hr
T8	1,000	Every 3 hr
T9	1,000	Every 4 hr

T1, T2 and T3 contained 250 mL of culture medium with feeding frequencies of every 2 hr, 3 hr and 4 hr, respectively; T4, T5 and T6 consisted of 500 mL of culture medium with feeding frequencies of every 2 hr, 3 hr and 4 hr, respectively; and T7, T8 and T9 consisted of 1,000 mL of culture medium with feeding frequencies of every 2 hr, 3 hr and 4 h, respectively.

Each treatment was repeated three times, with each repetition using 10 explants.

Survival rate and acclimatization of plantlets

After 1 mth of culture in the PGR-supplemented medium, the plantlets were transferred to half-strength MS medium without any PGRs for rooting. Plantlets that reached approximately 6 cm in height and developed well-established roots were selected for further acclimatization. The plantlets were cleaned gently and acclimatized following the protocol described by Suwal et al. (2020). Then, they were transplanted into a growing substrate composed of peat moss and coconut coir in a 1-to-1 ratio (weight per weight). In total, 20 replicates were maintained at room temperature (25 ± 2°C) under greenhouse conditions. After 4 wk, the survival rate of the plantlets was recorded. Subsequently, the successfully acclimatized plantlets were transplanted to their final planting site in the designated area of the Wianglor Wildlife Sanctuary, Phayao Province (Fig. 1)

Statistical analysis

Data were analyzed using analysis of variance, and means were compared using Duncan's new multiple range test at the 95% confidence level.

Results and Discussion

Effect of plant growth regulators on shoot and root formation

The plant growth regulators BAP (a cytokinin) and NAA (an auxin) are essential for promoting plant development both in natural and *in vitro* conditions (Dar et al., 2021). In the current study, micropropagation of *Dendrocalamus* sp. was successfully achieved on a semi-solid MS medium

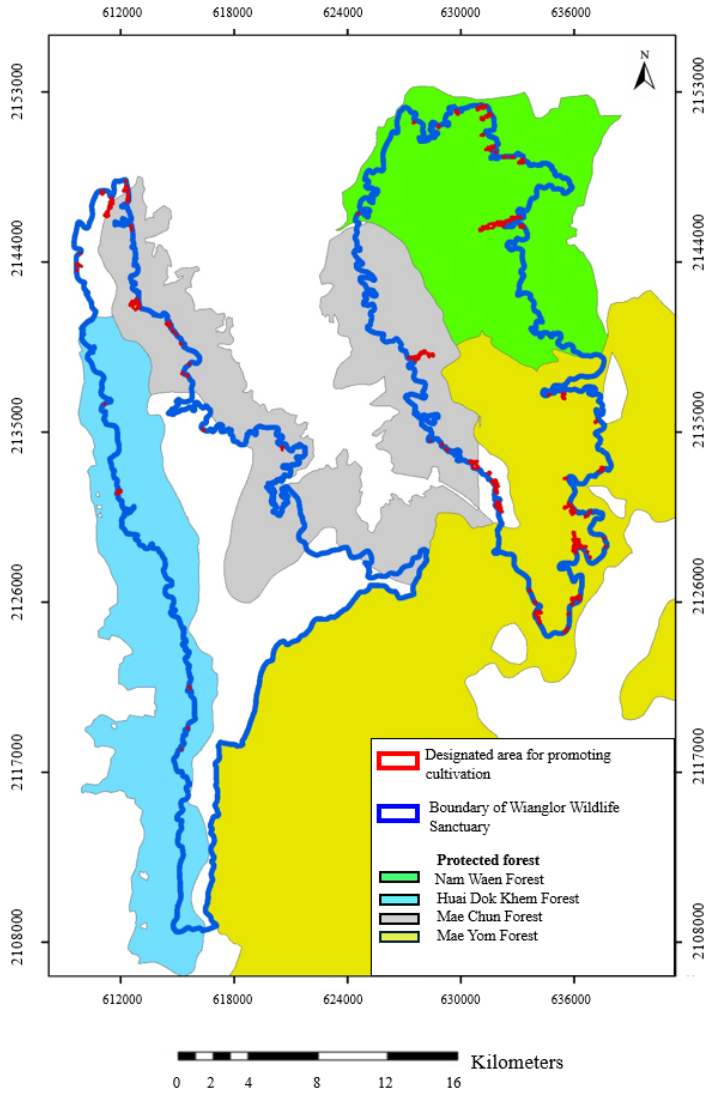

Fig . 1 Wianglor Wildlife Sanctuary in Phayao Province, with 40 ha (marked in red) allocated for bamboo cultivation

supplemented with 30 g/L sucrose, 8 g/L agar and varying concentrations of BAP and NAA. Based on the results, there were significant differences in shoot multiplication depending on the concentration of these PGRs. Combinations with higher concentrations of BAP and NAA yielded superior shoot induction compared to lower concentrations. Specifically, the combination of 3 mg/L BAP and 2 mg/L NAA produced the highest number of shoots, with an average of 24.66 shoots per explant. In addition, this treatment resulted in an average shoot length of 8.33 cm and 6.66 roots per explant after 8 wk of culture (Table 2). Microscopic observations under a stereomicroscope (SZ61; Olympus; Tokyo, Japan; 0.6× magnification) using the Epview software (EVIDENT; Waltham, MA, USA) further confirmed that axillary buds cultured on MS medium with PGRs began forming multiple shoots within 2 wk (Fig. 2A), whereas no such development was observed in explants cultured on hormone-free MS medium (Fig. 2B)

BAP, a cytokinin-type plant growth regulator, plays a crucial role in promoting cell division, stimulating shoot proliferation and enhancing shoot bud formation (Ario & Setiawan, 2020). In bamboo micropropagation, PGRs are commonly incorporated into MS medium to optimize shoot induction. For example, Venkatachalam et al. (2015) reported that a combination of 3 mg/L BAP and 0.5 mg/L IBA resulted in an average of 24.2 shoots per explant under optimized conditions.

