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MICROPROPAGATION OF *Dendrocalamus sericeus*

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Abstract: Micropropagation of Sang Mon bamboo tissue culture using bamboo node explant can increase the number of plants for rapid utilization. Therefore, this experiment aimed to study the sterilization of node explant and suitable medium for shoot induction and shoot multiplication. The method suitable for disinfecting node explant for bamboo was established. First, the node explant was washed and thoroughly rinsed with soap, then immersed in 70% alcohol for 2 min, in 10% Clorox® for 15 min, in 5% Clorox® for 10 min, (Add 1-2 drops of Tween20 to Clorox®) and in fungicide (Fosetyl-aluminium) at a concentration of 1.5 g L^{-1} for 1 hr. The bamboo node was washed three times of autoclaved distilled water. It was found that the leas microbial contamination was 25.89±6.22% and the number of shoots was 1.35±0.24 shoots/node. This disinfection method was applied before shoot induction and shoot multiplication. For shoot induction, nodes were cultured on solid MS medium supplemented with 0, 2, 4 and 6 mg L^{-1} BA, with 2, 4 and 6 mg L^{-1} BA and 1 mg L^{-1} KIN and with 2, 4 and 6 mg L^{-1} BA and 1 mg L^{-1} NAA for 4 weeks. The results showed that the explants cultured on MS medium supplemented with 4 mg L^{-1} BA had a maximum induced shoot at 67.53±7.80%, an average of shoot numbers at 4.03±0.23 shoots/node and shoot length of 3.52±0.44 cm. Then, three shoots per clump were sub-cultured onto solid medium or liquid MS medium supplemented with 0, 2, 3 and 4 mg L^{-1} BA for shoot multiplication. After cultured for two weeks the results showed that medium-stage and BA concentrations were not significantly different on shoot multiplications and shoot length. Hence, this protocol can be used for micropropagation of *D. sericeus* and a database for the tissue culture of other bamboo species.

Keywords: plant tissue culture, bamboo, node explant, growth regulators, shoot induction and shoot multiplication

Introduction

Bamboo is a monocotyledonous plant in the Poaceae family. It can be well adaptable and has a natural distribution in almost every part of the world. The areas with dense distribution are found in the southern and southeastern parts of Asia, including Thailand (Pattanawiboon *et al.*, 2001). At present, there are 47 genera of bamboo, divided into 1,250 species. In Thailand, where bamboo grows well, Sang Mon Bamboo (*D. sericeus*) is a native bamboo species found in the northern region. its stems are large, straight, strong and thornless. Young stems are covered with white powder, similar to flour, while mature stems are grayish-green. When fully grown, they are about 10-25 meters tall. Their thick wood is popularly used to produce furniture. This makes Sang Mon Bamboo be required in high demand in the market, resulting in a high price (Phuangchik *et al.*, 2013). However, there are many limitations to natural bamboo propagation, including the surroundings caused by nature, wild animals, or humans (Austin *et al.*, 1983), as well as the uncertain flowering of each bamboo type and the low germination rate. Its flowering period is approximately 30-100 years (Banik, 1980; Pattanawiboon *et al.*, 2001). In addition, asexual propagation of bamboo is popularly done by cutting and air layering, but there are often limitations in terms of the quality of the buds or complete branches, as well as pathogens that can enter the plant during propagation. Its growth takes a long time, has a low survival rate in thin bamboo (Hasan, 1980; Banik, 1985; Sing *et al.*, 2013), and cannot be propagated in large numbers. In many cases, such micropropagation techniques could present drawbacks, such as low

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efficiency, high costs, seasonality and problems with the transportation logistics of large-sized propagules (Gielis *et al.*, 2001; Singh *et al.*, 2013). Due to these problems, the plant tissue culture technique is used for propagation, which is a method that can produce a large number of plants, does not take a long time, and is free of pathogens, with even sizes (Kaveeta, 2002) and similar characteristics to the mother plant (Bekheet *et al.*, 2015; Sahu *et al.*, 2018). According to Songsoem *et al.* (2022) experiment, culturing disinfected explants revealed a relatively not high survival rate for sterile nodes (about 30-60%) due to the fact that the explants were derived from plants grown in natural conditions, which often resulted in fungi and bacteria adhering to the hairs and leaf sheaths, making sterilization difficult. Dongmanee (2006), disinfected *T. siamensis* by surface sterilization with 10% Haiter for 10 minutes, achieved the highest sterilization outcomes in March, April and May, with a survival rate ranging from 76.1 to 82.0%. However, in October, the survival percentage dropped to 46.6%. Bamboo propagation can use tissue culture. According to the research of Saroj *et al.* (2022), bamboo nodes cultured on MS medium supplemented with BA at a concentration of 1.5 mg L^{-1} together with TDZ at a concentration of 0.05 mg L^{-1} as well as the research of Suda *et al.* (2021) revealed that seed culture on MS medium supplemented with BA at a concentration of 2 mg L^{-1} together with NAA at a concentration of 0.5 mg L^{-1} can increase the number of shoots, etc. However, at the same time, most of tissue culture of the Sang Mon Bamboo using nodes from the plantation for cultivation still has a high problem of microbial contamination during the surface sterilization step, especially during the rainy season. Moreover, there is also a problem of not being able to increase the number of shoots in large quantities. Therefore, this research aimed to study the sterilization methods for the surface of initial tissues for culture and investigate methods for optimizing the medium for shoot induction and multiplication in bamboo. The guidelines will guide the development of efficient Sang Mon bamboo propagation protocols, beginning with the selection of bamboo explants, the use of disinfection techniques, appropriate medium formulas for inducing new shoots, multiplying new shoots, and the dissemination of knowledge to the public for further research. It serves as a database that provides tissue culture information for other decent bamboo species in the future.

