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Influence of media variations and growth regulators on in vitro propagation of *Dendrocalamus asper* bamboo

WAN NURFARZANA WAN MOHAMAD ZANI[1], NORRIZAH JAAFAR SIDIK[1,♥], NUR AFIQAH MOHD ISA[1], ASMAH AWAL[2], NURUL IZZATI OSMAN[3], NORALIZA ALIAS[4], MOHD KHAIRI NORDIN[5]

[1]Department of Biology, Faculty of Applied Science, Universiti Teknologi MARA Shah Alam. 40450 Shah Alam, Selangor, Malaysia. Tel.: +603-5560-5543, Fax.: +603-5543-4562, ♥email: norri536@uitm.edu.my

[2]Agricultural Biotechnology Research Group, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Jasin Campus. 77300, Merlimau, Malacca, Malaysia

[3]Department of Pharmacology and Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA Puncak Alam Campus. 42300 Bandar Puncak Alam, Selangor, Malaysia

[4]Forest Biotechnology Division, Forest Research Institute Malaysia. 52109 Kepong, Selangor, Malaysia

[5]School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA Shah Alam. 40450 Shah Alam, Selangor, Malaysia

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Abstract. *Zani WNWM, Sidik NJ, Isa NAM, Awal A, Osman NI, Alias N, Nordin MK. 2024. Influence of media variations and growth regulators on in vitro propagation of* Dendrocalamus asper *bamboo. Nusantara Bioscience 16: 237-244.* This study reports the effects of different types of media and combinations of plant growth regulators on the in vitro propagation of *Dendrocalamus asper* (Schult.f.) Backer bamboo species, a species known for its economic significance and challenges in traditional cultivation. The experiment was initiated through the cultivation of in vitro nodal segments as explants in various strengths (half-strength and full-strength) of Murashige and Skoog's (MS) and Vacin & Went (VW) media, supplemented with 1.0 mg L^{-1} 6-benzylaminopurine (BAP) hormone. Optimal results were achieved in full-strength MS media with 1.0 mg L^{-1} BAP, with the highest shoot number (5.6 shoots) and length (2.14±0.18 cm), surpassing outcomes in VW media. Shoot multiplication in full-strength MS media, with varying BAP and indole-3-butyric acid (IBA) hormone combinations (0.5, 1.0, 2.0, and 4.0 mg L^{-1}), was conducted. The combination of 4.0 mg L^{-1} BAP and 0.5 mg L^{-1} IBA yielded the highest number of shoots (5.17±3.97), while supplementation with 4.0 mg L^{-1} IBA alone resulted in the longest shoot with 1.89±1.15 cm. These findings underscore the significance of tailored conditions for optimal in vitro propagation of this species. Further investigations could explore additional factors influencing the propagation process for better refinement of bamboo cultivation techniques.

Keywords: Bamboo, *Dendrocalamus asper,* micropropagation, plant growth regulators

Abbreviations: BAP: 6-benzylaminopurine, IBA: Indole-3-butyric acid, MS: Murashige and Skoog, VW: Vacin & Went

INTRODUCTION

Bamboo is a versatile plant that is widely used for reforestation and has economic significance in various regions of the world. This is mainly due to its fibers' mechanical and physical properties, which allow it to be used in different industries such as food, textile, construction, furniture, handicrafts, and household products (Gusmiaty et al. 2020; Hartono et al. 2022). Moreover, bamboo serves as an effective carbon sequestrator while aiding in soil and water conservation (Emamverdian et al. 2020).

One of the species that possesses the mentioned qualities is *Dendrocalamus asper* (Schult.) Back. ex. Hyne, a tropical bamboo species that is native to China and falls under the subfamily Bambusoideae (Souza et al. 2020). It has several common names, known as *betong* bamboo, sweet bamboo, black bamboo, giant bamboo, or rough bamboo (Charoenphun and Pakeechai 2021; Gonçalves et al. 2023). The shoots are rich in secondary metabolites and consumed by many as part of delicacies, making them one of the most important export commodities (Chandramouli et al. 2015; Kong et al. 2020). In Malaysia, this species holds economic prominence (Hossain et al. 2018), and its mature culms are extensively being used as raw materials to make paper, handicrafts, and floorboards for construction (Zang et al. 2019).