Auxins are essential hormones that regulate a wide range of developmental processes in plant tissue culture. Unique among plant hormones, auxins exhibit polar transport, form concentration gradients and play a central role in determining cell fate (Pasternak and Steinmacher, 2024). As such, they are critical for both shoot and root morphogenesis. Local auxin biosynthesis within plant tissues has been shown to regulate various morphogenic processes (Brumos et al., 2018; Zhao, 2018; Lv et al., 2019). The most frequently applied auxins commonly used in regeneration studies are 2,4-dichlorophenoxyacetic acid (2,4-D) and α-naphthalene acetic acid (NAA) (Subotić et al., 2010; Petrić et al., 2015; Hazubska-Przybył et al., 2020; Zdravković-Korać et al., 2022). Cytokinins, when used in combination with auxins, enhance cell division and influence cell differentiation and are widely used in tissue culture to induce morphogenesis, promote the formation of axillary and adventitious shoots and stimulate cell proliferation from organs and callus tissues (Sugimoto and Meyerowitz, 2013). The most commonly used adenine-derived cytokinins in plant tissue culture include BAP, zeatin [6-(4-hydroxy-3-methylbut-2-enylamino) purine], kinetin (6-furfurylaminopurine) and isopentenyl adenine (2-iP) (Palee, 2018; Machua1 et al., 2024).

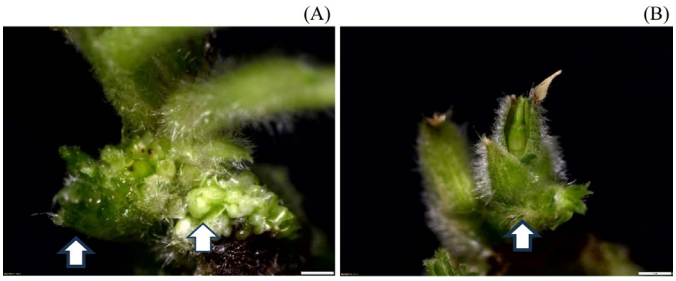

Fig. 2 Multiple shoot development of *Dendrocalamus* sp. on semi-solid Murashige and Skoog (MS) culture medium: (A) *in vitro* shoot formation from axillary buds on MS medium supplemented with 3 mg/L BAP and 2 mg/L NAA, with arrow showing multiple shoot induction after 2 wk of culture; (B) MS medium without hormone supplementation, showing no multiple shoot development. Scale bars = 1 mm.

Table 2 Average shoot and root numbers with treatments of Murashige and Skoog semi-solid medium supplemented with different combinations of plant growth regulators (PGRs)

PGR concentrations (mg/L)		Number of shoots per explant	Shoot length (cm)	Number of roots per explant	Root length (cm)
BAP	NAA				
0	0	2.00 ± 1.00 ^{e}	6.33 ± 0.35 ^{ab}	1.00 ± 1.00 ^{c}	1.77 ± 0.57 ^{a}
1	0.5	14.66 ± 2.08 ^{bc}	5.00 ± 1.00 ^{b}	5.33 ± 2.30 ^{ab}	2.33 ± 0.57 ^{a}
1	1	12.33 ± 2.51 ^{cd}	6.33 ± 0.57 ^{ab}	6.00 ± 2.00 ^{ab}	3.33 ± 0.57 ^{a}
1	2	10.66 ± 1.15 ^{c}	6.66 ± 1.52 ^{ab}	6.00 ± 2.64 ^{ab}	3.00 ± 2.00 ^{a}
2	0.5	11.33 ± 2.51 ^{cd}	7.33 ± 1.15 ^{ab}	5.00 ± 0.00 ^{b}	3.66 ± 2.08 ^{a}
2	1	9.33 ± 0.57 ^{c}	8.00 ± 1.00 ^{a}	7.00 ± 1.00 ^{b}	4.66 ± 1.15 ^{a}
2	2	12.00 ± 2.00 ^{cd}	7.33 ± 0.57 ^{ab}	5.00 ± 1.00 ^{b}	3.33 ± 2.08 ^{a}
3	0.5	18.00 ± 1.00 ^{b}	7.00 ± 1.00 ^{ab}	6.66 ± 0.57 ^{ab}	4.33 ± 2.51 ^{a}
3	1	16.33 ± 1.15 ^{b}	7.66 ± 2.08 ^{a}	8.33 ± 0.57 ^{a}	1.66 ± 0.57 ^{a}
3	2	24.66 ± 2.51 ^{a}	8.33 ± 1.52 ^{a}	6.66 ± 2.3^{0} ^{a}b	2.33 ± 0.57 ^{a}

Each treatment was repeated three times, with each repetition using 10 explants.