Materials and Methods

Origin of bamboo species utilized in experiment

The study chose a 3-year-old Sang Mon, derived from seed culture. Associate Professor Thanpisit Phuangchik from the Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University, Rangsit Campus, proposed and selected this bamboo species based on his research. The planting occurred at the Science and Technology Equipment Center, Suranaree University of Technology (SUT).

Study of the sterilization node explants

Sever bamboo nodes (2–4 cm length, 0.5–0.8 cm diameter) from the mother plant. Clean them with soap and water, then sterilize them using the methods in Table 1. Rinse with distilled water and sterilize thrice for 3 minutes each. Trim both ends, leaving 1–2 cm, and cultivate on MS media without growth regulators, adding 6 g L^{-1} agar, and 30 g L^{-1} sucrose, adjusting pH to 5.7, and autoclaving at 121 °C for 15 minutes. Incubate under 16-hour light (80–100 μmol m^{-2} s^{-1}) at 25 ± 2 °C for 4 weeks. The CRD experiment used 5 treatments, 3 replications, and 30 bottles (1 node each).

Data included fungal/bacterial contamination and shoot numbers, analyzed using DMRT at a 95% confidence level.

Table 1 The method fir sterilization the node explant

Treatments
T1; 70% alcohol for 2 min, 10% Clorox® for 15 min, 5% Clorox® for 10 min, 1.5 g/L concentration in fungicide for 1 hr.
T2; 70% alcohol for 1 min, 10% Clorox® for 1 min
T3; 70% alcohol for 1 min, 30% H_2O_2 for 15 min
T4; 70% alcohol for 1 min, 30% H_2O_2 for 15 min, 10% Clorox® for 10 min, 5% Clorox® for 5 min
T5; 30% H_2O_2 for 15 min

Study of the appropriate formula for shoot induction

The optimal approach from experiment 1 is to sterilize the bamboo shoot nodes. The experiment investigated suitable formulas for shoot induction in bamboo node tissue culture. MS medium was tested with 10 formulas: BA (2, 4, 6 mg L^{-1}), BA (2, 4, 6 mg L^{-1}) + KIN (1 mg L^{-1}), BA (2, 4, 6 mg L^{-1}) + NAA (1 mg L^{-1}), and MS without growth regulators as a control. Each formula contained 6 g L^{-1} agar, and 30 g L^{-1} sucrose, and was adjusted to pH 5.7 before autoclaving at 121 °C for 15 minutes. Cultures were maintained under 16-hour light (80–100 µmol m^{-2} s^{-1}) at 25 ± 2 °C for 4 weeks. Each treatment was tested in 10 bottles (1 node/bottle) with 3 replicates. The data collected included shoot emergence percentage, number of shoots emerging per lateral bud, and shoot height. We analyzed the data using CRD and evaluated mean differences using the DMRT method with a 95% confidence level.

Study the appropriate formula to induce multiple shoots.

Take the shoots from Experiment 2, Sang Mon bamboo node explants, which were about 1.5-2 cm tall. The shoots were under sterile conditions. After that, they were divided into clumps of three shoots. They were grown for two weeks, and the experiment was designed as a factorial in a complete randomized design with 3 replications. There were two variables. Factor 1 was the medium status, which consisted of two options: 1) solid medium status with 6 g L^{-1} agar powder and 2) liquid medium status. Factor 2 was the tissue culture medium, which included 1) MS control, 2) MS + 2 mg L^{-1} BA, 3) MS + 3 mg L^{-1} BA, and 4) MS + 4 mg L^{-1} BA. All formulae were combined with 30 g L^{-1} of sugar. The pH was adjusted to 5.7. They were cultured for two weeks, and the experimental outcomes were documented. The number of shoots produced per clump, shot length, and the proportion of shoot multiplication. The experimental findings were statistically examined, and the difference in mean values was calculated using the DMRT method at a 95% confidence level.