Rising demands for bamboo necessitate efficient propagation methods to ensure that there is a constant and sustainable supply of bamboo plants. Traditional propagation methods using seeds and culm cuttings face limitations in availability, transportation, and quantity (Sharothi et al. 2022). Additionally, the infrequent and season-dependent flowering that occurs between 25 to over 100 years poses challenges in obtaining seeds for propagation (Pasqualini et al. 2019; Zang et al. 2019). Therefore, to overcome these problems, micropropagation presents an alternative via in vitro techniques, enabling large-scale bamboo propagation. However, this method encounters challenges, particularly in optimizing growth through suitable media and Plant Growth Regulators (PGRs) to promote shoot proliferation and rooting of the explants.

The basal medium significantly impacts in vitro plant growth, with the majority of the species exhibiting preferences for MS (Murashige and Skoog 1962) medium. Although MS medium is commonly used, some plant species thrive better in alternative media formulations such as VW (Vacin and Went). This medium is known to be established for orchids, but previous studies have successfully utilized this medium to culture other plant species (Lestari et al. 2020; Menezes et al. 2016). The potential of this medium for bamboo growth, particularly for *D. asper*, remains unevaluated. In comparison, other types of media, such as B5 (Gamborg et al. 1968), SH (Schenk and Hildebrant 1972), and NN (Nitsch and Nitsch 1979) have been tested in the previous study (Singh et al. 2012). Apart from that, the salt strength in the media also impacts plant growth, in which reduced media strength resulted in superior growth compared to the full-strength media (Saad and Elshahed 2012). Hence, precise selection of medium type and concentration is vital to improve micropropagation efficiency in *D. asper* bamboo (Suwal et al. 2020).

Plant growth regulators (PGRs) like cytokinin (BAP, kinetin) and auxins (IAA, IBA) mimic natural hormones in micropropagation media, in which the former often promotes bud breaking and shoot multiplication while the latter induces rooting during bamboo micropropagation (Pratibha and Sarma 2014; Patel et al. 2015; Gantait et al. 2018; Lin et al. 2019). Previous studies recorded that the combination of auxin and cytokinin at specific concentrations could further enhance the explant's growth, in this case, *Bambusa bambos* (L.) Voss and *B. vulgaris* Schrad. ex J.C.Wendl. species, with the right ratio of auxin to cytokinin being crucial for optimal results (Desai et al. 2019). To our knowledge, no published studies have investigated the combination effects on *D. asper* species. Therefore, this study aims to evaluate the effectiveness of basal media formulations and the synergistic effects of BAP and IBA growth regulators on in vitro propagation, along with rooting and acclimatization, with the aim of optimizing protocols for enhanced propagation efficiency of *D. asper* species.

MATERIALS AND METHODS

Preparation of the culture medium

The media used in this study were MS (Murashige and Skoog 1962) (Duchefa Biochemies, The Netherlands) and VW (Vacin and Went) (Duchefa Biochemies, The Netherlands) media. Each medium was prepared either half-strength or full-strength, with the addition of 30 g/L sucrose (Systerm, Malaysia), 100 mg L^{-1} myo-inositol (Duchefa Biochemies, The Netherlands), 1 mg L^{-1} BAP hormone (Duchefa Biochemies, The Netherlands) and 3 g L^{-1} Gelrite (Sigma, St. Louis, USA). The media were adjusted to pH 5.7 prior to autoclaving at 121°C for 20 minutes. The autoclaved media were then poured into 100 mL sterile pill box containers at a volume of approximately 20 mL per container.

Culture in different types and strength of basal media

In this study, in vitro *D. asper* plantlets were utilized. The young, actively growing nodal segments were cut into 10-15 mm and cultured on different strengths of two different basal media, MS and VW media, with or without the addition of 1 mg L^{-1} BAP hormone, according to Table 1. The cultures were maintained in the culture room at 27±2°C under a 16 h light photoperiod of white cool fluorescent light (Philips, China). The observation for length and number of shoots were taken after the 8th week of culture.

Multiplication and shoot proliferation

The nodal explants were cultured in full-strength MS basal medium supplemented with a combination of BAP and IBA hormone to determine the most suitable hormone combination that supports the establishment of multiple shoots (Duchefa Biochemies, The Netherlands) at different concentrations from 0.5 to 4.0 mg L^{-1} in approximately 20 mL media. All media used in this study were supplemented with 3 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, and 0.3 g L^{-1} Gelrite as a gelling agent. The pH of the media was adjusted to 5.7 prior to autoclaving at 121°C for 20 min. All cultures were maintained in the culture room at 27±2°C under 16 h light photoperiod of white cool fluorescent light. The observation for length and number of shoots were taken after the 12th week of culture.