Values (mean ±SD) within each column with different lowercase superscripts are significantly different ($p < 0.05$).

In the current study, the micropropagation of *Dendrocalamus* sp. using semi-solid MS medium resulted in high numbers of shoot formation. The MS medium supplemented with 3 mg/L BAP induced 100% shoot formation, consistent with other reports highlighting the effectiveness of hormone-like substances in promoting shoot induction in *Dendrocalamus* sp. (Yasamut et al., 2022). Among the tested concentrations of BAP (1–3 mg/L) and NAA (0.5–2 mg/L), the combination of 3 mg/L BAP and 2 mg/L NAA yielded the highest shoot proliferation. Additionally, treatments containing PGRs produced a significantly greater number of roots than the control group, although no significant differences were observed in root length. The use of BAP—either alone or in combination with kinetin or NAA—in the basal MS medium has also been reported to enhance shoot multiplication in various bamboo species (Bag et al., 2000; Bag, 2001; Jimenez et al., 2006; Mahadtanapuk et al., 2006; Putalun et al., 2010; Perez et al., 2013).

Study of temporary immersion bioreactor conditions

Based on the results, the *Dendrocalamus* sp. Tissue samples produced optimal growth when feed every 2 hr in 500 mL of MS medium supplemented with 3 mg/L BAP and 2 mg/L NAA, using 10 explants per bioreactor bottle. Under these conditions, the significantly highest number of shoots was an average of 72.33 shoots per bottle and a mean shoot height of 18.00 cm. However, the greatest number of leaves and roots was recorded under different conditions—feeding with 250 mL of medium every 2 hr—which resulted in 34.66 leaves and 35.33 roots per bottle, respectively (Fig. 3)

Frequent feeding using the TIB system allowed the explants to be periodically immersed in liquid medium, ensuring uniform nutrient absorption by all plant tissues, thereby enhancing growth and multiplication (Teisson and Alvard, 1995). This finding was consistent with the study by Singhanukunkit (2022), who reported that feeding *Dendrocalamus* sp. every 6 hr in a TIB system produced the highest number of new shoots (3.33 shoots per explant) and the tallest shoot height (7.33 cm). Similarly, Gutiérrez et al. (2016) found that *Guadua angustifolia* (Nine Star Bamboo) cultured in a RITA® TIB system with 6 hr feeding intervals produced the greatest shoot and leaf development per explant. The finding from these studies suggested that optimizing the frequency and duration of the nutrient immersion significantly enhanced bamboo shoot proliferation. Consistent with García-Ramírez et al. (2014), who reported superior shoot multiplication in *Bambusa vulgaris* (black bamboo) using TIB compared to semi-solid medium, it can be supported that TIB systems offer a more effective platform for large-scale bamboo propagation.

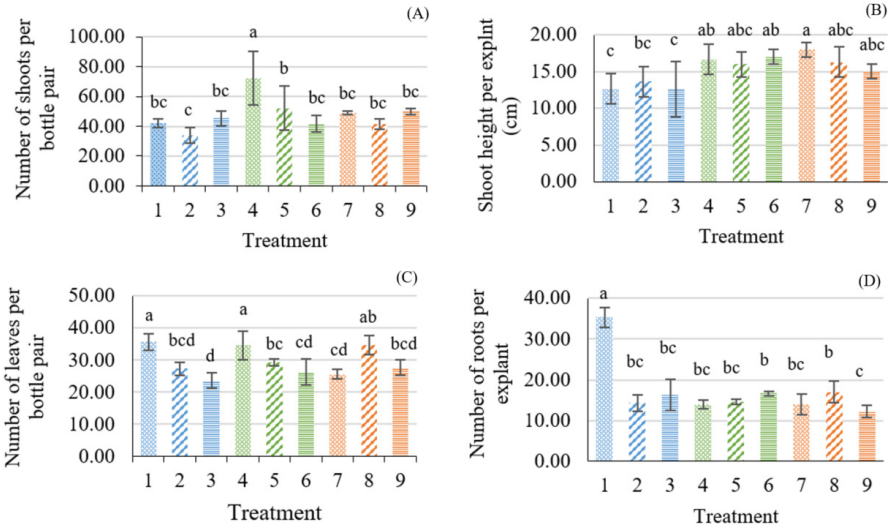

Fig. 3 Effects of culture medium volume and feeding frequency on (A) average number of shoots per bottle pair, (B) shoot height, (C) number of leaves, and (D) number of roots in *Dendrocalamus* sp. after 8 wk of culture in a temporary immersion bioreactor system. Error bars indicate mean ± SD. Different lowercase letters above columns indicate significant differences ($p < 0.05$). Treatments T1–T3 = 250 mL medium with feeding every 2, 3, and 4 hr, respectively; T4 –T6 = 500 mL medium with feeding every 2, 3, and 4 hr, respectively, respectively; T7–T9 = 1,000 mL medium with feeding every 2, 3, and 4 hr, respectively.

