Borneo International Journal of Biotechnology
(BIJB) Vol. 5 (December 2025), 143-156.

e-ISSN 2716-697X

Development of an *In Vitro* Culture Protocol for *Gigantochloa levis* Using Nodal Segments

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Received: 4 December 2025 | Accepted: 20 December 2025 | Published: 31 December 2025

ABSTRACT

Bamboo is a giant perennial arborescent grass in the Poaceae family and a fast-growing, economically important plant. However, conventional propagation is constrained by limited planting material, genetic variation, and the risk of disease and pest transmission. *In vitro* propagation using nodal segments has become an effective method for the large-scale multiplication of bamboo species. This study aimed to establish an *in vitro* culture system for the tropical bamboo *Gigantochloa levis*. Nodal segments containing axillary buds were selected as explants and surface-sterilized using an optimized protocol. Sterilized explants were cultured on full-strength semi-solid Murashige and Skoog (MS) medium for shoot initiation. MS medium supplemented with 6-benzylaminopurine (BAP) at 2, 3 and 4 mg/L was evaluated for shoot induction and multiplication. Medium containing 3 mg/L BAP produced the highest number of shoots (6.80 ± 0.49) after six weeks of culture. Rooting was optimized using liquid half-strength MS medium supplemented with 0.5 mg/L indole-3-butyric acid (IBA). The results provide a baseline protocol for efficient *in vitro* propagation of *G. levis*, supporting future efforts in bamboo multiplication and conservation.

Keywords: *Gigantochloa levis*, tropical bamboo, *in vitro* propagation, nodal segments, 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA)

e-ISSN 2716-697X

INTRODUCTION

Tissue culture, also known as *in vitro* propagation or micropropagation, is a widely used biotechnological approach in plant science. It involves cultivating plant cells, tissues, or organs on sterile synthetic media under controlled temperature, light, and humidity (Sudheer et al., 2022; Bansal et al., 2024). Beyond its role in plant propagation, tissue culture supports a broad range of basic and applied research applications, including studies on plant developmental processes, functional gene analysis, production of transgenic plants with desirable traits, and crop improvement through plant breeding. It is also employed for germplasm conservation, long-term preservation of vegetatively propagated crops, and the rescue of rare, threatened, or endangered species (Espinosa-Leal et al., 2018; Loyola-Vargas & Ochoa-Alejo, 2018). Furthermore, tissue culture enables mass multiplication of elite plants, production of disease-free planting material, and propagation of species that are difficult to multiply using seeds or cuttings (Espinosa-Leal et al., 2018; Bansal et al., 2024; Bao et al., 2024; Eliwa et al., 2024). It also promotes the rapid generation of genetically uniform plants, supporting vigorous growth in subsequent developmental stages (Permadi et al., 2023).

Seed-based bamboo propagation is constrained by sporadic flowering, long flowering cycles, seed recalcitrance, and vulnerability to predation. Vegetative propagation techniques such as culm cuttings and air layering are widely practiced but are limited to small-scale applications because they damage mother plants, require bulky planting material, and depend on specific seasons (Sandhu et al., 2018). *In vitro* propagation has therefore emerged as a practical alternative, enabling large-scale clonal multiplication, providing planting material for breeding programmes, and supporting germplasm conservation. Across forestry, agriculture, and horticulture, *in vitro* culture plays a crucial role not only in propagation but also in genetic improvement and conservation strategies (Ahmed, 2022; Liu et al., 2024)

In recent years, substantial progress has been made in developing *in vitro* culture systems for many bamboo species, offering an effective alternative to conventional propagation. Successful micropropagation protocols have been reported for *Dendrocalamus asper* (Gunasena et al., 2024), *Bambusa vulgaris* (Gonçalves et al., 2023; Sarker et al., 2024), *Bambusa tulda* and *Dendrocalamus stocksii* (Choudhary et al., 2022), *Bambusa tuldoides* (Sharothi et al., 2022), *Bambusa balcooa* (Rajput et al., 2020), *Melocanna baccifera* (Waikhom & Louis, 2014), and *Gigantochloa atroviolaceae* (Bisht et al., 2010). However, despite these advances, studies on *Gigantochloa levis* remain scarce. This species is highly valued for its strong culms, extensive use in construction and handicrafts, and its emerging potential in the development of sustainable biomaterials.