Rooting and acclimatization

During this stage, propagules consisting of 3 to 5 shoots were transferred in liquid MS media supplemented with different concentrations of IBA hormone (1.0-5.0 mg L^{-1}) for rooting induction. The culture was maintained in the culture room at 27±2°C under 16 h light photoperiod of white cool fluorescent light. After four weeks of culture, the rooted plantlets underwent pre-hardening, where they were transferred to seedling trays containing peat moss and maintained in the culture room for three weeks. The plants were then removed from the trays and transferred to bigger trays containing a mixture of soil, vermiculite, and vermicompost at (1:1:1) ratio. The trays were placed in the greenhouse, and the survival of the plantlets was observed after a month.

Table 1. Experimental treatments and corresponding media specifications

Treatment	Media type	Strength	BAP concentration (mg L^{-1})
1	MS	Half	0
2	MS	Half	1
3	MS	Full	0
4	MS	Full	1
5	VW	Half	0
6	VW	Half	1
7	VW	Full	0
8	VW	Full	1

Statistical analysis

Six replicates were utilized for each treatment, and the experiment was repeated twice. All collected data were analyzed and subjected to one-way ANOVA to test for statistical significance, followed by Tukey's post hoc test at $p<0.05$ if significant data was obtained. Correlations between the PGRs used and shoot data were evaluated using Pearson's correlation coefficient. All analyses were performed using IBM SPSS statistical software v.28.0.

RESULTS AND DISCUSSION

Effect of different types and strength of basal media

Number of shoots

The highest average number of shoots was observed in full-strength MS with BAP hormone (5.6 shoots), followed by full-strength VW with added BAP hormone (4.57 shoots) (Figure 1). Half-strength VW without hormone resulted in the lowest shoot number with an average of 1 shoot per explant. The findings from ANOVA analysis indicated that the treatments had no significant effect (at $p<0.05$) on the overall average number of shoots of *D. asper* as compared to the length of shoots.

The result obtained was in line with a previous study in which *D. asper* nodal explants responded better in MS media, producing an average of 5.33 shoots per explant compared to other media tested such as SH (Schenk and Hildebrandt 1972), NN (Nitsch and Nitsch 1969) and B5 (Gamborg et al. 1968) media (Singh et al. 2012). However, another study conducted on the same bamboo species reported that MS media with the strength of three-fourths (¾) supplemented with 3 ppm TDZ resulted in the highest number of shoots (Gusmiaty et al. 2020). Another study conducted on *D. strictus* (Roxb.) Nees had shown the maximum shoot with the incorporation of 0.5 mg L^{-1} TDZ into the half-strength MS liquid media (Singh et al. 2001).

The comparison between MS and VW media showed that the length of shoots in full-strength MS media with or without 1.0 mg L^{-1} BAP was higher compared to the full-strength VW media with and without the hormone. The presence of 1 mg L^{-1} BAP hormone in the media played a vital role in improving the growth of explants, and all media with this hormone showed better responses than those without it. The effect of BAP in improving shoot proliferation has been observed in previous studies on the same species (Ojha et al. 2009).

While VW medium is prominently used in orchid culture, this medium is not limited exclusively to orchids. In fact, many previous studies have utilized this medium in culturing different plant species. Suryaningsih et al. (2018) cultured sorghum, a plant from the grass family in VW media to induce callus formation. Pal et al. (2022) remarked that VW medium promoted indirect organogenesis of *Eleusine coracana* (L.) Gaertn. seeds by formation of callus compared to other media. In our study, however, the observed outcomes suggested that the growth and development of bamboo were less favorable in the VW medium compared to the MS medium (Figure 2). This underscores the importance of selecting an appropriate culture medium tailored to the specific needs of bamboo species, and our finding recommends MS medium as a more effective choice for successful tissue culture.

Length of shoots

From the result, it was found that the highest shoot length was obtained from explants in full-strength MS media with the addition of 1.0 mg L^{-1} BAP hormone (Treatment 4) with 2.14±0.18 cm (Figure 3). This treatment was significantly different ($p<0.05$) from others except for full-strength MS without BAP (Treatment 3), suggesting that full-strength MS resulted in a distinct impact on shoot length compared to other treatments. ANOVA analysis revealed that treatments of different media types and strengths significantly affected the length of shoots of the *D. asper* explants. The length of shoots observed in all VW media was overall relatively lower compared to the one cultured in MS media.

