Research Article

In vitro Culture of *Dendrocalamus asper* Bamboo in Liquid and Semi-Solid MS Media

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Abstract | *Dendrocalamus asper*, a tropical bamboo variety renowned for its economic significance across industries like food, construction, and handicrafts, is presently a surge in demand for large-scale propagation and sustained supply. Traditional propagation methods are inconvenient and time-intensive. As an alternative, micropropagation techniques are opted to overcome these challenges. This research aimed to develop a micropropagation protocol for expanding *D. asper* bamboo through the utilization of various propagule sizes and a comparative analysis of their growth on liquid and semi-solid MS media. *In vitro* nodal segments were initiated on MS media supplemented with 4 mg L^{-1} BAP and 0.5 mg L^{-1} IBA. Subsequently, after four weeks, a cluster of shoots, varying in numbers (3, 4, and 5 shoots per propagule), were cultured in the same media for shoot multiplication. Propagules with three shoots exhibited the highest multiplication rate, showing a 4.9-fold increased and an average of 14.8 ± 3.5 shoots after a 5-week culture period. Following this, three shoots/clumps were transferred to liquid and solid MS media with varied concentrations of BAP (0.5, 1.0, 2.0, and 4.0 mg L^{-1}) to assess their growth rates. Cultures in liquid media demonstrated superior shoot proliferation compared to semi-solid media, recording the highest mean shoot number of 3.5 ± 0.8 shoots per explant in media supplemented with 0.5 and 4.0 mg L^{-1} BAP. The longest shoots were observed in liquid media with 0.5 mg L^{-1} BAP, with an average length of 3.57 ± 0.44 cm. Subsequently, the explants underwent rooting in both semi-solid and liquid MS media, supplemented with various IBA concentrations over a 5-week period, with rooting observed solely in the cultures in liquid media. The rooted plantlets were 100% survived when acclimatized in a greenhouse using a mixture of soil, sand and compost.

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Introduction

Bamboo, a member of the grass family Poaceae, encompasses over 1,600 species with a predominantly tropical and subtropical distribution (Soreng *et al.*, 2017). The economic viability of this plant is relatively high in the global forestry economy due to its silvicultural and morphological features, making it an efficient and environmentally friendly substitute for wood (Gonçalves *et al.*, 2023; Admas, 2024). Apart from that, this plant could sequester more CO_2 and lock the carbon source in its fibre and soil where it grows compared to other trees, which eventually could help in mitigating global warming issues (Lou *et al.*, 2010; Patel *et al.*, 2015). Bamboo can also help in reducing soil erosion by having extensive rhizomes and roots which bind to the soil, and the presence of its evergreen canopy and leaf litter helps in intercepting rain as well as reducing its impact on the ground (Kuehl *et al.*, 2011).

One of the prominent tropical bamboo species that is commonly planted in Southeast Asia countries such as Malaysia, Thailand, Indonesia, Vietnam, and the Philippines is *Dendrocalamus asper* (Mustafa *et al.*, 2021). Its economic significance is multifaceted. It is valued for its edible shoots that are consumed as food, and its mature culms that have been used in furniture, construction, biofuel and handicraft industries (Hartono *et al.*, 2022). Due to this, the demand for bamboo continues to rise, necessitates for efficient and sustainable propagation methods.

Conventional methods such as propagation from rhizomes or culm cuttings are often time-consuming, labour-intensive and have low survival rates (Singh *et al.*, 2013). Propagation using seeds is unreliable since the flowering is irregular and in a long cycle, plus the seeds are mostly sterile and have a short viability period, further hindering propagation efforts (Sandhu *et al.*, 2017). In order to overcome these challenges to fulfil the demand, micropropagation techniques offer a compelling solution to address the limitations of conventional propagation methods. This *in vitro* approach enables rapid and controlled multiplication of high-quality *D. asper* plantlets.