Survival and acclimatization of plantlets

In total, 20 plantlets cultured on semi-solid MS medium, along with donor plants, were transferred to a nursery following the hardening protocol described by Marulanda et al. (2005) (Fig.4) There were no significant morphological differences between the tissue culture-derived plantlets and the donor plants during acclimatization. DNA-based molecular analysis using polymerase chain reaction amplification was performed to assess genetic uniformity. All primers produced clear bands in the range 200–3000 bp and no polymorphic bands were observed among the tissue culture-derived samples and donor plant controls, including those amplified with the OPA11 primer (data not shown). These findings confirmed the genetic stability of the regenerated plants and supported the reliability of the micropropagation protocol used for the *Dendrocalamus* sp. Furthermore, despite the influence of physiological, morphological and environmental factors on plant growth, the results of this study demonstrated the protocol's potential for future large-scale propagation, achieving a 99% survival rate under both greenhouse and field conditions (data not shown).

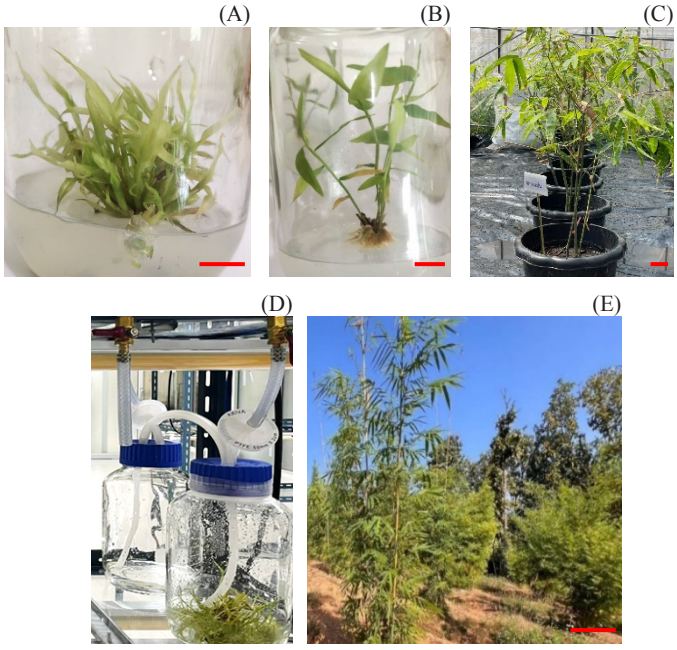

Fig. 4 Micropropagation and acclimatization stages of *Dendrocalamus* sp.: (A) shoot multiplication on semi-solid Murashige and Skoog (MS) medium supplemented with 3 mg/L BAP and 2 mg/L NAA (scale bar = 1 cm); (B) root induction on half-strength MS medium without plant growth regulators (scale bar = 1 cm); (C) plantlets acclimatized under greenhouse conditions (scale bar = 10 cm); (D) temporary immersion bioreactor system used for *in vitro* propagation; (E) transplanted *Dendrocalamus* sp. in field conditions at Wianglor Wildlife Sanctuary (scale bar = 50 cm)

Other studies have reported survival rates of over 70% for various bamboo species during the hardening phase (Gielis and Oprins, 2002; Sharma et al., 2022). In the current study, the *Dendrocalamus* sp. plantlets aged 1 yr from all treatments were successfully transferred to field conditions at the Wianglor Wildlife Sanctuary, under the management of the Department of National Parks, Wildlife and Plant Conservation in Phayao Province, Thailand. The planting site was in a dry, degraded forest area covering approximately 287 ha, of which 40 ha have been designated specifically for bamboo cultivation. Plant growth and wood yield were monitored to assess the suitability of the tissue culture-derived plantlets for reforestation purposes. Given the scale of the reforestation effort and the need for a substantial number of bamboo plants, the protocol developed in the current study should have considerable practical value by offering a viable solution for both commercial bamboo production and forest restoration initiatives.

Based on the results the semi-solid tissue culture method produced 1,382 times more *Dendrocalamus* sp. plantlets than the conventional stem cutting technique. In addition, the tissue culture approach required only 4 mth—two subculture cycles less than the time needed for stem cutting—to achieve mature, transplantable bamboo plants. A comparison of the two propagation methods is illustrated in Fig. 5 The stem cutting method involves the selection of donor plants, explant preparation, root induction and acclimatization, resulting in the production of approximately 10 bamboo plants per cycle. In contrast, the tissue culture technique involves the surface sterilization of the seeds or explants, shoot and root induction, shoot elongation and acclimatization, ultimately yielding approximately 13,824 plantlets per cycle. This highlights the considerably higher efficiency and scalability of tissue culture for bamboo propagation.

Furthermore, the coordination between *in vitro* culture conditions and the environment-controlled room is critical, as plantlets exhibiting vigor *in vitro* must also demonstrate robust growth during acclimatization under controlled *ex vitro* conditions (Sharma et al., 2022). For example, the cultivation of *Anubias barteri* broadleaf shoots in a TIB system significantly outperformed semi-solid medium cultures, with notable improvements in seedling count, leaf number, shoot height, fresh weight and dry weight. Then, upon transfer to *ex vitro* environments, the plantlets derived from the TIB system achieved a 100% survival rate, likely due to their enhanced vigor and absence of hyperhydricity, allowing for effective acclimatization (Teanchartsakul et al., 2019).