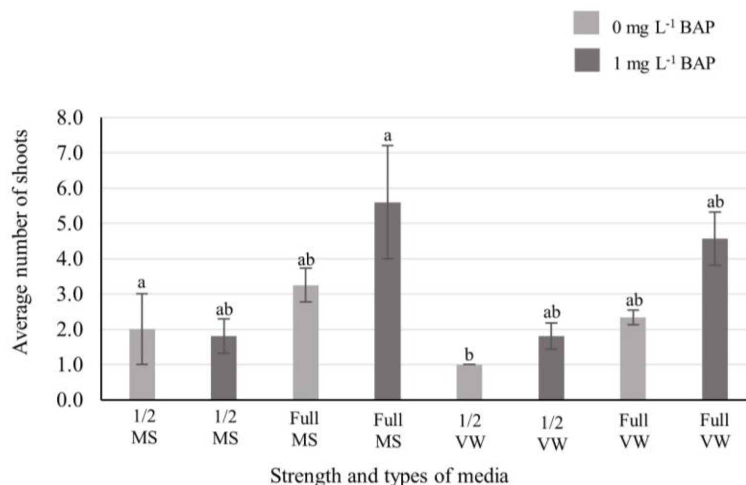

Figure 1. Effects of different treatments (types and strength of media) on shoot number of *Dendrocalamus asper*

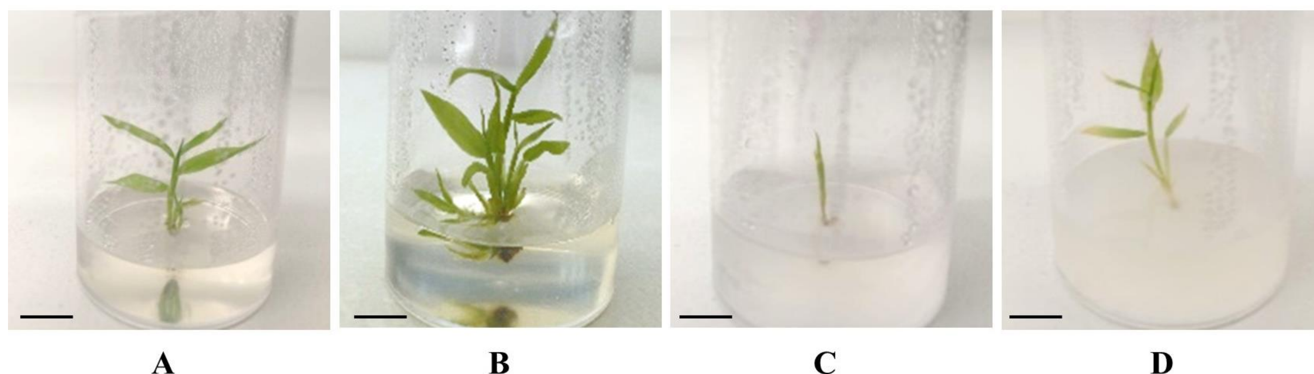

Figure 2. Observation for culture initiation of *Dendrocalamus asper* in different types of media after 8 weeks of culture. A. Culture in full-strength MS without BAP, B. Culture in full-strength MS with 1 mg L^{-1} BAP, C. Culture in full-strength VW media without BAP, D. Culture in full-strength VW media with 1 mg L^{-1} BAP. Bar = 1 cm

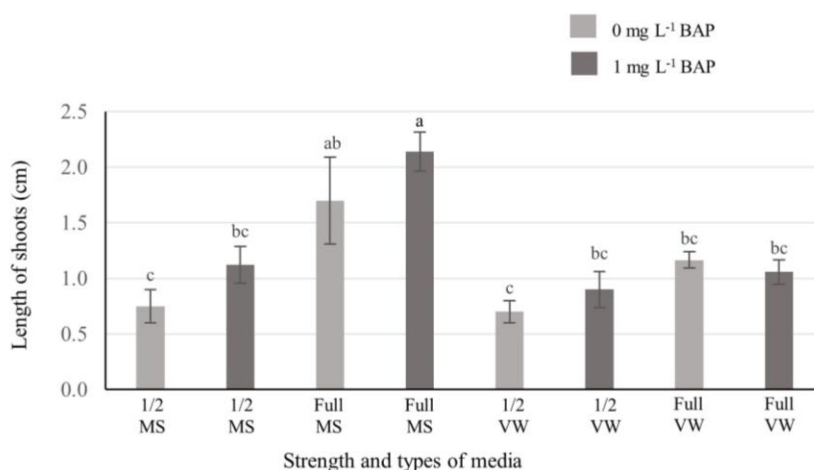

Figure 3. Effects of different treatments (types and strength of media) on shoot length of *Dendrocalamus asper*