Results and Discussion

Results of the study from the sterilized node explants.

Bamboo nodes were sterilized and cultured on MS medium without growth regulators for **4** weeks with different solutions and times; the microbial contamination was significantly different. It was

found that Treatment **1** had the fewest microbes at **25.89±6.22%** and the most shoots at **1.351±0.24** shoots (Table **2).** This matches Songsoem *et al.* (**20**22**)** research report, which washed the nodes of *D. sericeus* with **10%** Clorox® for 1**0** minutes. It yielded the most appropriate survival percentage, with the highest node survival at **66.67%.** Additionally, Jirakiattikul *et al.* (**2010)** cultivated the sterilized nodes of *Bambusa nana* on the medium after sterilizing the bamboo nodes with Clorox® at a concentration of **10%** for **15** minutes and **5%** for **10** minutes. The experiment discovered that the sterilization method, duration, concentration, type, and also the season affect the sterilization. Dongmanee (2006) disinfected bamboo nodes of *Thyrsostachys siamensis* with Clorox® at a concentration of **10%** for **10** minutes and found that the nodes were most sterile in March, April and May at **82.00, 76.10** and **78.70%,** respectively. However, during the rainy season from June to October, the disinfection efficiency decreased to **32.50-46.60%** due to the high humidity, which caused microorganisms to stick to the hairs and grow in the leaf sheaths, leading to a higher percentage of contamination and problematic disinfection. It was found that Treatment **5** had the most microbial contamination at **75.41±4.38%** and the fewest shoots on average at **0.38±0.14** shoots. Treatment **2** the most microbial contamination at **72.04%±5.54%** and the fewest shoots on average at **0.24 ± 0.11 (**Table **2).** The use of one type of disinfectant resulted in the highest microbial contamination, likely due to the low disinfectant concentration on the surface and the inappropriate choice of bamboo species. This information is based on research reports by Aasim *et al.* (**2013)**; Mihaljević *et al.* (**2013);** and Barampuram *et al.* (**2014).** To disinfect with H_2O_2, the concentration was between **10** and **12%,** the time duration was between **5** and **10** minutes, and the efficiency was high. This depends on various factors, such as tissue sizes, environmental conditions, and the selection of explants, all of which influence the response to time and the concentration of the substance used. Due to its market availability, most people sterilize bamboo, including nodes, seeds, and inflorescences, using a concentration of **10-14%** of Clorox® (Kaveeta, **2002).** Upon testing for microbial sterilization in five bleaching formulas, the characteristics of bamboo nodes revealed a change in color to pale green on both sides. However, the fact that the bud explants were able to grow into shoots shows that the amount of fungal contamination by the sterilization agents and time varies, which suggests that node survival varies. Moreover, dropping liquid soap like polyoxyethylene sorbitan monolaurate (Tween **20)** during sterilization makes the surface tension between the water and the explant tissues less. Then, rinse them several times with distilled water to eliminate any disinfectant residues. Remove the damaged tissue, usually at the very end of them, and use only the healthy tissues for culture in a medium (Kitwichan, 2011**).** However, surface sterilization is one of the most important steps in pursuing tissue culture. The success of obtaining sterile explants is dependent on several factors, including sterilization time, explants, methods, types, and concentration of disinfectants; the selection of explants is significant step.

Table 2 Contamination percentage and number of shoots in D. sericeu cultured for 4 weeks after sterilization treatment

Treatments	Contamination (%)	Number of shoots (shoot)
1	25.89±6.22^{c}	1.35±0.24^{a}
2	72.04±5.54^{a}	0.24±0.11^{c}
3	54.03±6.49^{b}	0.75±0.19^{bc}
4	60.14±6.62^{ab}	1.01±0.18^{ab}
5	75.41±4.38^{a}	0.38±0.14^{c}
F-test	**	**
CV (%)	35.32	76.02

Data shows mean ± SE, different characters mean statistically different at 0.05 level compared by DMRT (** = *highly significant difference at* $p < 0.01$)

Results of the study the appropriate formula for shoot induction.