Previous studies have been conducted to develop proper protocols for micropropagation of *D. asper*. During this stage, the frequency of shoot growth is significantly influenced by the physical status of the media, either liquid or semi-solid (Sandhu *et al.*, 2017). In semi-solid media, often solidifying agent such as agar and Gelrite are added, aiming to provide three-dimensional support for the growing shoots and allow for nutrient uptake (Raju *et al.*, 2023). Liquid culture systems, on the other hand, eliminate the need for a solidifying agent and provide a more homogenous distribution of nutrients and growth factors (Sandhu *et al.*, 2017). However, the cons of this system include the lack of physical support for the growing shoots, as well as the risk of hyperhydricity due to continuous immersion in the liquid medium (Polivanova and Bedarev, 2022). Interestingly, despite the possible risk of hyperhydricity, study in the past recorded that culture of some bamboo species in liquid media resulted in better growth compared to semi-solid media (Arshad *et al.*, 2005; Ogita *et al.*, 2008; Rathore *et al.*, 2009). Other than this, multiplication *in vitro* is also influenced by the number of shoot propagules used during subsequent subculturing stage. During this stage, the shoot clusters were divided into smaller clumps consists of three to ten shoots (Jimenéz *et al.*, 2021). By adopting this method, higher multiplication rate was achieved (Jiménez *et al.*, 2006; Ornellas *et al.*, 2019), but optimum number of shoot clusters that support the highest multiplication rate might vary between different bamboo species.

Hence, in this study, we aim to investigate the growth of *D. asper* bamboo species in both liquid and semi-solid MS media, along with optimization of number of shoot propagules during multiplication stage. The growth patterns were evaluated and documented over a specified period of time to determine the most effective medium and optimum number of propagules for bamboo micropropagation.

Materials and Methods

Establishment of initial culture

In vitro plantlets of *D. asper* were being utilized as initial explants, in which the nodal segments without axillary buds were cut into 1.0 – 1.5 cm in size. These nodes were cultured in Murashige and Skoog (MS) medium (Duchefa Biochemies, The Netherlands) with an addition of 100 mgL^{-1} myoinositol (Duchefa Biochemies, The Netherlands), 30 gL^{-1} sucrose (Systerm, Malaysia) and 3 gL^{-1} Gelrite (Sigma, St. Louis, USA). Plant growth regulators (PGRs) were added, 4 mgL^{-1} 6-benzylaminopurine (BAP) and 0.5 mgL^{-1} indole-3-butyric acid (IBA) (Duchefa

Biochemies, The Netherlands) for culture initiation. The cultures were maintained under 16-hour photoperiod under white cool fluorescent lights (Philips, China) in the culture room with the temperature of 27±2°C. After 6 weeks, the shoots were subcultured at different number of propagules for multiplication of shoot.

Optimization of propagules sizes for shoot multiplication

To study the impact of propagules sizes towards shoot multiplication, group of 3-, 4- and 5- shoots were transferred to multiplication medium having the same hormone concentrations from previous culture, which consisted of solidified MS medium with addition of 4.0 mgL^{-1} BAP and 0.5 mgL^{-1} IBA hormone. Observation was recorded for 5 weeks period for number of shoots and multiplication rate.

Effect of semi-solid and liquid culture on shoot multiplication

To determine the effect of different culture media towards the growth of explants, propagules containing 3 shoots each were transferred in liquid (without addition of Gelrite) and semi-solid (addition of 3 gL^{-1} Gelrite) media supplemented with different concentrations of BAP (0.5, 1.0, 2.0 and 4.0 mg L^{-1}). For liquid media, sterile filter papers were used to support the explants, preventing them from fully soaked in the media. Observations for number and length of shoots were recorded after 4 weeks of culture.

Effect of semi-solid and liquid culture on rooting induction

Propagules of three to five shoots were transferred to both semi-solid and liquid MS media supplemented with different IBA concentrations (1.0 – 5.0 mg L^{-1}) for rooting. During this stage, sterile filter paper was not used as support in the liquid media. Observations for rooting response, number and length of shoots were recorded after 5 weeks of culture.

Media preparation

MS media in the present study were prepared at 4.4 g L^{-1} supplemented with 30 g L^{-1} sucrose and 100 mg L^{-1} myoinositol. Solidified MS medium was prepared by adding 3 gL^{-1} Gelrite. The pH of the media was adjusted to 5.6–5.8 prior to autoclaving at 121°C at 0.1 kPa for 20 min. All the cultures were maintained in the culture room at temperature of 27±2°C with 16hr photoperiod under cool white, fluorescent light.

Pre-hardening and acclimatization

The rooted plantlets were then removed from the culture jars and washed thoroughly under running tap water to remove medium traces from the roots. Plantlets were transferred to root trainers filled with mixture of autoclaved vermicompost and vermiculite at 1:1 ratio and maintained in culture room for 2 weeks. Afterwards, the plantlets were acclimatized in mixture of different substrates: soil, cocopeat and compost (1:1:1) for a month in the greenhouse.

Statistical analysis

All collected data were subjected to statistical analysis for Analysis of Variance (ANOVA) at $p < 0.05$ and standard error using IPM SPSS statistical software Version 28.0.