A previous study conducted on *Phyllostachys meyeri* McClure nodes revealed that cultivation in half-strength liquid MS without the addition of phytohormone improved the elongation of shoots (Ogita et al. 2008). Another study conducted by Patel et al. (2015) using *B. balcooa* Roxb. species showed that the highest shoot elongation was recorded in half-strength MS with an additional 3 mg L^{-1} BAP during the pre-hardening stage. In the present experiment, full-strength MS showed better performance than half-strength MS in terms of shoot length. The strength of media refers to the concentration of nutrients and salts present in the media. A higher strength of MS media possessed high levels of macro elements, especially nitrogen content (KNO3 and NH4NO3) in the form of ammonium and nitrate that helps to improve the regeneration of explants compared to other basal media (Arab et al. 2014; Phillips and Garda 2019). Nucleic acids, proteins, and secondary metabolites were also highly influenced by nitrogen concentration during plant development (Sidek et al. 2018). This difference in results emphasized the need for tailored approaches considering species-specific requirements in tissue culture studies.

Effect of different combinations of hormones during shoot multiplication

Number of shoots

The results of ANOVA analysis showed that the different treatments of hormones significantly affected ($p<0.05$) the number of shoots produced per explant. Concentration of 4.0 mg L^{-1} BAP combined with 0.5 mg L^{-1} IBA was shown to produce the highest number of shoots compared to other treatments with 5.17±3.97 (Table 2). Other combinations of BAP and IBA also displayed a high number of shoots in vitro, such as the combination of 4.0 mg L^{-1} BAP with 1.0 mg L^{-1} IBA and 4.0 mg L^{-1} IBA, resulting in 4.75±1.91 and 4.8±2.95 shoot number respectively. The lowest shoot number was obtained in the treatment of 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} IBA.

Table 2. Effect of different BAP and IBA hormone concentrations in MS medium on length and number of shoots of *Dendrocalamus asper* after 12 weeks

Concentration (mg L^{-1}) BAP	IBA	Length of shoots (cm)	No of shoots
0	0	1.59±1.20^{abcde}	2.00±1.00^{ab}
0.5	0	1.25±0.44^{ab}	3.43±1.81^{ab}
1.0	0	1.11±0.65^{efg}	3.56±2.35^{ab}
2.0	0	0.82±0.32^{fg}	2.86±1.35^{ab}
4.0	0	0.93±0.55^{cdefg}	3.00±2.16^{ab}
0	0.5	1.13±0.84^{efg}	2.14±1.35^{ab}
0	1.0	1.27±0.50^{efg}	1.89±1.05^{ab}
0	2.0	1.77±1.33^{abcd}	2.29±1.50^{ab}
0	4.0	1.89±1.15^{a}	1.75±0.89^{ab}
0.5	0.5	1.47±0.93^{abcd}	2.14±0.70^{ab}
0.5	1.0	1.75±0.10^{abcdef}	1.56±0.73^{b}
0.5	2.0	1.26±0.65^{abc}	2.33±0.87^{ab}
0.5	4.0	0.87±0.31^{efg}	3.00±0.82^{ab}
1.0	0.5	0.78±0.45^{defg}	2.25±1.75^{ab}
1.0	1.0	1.13±0.55^{bcdefg}	2.43±1.62^{ab}
1.0	2.0	1.03±0.76^{bcdefg}	1.90±1.29^{ab}
1.0	4.0	1.07±0.49^{bcdefg}	3.71±1.98^{ab}
2.0	0.5	1.35±1.01^{bcdefg}	2.33±1.75^{ab}
2.0	1.0	0.65±0.32^{efg}	3.50±2.67^{ab}
2.0	2.0	1.02±0.81^{defg}	4.09±2.26^{ab}
2.0	4.0	0.80±0.31^{efg}	2.67±1.22^{ab}
4.0	0.5	1.01±0.33^{cdefg}	5.17±3.97^{a}
4.0	1.0	0.62±0.27^{g}	4.75±1.91^{ab}
4.0	2.0	0.85±0.71^{fg}	2.71±1.60^{ab}
4.0	4.0	1.06±0.91^{fg}	4.80±2.95^{ab}