From the culture of sterile bamboo nodes on media supplemented with BA, KIN, and NAA at various concentrations for 4 weeks, it was found that the nodes cultured on all 10 formulas of media had green stems and leaves (Figure 1) and developed into shoots, indicating that the lateral buds of bamboo have the potential to grow and develop into shoots. Chanthanurak (1991) stated that tissue from node explants will grow and can develop into shoots. There are also reports of the development of lateral buds into shoots in many bamboo species when the nodal explants are cultured in tissue culture, such as *Thyrsostachys siamensis* (Dongmanee, 2006), and *Bambusa vulgaris* (Kaladhar *et al.*, 2017). The percentage of lateral buds that developed into shoots, the number of shoots, and the height of the shoots were significantly different. The MS formula supplemented with BA at a concentration of 4 mg L^{-1} had the highest percentage of lateral buds developed at 67.53±7.80%, with an average shoot formation of 4.03±0.23 shoots and a shoot height of 3.52±0.44 cm (Table 3), which is consistent with the research study of Dongmanee (2006), finding that synthetic media had an effect on the induction of shoot formation from bamboo node grown on solid media supplemented with benzyl adenine (BA) at 2.5 mg L^{-1}, induced the highest average shoot spurt from lateral buds, approximately 13.50 shoots/node within 1 month, and propagated Tong Bamboo by growing on MS medium supplemented with 0-5 mg L^{-1} BA for 15 days. It was found that the medium supplemented with BA 4 mg L^{-1} was the most suitable for increasing the amount of bamboo shoots (Kentri, 1999), indicating that the use of hormones depends on the plant species. It was also found that *Bambusa balcooa*, cultured on MS medium supplemented with 2 mg L^{-1} BA, induced the highest shoot spurt, and the average shoot length was 3.9 shoots/node (Khileshwar *et al.*, 2018). This experiment showed that adding BA, which is a growth regulator in the cytokinin group, had a positive effect on the shoot increase of Sang Mon Bamboo as it promotes cell division, stimulates shoot growth, increases the number of shoots, and increases cytokinin levels (Kieber and Schaller, 2018; Ario & Setiawan, 2020). BA concentration levels affect shoot number and shoot length (Pandey & Singh, 2012; Goyal *et al.*, 2015). Prutpongse & Gavinlertvatana (1987) reported that the culturing of branch buds of various bamboo species on MS medium supplemented with BA at concentrations in the range of 1-25 mg L^{-1} showed different results depending on the bamboo species cultured.

For tissue culture, Sang Mon bamboo nodes cultured on MS medium supplemented with different concentrations of BA and KIN at 1 mg L^{-1} showed a tendency to increase the number of bamboo shoots by 3.81±0.66 shoots (Table 3; Figure 1). Since KIN has a similar effect to BA, resulting in thin and non-succulent shoots, it is relatively used as a supplement with BA after increasing the concentration of BA until the shoots are plump. However, each plant species responds differently to each cytokinin; therefore, it should be first tested by culturing the interesting plants on media containing different cytokinins. There have been reports of lateral bud developing into shoots in several bamboo species when BA is added together with KIN when the node explants are cultured in tissue, such as *M. baccifera* (Waikhom and Louis, 2014), *D. strictus* (Ravikumar *et al.*, 1998), *D. giganteus* (Arya *et al.*, 2002), *B. wamin* (Arshad *et al.*, 2005), and Sang Mon bamboo node explants cultured on MS culture medium supplemented with various concentrations of BA together with NAA at 1 mg L^{-1}. The tendency towards an increase in shoot number of Sang Mon bamboo was equal to 3.12±0.43 shoots (Table 3; Figure 1) because the addition of BA and NAA affects the increase in bamboo shoot number. As BA is a growth regulator in the cytokinin group, having the property of stimulating A is a growth auxin group that is commonly used together with cytokinin to increase shoot number (Kaveeta, 2002). cell division, stimulating stems, and lateral bud growth. NAA is a growth regulator in the auxin group that is commonly used together with cytokinin to increase shoot number (Kaveeta, 2002). Auxin is usually added at a lower concentration than cytokinin to increase shoot number. If added at a concentration that is too high, it will inhibit shoot growth (Chuichai, 2010). This experimental result is consistent with the culture of many bamboo tissue types that commonly use BA alone or together with NAA to induce shoot formation and increase shoot number. However, they were used in different concentrations; for example, the shoot number of bamboo shoots from Hok bamboos and Tong bamboos could increase well on MS solid medium supplemented with BA at a concentration of 2.5 mg L^{-1} (Godbole *et al.*, 2002) and 5 mg L^{-1} (Prutpongse & Gavinlertvatana, 1992), respectively.

Observations from experiments indicated that the addition of BA alone produced more robust shoots compared to the combination of KIN and NAA. Following over four weeks of tissue culture, the shoots became yellow and perished. The addition of KIN may enhance shoot induction in Sang Mon Bamboo; however, KIN exhibits effects akin to BA, resulting in shoots that are slender rather than robust. Consequently, it was utilized to augment BA. Nonetheless, each plant exhibits a distinct response to various types of cytokinins. The inclusion of KIN is contingent upon variables such as the species of bamboo. Various plants and species predominantly necessitate distinct nutrients. Despite being the same species, variations in age and developmental stage may necessitate distinct nutrient requirements (Kaveeta, 2002). NAA, in accordance with the cytokinin to auxin balance ratio theory, influences organogenesis and is essential for the growth and development of cultured cells, including callus, roots, or shoots (Kaveeta, 2002). A higher ratio of cytokinin to auxin promotes cell division and the development of shoots, stems, and leaves. Nevertheless, a lower ratio of cytokinin to auxin facilitates superior root formation.