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Increasing demand has placed pressure on natural stands, yet large-scale cultivation is limited by inadequate availability of high-quality planting material. Therefore, establishing a reliable *in vitro* propagation protocol for *G. levis* is essential to support commercial-scale cultivation, ensure genetic uniformity, and promote conservation and sustainable utilization of this economically important tropical bamboo.

MATERIALS AND METHODS

Sample Collection

Culms of *Gigantochloa levis* were collected from the Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah (UMS). Species identity was confirmed by an authorized plant taxonomist. Culms and branches containing internodes were selected and transported to the climate-controlled greenhouse at the Biotechnology Research Institute (BRI), UMS. The culms were established in polybags for acclimatization prior to explant preparation (Figure 1).

Figure 1 (a) *Gigantochloa levis* at the Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah (UMS); **(b)** culms of *G. levis* established in polybags for acclimatization in the greenhouse

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Explants Surface Sterilization

Nodal segments of *G. levis* containing axillary buds were used as explants. Explants were trimmed to 4-5 cm using a sterilized blade, and the outer layers were gently scrubbed to remove dust and wax. The explants were first washed three times with sterile distilled water for 3 minutes each, followed by washing in 0.5% commercial detergent for 3 minutes and rinsing 4-5 times with sterile distilled water. Explants were then soaked in 1.5% Antracol 70 WP (fungicide) for 30 minutes on a rotary shaker and rinsed three times with sterile distilled water. This was followed by washing in 2.5% commercial detergent for 5 minutes and rinsing 5-6 times with sterile distilled water. Surface disinfection was carried out using 70% ethanol for 1 minute, followed by three rinses with sterile distilled water. Explants were then sterilized in 0.1% (v/v) mercuric chloride ($HgCl_2$) for 5 minutes and washed four times, with each wash lasting 5 minutes. Sterilized explants were air-dried in a laminar airflow workstation. The sterilization protocol was adapted and optimized based on Kaladhar et al. (2017).

Media and Culture Conditions

Murashige and Skoog (MS) medium was prepared in glass jar and used as the basal medium. The medium contained 3% (w/v) sucrose and was supplemented with varying concentrations of 6-benzylaminopurine (BAP). The pH was adjusted to 5.8 ± 0.1 before adding 3% sucrose, 1.0 g/L gelrite, and autoclaving at 121°C for 15 minutes. Cultures were maintained under a 16/8-hour (light/dark) photoperiod at 26 ± 2°C and 70% relative humidity in a SANYO Versatile Environmental Test Chamber (SANYO Electric Co., Ltd., Japan) (Mustafa et al., 2023).

Shoot Initiation (Bud Break)

A total of 36 nodal explants containing axillary buds were used for shoot initiation, with 12 explants allocated to each BAP concentration (2.0, 3.0, and 4.0 mg/L). Sterilized explants were inoculated onto MS medium supplemented with 3% sucrose, 1.0 g/L gelrite, and the respective BAP concentrations (Figure 2). All procedures were carried out under aseptic conditions in an ESCO laminar flow cabinet (ESCO Micro Pte. Ltd., Singapore). The percentage of shoot initiation (bud break) was recorded after four weeks (Mustafa et al., 2023).

