Propagation of high-density carbon bamboo to support smart ecotourism at the Lake Toba, North Sumatera

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Abstract. Appropriate propagation technology to promote bamboo sustainability is important in supporting conservation and industrial needs. As part of smart ecotourism package based on the integrated charcoal and bamboo tourism at Lake Toba, this research aimed to develop appropriate propagation for the selected bamboo species. Various bamboo species were collected and propagated by both ex vitro and in vitro propagation. Survival rate of the selected bamboo shoots after 3 months in the ex vitro planting medium was in the range of 25 to 50% of 10 – 25 planted shoots, from the highest to the lowest rate were Bambusa sp., Dendrocalamus asper, Oxythenantera abyssinica, and Balcoa 36. Meanwhile, the efficiency of the shoot formation in the in vitro medium was in the range of 7 to 13%, from the highest to the lowest percentage were D. asper, Gigantochloa cf robusta, O. abyssinica and Bambusa sp. Various types of explant (leaves disc, shoots tips, and nodal segment) from G. robusta and O. abyssinica were induced in six different combinations and concentrations of induction callus medium. Explant from nodal segment of G. robusta and O. abyssinica showed a positive response, i.e., 6% and 20% consecutively, in the Murashige & Skoog (MS) medium containing 4 mg/L of 2,4-D and 0.5 mg/L of IBA. The nodal segment explant of G. robusta was also able to form callus (13%) on the MS medium containing 6 mg/L of kinetin. A similar efficiency percentage was also obtained from O. abyssinica nodal segment explant on the MS medium containing 3 mg/L of 2,4-D and 2 mg/L of kinetin. This research opens a possibility of cultivating bamboo by in vitro propagation and generating new variety of bamboo with desirable characteristics.

1. Introduction

Bamboo is one of the lignocellulosic materials from the group of grasses (Gramineae). Bamboo as fiber is versatile and becomes an alternative material to wood [1, 2]. Different forms of bamboo fiber, i.e., macro, micro and cellulose nanofiber [3] have been developed into various bioproducts such as pulp and paper [4], planting media [5], sound absorbers [6, 7], bamboo layers for furniture and construction [8-10], laminated bamboo [11], bamboo blocks [12], outdoor utilities, and polyurethane composites [13]. In the form of powder or particles, bamboo can be utilized as car door panels and
cement boards [14]. Bamboo can also be converted to lactic acid [15], reinforcing composite materials [16], methane [17], textiles [18], carbon and/or activated carbon [19], liquid smoke [20, 21], supercapacitors [22, 23] and bioenergy [24].

In Indonesia, there are at least ± 160 types of bamboo which 88 of them are endemic species, and 38 of them are introduced species [25]. Bamboo has a very unique growth character. It has a very fast-growing period (5-20 meters in 2-4 months) and a short cutting cycle, i.e., 3-5 years [26]. It can produce an easily harvested culm (stem) [27]. Bamboo plant naturally has been propagated by a conventional seedlings cultivation, or ex vitro, derived from seed and organ cuttings such as branch, culm, and rhizome [28]. However, bamboo has an uncertain flowering period (40-120 years), which is an inhibiting factor for the conventional bamboo propagation [29]. Studies reported that bamboo can also be propagated by tissue culture or in vitro regeneration. In vitro regeneration in bamboo has been carried out both by using the reproductive and vegetative tissues [29]. Recent studies show a high level of efficiency (50%) using bamboo shoots as a source of explant for in vitro regeneration [30]. Identification of bamboo species needs to be critically examined before using any of these methods for plantations or farms [28].

Appropriate propagation technology to promote bamboo sustainability is important in supporting conservation and industrial needs. This research is a part of the "Smart Ecotourism" project in the Lake Toba region, North Sumatra, by The Ministry of Environment and Forestry of Indonesia which integrates bamboo-based charcoal technology and bamboo tourism for education and health [31]. The application of research results can be used as branding in the tourist area of Lake Toba and its surroundings (Figure 1). The concept of integrating smart tourism based on research, education, and health is paramount in contemporary tourism branding. This is in accordance with the definition of ecotourism by The International Ecotourism Society [32], which states that ecotourism is "responsible travel to natural places that preserve the environment, improve the welfare of local communities and involve interpretation and education". As reported in Darmawan et.al [31], integrated charcoal technology has created charcoal products, liquid smoke, and bioactive compost charcoal (ARKOBA). The technology supports organic cultivation systems that are environmentally friendly when continuously applied and it replaces the role of chemical fertilizers and pesticides. The present research projects also integrate the use of activated carbon as an advanced material for cancer healing therapy and drug delivery systems by utilizing bamboo and Taxus sumatrana plants that grow around the Lake Toba region as raw material (Figure 1). The activity is intended to provide alternative solutions for cancer prevention, especially in the therapy, rehabilitation, and palliative stages, and to assist cancer services by developing bamboo-based nano porous carbon materials and taxus-based drug delivery systems. Various species of bamboo and taxus plants grow very well around the Lake Toba region. Another important breakthrough in the project is the identification and characterization of high carbon-containing bamboo types [31]. Cultivation of these bamboo species contributes to reducing erosion and enhancing protection in the Lake Toba ecosystem.