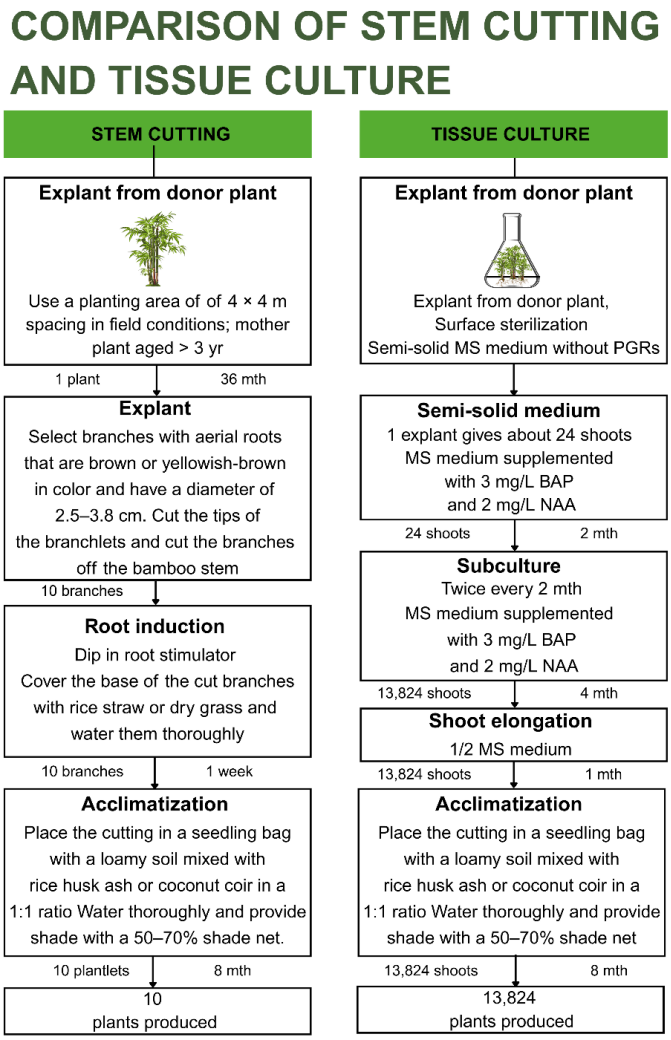

Fig. 5 Schematic representation of tissue culture in semi-solid medium and stem cutting propagation, where BAP = 6-benzylaminopurine, MS = Murashige & Skoog medium, NAA = naphthaleneacetic acid, PSG = Penicillin–Streptomycin–Glutamine.

Furthermore, plants propagated in TIB systems have been shown to exhibit improved physiological functions—including enhanced photosynthesis, respiration, chlorophyll synthesis and stomatal regulation—which contribute to their successful adaptation during the acclimatization phase (Aragón et al., 2014).

Bamboo plants with strong root systems and well-developed branches can be propagated using cuttings to produce numerous genetically identical offspring (Suwal et al., 2020). However, TIB systems enable much faster and more efficient propagation than conventional methods (Etienne and Berthouly, 2002). Gómez-Cruz et al. (2024) reported that by the third production cycle using TIB, the unit cost per CP-Jacona mother plant was lower than that of imported *Festival* or *Camarosa* strawberry varieties. This reduction in cost was attributed to the high proliferation rate and the minimal loss during handling in the TIB system, making it a more economical alternative. Similarly, Pożoga et al. (2024) found that TIB was more cost-effective than agar-based cultures, primarily due to reduced labor requirements. In agar-based systems, labor accounted for 43% of total production costs, followed by materials and reagents, which represented 25%. These findings supported the economic advantages of adopting TIB for large-scale plant propagation, including bamboo.

In the TIB system, production materials and reagents account for the largest share of total costs, followed by labor. Despite these input costs, TIB enables considerably faster plant multiplication compared to agar-based cultures. In fact, plants produced during the multiplication phase using TIB are approximately twice as cost-effective as those generated through agar culture . While rooting contributes a substantial portion of production costs in both systems, TIB has demonstrated superior overall efficiency and cost-effectiveness (Pożoga et al., 2024). In the current study, the estimated cost of producing *Dendrocalamus* sp. via semi-solid medium culture was approximately THB 5.88 per plant (USD 1 = THB 32), compared to THB 3.02 per plant using the TIB system. In contrast, the cost of propagation via stem cutting was around THB 3.00 per plant (data not shown). However, stem cutting is limited by its low propagation rate, as a single mother plant can typically yield only 5–10 branches (Fig. 5) Tissue culture, particularly via the TIB system, offers a more time-efficient and scalable solution, making it highly suitable for meeting the large-scale propagation demands of the bamboo industry.

Conclusions

An efficient micropropagation protocol was presented for *Dendrocalamus* sp., demonstrating the effectiveness of specific PGR combinations and culture systems. Shoot multiplication was induced successfully, with a maximum average of 24.66 shoots per explant observed on semi-solid MS medium supplemented with 3 mg/L BAP and 2 mg/L NAA after 8 wk of culture. The TIB system enhanced production. The optimal condition—feeding every 2 hr with 500 mL of MS medium and 10 explants per bottle—resulted in a significantly higher shoot yield, reaching 72.33 shoots per bottle pair. Furthermore, the protocol achieved a 99% survival rate across all substrate types under both greenhouse and field conditions.