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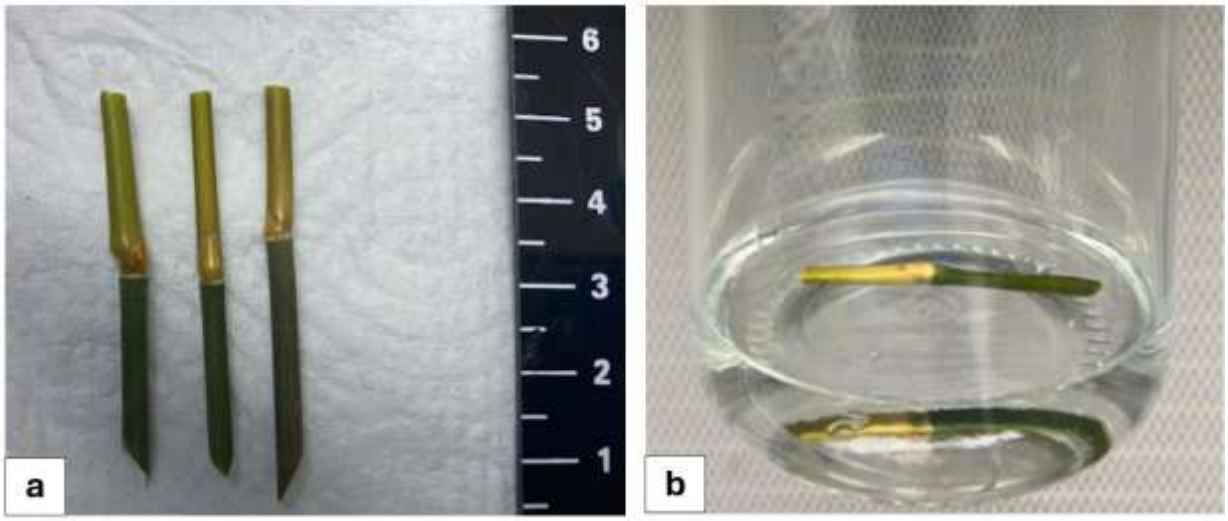

Figure 2 (a) Nodal segments of *Gigantochloa levis* containing axillary buds; **(b)** inoculation of explant onto Murashige and Skoog (MS) medium.

Shoot Multiplication

Proliferated axillary shoots were transferred to MS medium containing 2.0, 3.0, or 4.0 mg/L BAP supplemented with 3% sucrose and 1.0 g/L gelrite. Subculturing was carried out every seven days under sterile conditions to minimize browning and maintain healthy cultures. Shoot multiplication performance (n=5) was evaluated after six weeks by recording the number of shoots per explant (Choudhary et al., 2022). The percentage of microbial contamination and browning was also assessed.

Rooting and Acclimatization

After six weeks of culture, axillary shoots were excised and transferred to liquid half-strength MS medium supplemented with indole-3-butyric acid (IBA) at concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/L. Rooting response was evaluated after eight weeks by recording the rooting percentage, average number of roots, and mean root length. Rooted plantlets were acclimatized in peat moss (Kekkilä, Finland) for four weeks and maintained at 25 ± 2°C in the greenhouse (Mustafa et al., 2023).

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RESULTS AND DISCUSSION

Establishment of *In Vitro* Shoots of *Gigantochloa levis*

Shoot Initiation (Bud Break)

Nodal segments containing axillary buds successfully initiated shoot development across all BAP concentrations tested. Shoot bud initiation was observed between 4 and 7 days after inoculation. After 14 days, visible bud sprouting was recorded at 2.0, 3.0, and 4.0 mg/L BAP (Figure 3). After four weeks, 3.0 mg/L BAP produced the highest shoot initiation percentage (100%), followed by 2.0 mg/L BAP, while the lowest initiation (75%) occurred at 4.0 mg/L BAP (Figure 4). No buds initiated after the fourth week, suggesting a limited response window.