The results of the experiment showed that the number of shoots developed from the bamboo node explants 2.18–4.03 shoots was not particularly high. Further study is required to increase the number of shoots. Also, when the bamboo shoots were moved to a new medium using the same MS formula and BA added at a concentration of 4 mg L^{-1} to increase the number of shoots, the browning and black substances showed up on the culture medium around the shoots. This led to the shoots turning brown

and ultimately dying. Therefore, additional research on various factors is necessary to boost the production of Sang Mon shoots. Additionally, the bamboo shoots developed in this sterile environment could successfully grow. In the initial experiment, we added 0.01% of charcoal powder to reduce browning symptoms. This was due to its ability to absorb phenols or black toxins released by the plants, preventing them from reacting with the culture medium (Johansson, 1983). The experiment revealed that the bamboo shoots were unable to grow, resulting in their leaves and plants turning yellow and ultimately dying. The charcoal powder likely absorbs not only toxins but also growth regulators for the medium. Therefore, it had an effect on plant growth (Kitwijan, 1997). However, the addition of liquid and solid nutrients to the medium resulted in an increase in the number of shoots for some bamboos, such as Pai Poh (Ramanayake & Yakandawala, 1997) and Pai Ruak (Dongmanee, 2006). These factors could potentially contribute positively to the growth of Pai Sang Mon. Consequently, we conducted this study to investigate the influence of medium status.

Table 3. Percentage (%) induce of shoot, number of shoots and shoot length (cm) of D. sericeus after cultured for 4 weeks on shoot induction medium

Treatment	Percentage (%) induce of shoot	Number of shoots (shoots)	Shoot length (cm)
MS (Control)	56.28±7.69^{ab}	3.32±0.11^{abc}	2.37±0.10^{ab}
MS+2 mg L^{-1} BA	57.53±7.33^{ab}	3.35±0.31^{abc}	2.44±0.21^{ab}
MS+4 mg L^{-1} BA	67.53±7.80^{a}	4.03±0.23^{a}	3.52±0.44^{a}
MS+6 mg L^{-1} BA	56.28±5.67^{ab}	2.35±0.18^{c}	2.49±0.36^{ab}
MS+2 mg L^{-1} BA+1 mg L^{-1} KIN	49.53±9.37^{ab}	2.68±0.42^{abc}	1.49±0.17^{bc}
MS+4 mg L^{-1} BA+1 mg L^{-1} KIN	51.78±7.69^{ab}	2.85±0.53^{abc}	1.85±0.20^{bc}
MS+6 mg L^{-1} BA+1 mg L^{-1} KIN	40.52±2.60^{bc}	3.81±0.66^{ab}	2.45±0.20^{ab}
MS+2 mg L^{-1} BA+1 mg L^{-1} NAA	33.77±9.99^{bc}	2.18±0.62^{c}	0.98±0.26^{c}
MS+4 mg L^{-1} BA+1 mg L^{-1} NAA	38.27±4.31^{bc}	3.12±0.43^{abc}	1.40±0.22^{bc}
MS+6 mg L^{-1} BA+1 mg L^{-1} NAA	24.76±6.75^{c}	2.50±0.51^{bc}	2.62±0.94^{ab}
F-test	**	**	**
%CV	31.80	29.00	34.03

*Data shows mean ± SE, different characters mean statistically different at 0.05 level compared by DMRT(** = highly significant difference at $p<0.01$)*