Results and Discussion

Effect of propagule sizes on shoot multiplication

Propagules of three shoots resulted in the highest multiplication rate with 4.9-fold and shoot number (average 14.8) after 5 weeks of culture (Table 1). As the number of propagules increased, the multiplication rate decreased over time, though the number of shoots produced from each propagule did not differ significantly ($p < 0.05$). In this study, single or group of 2-shoots were not being utilized since most of them did not manage to multiply and produce new shoots over time. In addition, they showed some browning effects and most of the leaves turned brown (result was not shown).

Table 1: *The number of shoots and shoot multiplication rate obtained from different sizes of propagules cultured in MS + 4.0 mg L^{-1} BAP + 0.5 mg L^{-1} IBA.*

Size of propagules used	2 weeks		3 weeks		5 weeks	
	No of shoots	Multiplication rate	No of shoots	Multiplication rate	No of shoots	Multiplication rate
Three shoots	6.8 ± 0.2[a]	2.3	11.0 ± 2.1[a]	3.7	14.8 ± 3.9[a]	4.9
Four shoots	6.2 ± 0.7[a]	1.5	12.0 ± 1.5[a]	3.0	14.0 ± 1.7[a]	3.5
Five shoots	8.0 ± 1.0[a]	1.6	9.5 ± 0.9[a]	1.9	13.0 ± 1.6[a]	2.6

Values = Mean ± SE.

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Previous study on the same species revealed that propagules with 8 shoots cultured in MS medium fortified with 10 µM BAP and 75 µM adenine sulphate (Ads) produced the highest shoot multiplication fold at 3.9 (Singh *et al.*, 2012a). However, most of the studies in the past had utilized propagules of 3 shoots, maximum was 4 shoots, during shoot multiplication stage in different bamboo species such as *P. stocksii* (Sanjaya *et al.*, 2005), *D. strictus* (Pandey and Singh, 2012), *B. balcooa, B. bambos, D. stocksii* and *G. angustifolia* (Rathore *et al.*, 2009). Our study showed a decrease in multiplication rate as bigger propagule sizes (more shoots) were being utilized. This finding was in concordance with those reported by previous study in which propagules with more than 3 shoots displayed a decline in multiplication rate after 4 weeks in *D. asper* (Arya *et al.*, 1999). The same authors also concluded that three-shoot propagules were the most suitable propagule size for shoot multiplication.

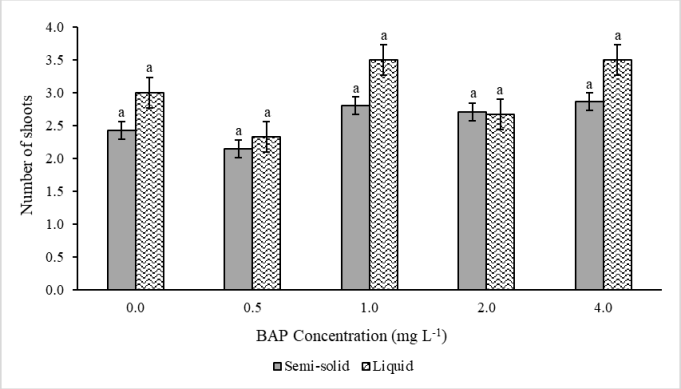

Figure 1: *Effect of different types of media towards number of shoots of D. asper.*

Effect of the state of culture medium during shoot multiplication and rooting

During shoot multiplication, clusters of three shoots were transferred into semi-solid and liquid MS media containing different concentrations of BAP hormone. After four weeks, the results displayed that the means of length and number of shoots obtained from the explants cultured in liquid media were overall higher compared to semi-solid media. The overall highest number of shoots was obtained from the liquid culture supplemented with 1.0 and 4.0 mg L^{-1} BAP with an average of 3.5 shoots per explant (Figure 1). The same culture supplemented with 0.5 mg L^{-1} BAP in a liquid medium resulted in the highest length of shoots (3.09 ± 0.19 cm) compared to other treatments (Figure 2). Meanwhile, in a semi-solid medium, the number of shoots obtained in 4.0 mg L^{-1} BAP was the highest with an average of 2.9 ± 0.6 shoots. In the same medium type, the highest shoot length was observed in the treatment with only MS media without supplementation of plant growth regulators at 2.13 ± 0.09 cm. In short, D. asper explants in liquid medium had better growth performance compared to in semi-solid medium (Figure 3). Overall, the mean number and length of shoots did not differ significantly ($p < 0.05$) for both media types and different BAP concentrations.