No morphological differences were observed between regenerated plantlets and donor stock plants, confirming the genetic and phenotypic stability of the propagated material. This protocol offers a scalable, cost-effective solution for large-scale propagation and reforestation of *Dendrocalamus* sp.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Aragón, C.E., Sánchez, C., Gonzalez-Olmedo, J., Escalona, M., Carvalho, L., Amâncio, S. 2014. Comparison of plantain plantlets propagated in temporary immersion bioreactors and gelled medium during *in vitro* growth and acclimatization . Biol. Plant. 58: 29–38. doi:10.1007/s10535-013-0381-6

Ario, A., Setiawan, S. 2020. The effect of benzyl amino purine (BAP) concentration on the growth amount of the explant of *Dendrobium spectabile* orchid by *in-vitro*. Int. J. Multi Discip. Sci. 3: 33–38. doi.org/10.26737/ij-mds.v3i2.2397

Bag, N. 2001. Mass propagation of tea, maggar bamboo and dev ringal. Ph.D. Thesis. Hemvati Nandan Bahuguna Garhwal University. Uttarakhand, India.

Bag, N., Chandra, S., Palni, L.M., Nandi, S.K. 2000. Micropropagation of dev-ringal. Plant Sci. 156: 125–135. doi: 10.1016/s0168-9452(00)00212-0

Brumos, J., Robles, L.M., Yun, J., Vu, T.C., Jackson, S., Alonso, J.M., Stepanova, A.N. 2018. Local auxin biosynthesis is a key regulator of plant development. Dev. Cell. 47: 306–318. doi.org/10.1016/j.devcel.2018.09.022

Cardoso, J.C., Gerald, L.T.S, da Silva, J.A.T. 2018. Micropropagation in the Twenty-First Century, 4th Ed. In: Loyola-Vargas, V., Ochoa-Alejo, N. (Eds.). Plant Cell Culture Protocols (Methods in Molecular Biology, 1815). Springer. New York, NY, USA. pp. 17–46.

Cassells, A., Curry, R. 2001. Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers. Plant Cell Tiss. and Org. 64: 145-157. doi:10.1023/A:1010692104861

Dar, S.A., Nawchoo, I.A., Tyub, S., Kamili, A.N. 2021. Effect of plant growth regulators on *in vitro* induction and maintenance of callus from leaf and root explants of *Atropa acuminata* Royle ex Lindl. Biotechnol. Rep. 32: e00688. doi.org/10.1016/j.btre.2021.e00688

Etienne, H., Berthouly, M. 2002. Temporary immersion systems in plant micropropagation. Plant Cell Tissue Organ Cult. 69: 215–231. doi:10.1023/A:1015668610465

Frometa, O.M., Morgado, M.M.E., Silva, J.A.T., Morgado, D.T.P., Gradaille, M.A.D. 2017. *In vitro* propagation of *Gerbera jamesonii* Bolus ex Hooker f. in a temporary immersion bioreactor. Plant Cell Tissue Organ Cult. 129: 543–551. doi:10.1007/s11240-017-1186-7

Gao, H., Li, J., Ji, H., An, L., Xia, X. 2018. Hyperhydricity-induced ultrastructural and physiological changes in blueberry (*Vaccinium* spp.). Plant Cell Tissue Organ Cult. 133: 65–76. doi: 10.1007/s11240-017-1361-x

García-Ramírez, Y., Gonzáles, M., Mendoza, E., Seijo, M., Cárdenas, M., Moreno-Bermúdez, L., Ribalta, O. 2014. Effect of BA treatments on morphology and physiology of proliferated shoots of *Bambusa vulgaris* Schrad. Ex Wendl in temporary immersion. Am. J. Plant Sci. 5: 205–211. doi.org/10.4236/ajps.2014.52027

Gielis, J., Oprins, J. 2002. Micropropagation of temperate and tropical woody bamboos-from biotechnological dream to commercial reality. Bamboo for sustainable development. In: Proceedings of the Vth International Bamboo Congress and the Vth International Bamboo Workshop. San Jose, Costa Rica. pp. 333–344.

Gómez-Cruz, M. A., Arana-Coronado, J. J., Arellano-Ostoa, G., Omaña-Silvestre, J. M., García-Sánchez, R. C. 2024. Cost efficiency in the production of strawberry mother plants (CP Jacona) using temporary immersion systems versus traditional systems. Agro Productividad. 17: 109–117. doi.org/10.22004/ag.econ.347917

Gutiérrez, L.G., López-Franco, R., Morales-Pinzón, T. 2016. Micropropagation of Guadua. Afr. J. Biotechnol. 15: 1503-1510. doi.org/10.5897/AJB2016.15390

Hazubska-Przybý, T., Ratajczak, E., Obarska, A., Pers-Kamczyc, E. 2020. Different roles of auxins in somatic embryogenesis efficiency in two *Picea* species. Int. J. Mol. Sci. 21: 3394. doi.org/10.3390/ijms21093394

Jimenez, V.M., Castillo, J., Tavares, E., Guevara, E., Montiel, M. 2006. *In vitro* propagation of the neotropical giant bamboo, *Guadua angustifolia* Kunth, through axillary shoot proliferation. Plant Cell Tissue Organ Cult. 86: 389–395. doi.org/10.1007/s11240-006-9120-4

Kei-ichiro, U, Susan, C., Kalidas, S. 1998. Reduced hyperhydricity and enhanced growth of tissue culture-generated raspberry (*Rubus* sp.) clonal lines by *Pseudomonas* sp. isolated from oregano. Process Biochem. 33: 441-445. doi.org/10.1016/S0032-9592(98)00011-9

Kevers, C., Franck, T., Strasser, R.J., Dommes, S., Gaspar, T. 2004. Hyperhydricity of micropropagated shoots: A typically stress-induced change of physiological state. Plant Cell Tissue Organ Cult. 77: 181–191. doi: 10.1023/B:TICU.0000016825.18930.e4.