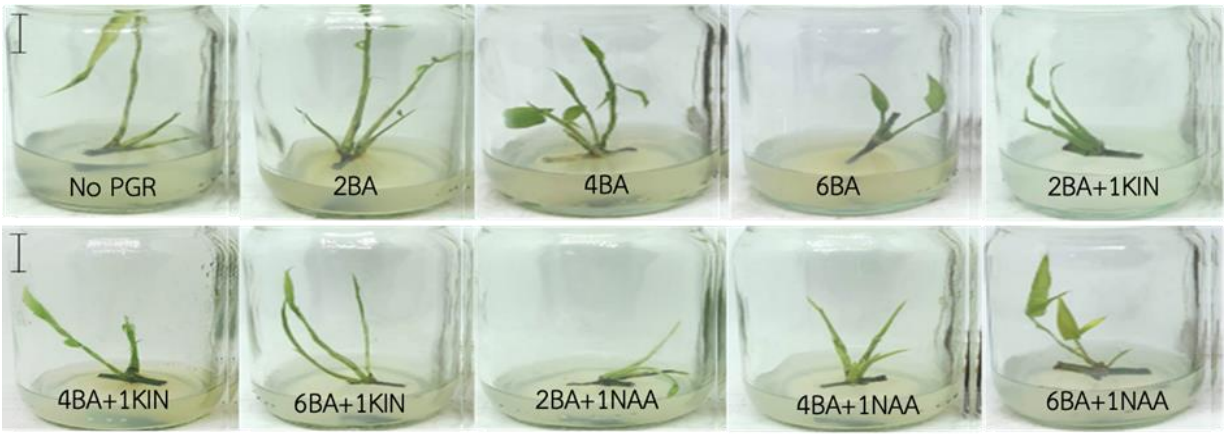

Figure 1. Shoot development of D. sericeus cultured for 4 weeks, the node was grown on MS solid medium with BA concentrations of 2-6 mg L^{-1} , KIN concentrations of 1 mg L^{-1} , and NAA concentrations of 1 mg L^{-1} . (No PGR, growth regulator was added.) (Scale bar=1 cm)

Results of the study the appropriate formula to induce multiple shoots.

Take shoot groups from the nodal explant with a height of approximately 1.5-2.5 cm obtained from tissue culture on solid MS medium containing 4 mg L^{-1} BA from experiment 2 to be cut and separated into 3 shoots/clump. They were sub cultured on MS medium containing 2-4 mg L^{-1} BA and control without growth regulators. They were cultured in different mediums liquid medium and solid medium for 2 weeks. It was found that the medium status did not have any statistically significant differences in shoot multiplication, shoot height, or percentage of shoot multiplication. However, it was found that the plants cultured on liquid medium had the highest number of shoots, shoot height, and percentage of shoot multiplication at 7.15±0.22 shoots, 2.35±0.16 cm, and 138.33±7.22%, respectively (Table 4) The physical state of the medium (liquid or semi-solid) also affects the frequency of shoot initiation and development in various bamboo species. Numerous data indicate elevated rates of bamboo shoot proliferation and enhanced development in liquid medium relative to solid medium. **(**Arshad *et al.*, 2005; Sanjaya & Rai, 2005; Arya *et al.*, 2006; Ogita *et al.*, 2008; Kabade, 2009;**)**. Which was consistent with the research of Gunasena *et al.* (2024), who cultured *D. asper* onto MS liquid medium supplemented with BA at a concentration of 2 mg L^{-1} for 6 weeks and found that the number of shoots was 21.30±1.53 shoots and the shoot height was 12.15±0.60 cm, more than those cultured on solid medium, which was 13.60±1.36 shoots with the shoot height 10.65±0.45 cm. Similat to Mohamad Zani *et al.* **(2024)** The culture of *D. asper* on MS liquid medium supplemented with BA at concentrations of 1 and 4 mg L^{-1} for 4 weeks yielded 3.5 shoots, which is more than the culture on solid medium. According to Gonçalves *et al.* (2023), a semisolid medium recorded the highest mean survival rate of *Bambusa vulgaris* in vitro cultures at 65%, but this did not significantly differ from the survival rate in a liquid medium at 40%. Interspecies variations may account for the variation in the optimal physical condition of the medium. From this study, it can be seen that the best liquid and solid medium formulas gave the same number of shoot multiplications and shoot height. However, the shoots cultured in liquid medium had better external characteristics, color, and shoot height than those in solid medium. Explants frequently exhibit superior development in liquid media due to direct contact with the medium, facilitating enhanced absorption of its constituents and subsequently promoting proliferation (Rai *et al.*, 2022). The partially submerged shoots in the media offer extensive surface absorption, facilitating the effective assimilation of plant

growth regulators (PGRs) like BAP and other hormones into the explants (Rathore *et al.*, 2009). In contrast to liquid medium, the inclusion of a gelling agent may result in a slower release of nutrients and plant growth regulators, leading to delayed absorption of these substances, which ultimately impacts the growth rate of the explants (Singh *et al.*, 2013). While the use of liquid media provides more advantages than semi-solid media, extended immersion in liquid may result in hyperhydricity, a physiological disease that induces biochemical alterations and disrupts the structural integrity of the explant's tissues (Polivanova & Bedarev, 2022) This is consistent with the research report that bamboo *B. nutans* subsp. cupulata cultured onto liquid media and MS solid medium supplemented with BA at a concentration of 1-6 mg L^{-1} for 4 weeks, it was found that the MS formula on liquid medium and solid medium supplemented with BA at a concentration of 3 mg L^{-1} had the highest shoot multiplication and shoot height of 4.70±0.15 shoots, 4.38±0.20 cm and 4.10±0.10 shoots, and 4.90±0.31 cm, respectively (Suwal *et al.*, 2021). This is because the agar added to the solid medium will bind with water and growth regulators, resulting in a decrease in the plant's nutrient absorption capacity. In addition, over time, in the solid medium, the plant will accumulate phenolic substances at the base of the shoot and the plant, increasing toxicity to the plant and causing the plant to grow less, produce fewer shoots, and have yellow leaves that fall earlier than normal. In the liquid medium, there is less nutrient absorption and accumulation of phenolic substances. It can grow and have better plant characteristics (Arshad *et al.*, 2005). In addition, liquid medium can induce more shoot induction because the culture in liquid media provides more surface area for plant tissue than the culture in solid medium (Sookwanna and Pussorn, 2016). The culture of many plant species has the same results (Earle and Langhans, 1975). For example, the culture of vanilla found that MS liquid medium added with BA at a concentration of 2.15 mg L^{-1} after 4 weeks can increase the number of shoots by 18.60 shoots (Lee-Espinosa *et al.* 2008).

