

# The optimization of in vitro micropropagation of betung bamboo

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## The optimization of in vitro micropropagation of betung bamboo (*Dendrocalamus asper* backer) by medium concentrations and plant growth regulators

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## The optimization of in vitro micropropagation of betung bamboo (*Dendrocalamus asper* backer) by medium concentrations and plant growth regulators

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**Abstract.** Bamboo has excellent potential to be developed as a building material, pulp, textile, carbon sink, water-storing, and water-holding. An alternative method for plant propagation is by in vitro micropropagation (tissue culture). The cultured explants were able to grow to be a new individual having shoots and leaves. Murashige and Skoog (MS) medium concentrations solely were significantly affected by the number of shoots. The addition of thidiazuron (TDZ) into media resulted in the best percentage of shoot number (80%) in all concentrations of media. Using low concentration, TDZ was able to produce more leaves than other cytokinins. The appropriate concentration of MS medium and the plant growth regulator for Betung bamboo's tissue culture is ¼ MS with the addition of 3 ppm TDZ.

### 1. Introduction

Bamboo is one of the plants classified in the grass family (*Poaceae*). It has enormous potential to be developed as a building material, pulp, textile, carbon sink, water-storing, and water-holding. Root and stem of bamboo also possess benefits to be used as traditional herbs. The need for bamboo is massively increasing. However, it has not been balanced between exploitation and innovative cultivations, which consequence in the reduction of its species in nature. One of the species of bamboo that is widely used and developed is betung bamboo (*Dendrocalamus asper*).

Betung bamboo is able to grow from the lowlands to an altitude of 1.500 masl and also capable of growing in all types of soil. Thus this species can be an alternative plant for the rehabilitation of critical land and watersheds. The initial stage of using bamboo as rehabilitation for critical land is by increasing the supply of superior seedlings [1]. The need for superior seedlings commonly cannot be fulfilled by only generative propagation due to several limitations, such as limited of fruiting season time, variation in hereditary traits, required a large space for cultivation and a limited number of seedlings produced, hence plant alternative propagation is needed in order to fill the need of seedlings [2]. The alternative method for plant propagation that can be used is tissue culture techniques [3]. Tissue culture techniques is a method for isolating parts of plants such as protoplasts, cells, tissues, organs, and growing them in aseptic conditions [4,5].

The study by Ruhiyat (1998) [6] revealed that the use of basic media WPM and MS with various plant growth regulators (PGR) in betung bamboo's node as the explant obtained very limited plantlet quantity. Based on the reason mentioned before, this study aimed to optimize the in vitro



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micropropagation protocol of betung bamboo by using node explants on various concentrations of MS medium and types of cytokinin.

## 2. Methodology <sup>4</sup>

This research was conducted at the Biotechnology and Tree breeding laboratory, Faculty of Forestry, Universitas Hasanuddin, Makassar. The explants used in the study were nodes from the mature bamboo branches at age  $\pm$  6-month-old.

### 2.1. Research procedure

2.1.1. *Explant sterilization.* The following procedure performed explant sterilization steps:

1. Cut node part with a size  $\pm$ 4 cm
2. The node was cleaned from sheath leaves by using a cutter. Brushed the node under running water, then soaked it using detergent by adding two drops of tween 80 for 20 minutes.
3. The node was rinsed once with an aquadest.
4. The node was soaked for the second time in 2% of Masalgin added two drops of tween 80 for an hour.
5. The node was then rinsed again with sterile distilled water for three times.
6. Then the node was soaked in 2% of Agrept added two drops tween 80 for an hour.
7. Afterward, the node was rinsed with sterile distilled water for three times.
8. Sterilization was continued inside Laminar Air Flow Cabinet (LAFC) by using 70% alcohol added two drops tween 80 for 10 minutes.
9. Furthermore, the node was soaked in 85% of commercial bleaching liquid for 20 minutes.
10. The node was rinsed by using sterile aquadest three times and placed inside a petri dish containing filter paper (Whatman 3MM) for drying purposes.
11. After the residues of the sterilized solutions were dried, both of the ends of the node that had direct contact with the sterilized solutions were cut using pruning scissors until the node reached  $\pm$  2 cm in length.
12. The node was ready to plant on the media treatments. The planted nodes were then incubated at 24-25<sup>0</sup>C in the incubation room with 12 hours of light.

