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In Vitro Propagation of *Bambusa balcooa* as Alternative Material of Wood

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Abstract

A diversion of raw material from wood to bamboo is necessary. *In vitro* culture of bamboo can be used to provide a high number of seedling. The aim of this study was to increase the multiplication of a high quality *Bambusa balcooa* as a wood alternative material. Part of plants used was the sterile axillary shoot. The explants were planted on MS0 medium for 2 weeks and later on multiplication medium MS+0.3 mg/l BAP + 0.3mg/l TDZ. The shoots obtained were fragmented into clusters (3-5 shoots) used for the next multiplication stage using five different medium formulas: (1) MS0; MS containing: (2) 0.1 mg/l BAP, (3) 0.3 mg/l BAP, (4) 0.1 mg/l BAP + 0.1 mg/l TDZ and (5) 0.3 mg/l BAP + 0.1 mg/l TDZ. The results showed that MS medium containing 0.1 mg/l BAP + 0.1mg/l TDZ was the best medium for *B. balcooa* propagation. The shoots produced from aforementioned medium had a better quality compared to the other medium. Forty days after planting, the average number of shoots in this medium was 14.25. MS medium containing 0.3 mg/l BAP + 0.1 mg/l TDZ produced the highest number of shoot but in lower quality. Rooting medium containing 10 mg/l IBA + 5 mg/l NAA produced 9-16 root in 8 weeks. Vermicompost was more prevalent for the acclimatization of *B. balcooa* compared to compost. The use of *B.balcooa* resulted in *in vitro* propagation as a substitute alternative for wood is expected to save the environment from illegal logging.

How to Cite

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INTRODUCTION

Indonesia is the third country in the world with the largest forest area. It consists of huge forested area reaches130 million hectares. About 2% of the forest undergoes the destruction every year due to several factors. The destruction of the forest resulted in erosion and flooding. Illegal logging is the biggest problem related to forest damages. People need woods as building materials and raw material of paper.

Bamboo can be used as a building material and raw material for paper. It is also functioned to overcome the soil erosion, increase the carbon dioxide absorption as well as produce the biofuel. Therefore, bamboo can be used as an alternative material for wood. In general, bamboos found in Indonesia have a thin stem with small diameter, for example is *Bambusa vulgaris* with 6-15 mm (Widjaja, 2001).

B. balcooa is a native plant of India (Widjaja, 2001). The superior traits of this species are its strong stem and root as well as its large diameter of stem. This bamboo can reach 25 m in height and 15 cm in diameter of stem (Negi & Saxena, 2011). According to Gillis *et al.* (2007) *B. balcooa* is categorized as the best type in *Bambusa* genus.

Bamboo is usually propagated vegetatively. Propagation using the bamboo seed has a limitation because the flowering time of bamboo cannot be predicted easily and the flowering phase takes a long time which is about 55-60 years (Negi & Saxena, 2011). Flowering type of bamboo is categorized as gregarious type, the plant will die after flowering without setting the seeds (Brar *et al.*, 2014).

B. balcooa has not been widely distributed in Indonesia, allowing the needs to propagate this species in a large quantity. Tissue culture technique can be used to multiply the plants rapidly and produce the clones of the plant. This method is expected to provide *B. balcooa* seedling that can be used immensely in Indonesia.

B. balcooa propagated from branch cutting has many disadvantages as shoots are produced and a lot of matterial is needed. Irvanti *et al.* (2014) found that in *Gigantochloa atroviolacea,* number of shoot produced depends on the number of nodes used as an explant. As the best result, they found that explant of four nodes produced only four shoots in two months. In contrast, *in vitro* propagation method can produce more shoots within a short periode of time.

The aims of this study were to analyze the effect of cytokinin concentration on the growth of *B. balcooa in vitro* to find the best medium for multiplication and acclimatization. This paper was expected to give an information on multiplication of a high quality as a wood alternatif material. Therefore, the the use of wood could be reduced in the future

METHODS

Sterilization

The material used was the axillary shoot of *B. balcooa* taken from the field as a collection of Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) Bogor. The explants were first sterilized using surfactan (30 minutes), streptomicin sulfat 25% and benomil 50% (two hours). Then the explant were desterilized using 70 % alcohol for five minutes, 30 % natrium hipoclorit for five minutes, and 20 % natrium hipoclorit for seven minutes. After that, the axillary shoots were then rinsed three times using sterilized distilled water.