When considering the formula of MS medium supplemented with BA at a concentration of 2-4 mg L^{-1} and the control without a growth regulator, it was found that the shoot multiplication, shoot height, and percentage of shoot multiplication were not significantly different. However, it was likely that the plants grown on the MS medium supplemented with BA at a concentration of 3 mg L^{-1} had the highest number of shoots, shoot height, and percentage of shoot multiplication, which were 6.90±0.52 shoots, 2.69±0.22 cm, and 130.00±17.25%, respectively (Table 4). It was also found that the shoots of Sang Mon Bamboo cultured on the MS medium supplemented with BA at a concentration of 4 mg L^{-1} had the number of shoots as 6.90±0.28 shoots and the height as 2.27±0.21 cm (Table 4). When considering the two factors together, there are no interaction on the number of shoots, the shoot height, or the percentage of shoot multiplication (Table 4), indicating that the medium status and BA concentration can be used together. This is consistent with the research of Deelom (2019), it was found that the culture of Beijing Bamboo for 2 weeks on liquid medium and solid MS medium supplemented with BA at a concentration of 2-4 mg L^{-1} tended to have the highest shoot multiplication at 4.60±1.20 shoots. In addition to medium status and growth regulators, the results of shoot multiplication also depend on the plant species. Prutpongse and Gavinlertvatana (1987) reported that the culture of many bamboo species on MS medium supplemented with BA at a concentration of 1-25 mg L^{-1} had different results depending on the bamboo type used for cultivation. Some bamboo species can expand quantities a lot, while others are not successful in cultivation for expansion. This is consistent with the research of Songsoem *et al.* (2022): culturing on MS medium supplemented with BA at a concentration of 4 mg L^{-1} together with IBA at a concentration of 0.3 mg L^{-1} showed a tendency to increase the number of shoots to a maximum of 2.8 shoots, and it was also

found that *B. balcooa* bamboo culturing on MS medium supplemented with BA at a concentration of 2 mg L^{-1} induced the most shoots, and the average shoot length was 3.9 shoots/node (Khileshwar *et al.*, 2018) The size, stage of development, and quantity of explants in each culture container significantly influence the achievement of high multiplication rates, improved shoot growth, and higher-quality shoots per culture container. This is a crucial factor in determining the cost of large-scale production. Most papers evidence advantage of using several shoots instead of single ones for shoot multiplication of different bamboo species; viz., **2-3** shoots in *P. stocksii* (Sanjaya and Rai, **2005)** and *B. nutans* (Negi and Saxena, **2011**a); three shoots in *A. callosa* (Waikhom and Sharma, **2009)** and *Gigantochloa atroviolacea* (Bisht *et al.,* **2010);3-4** shoots in *D. hamiltonii* (Sood *et al.,* **2002**a); **3-5** shoots in *B. wamin* (Arshad *et al.,* **2005)** and *G. angustifolia* (Jiménez *et al.,* **2006)**; **5-7** shoots in *D. strictus* (Ravikumar *et al.,* **1998)**. Researchers found that three to four propagules work better for each culture to increase **(**Singh *et al.,* 2013; Mudoi *et al.*, 2014; Kapruwan *et al.*, 2014**)** compared to one propagule grown **(**Thakur *et al.*, 20**06).** In a lab, researchers frequently used BAP to grow various types of bamboo shoots **(**Somashekar *et al.,* 2008; Kavitha and Kiran, 2014; Thapa *et al.*, 2018**).** More BAP in the air reduced shoot number and length.

Table 4 Number of shoots multiplications, shoot length (cm) and multiplication percentage (%) of D. sericeus after cultured for 2 weeks on shoot multiplications medium.