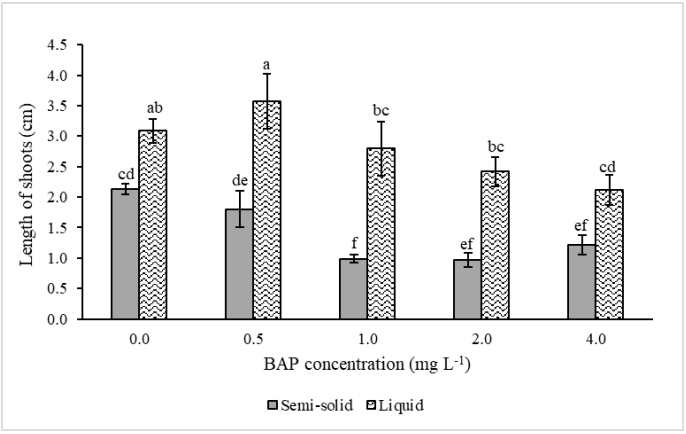

Figure 2: *Effect of different types of media towards length of shoots (cm) of D. asper.*

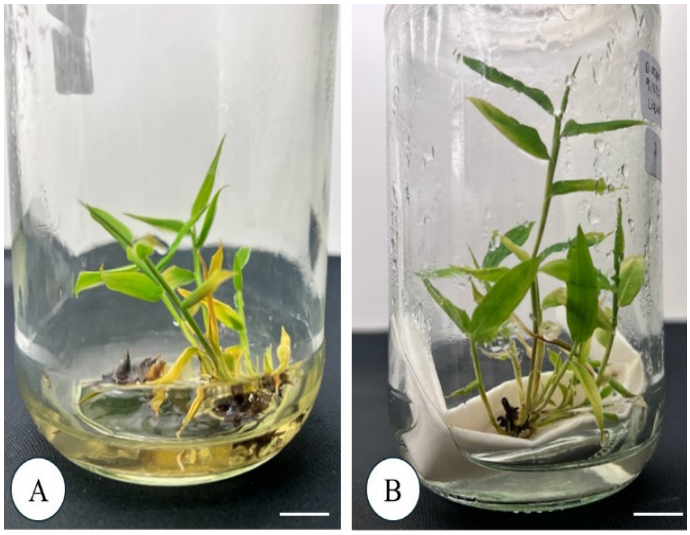

Figure 3: *Observation for shoot multiplication of D. asper in different types of media after 4 weeks of culture. (A) Culture in semi-solid MS medium (B) Culture in liquid MS medium. Bar = 1 cm.*

Previous studies have also reported similar findings, highlighting the benefits of utilizing liquid media in bamboo tissue culture. Ogita *et al.* (2008) suggested that half-strength liquid MS media had a positive effect on the elongation of axillary buds of *P. meyeri*. The multiplication of *D. hamiltonii* explants was also more efficient in liquid culture compared to semi-solid media (Sood *et al.*, 2002). Another species, *B. bambos* favoured liquid MS medium supplemented with 0.1 mg L^{-1} BAP, 0.1 mg L^{-1} NAA and additives during

shoot multiplication stage (Rathore *et al.*, 2009). Dos Santos *et al.* (2019) cultured the *D. asper* species in both semi-solid and liquid media supplemented with Plant Preservative Mixture (PPM) and discovered that culture in liquid medium resulted in lower contamination rates. However, study on the same bamboo species by Singh *et al.* (2012b) revealed that culturing in liquid medium was not as effective as compared to semi-solid medium for multiplication of shoots.

In tissue culture, the *in vitro* shoots are often transferred to medium containing certain concentration of auxin to induce rooting (Ribeiro *et al.*, 2020). Without supplementation of this type of hormone, the formation of roots in the explants might become challenging. In this study, the induction of rooting was performed in both semi-solid and liquid media supplemented with different range of IBA concentrations (1.0–5.0 mgL^{-1}) using propagules of three to five shoots. After five weeks, the observation of rooting response and root growth were observed where explant cultures across all IBA concentrations tested in semi-solid media failed to exhibit root development (data not shown). Meanwhile, in liquid media, rooting response was observed in at least 50% of the explants aside from culture supplemented with 1 mg L^{-1} IBA (Table 2). Mean root length ranged from 8.22 cm to 11.36 cm and showed a trend of increasing with increasing IBA concentration, however the highest length can be observed in culture supplemented with 1 mg L^{-1} IBA hormone. The same trend was observed in the mean number of roots, with the highest number of roots (5.20) being observed in the culture supplemented with 5 mg L^{-1} IBA.