Klaocheed, S., Rittirat, S., Kalawong, S., Thammasiri, K. 2024a. Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Lobelia cardinalis* L., an ornamental aquatic plant. Trends Sci. 21: 7690. doi.org/10.48048/tis.2024.7690

Klaocheed, S., Rittirat, S., Kalawong, S., Thammasiri, K. 2024b. Maximization of micropropagule production in an attractive ornamental plant, *Oxalis triangularis* A.st.-Hil. Trends Sci. 21: 7917. doi.org/10.48048/tis.2024.7917

Kumar, D., Mandel, A. 2022. Review on manufacturing and fundamental aspects of laminated bamboo products for structural applications. Constr. Build. Mater. 348: 128691. doi.org/10.1016/j.conbuildmat.2022.128691

Lee, T.J., Zobayed, S.M.A., Firmani, F., Park, E.J. 2019. A novel automated transplanting system for plant tissue culture. Biosyst. Eng. 182: 122–135. doi.org/10.1016/j.biosystemseng.2019.02.012

Lv, B., Yan, Z., Tian, H., Zhang, X., Ding, Z. 2019. Local auxin biosynthesis mediates plant growth and development. Trends Plant Sci. 24: 6–9. doi.org/10.1016/j.tplants.2018.10.014

Machua1, J., Kimani1, P., Karani1, S., et al. 2024. Technical Paper Technical Manual on Tissue Culture Protocol for Bamboo. International Bamboo and Rattan Organization. Beijing, China.

Mahadtanapuk, S., Topoonyanont, N., Handa, T., Sanguansermsri, M., Anuntalabhochai, S. 2006. Genetic transformation of *Curcuma alismatifolia* Gagnep. using retarded shoots. Plant Biotechnol. 23: 233–237. doi.org/10.5511/plantbiotechnology.23.233

Marueng, V., Tanawat, T., Pakdeelun, W., Klung–ngoen, W. 2021. Bamboo value chain analysis in Thailand. https://www.tei.or.th/file/Library/2021-bamboo-eng_58.pdf, 24 May 2024.

Marulanda, M.M., Gutierrez, L.G., Márquez, M. 2005. Micropropagación de *Guadua angustifolia* Kunth. Actual. Biol. 27: 5–15. doi.org/10.17533/udea.acbi.329424 [in Spanish]

Mirzabe, A.H., Hajiahmad, A., Fadavi, A., Rafiee, S. 2022. Temporary immersion systems (TISs): A comprehensive review. J. Biotechnol. 357: 56–83. doi.org/10.1016/j.jbiotec.2022.08.003

Mudoi, K.D., Saikia, S.P., Goswami, A., Gogoi, A., Bora, D., Borthakur, M. 2013. Micropropagation of important bamboos: A review. Afr J Biotechnol. 12(20):2770-2785. doi:10.5897/AJB12.2122

Mustafa, A.A., Yong, W.T.L., Kulip, J., Rodrigues, K.F. 2023. Establishment of an *in vitro* mass propagation system for *Dendrocalamus asper*. J. Trop. Biol. Conserv. 20: 193–202. doi.org/10.51200/jtbc.v20i.4644

Nadgauda, R.S., John, C.K., Joshi, M.S., Parasharami, V.A., Mascarenhas, A.F. 1997. A comparison of *in vitro* with *in vivo* flowering in bamboo: *Bambusa arundinacea*. Plant Cell Tissue Organ Cult. 48: 181–188. doi.org/10.1023/A:1005800700024

Nadgauda, R.S., Parashavami, V.A., Mascarenhas, A.F. 1990. Precocious flowering and seeding behaviour in tissue-cultured bamboos. Nature 344: 335–336. doi.org/10.1038/344335a0

Palee, J. 2018. *In vitro* shoot cultures of *Tupistra albiflora* K. Larsen. Walailak J. Sci. Technol. 17: 405–411. doi.org/10.48048/wjst.2020.4182

Pasternak, T.P., Steinmacher, D. 2024. Plant growth regulation in cell and tissue culture *in vitro*. *Plants*. 13(2):327. doi:10.3390/plants13020327

Pérez, M., Bueno, M.A., Escalona, M., Toorop, P.E., Cañal, M.J., Rodríguez, M. 2013. Temporary immersion systems (RITA®) for the improvement of cork oak somatic embryogenic culture proliferation and somatic embryo production. Trees 27: 1277–1284. doi.org/10.1007/s00468-013-0876-y

Petrić, M., Subotić, A., Jevremović, S., Trifunović-Momčilov, M., Tadić, V., Grujić, M., Vujčić, Z. 2015. Esterase end peroxidase isoforms in different stages of morphogenesis in *Fritillaria meleagris* L. in bulb scale culture. Comptes Rendus Biol. 338: 793–802. doi.org/10.1016/j.crvi.2015.08.002

Pożoga, M., Olewnicki, D., Latocha, P. 2024. A temporary immersion system as a tool for lowering planting material production costs using the example of *Pennisetum* × *advena* 'Rubrum'. Agriculture 14: 1177. doi.org/10.3390/agriculture14071177

Preeti, V., Nikita, M. 2018. A review: *in vitro* regeneration of bamboo plants by plant culture techniques. Int. J. Adv. Sci. Eng. Technol. 6: 58–67.