Note: Data shown as mean ± SE followed by the same letter are not significantly different according to Tukey's post hoc test at $p<0.05$

Table 3. Pearson correlation between BAP concentration on shoot length and shoot number of *Dendrocalamus asper*

Variables	BAP Concentration	Shoot length	Shoot number
BAP concentration	1	-0.562**	0.103
Shoot length	-0.562**	1	-0.417**
Shoot number	0.103	-0.417**	1

Note: Correlation was significant at the 0.05 level (2-tailed)

Table 4. Pearson correlation between IBA concentration on shoot length and shoot number of *Dendrocalamus asper*

Variables	IBA Concentration	Shoot length	Shoot number
IBA concentration	1	-.030	.147
Shoot length	-.030	1	-.388**
Shoot number	.147	-.388**	1

Note: Correlation was significant at the 0.05 level (2-tailed)

Our finding was closely aligned with Devi and Sharma (2009), in which a concentration of 13.3 µM BAP and 1.0 µM IBA used together in the media resulted in an enhanced multiplication rate in *Arundinaria callosa* Munro bamboo species. A previous study has reported the combination of 4.0 mg L^{-1} BAP, and 1.0 mg L^{-1} 1-Naphthaleneacetic acid (NAA) resulted in an average of 62 shoots per explants in *B. balcooa* Roxb. culture (Rajput et al. 2020). In another study, instead of increasing shoot multiplication, media enriched 8.0 µM BAP and 1.0 µM NAA led to an expanded rhizomatous section of the explants of *D. hamiltonii* Nees & Arn. Ex. Munro (Agnihotri and Nandi 2009). Other studies have also documented impressive outcomes when utilizing the combined influence of cytokinin and auxin together (Venkatachalam et al. 2015; Rajput et al. 2019; Huang et al. 2024). Apart from that, a combination of two cytokinins has been reported to enhance the proliferation of shoots in certain bamboo species. Utilizing 2 mg L^{-1} kinetin (Kn) and 3 mg L^{-1} BAP together led to the highest average shoots per explant in two different bamboo species, *Melocanna baccifera* (Roxb.) Kurz and *B. tulda* Roxb. (Waikhom and Louis 2014).

Apart from the combination of BAP and IBA hormone, explants cultured in single hormone BAP produced a relatively higher number of shoots compared to single hormone IBA. The role of BAP as a shoot-inducing hormone can be observed from this, and among all treatments with single hormone BAP, a concentration of 1.0 mg L^{-1} resulted in the highest number of shoots. A previous study by Arya et al. (1999) showed that 3.0 mg L-1 BAP added in MS medium produced up to a 16-fold multiplication rate of *D. asper* nodal explants. Favorable responses from single BAP treatments have also been observed in *B. balcooa* (Gantait et al. 2018; Pratibha and Sarma 2014), in which a maximum number of shoots were obtained in their study.

Length of shoots

The highest length of shoots was observed in MS with 4.0 mg L^{-1} IBA with 1.89±1.15 cm, followed by 2.0 mg L^{-1} IBA with 1.77±1.33 cm (Table 2). The shortest shoot length was recorded in treatment combining 4.0 mg L^{-1} BAP and 1.0 mg L^{-1} IBA with an average of 0.62±0.27 cm. Based on the statistical analysis conducted, the hormone concentration also significantly affected ($p<0.05$) the length of shoots.

IBA is a type of hormone that is commonly used as a rooting hormone in woody plants (Zhao et al. 2022) and is responsible for enhancing cell elongation, especially in root cells (Pacheco-Villalobos et al. 2016). The result of this study was slightly different from most previous publications, reported a significant increase in shoot length in media with a combination of BAP and IBA compared to individual hormone treatments in different bamboo species (Venkatachalam et al. 2015; Desai et al. 2019; Rajput et al. 2019). Plus, compared to IBA, most of the bamboo explants produced higher lengths of shoots when treated with a single BAP hormone or other cytokinin only, such as Kn and thidiazurone (TDZ) (Ornellas et al. 2017; Gusmiaty et al. 2020; Choudhary et al. 2022; Gonçalves et al. 2023) instead of IBA alone.