Table 2: *Effect of different IBA concentrations on D. asper growth in liquid media after 4 weeks.*

IBA concentration (mg L^{-1})	Rooting response (%)	Mean root length (cm)	Mean root number
0	50%	8.22 ± 0.57^{a}	1.00 ± 0.63^{a}
1	25%	11.68 ± 1.39^{b}	2.20 ± 2.20^{a}
2	50%	10.53 ± 0.74^{ab}	3.00 ± 2.32^{a}
3	50%	10.3 ± 0.74^{ab}	3.20 ± 2.52^{a}
4	50%	11.01 ± 0.96^{ab}	4.80 ± 3.01^{a}
5	75%	11.36 ± 0.64^{b}	5.20 ± 2.96^{a}

Mean with the same letters does not differ significantly at $p < 0.05$

The result obtained for this study was corroborated with a study conducted by Shirin and Rana (2007), where the best concentration that supported induction of rooting in *Bambusa glaucescens* was 5 mg L^{-1} IBA in MS liquid medium. However, there were previous studies that reported that utilizing lower IBA concentration (1 mgL^{-1}) managed to result in the highest number of roots in the same species (Banerjee *et al.*, 2011; Kumar and Banerjee, 2014). Another study utilized half-strength MS medium instead and managed to obtain the highest growth rate along with elongated shoots in the liquid culture supplemented with 2 mg L^{-1} IBA hormone. Though no root growth in the semi-solid media was observed in the current study, past research had recorded otherwise. Rooting in *Bambusa tuldoides* and *Bambusa vulgaris* explants were successful in half-strength MS semi-solid media supplemented with IBA hormones (Desai *et al.*, 2019; Sharoti *et al.*, 2022). The same species with the current study also managed to form roots in semi-solid media with addition of 1.0 mg L^{-1} IBA, resulted in the highest length and number of roots (Kumar *et al.*, 2018).

Explants often thrive better in liquid media since they are directly in contact with the media, improving the absorption of the media content which further enhances the growth proliferation (Rai *et al.*, 2022). Particularly, the partially submerged shoots in the media provide large surface absorption, allowing PGRs such as BAP and other hormones to be efficiently assimilated into the explants (Rathore *et al.*, 2009). Compared to liquid media, the presence of gelling agent might cause the nutrients and PGRS to be released at a slower rate, causing the absorption of those chemicals to delay which eventually affects the growth rate of the explants (Singh *et al.*, 2013). Though utilizing liquid media offers more benefits as compared to semi-solid, prolong submersion in liquid could lead to hyperhydricity, a physiological disorder causing biochemical changes and disturbance of the structure of the explant's tissues (Polivanova and Bedarev, 2022). To reduce the occurrence of this event, sterile filter paper bridge was being used to prevent the whole explants from being fully immersed in the liquid medium, along with providing support to the explants in the culture system.

Acclimatization

The rooted plantlets were pre-hardened in a mixture of vermicompost and vermiculite before being transferred to the greenhouse for acclimatization. The use of a combination mixture of soil, cocopeat and

compost resulted in 100% survival of the plantlets after 4 weeks.

Conclusions and Recommendations

In conclusion, the micropropagation protocol developed for the multiplication of *D. asper* bamboo using different sizes of propagules and comparing their growth in liquid and semi-solid MS media has provided valuable insights. The study found that propagules containing three shoots produced the highest multiplication rate, and cultures in liquid media showed a higher shoot proliferation and rooting induction compared to semi-solid media. Additionally, the rooted plantlets were successfully pre-hardened and acclimatized in a greenhouse, demonstrating the potential for large-scale propagation of *D. asper* bamboo using micropropagation techniques. Further studies could explore the long-term growth and development of the propagated bamboo plants in different environmental conditions, as well as the potential application of these micropropagation techniques on a commercial scale.

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Novelty Statement

The study demonstrates a significant enhancement in the *in vitro* growth of *Dendrocalamus asper* when cultured in liquid media as compared to semi-solid media, providing critical insights into optimizing propagation techniques for this economically and ecologically valuable bamboo species.

Author's Contribution

Wan Nurfarzana Wan Mohamad Zani: Wrote the manuscript and data analysis.
Norrizah Jaafar Sidik: Assisted in data analysis, funding acquisition and editing the manuscript.
Asmah Awal, Nurul Izzati Osman, Mohd Khairi Nordin, Lyena Watty Zuraine Ahmad: Reviewed the manuscript.

Conflict of interest
The authors declared no conflict of interest.

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