This research was arranged in a completely randomized design (CRD) factorial experiment, as follows :

- a) The first factor was the concentration of MS basic medium, which consisted of four levels, namely :
  1.  $\frac{1}{4}$  strength of MS concentration (M1)
  2.  $\frac{1}{2}$  strength of MS concentration (M2)
  3.  $\frac{3}{4}$  strength of MS concentration (M3)
  4. 1 strength of MS concentration (M4)
- b) The second factor was the types of cytokinin, which consisted of three levels:
  1. 6-Benzyl Amino Purine (BAP) 3 ppm (S1)
  2. N6-furfuryladenine (Kinetin) 3 ppm (S2)
  3. Thidiazuron (TDZ) 3 ppm (S3)

Total treatment combinations were 12, and each treatment was repeated five times, each repetition consisted of one observation bottle. Each observation bottle was planted a node explant. Hence all observation units were 60 observation units. The observation was conducted in 60 days after planting (DAP). Particularly for the day when the shoot formed, the observation was conducted every day until the end of observation. The observation variables were :

- a. The day of shoot formation (DAP), was conducted by counting the first day of shoot formed at each explant in every treatment.
- b. The percentage of the shoots (%) at each treatment, was calculated using the following formula:

$$\frac{\sum \text{Number of shoots at each explant} \times 100\%}{\sum \text{Number of total planted explant}}$$

- c. The number of shoots was performed by counting the shoot number formed at each explant.
  - d. The number of leaves was conducted by counting the number of leaves formed at each explant.
- The data obtained were analyzed by using the F-test at a significant level of 5%. If the results show significant differences, then further tests would be performed by the Duncan's Multiple Range Test (DMRT). The data were analyzed using the SPSS program.

**3. Results and discussion**

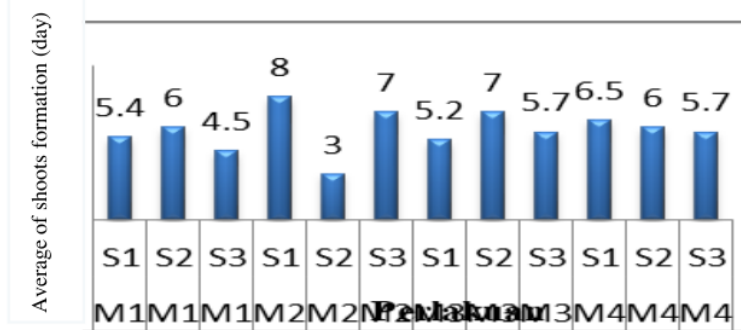
*3.1. Time of Shoot Formation*

The observation showed that the combination of treatments did not affect the day of the shoot formed. The average observation result for shoot formation, as depicted in figure 1.



**Figure 1.** Condition of shoot explant at seven days after planting

Figure 1 presents the betung bamboos' node that was capable of growing and developing into new individuals with a good growth response by showing the formation of the shoot. Figure 2 shows that the fastest average for a shoot to be formed at explant was on M2S2 (1/2 MS Kinetin) (3 DAP), followed by M1S3 (1/4 MS TDZ) (4,5 DAP) and M3S1 (3/4 MS BAP) (5.2 DAP). M2S1 (1/4 MS DAP) had the slowest response to induce the formation of the shoot, namely 8 DAP. These results were indicated that Kinetin had an effect on rapidly stimulating the formation of shoot compared to BAP and TDZ.



**Figure 2.** The averages day of shoots formation on node explants of betung bamboo

3.2. Percentage of shoots

The observation presented that the total average of the shoot was 72% (43 explants) out of a total of 12 treatments (60 explants). The average percentage of shoots from each treatment can be seen in figure 3.

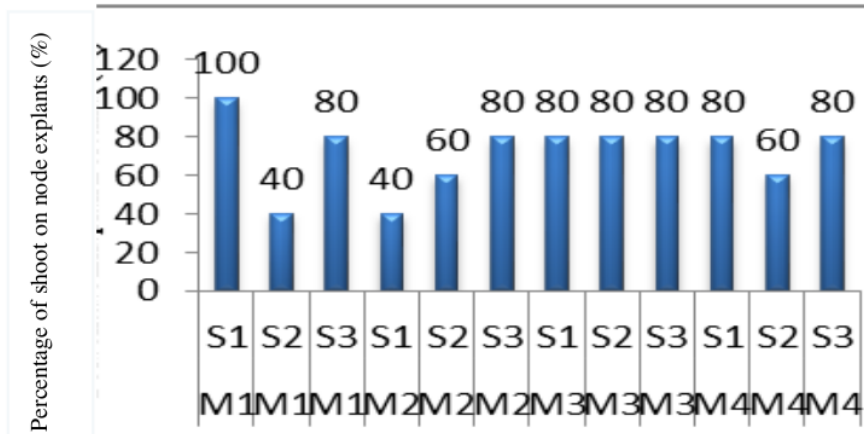


Figure 3. The averages percentage of shoot on node explants of betung bamboo

The highest percentage of shoots was M1S1 (1/4 MS BAP), with 100%. That of the lowest was M1S2 (1/4 MS Kinetin) and M2S1 (1/2 MS BAP), which were 40%, respectively. The media with the addition of TDZ is the best way for inducing shoot on betung bamboos' nodes tissue culture, as seen on this study, which was the TDZ could obtain the percentages of shoot formation in each treatment up to 80%