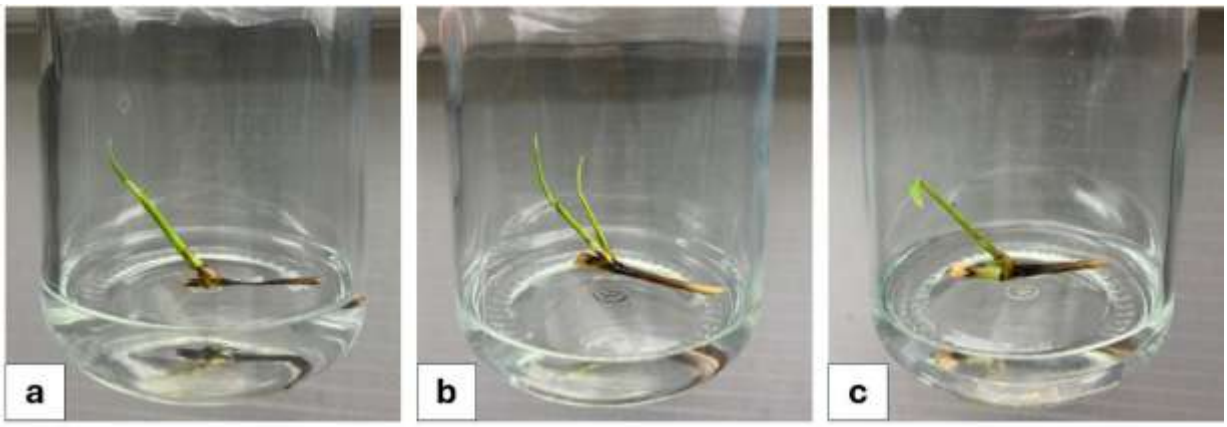

Figure 3 Bud sprouting of *Gigantochloa levis* at 14 days: (a) 2.0 mg/L BAP; (b) 3.0 mg/L BAP; (c) 4.0 mg/L BAP.

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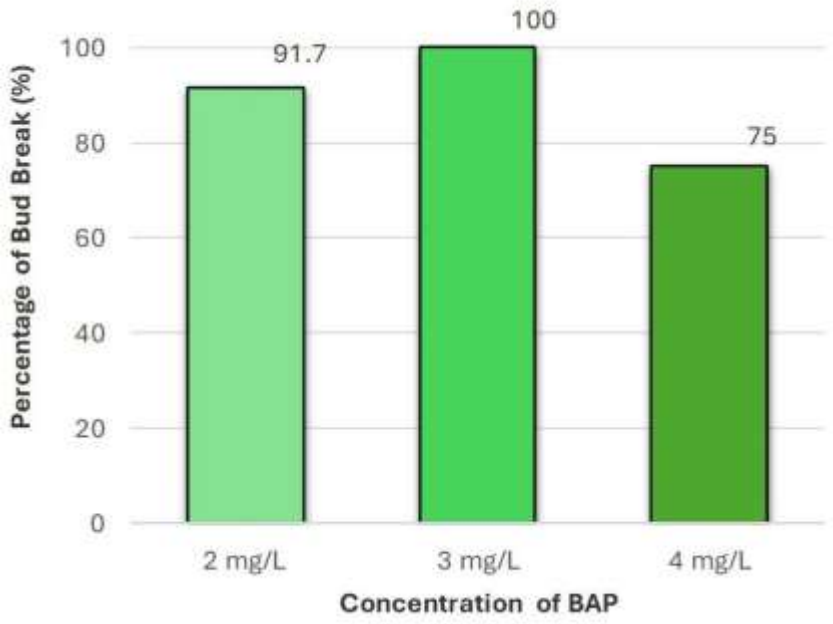

Figure 4 Percentage of shoot bud initiation after four weeks.

The successful and rapid bud break (4-7 days) demonstrates the strong responsiveness of *G. levis* nodal explants to BAP, consistent with established findings for bamboo tissue culture. Nodal segments perform well because they contain organized meristems with high regenerative capacity (Waikhom & Louis, 2014), which also exhibit greater genetic stability than disorganized tissues (Nowakowska et al., 2020; Duta-Cornescu et al., 2023). BAP is widely reported as the most effective cytokinin for bud break in bamboo, including *Bambusa nutans*, *B. tulda*, *Dendrocalamus strictus*, and *D. asper* (Ray & Ali, 2017). The optimal concentration of 3.0 mg/L BAP identified in this study aligns with reports for *Guadua angustifolia* (Jiménez et al., 2006) and several other bamboo species. Higher BAP concentrations reduced bud initiation, likely due to cytokinin-induced stress or hormonal imbalance (Mahardhini et al., 2023).

Shoot Multiplication

Shoot multiplication varied significantly among BAP concentrations (Figure 5). The highest shoot number (6.80 ± 0.49) was obtained with 3.0 mg/L BAP, while the lowest (5.40 ± 0.51) occurred at 4.0 mg/L BAP (Figure 6). BAP concentrations above 3.0 mg/L reduced shoot proliferation. MS medium containing 3.0 mg/L BAP produced vigorous shoots with minimal browning and no contamination, making it the most suitable treatment.