**Figure 1.** Integrated bamboo-based charcoal technology project and bamboo tourism aims to support scientific tourism in the Lake Toba area that integrates research, education, and health as one "Smart Ecotourism" package.
The selection of bamboo species for propagation in this current research was based on the preliminary study of the activated carbon characterization of five collected bamboo from the nursery at the Center for Research and Development of the Environment and Forestry, Aek Nauli, North Sumatera, i.e. *Bambusa vulgaris*, *Bambusa* sp., *Gigantochloa cf robusta* Kurz, *Gigantochloa* sp and *Dendrocalamus asper* [31]. The result showed that three of the species, namely Mayan bamboo (*Gigantochloa cf robusta* Kurz and *Gigantochloa* sp.) and Betung bamboo (*Dendrocalamus asper*) have exceeded the Indonesia National Standard for iodine absorption above 750 mg/g [33], i.e. 796.90 mg/g, 757.30 mg/g and 927.80 mg/g respectively. This finding confirmed that these particular bamboo species are qualified for charcoal or carbon products raw material. Further result also showed that *G. cf. robusta*, has the highest value on fixed carbon value (i.e. 80.90%). Based on the findings, those bamboo species were further propagated by both the *ex vitro* and *in vitro* propagations.

In addition to the obtained preliminary data from the project [31], cross-referencing of bamboo-based activated charcoal from previous studies was also taken into consideration. Andong bamboo (*G. pseudoarundinacea*) produces a high yield of carbon (up to 80.1%) by hot alkaline method and has the highest iodine absorption (1150 mg/g) compared to other bamboo species, namely Rope bamboo (*G. apus*), Betung bamboo (*D. asper* Back) and Ater bamboo (*G. atter*) [34]. Lastly, information from the private bamboo nursery, PT. Tani Pertiwi Jaya, was also noted (personal communication). The nursery bamboo species that have potential as activated carbon raw material are namely Ampel bamboo (*Bambusa vulgaris*), Betung bamboo (*D. asper*), Andong bamboo or also known as Gombong bamboo (*G. pseudoarundinacea*) and Siam bamboo (*T. siamensis*).

In general, this current research was conducted to support the smart ecotourism integrated charcoal technology project. In specific, this research was aimed to (1) initiate bamboo propagation by both *ex vitro* and *in vitro* propagation for various species of bamboo, (2) measure the efficiency of shoot induction in the *ex vitro* propagation, (3) observe and measure the efficiency of callus formation in the various combination of callus induction medium.

2. Materials and method

2.1. Plant material collection

Bamboo plants were originally collected from the private bamboo nursery, i.e. PT. Tani Pertiwi Jaya (Bogor, West Java) in December 2018. They are namely *Oxythenantera abyssinica*, *Dendrocalamus asper*, Balcoa, Balcoa 36, *Bambusa* sp. Another species of bamboo, namely *Gigantochloa cf. robusta* was collected from the government bamboo nursery, i.e. Balai Penelitian dan Pengembangan Lingkungan Hidup dan Kehutanan (BP2LHK) or Environment and Forestry Research and Development Institute (Aek Nauli, North Sumatera) in July 2019 (Figure 2).

2.2. Bamboo seedlings cultivation

Collected bamboo plants, shoots, and stem shoots were relocated and replanted at the Research Center for Biotechnology LIPI nursery (Bogor, Indonesia). Total number of 10, 15, 25, 20 shoots of *Oxythenantera abyssinica*, *Dendrocalamus asper*, Balcoa 36, *Bambusa* sp respectively was acclimatized and propagated *ex vitro* in a nursery house from December 2018 until September 2019 under nursery condition of 26 – 32°C and 50 – 75% humidity. In addition, total number of 11 shoots of
Gigantochloa cf. robusta was also acclimatized and propagated from July 2019 under similar nursery condition (Figure 3). Acclimatized bamboo stem shoots were collected and cultivated \textit{ex vitro} in planting medium (husk charcoal: coco peat: compost in 1:1:1 ratio) in pots (Figure 3). The young shoots with nodule, shoots tip, young leaves, were also collected and prepared for \textit{in vitro} propagation (Figure 4).

![Figure 3](image1.jpg)

**Figure 3.** Bamboo shoots acclimatization in nursery house at the RC for Biotechnology LIPI. (a) 17 days seedling acclimatization, (b) 3 months old bamboo shoots grown planting medium, (c) more than 6 months old bamboo shoots grown in planting medium.

![Figure 4](image2.jpg)

**Figure 4.** Preparation of \textit{O. abyssinica} bamboo in the \textit{in vitro} propagation. (a) Collected stem shoots. (b) Nodal segment for shoots initiation explant. (c) Sterile shoots tip, leaves disk, young stems node for callus induction explants.