Experimental factors		Number of shoots multiplications (shoot)	Shoot length (cm)	Multiplication percentage (%)
Medium status (A)	Liquid medium	7.15±0.22	2.35±0.16	138.33±7.22
	Solid medium	6.50±0.33	2.27±0.13	116.67±11.13
	control	6.60±0.37	2.18±0.24	120.00±12.43
Formula medium (B)	MS+2 mg L^{-1} BA	6.90±0.43	2.30±0.12	130.00±14.29
	MS+3 mg L^{-1} BA	6.90±0.52	2.69±0.22	130.00±17.25
	MS+4 mg L^{-1} BA	6.90±0.28	2.27±0.21	130.00±9.33
F-test	A	ns	ns	ns
	B	ns	ns	ns
	A*B	ns	ns	ns
CV(%)		26.48	38.72	47.25

Data shows mean ± SE, different characters mean statistically different at 0.05 level compared by DMRT (ns = non-significant at p>0.05)

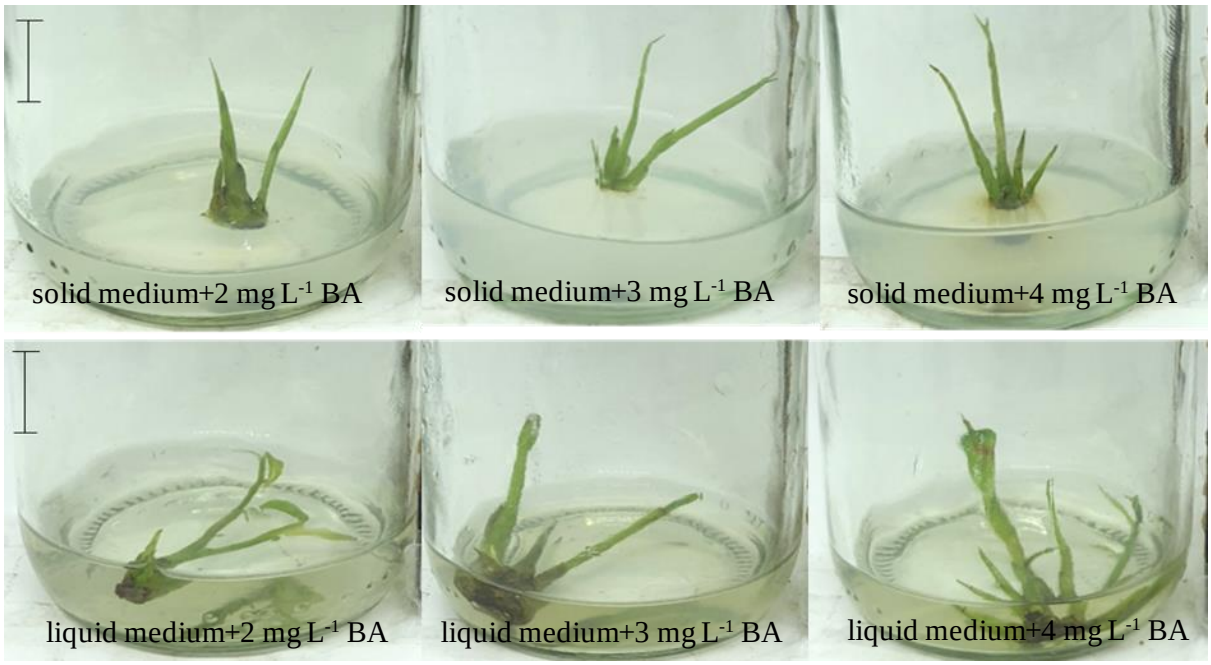

Figure 2 Shoot multiplication of D. sericeus cultured for 2 weeks, the group of shoots are cultured on solid medium (top row) and in liquid medium (bottom row). (Scale bar=1 cm)

Conclusion

D. sericeus, the disinfection was performed by bleaching and sterilizing the surface, the branch nodes were washed with soap, and rinsed thoroughly. After that, they were immersed in 70% alcohol for 2 min, in 10% NaOCl for 15 min, and 5% NaOCl for 10 min, and soaked fungicide at a concentration of 1.5 g/L for 1 hr, respectively. Soaking in sodium hypochlorite in all concentrations, 1- 2 drops of Tween20 were added. The nodes were then washed with sterile distilled water three times, for 3 minutes each, resulting in the least contaminant effect at 25.89%

Micropropagation of *D. sericeus* using bamboo node explants should be done on MS medium supplemented with 4 mg L^{-1} BA.

Suitable formula to increase the number of shoots multiplication can be chosen from any formula and the medium status of the culture should be liquid medium

Recommendations

1.The bamboo nodes used for sterilization in different seasons have varying survival rates. Therefore, it is necessary to study the appropriate timing for sterilizing the bamboo nodes over a period of one year or several years, including the size of the bamboo nodes.

2. Constraints of bamboo species within the research and agricultural regions.

3.The constraints on laboratory space owing to the high student population render it inadequate for practical usage, coupled with the lack of financial research support, since Thailand has very few studies on tissue culture related to bamboo.

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