Putalun, W., Udomsin, O., Yusakul, G., Juengwatanatrakul, T., Sakamoto, S., Tanaka, H. 2010. Enhanced plumbagin production from *in vitro* cultures of *Drosera burmanii* using elicitation. Biotechnol. Lett. 32: 721–724. doi:10.1007/s10529-010-0202-3

Ramanayake, S.M.S.D., Meemaduma, V.N., Weerawardene, T.E. 2006. *In vitro* shoot proliferation and enhancement of rooting for the large-scale propagation of yellow bamboo (*Bambusa vulgaris* Striata). Sci. Hortic. 110: 109–113. doi.org/10.1016/j.scienta.2006.06.016

Sharma, A., Manpoong, C., Gohain, A., Pandey, H., Padu, G., Aku, H. 2022. Tissue-cultured regeneration and ecological values in major bamboo species. J. Ecol. Environ. 46: 1–49. doi.org/10.5141/jee.22.044

Singhanukunkit, P. 2022. Development of Large-Scale Micropropagation System for *Dendrocalamus sericeus* by Using Temporary-Immersion Bioreactor System. M.Sc. thesis. Maejo University. Chiang Mai, Thailand.

Schuchovski, C., Sant'Anna-Santos, B., Marra, R., Biasi, L. 2020. Morphological and anatomical insights into *de novo* shoot organogenesis of *in vitro* 'Delite rabbiteye' blueberries. Heliyon 6: e05468. doi: 10.1016/j.heliyon.2020.e54468

Sood, A., Ahuja, P.S., Sharma, M., Sharma, O.P., Godbole, S. 2002. *In vitro* protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. Plant Cell Tissue Organ Cult. 71: 55–63. doi: 10.1023/A:1016582732531

Subotić, A., Trifunović, M., Jevremović, S., Petrić, M. 2010. Morpho-histological study of direct somatic embryogenesis in endangered species *Fritillaria meleagris*. Biol. Plant. 54: 592–596.

Suwal, M.M., Lamichhane, J., Gauchan, D.P. 2020. Regeneration technique of bamboo species through nodal segments: A review. Nepal Journal of Biotechnology 8: 54–68. doi.org/10.3126/njb.v8i1.30209

Teanchartsakul, N., Laohavisuti, N., Seesanong, S. 2019. Temporary immersion bioreactor for micropropagation of *Anubias barteri* 'Broad leaf'. King Mongkut's Agric. J. 37: 23–31.

Teisson, C., Alvard, D. 1995. A new concept of plant *in vitro* cultivation liquid medium: temporary immersion. In: Terzi, M., Cella, R., Falavigna, A. (Eds.). Current issues in plant molecular and cellular biology. Spinger. Dordrecht, the Netherlands. pp. 105–110.

United Nations Conference on Trade and Development. 2022. Commodities at a glance. Special issue on bamboo. United Nations. Geneva, Switzerland.

Venkatachalam, P., Kalaiarasi, K., Sreeramanan, S. 2015. Influence of plant growth regulators (PGRs) and various additives on *in vitro* plant propagation of *Bambusa arundinacea* (Retz.) Wild: A recalcitrant bamboo species. J. Genet. Eng. Biotechnol. 13: 193–200. doi: 10.1016/j.jgeb.2015.09.006

Waraporn, H., Sompong, T., Chakriya, N., Nucharee, C., Sureerat, Y. 2022. Enhanced efficiency of *in vitro* plant regeneration of caladium (*Caladium biocolor* cv. "Khum Thong") through young leaf culture. Sci. Asia. 48: 740–745. doi: 10.2306/scienceasia1513-1874.2022.112

Wongchaya, P., Bansra, T., Mahadtanapuk, S. 2024. Rapid micro-propagation of disease-free hemp (*Cannabis sativa* L.) to improve industrial production. Trends Sci. 22: 9057. doi.org/10.48048/tis.2025.9057

Yasamut, A., Teeraarunsiri, W., Mahatthanapuk, S. 2022. Micropropagation of *Dendrocalamus* sp. by tissue culture and temporary immersion bioreactor system. In: Proceeding of the 33rd TSU Conference. Songkhla, Thailand. pp. 47–55.

Zdravković-Korać, S., Milojević, J., Belić, M., Ćalić, D. 2022. Tissue culture response of ornamental and medicinal *Aesculus* species—a review. Plants. 11: 277. doi.org/10.3390/plants11030277

Zhang, B., Sarsaiya, S., Pan, X., et al. 2018. Optimization of nutritional conditions using a temporary immersion bioreactor system for the growth of *Bletilla striata* pseudobulbs and accumulation of polysaccharides. Sci. Hortic. 240: 155–161. doi.org/10.1016/j.scienta.2018.06.010

Zhao, Y. 2018. Essential roles of local auxin biosynthesis in plant development and in adaptation to environmental changes. Annu. Rev. Plant Biol. 69: 417–435. doi.org/10.1146/annurev-arplant-042817-040226