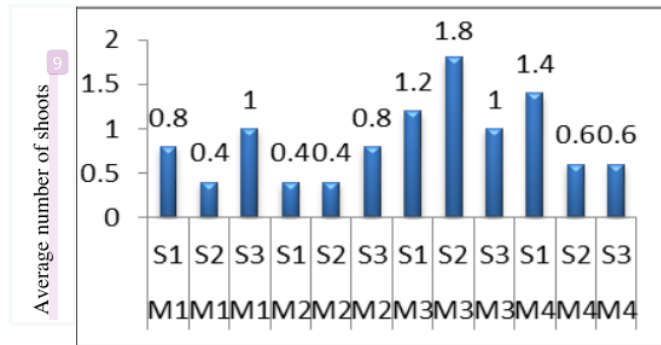
3.3. The number of shoots

The shoots formation is a crucial matter in in vitro micropropagation. The shoots formed can be multiplied by this technique, as it produces new shoots in large quantities. In this study, M3 (3/4 MS) added 3 ppm cytokinins (BAP, Kinetin, or TDZ) gave the best number of shoots compared to M1 (1/4 MS), M2 (1/2 MS), and M4 (1MS). The effect of exogenous hormones became the main factor in that multiplication activity in order to obtain the optimal level of plant multiplication [7].

Table 1. Duncan's Multiple Range Test on number of shoots

Treatment	Average	Note
M3	1.333	b
M4	0.867	ab
M1	0.733	a
M2	0.533	a

Note: Different letters in the same column mean significantly different at level 5%.

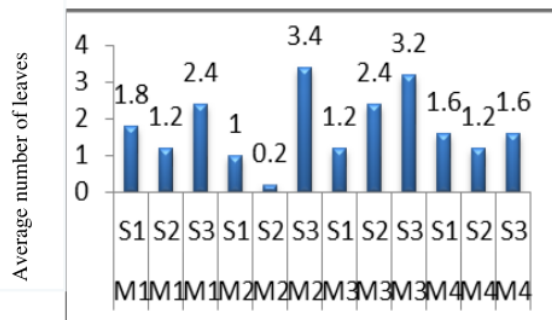


**Figure 4.** The averages number of shoots on node explants of betung bamboo

Figure 4 presents the highest shoots number was obtained by M3S2 ( $\frac{3}{4}$  MS + 3 ppm Kinetin), which was 1,8. M1S2 (1/4 MS + 3 ppm Kinetin), M2S1 (1/2 MS + 3 ppm BAP), M2S2 (1/2 MS + 3 ppm Kinetin) had the lowest average number of shoots, 0,4 shoot, respectively. George *et al.* (2008) [8] stated that the implementation of cytokinins in the media is able to produce maximum shoots formation; however, at a particular concentration, it induces the abnormality forms on the shoots produced. M3 ( $\frac{3}{4}$  MS) and S1 (BAP) were the best concentration of medium and plant growth regulator for stimulating the number of shoots with the averages of 1,3 shoot and 1,0 shoot, respectively. The different formed shoots number were suspected due to the explant capability in absorbing nutrients that presented in MS media and plant growth regulator [9]. M2S1 (1/2 MS+ 3 ppm BAP) possessed the lowest average number of shoots, which was 0,4 shoot. It was due to the explants used in the study that were not in the optimal conditions. The contamination still occurred because explant sterilization that supposed to eliminate the microorganisms was not adequately worked out. Rinaldi (2011) [10] explained that the sterilization technique is the success key in tissue culture. The most challenging contamination to be sterilized is from the explant itself [11].

**3.4. The Number of Leaves**

The calculation of leaves number is starting at the time of leaves formed until the end of observation. The ANOVA showed that media treatments did not affect the leaves number (Figure 5). The averages number of leaves is presented in figure 5.



**Figure 5.** The averages number of leaves on node explants of betung bamboo

Figure 5 shows that M2S3 (1/2 MS + 3 ppm TDZ) had the highest average number of leaves, 3,4 leaves. Meanwhile, M2S2 (1/2 MS + 3 ppm Kinetin) produced 0,2 leaves. The results obtained that TDZ is the best for producing leaves at the explants. TDZ in all MS concentrations performed the best response on leaves number compared to others. TDZ

produced an average leaves number of 2,7 leaves. Meanwhile, that of on media concentrations,  $\frac{3}{4}$  MS media obtained the highest leaves number (2,3 leaves). Kusmianto (2008) [12] and Behera et al (2018) [9] reported TDZ has better activity than other cytokinins as its vital role in stimulating the production of endogenous cytokinin in the cells.

#### 4. Conclusion

1. The appropriate concentration of MS media and plant growth regulator for betung bamboo tissue culture was  $\frac{3}{4}$  MS + 3 ppm TDZ.
2. M3 ( $\frac{3}{4}$  MS) obtained the best average number of shoots (1,3 shoot), the number of leaves (2,3 leaves), and the percentage of shoots (80 %).
3. TDZ had the highest number of leaves (2,7 leaves) and the percentage of shoot (80 %).
4. M1S1 ( $\frac{1}{4}$  MS + 3 ppm BAP) was the best medium to stimulate the average percentage of shoot (100%).
5. M3S2 had the highest average number of shoots (1,8 shoot).
6. Explant sterilization procedure is the most critical step in performing tissue culture of betung bamboo.

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