Shoots multiplication

The sterilized explants (shoots sized 1cm) were planted on MS (Murashige and Skoog) adaptation medium containing no ZPT (MS0) for two weeks. After that, shoots were cultured on multiplication medium MS containing 0.3 mg/l BAP + 0.3mg/l TDZ with 30 gr/l sucrose and 2.4 gr/L phytagel. Incubation was done at culture room with 1000-4000 lux light intensity for 16 hours at 22°C. The shoots obtained was fragmented into shoots clusters (3-5 shoots) and subcultured three times every three weeks until enough materials for medium test stage were produced. Before applying to the medium test, the explants each as 3-5 shoots cluster were cultured on medium MS0 to remove the effect of multiplication medium.

Test on shoots multiplication medium.

The formulation of media used were MS basal media combined with (a) 0.1 mg/l BAP, (b) 0.3 mg/l BAP, (c) 0.1 mg/l BAP + 0.1mg/l TDZ, (d) 0.3mg/l BAP + 0.1mg/l TDZ, (e) MS0. The growth of bamboo was observed every 10 days for 40 days after planting. Parameters observed were the number of shoots, number of leaves, height of shoots and culture visual. This experiment was designed using Completely Randomized Design with 10 replications for each treatment. Duncan test was used for advance analysis.

Roots induction

After assessing the best medium for shoot

multiplication, the shoots were then propagated in basal medium (MS) containing IBA 10mg / l + NAA 5mg / l as a rooting medium.

Acclimatization

Acclimatization was done on plantletplantlets one month after the root induction. The media used were: 1) soil + husks rice + compost (1:1:1), 2) soil + husks rice + vermicompost (1:1:1) in polybag size 12cm X 12 cm. PlantletsPlantlet on polybags were kept in a green house with 75-80% of sun light and the flushing was done every three days.

RESULT AND DISCUSSION

Shoots multiplication

The explant planted on medium of MS+0.3 mg/l BAP + 0.3 mg/l TDZ produced shoots on the first week followed by abundant crumple shoots after three weeks. The *B. balcooa* shoots obtainedwere then subcultured by fragmanted shoot cluster every three weeks for three times. *B. balcooa* is a sensitive plant in cutting due to its high phenol content. Cutting shoot in cluster could caused *B. balcooa* shoots browning and died. Negi & Saxena (2011) used 5-8 shoots on one cluster as explants for multiplication *B. balcooa*. In our experiment, explant of *B. balcooa* used was of 3-5 shoots in one cluster.

Multiplication medium containing MS+0.3 mg/l BAP + 0.3 mg/l TDZ could increase the number of *B. Balcooa* shoot. However, shoots produced were very crumple and dwarf. This maybe due to a high cytokinin concentration used. The shoots with these condition were unfavourabel for root induction. So that, the test of multiplication medium was applied to found the best medium for multiplication to produce high quality *B. balcooa* shoots.

Test on shoot multiplication medium

The use of basal medium (MS0), without any additional plant growth regulator (ZPT) was unable to support the bud growth of *B. balcooa*. Without the ZPT, the explants began to produce phenols on the 3rd day which was then increasing gradually. Nevertheless, on the 10th days, about four shoots were produced. The shoots growing in this medium only survived until the 20th days after planting. The explant died due to the accumulation of high phenol production.

Explants growth required the addition of BAP and TDZ to grow intensively. A significant increase in the number of shoots and leaves, as well as shoot height was observed upon the addition of BAP and TDZ as well as the combination of both cytokinins. It can be shown by the observed variables i.e. the number of shoot, the number of leaves and the shoot height compared to control (Table 1). Usually, the addition of ZPT of cytokinins group such as BAP and TDZ is necessary for shoots development of plant (George & Sherrington, 1984). Without cytokinin, mitosis will be inhibited (Wattimena, 1992). Cytokinin affected the cell division by increasing the transition of G2 phase to the mitosis. This can be happened because cytokinin increases the protein synthesis rate for mitosis. Protein synthesis can be increased by triggering the formation of RNA (Fosket *et al.*, 1977). The highest number of shoot was found on medium containing MS + 0.3 mg/l BAP + 0.1mg/l TDZ which reaches up 21 shoots, however shoots appeared roset. The plantlet was grow normally on medium MS + 0.1 mg/l BAP + 0.1mg/l TDZ which produced 14.25 shoots.