Correlation between BAP and IBA hormone concentrations on the length and number of shoots

Pearson's correlation coefficients were calculated for the length and number of shoots in relation to BAP and IBA hormones to assess the relationship between PGR concentration and shoot morphology. Our results indicated a significant, strong negative correlation between BAP

concentration and shoot length (-0.562) (Table 3), suggesting that elevated BAP levels promote shorter shoot development. This finding is consistent with a previous study, where an increased concentration of BAP also resulted in an overall downward increment in the mean shoot length of this species, as reported by Gunasena et al. (2024).

Meanwhile, IBA concentration exhibited a weak, non-significant negative correlation with shoot length (-0.030) (Table 4), implying a minimal influence on shoot elongation under the experimental conditions, though the highest mean shoot length was observed in cultures supplemented with the maximum IBA concentration (4.0 mg/L). This somehow indicated that, under the specific conditions of this study, IBA either minimally influenced or potentially inhibited cell elongation shoots; however, the effect was very minimal and not significant. This apparent contradiction might be attributed to several factors, such as species-specific response by this plant species and other experimental conditions, such as light and nutrient availability in the media (Long et al. 2022).

Both BAP and IBA hormones displayed a very weak, positive correlation with the number of shoots formed (0.103 and 0.147, respectively). These results contrast with previous findings in *Guadua angustifolia* Kunth bamboo, where a modest positive correlation between BAP concentration and shoot formation was reported (Jiménez et al. 2006). The discrepancy might be attributed to the same factors, such as species-specific responses to PGRs or variations in experimental conditions.

Rooting and acclimatization

The effect of different concentrations of IBA hormone on *D. asper* plantlet rooting was evaluated after 4 weeks of culture. All IBA concentrations (1, 2, 3, 4, and 5 mg L^{-1}) resulted in some degree of rooting. The concentration of 5 mg L^{-1} IBA resulted in the highest percentage of rooting success (60%), while the concentration of 1 mg L^{-1} IBA resulted in the lowest rooting success (20%). Despite varying rooting performance across treatments, all replicates in nearly all treatments had over 10 roots, and the average length of roots exceeded 7 cm for most of the roots.

The observed positive correlation between IBA concentration and rooting success aligns with established knowledge regarding auxin's role in root initiation (Mustafa et al. 2021). Furthermore, the optimal concentration for rooting in our study (5 mg L^{-1} IBA) was consistent with the findings of a previous study that reported successful rooting in another bamboo species, *Oxytenanthera abyssinica* (A.Rich.) Munro uses the same IBA concentration (Admas 2024).

Following successful rooting, plantlets were subjected to a pre-hardening stage to facilitate acclimatization to ex-vitro conditions. This transfer occurred within the controlled environment of the culture room, where key parameters like humidity, light intensity, and temperature were maintained for an additional three weeks. This stage allowed the plantlets to gradually adjust to a slightly less humid environment and develop a more robust root system suited for independent growth in the greenhouse. The formation of new white secondary roots was observed (Figure 4.C) after this stage. The formation of these roots is vital to ensure the highest survival frequency during the acclimatization stage (Patel et al. 2015). All plantlets displayed healthy morphology and 100% successfully established themselves within the chosen substrate mixture (soil, vermiculite, and vermicompost).

In conclusion, the results of this study demonstrated that MS media at full strength is the best media for the initiation of culture in *D. asper* bamboo plants. Shoot multiplication was most successful on full-strength MS media supplemented with 4 mg L^{-1} BAP and 0.5 mg L^{-1} IBA, resulting in the highest number of shoots with satisfactory lengths. This finding suggests that the combination of these hormones has a positive impact on the growth and development of *D. asper* bamboo plants. For rooting, liquid MS media containing 5 mg L^{-1} IBA achieved the highest percentage of rooting success, and all rooted plantlets survived during the acclimatization stage. The findings of this study are of utmost importance for the successful in-vitro propagation of *D. asper* bamboo species, which can contribute to the development of efficient and sustainable bamboo cultivation practices for this economically important species.

Figure 4. Rooting and acclimatization of *Dendrocalamus asper* plantlets. A. Rooted plantlets cultured in liquid MS media supplemented with 5 mg L^{-1} IBA, B. Plantlets during pre-hardening stage after three weeks in the culture room, C. Rooted plantlets after pre-hardening, ready for transfer to new substrate mixture, D. Plantlets established in the chosen substrate mixture (soil, vermiculite and vermicompost) and maintained in the greenhouse

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