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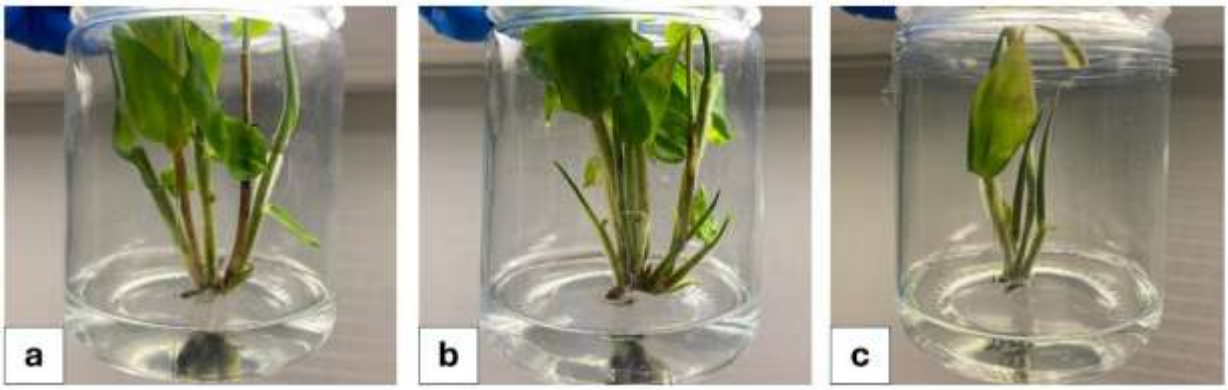

Figure 5 Shoot multiplication after six weeks at **(a)** 2.0 mg/L BAP; **(b)** 3.0 mg/L BAP; **(c)** 4.0 mg/L BAP.

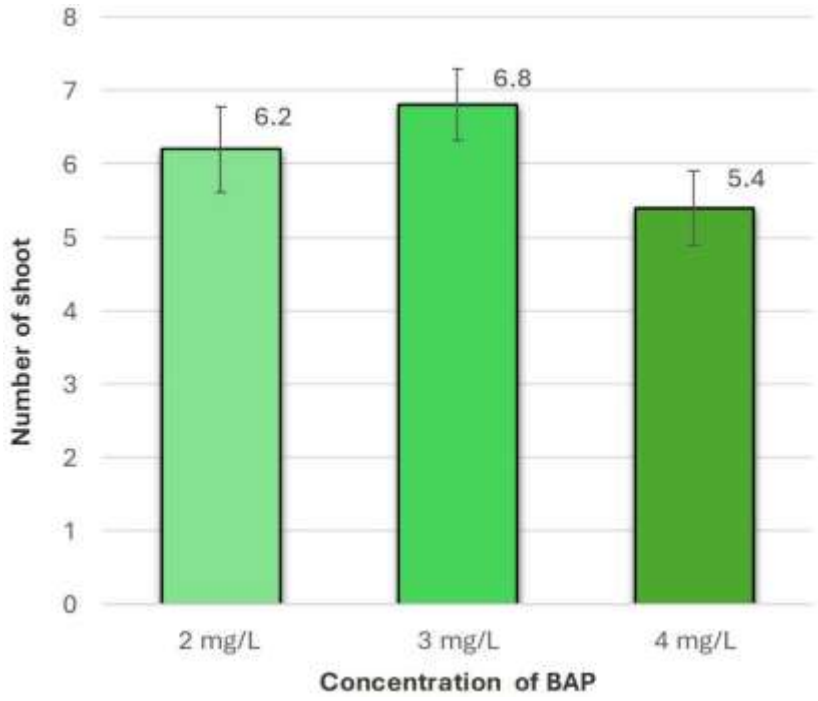

Figure 6 Number of shoots formed at different BAP concentrations.

The superior performance of 3.0 mg/L BAP is consistent with findings in *B. tulda*, *M. baccifera* (Waikhom & Louis, 2014), and *Bambusa bambos* (Muthukumaran et al., 2018), reinforcing the suitability of this concentration for bamboo shoot proliferation. Higher BAP levels typically reduce shoot multiplication, as reported in *D. strictus* and other bamboo species (Pandey & Singh, 2012). Excess cytokinin may inhibit bud development by disrupting hormonal balance (Wu et al., 2021). The results suggest that3.0 mg/L BAP provides a favourable hormonal environment for *G. levis*, supporting both bud break and shoot multiplication.

e-ISSN 2716-697

Contamination and Browning of *In Vitro* Cultures

Application of the optimized surface sterilization protocol significantly improved culture establishment. After six weeks, only 11.11% of *G. levis* explants showed microbial contamination, whereas the original method described by Kaladhar et al. (2017), when applied without modification, resulted in 94.44% contamination. Browning was observed in 19.44% of cultures during both the initiation and multiplication stages. Regular subculturing at seven-day intervals reduced browning symptoms and supported healthier explant growth.