The growth of *B. balcooa* shoots was influenced by the addition of thidiazuron (TDZ). The average number of *B. balcooa* shoot planted in media containing TDZ was higher than the shoot planted in media without TDZ. Table 1 showed that the number of shoots of explant planted on MS medium containing 0.1 mg/l BAP + 0.1 mg/l TDZ is significantly higher compared to those on MS medium containing 0.1 mg/l BAP only. The same results was found on MS medium containing 0.3 mg/l BAP + 0.1 mg/l TDZ compared to those on MS medium with 0.3 mg/l BAP. TDZ induce the formation of adventitious bud and proliferation of axillary shoot (George & Sherrington, 1984). This ZPT is a synthetic phenylurea cytokinin which is also very effective to regulate the formation of *Populus ciliata* shoots (Aggarwal *et al.*, 2012). Utami & Hariyanto (2016) found 1mg/L TDZ could increased the shoot differentiation of *Dendrobium antennatum*. The number of shoot was increased as the effect of combination between BAP and TDZ due to their function in triggering the bud formation.

In this experiment, the function of BAP was to increase the growth of *B. balcooa*. Plantlet on medium containing MS+ 0.3 mg/l BAP produced a higher number of shoot compared to plantlet on medium MS+ 0.1 mg/l BAP. Cytokinin such as BAP effectively increases the number of shoot by enhancing the cell proliferation (Wattimena, 1992). Niranjan *et al.* (2010) found that BAP increased the number of shoots of *Lagerstroemia indica* (L). The same result was found also in white turmeric shoots which planted in media containing 1.5 mg/l BAP produced 66 shoots twice as much as the control (33 shoots) (Yulizar

Table 1. *B. balcooa* growth on different medium formulations after 40 days.

Media Formulation (mg/l)	ΣShoot	ΣLeaf	Height(cm)	Shoot visual
MS	4.37 ± 0.52^{a*}	3.00 ± 0.75^{a*}	1.03 ± 0.07^{a*}	Dead
MS + BAP 0.1	11.50 ± 1.05^{b}	12.17 ± 0.98^{c}	1.58 ± 0.16^{b}	Rare
MS + BAP 0.3	15.00 ± 0.89^{c}	9.33 ± 1.21^{b}	1.65 ± 0.12^{b}	Roset
MS + BAP 0.1TDZ 0.1	14.25 ± 1.17^{c}	13.75 ± 1.83^{c}	1.56 ± 0.11^{b}	Normal
MS + BAP 0.3TDZ 0.1	21.00 ± 0.76^{d}	16.50 ± 2.14^{d}	1.79 ± 0.01^{b}	Roset

*At 10th days. The mean values in the column with the same letter are not statistically significant (p=0.05) according to Duncans Multiple range test.

et al., 2014).

The production of *B. balcooa* leaves was significantly different in every treatment. Cytokinin affects the growth of leaves compared to control. Table 1 showed that addition of TDZ produces ahigher number of leaves compared to the medium without TDZ (BAP only). The highest number of leaves was found on medium containing MS + 0.3 mg/l BAP + 0.1mg/l TDZ which also produced the highest number of shoots.

Different results were obtained from shoots height (Table 1). There was a significant difference observed upon the use of plant growth regulator such as BAP, TDZ, and the combination of them compared to the control group.

Moreover, the growth pattern of shoot was found different visually among the five formulations medium. Shoots growth was significantly affected by medium formulation. The induction of shoots formation was ceased after 10th days on medium without cytokinin MS0 (Figure 1E). The plantlet growth on medium MS+BAP 0.1 mg/l produced a few number of rare shoots (Figur 1A). Cytokinin of 0.3 mg/l or higher concentration enhanced the number of *B. balcooa*'s shoots, but in crumple, dwarft and roset shoots appearance. This condition occured in medium containing 0.3 mg/l BAP + 0.1 mg/l TDZ and in medium containing 0.3 mg/l BAP (Figure 1D, 1B). On the other hand, shoots planting in medium containing 0.1 mg/l BAP + 0.1 mg/l TDZ grew normally (Figure 1C).

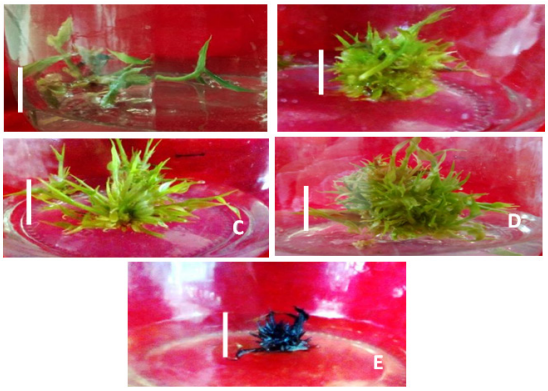

Figure 1. *B. balcooa* development on different medium formulations on the 40th day. (A) Rare, (B) Roset, (C) Normal, (D) Roset, (E) dead. White line :1cm.

Root induction

Rooting stage is important because bamboo plant growing from tissue culture must be able to survive on the field. The rooting induction was done on the plantlet that was produced from the best propagation medium (0.1 mg/l BAP + 0.1 mg/l TDZ). This medium was choosen because the plantlet can grow in a high number of shoots (Figure 1C).

The growth of roots can be triggered by auxin hormone. In this research, combination of 10 mg/l IBA + 5 mg/l NAA effectively induced roots formation. IBA 10 mg/l has been used also in rooting medium of *Garcinia mangostana* L. (Joni *et al.*, 2015), mangosteen (Roostika *et al.*, 2008), *Bambusa vulgaris* (Astuti, 2014) and *Bambusa tulda* (Sharma & Sarma, 2013), while the application of 5mg/l NAA concentration has been used in *Bambusa tulda* (Sharma & Sarma, 2013). Auxin affects the plant tissue by two ways: 1) Induction of the secretion of H^+ ion from the cell to the cell wall, make the cell wall being acid. Acidification of the cell wall causes the intake of K^+ ion so that the water potential is decreased. The decreasing of water potential drives the water went into the cell and enlarged the cell. 2) Auxin affects RNA metabolism by using the RNA transcription molecule (Heddy, 1996).

Rooting induction has long been known as a diffucult stage on bamboo micropopagation. Synthetic auxin is needed on rooting proccess because the accumulation of endogenous auxin will be degradated by the plant. Synthetic auxin is difficult to be degraded, so that the use of synthetic auxin remains capable in inducing roots formation (George & Sherrington, 1998). On the other

hand, auxin accumulation can induce the synthesis of ethylene (Muday *et al.*, 2012), so the leaves senesense occur before the root grow. In this experiment, the shoot of *B. balcooa* was dead before root induction stage was succeed, although it still could induce root. The roots started to grow on the 4th week after planting. The number of root produced on the 8th week ranges from 9-16 roots (Table 2). The roots colour was dominated by brown derived from the phenolic compound found in the shoots. In general, bamboo plantlet has to be moved to the new medium after four weeks.

Tabel 2. Number of root *Bambusa balcooa* in 8 weeks of planting on MS + 10mg/l IBA + 5 mg/l NAA.

Repetition	Weeks after planting							
	1	2	3	4	5	6	7	8
1	0	0	3	4	6	8	12	16
2	0	0	0	2	4	5	7	9
3	0	0	0	1	3	7	10	13
4	0	0	0	3	5	7	8	13
5	0	0	2	4	7	9	11	14

This study also showed that the roots grown on the same medium showed different diameter and length. Some plantlet produced a longer root with smaller diameter (Figure 2A-B). Eventhough, most of the plantlet produced roots with bigger diameter (Figure 2C-D).

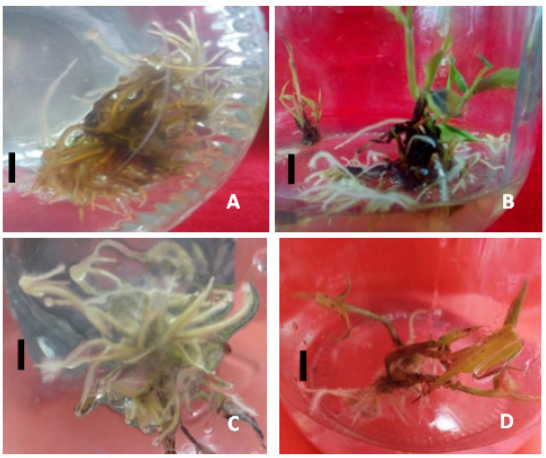

Figure 2. Roots of *B. balcooa* developed on MS medium containing 10 mg/l IBA + 5mg/l NAA. (A) smaller diameter and browning roots, (B) blackening base shoot produced brown roots, (C) larger diameter and white roots, (D) browning shoots produced white roots. Black line: 2mm.