Bamboo explants are highly susceptible to microbial contamination due to the presence of both epiphytic and endophytic microorganisms that inhabit their woody culm and nodal tissues (Ray & Ali, 2017; Pasqualini et al., 2019). The optimized sterilization protocol effectively addressed these challenges through several key improvements. Replacing tap water with sterile distilled water during explant washing eliminated a major source of contamination. The two-step detergent wash enhanced the removal of debris and microbial residues, while extended fungicide exposure combined with rotary shaking improved contact between the explant surface and sterilizing agents. Furthermore, thorough rinsing following the 0.1% $HgCl_2$ treatment helped prevent phytotoxic damage to the tissues (Kodape et al., 2024). These refinements contributed to the substantial reduction in contamination rates and improved the overall success of culture establishment.

Browning, a common physiological disorder in bamboo tissue culture, results from the oxidation of phenolic compounds released during explant wounding (Permadi et al., 2023). The trimming of explants to 4-5 cm likely increased phenolic exudation, contributing to the browning observed in this study. Regular subculturing to fresh medium was effective in mitigating browning by diluting oxidized phenolics and maintaining a more favorable environment for growth (Amente & Chimdessa, 2021). This approach is widely recommended in bamboo micropropagation to prevent phenolic accumulation and maintain culture viability.

Rooting and Acclimatization

Rooting of *G. levis* shoots occurred only on half-strength MS medium supplemented with 0.5 mg/L IBA. At this concentration, 80% of the shoots produced roots, with an average of 7.60 ± 1.96 roots per shoot and a mean root length of 8.96 ± 2.39 cm (**Figure 7**). No rooting was observed at higher IBA concentrations ranging from 1.0 to 5.0 mg/L. The rooted plantlets were successfully acclimatized in peat moss under greenhouse conditions, producing healthy and vigorous young bamboo plants (**Figure 8**).

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Figure 7 Root induction on half-strength MS medium supplemented with 0.5 mg/L IBA.

Figure 8 Hardened *G. levis* plantlets acclimatized in the greenhouse.

The finding that rooting occurred only at the lowest IBA concentration demonstrates the high auxin sensitivity of *G. levis*. A similar response has been reported in *D. asper*, where elevated auxin concentrations inhibit root formation rather than promote it (Mustafa et al., 2023). Excessive auxin can disrupt root initiation by causing hormonal imbalances or inducing tissue stress, leading to suppression of root primordia development.

The effectiveness of half-strength MS medium supplemented with 0.5 mg/L IBA is consistent with reports from other bamboo species, confirming its suitability for promoting root induction in *G. levis*. The high rooting percentage, coupled with the robust and well-developed root system, indicates that this protocol provides favorable physiological conditions for root formation. Successful acclimatization of rooted plantlets further supports the reliability of this rooting method, suggesting its potential for efficient nursery production and subsequent field establishment of *G. levis*.

CONCLUSION

This study established an efficient and reliable *in vitro* propagation protocol for *G. levis*, providing a practical solution to the limitations of its conventional propagation. The optimized sterilization method, together with the identified culture conditions for shoot induction, multiplication, and rooting, enabled consistent production of healthy plantletsthat acclimatized successfully under greenhouse conditions. The protocol offers a feasible approach for large-scale propagation, supporting the conservation, commercial cultivation, and sustainable utilization of this economically important bamboo species. Future work may focus on confirming genetic fidelity and refining acclimatization techniques to enhance field performance .

ACKNOWLEDGEMENTS

This research was supported by the Malaysian Ministry of Higher Education through the Fundamental Research Grant Scheme (Grant No. FRGS/1/2020/STG01/UMS/02/4).

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