Roots condition was affected by the shoots condition. Shoots experiencing browning will produce brown roots. In Figure 2A-B, blackening was found on the base part of the shoots as a result of high accumulation of phenolic compound. This phenolic compound was transported to the roots resulted in the roots turning brown. The accumulation of phenolic compound is directly correlated with the activity of polyphenol oxidase (PPO) (Poudyal *et al.*, 2008).

Tissue culture technique is a method for producing a big mass of plants in a short time. Tissue culture of axillary shoot produced more than 21 shoots on 40 days. On the other hand, vegetative propagation of *Gigantochloa atroviolacea* through cutting of four nodes as material resulted only four shoots in two month (Irvantia *et al.*, 2014).

Acclimatization

Acclimatization is the last stage which is necessary to assure the ability of the plants produced from tissue culture to survive in natural/ field condition. Number and diameters of roots could effect the acclimatization stage. The plantlet with a high number of shoot have a chance to survive by extending the absorption of water and nutrient on soil. The root with small diameter was better compared to root with large diameter, since root with large diameter would easily decay on the soil.

The plantlets were acclimatizated one month after roots induction. Fertilizer with a high nitrogen source is required for bamboo acclimatization. The result showed that the use of compost fertilizer was unable to produced new shoots of *B. balcooa*. The plantlets planted in soil medium with compost fertilizer were dead at the first and second week after planting. This condition was started by leaves senescence, followed by the stems, leading to the death of the plant (Figure 3B). While, one month after acclimatization in soil medium with vermicompost , the height of *B. Balcooa* can reach approximately up to 7 cm and the number of leaves was 8 (Figure 3A). According to Hernandez *et al.* (2010), N, C, Ca Zn and Cu content in vermicompost is higher than in compost. Sinda et al, (2015) also stated that vermicompost contains various materials necessary for the plant growth like nutrient content such as N, P, K, Mg and Ca.

Vermicompost can be used as another solution for *B. balcooa* seedling propagation. Husks rice was used to be added to vermicompost which is suitable for *B. balcooa* acclimatitation because husks rice function is holding water in order to

prevent the root decay. The use of *in vitro* technique and vermicompos as a fertilizer on acclimatization stage is very effective in *B. balcooa* propagation within a short period.

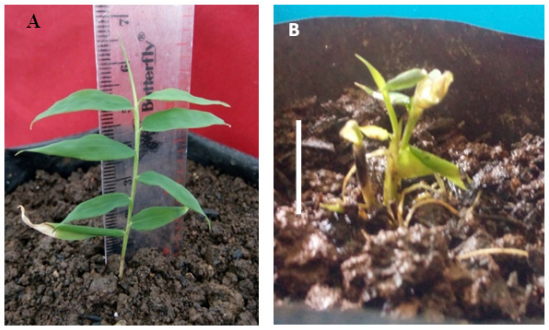

Figure 3. Acclimatization of B. balcooa on the first month. (A) planted on vermicompost fertilizer, (B) planted on compost fertilizer. White line : 1 cm.

Use of wood as a raw matterial for instance in building, manufacture of paper need material from tree that take a long time to grow. Therefore, use of wood should be replaced. This paper was expected to unveil the problem in seeking wood alternative material using faster and better *in vitro* technique in bamboo.

CONCLUSION

Combination of BAP and TDZ was significantly affecting the growth of *B. balcooa* shoots. The number of shoots in media containing BAP, TDZ and combination of both treatment was higher compared to that of control (without hormone). Medium containing 0.3 mg/l BAP + 0.1 mg/l TDZ produced more shoots than those of other media, but the shoots grew abnormally. Shoots which was planted on medium containing 0.1 mg/l BAP + 0.1 mg/l TDZ had the best quality of shoots which grew normally and uncrumpled. Medium containing 10 mg/l IBA + 5 mg/l NAA was capable to induce 9-16 roots of *B. balcooa* plantlet in two weeks. Vermicompost fertilizer was more suitable for *B. balcooa* growth under acclimatization compared to compost fertilizer.

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