### 2.3. Tissue culture initiation

Bamboo stem shoots were collected and cleaned from its fine hairs (Figure 4a). Explants disinfection was conducted by surface sterilization of the young stem (Figure 4b) in a series of washing step by solution containing active ingredients, i.e. fungicide. In this study, two different methods of sterilization were conducted. Double active ingredients were applied in one of the methods (find details in the sub section). After completion of the sterilization step, the sterile stem shoot explants were subsequently planted in Murashige & Skoog (MS) medium without addition of growth regulators (MS0) for shoot induction. The sterile explants were regenerated to plantlet and maintained in a controlled room.

#### 2.3.1. Sterilization method 1

Stem shoots were firstly washed by detergent under water flow for 30 minutes. The explants were then soaked in a solution containing 2 \% streptomycin sulfate for 30 minutes and rinsed by water. The shoots were continued soaked in solution containing 4 \% fungicide from 80\% of Mancozeb for 30 minutes. The next step of sterilization was carried out in a laminar air flow cabinet. The explants were soaked and shaken in a solution containing 30\% from 5.25\% commercial Natrium hypochlorit for 7 minutes and rinsed 5 times with sterile distilled water. The explants were then soaked in 70\% Alcohol for 3 minutes and rinsed 5 times with sterile distilled water.
2.3.2. **Sterilization method 2.** An additional step of sterilization was applied to the stem shoots. The stem shoots were immersed and shaken in a solution containing 50% benomyl from 4% commercial fungicide (i.e. 4 % Benlox) for 30 minutes prior to the application of solution containing 4 % mancozeb fungicide.

2.3.3. **Callus induction.** The nodal segment, young leaves, and shoot tip of *O. abyssinica* and *G. robusta* were used as explants for callus induction (Figure 4c). All explants were surface sterilized with the best method obtained for shoot induction. The explants were cut into appropriate size (Figure 4b 5b) and then directly inoculated on MS (Murashige Skoog 1962) medium supplemented with various types and concentrations of plant growth regulator. Six types of medium that used for callus induction were M1 (MS + 3mg/L 2,4D + 2 mg/L kinetin), M2 (MS + 4 mg/L 2,4D + 0.5 mg/L IBA), M3 (MS + 8 mg/L 2,4D + 0.5 mg/L IBA), M4 (MS + 8 mg/L 2,4 D + 0.5 mg/L NAA), M5 (MS + 4 mg/L 2.4 D + 0.5 mg/L NAA), M6 (MS + 4 mg/L kinetin) of medium the pH of medium was 5.8. The cultures were incubated in a culture room at 26°C.

3. **Results**

3.1. **Shoots induction in the ex vitro propagation**

Induction of shoots from bamboo branches (shoots) of the PT Tani Pertiwi Jaya and BP2LHK Aek Nauli bamboo collections were conducted on the husk charcoal: coco peat (1: 1) planting medium. Shoots were periodically observed at 17 days and 3 months after planting (Table 1). Survival rate at 17 days after planting was in a range of 30 to 100 % (Table 1). After 3 months, survival rate of the shoots to grow was declined in a range of 25 to 50 %. Shoots of *G. robusta* and Balcoa grew until 2 months after planting and then wilted. Three of the bamboo species, namely *O. abyssinica*, *D. asper* and *Bambusa* sp, survived and produced shoots in a range of 1 to 7 shoots per branch (Table 1). Physical performance of the shoots at 17 day and 3 months old are shown on Figure 5 and Figure 6, consecutively.

**Table 1.** Survival rate of bamboo shoots on the ex vitro planting medium.

| Bamboo types | Origin     | Percentage of survival plants at 17 days after planting (%) | Percentage of survival plants at 3 months after planting (%) | Range of shoots number |
|--------------|------------|--------------------------------------------------------------|--------------------------------------------------------------|------------------------|
| *G. robusta* | Aek Nauli  | 100                                                          | 0                                                            | 0                      |
| *O. abyssinica* | Bogor    | 40                                                           | 40                                                           | 2 – 3                  |
| *Balcoa*     | Bogor     | 30                                                           | 0                                                            | 0                      |
| *D. asper*   | Bogor     | 30                                                           | 33                                                           | 1 - 4                  |
| *Balcoa 36*  | Bogor     | 30                                                           | 50                                                           | 0                      |
| *Bambusa* sp | Bogor     | 95                                                           | 25                                                           | 1 - 7                  |

*Figure 5.* The seventeen (17) days old shoots grown on the ex vitro planting medium. A pot of (a) *G. robusta*, (b) *O. abyssinica* and Balcoa, (c) *Bambusa* sp bamboo shoots.

3.2. **Shoots induction of in vitro propagation**

Sterilization by method 2 was better performed than that by method 1. Additional fungicide of 50% benomyl in method 2 resulted in higher number of aseptic culture. Aseptic culture of bamboo was obtained by conducting surface sterilization steps with the absence of mercuric chloride. It has
succeeded to eliminate bacterial and fungal contamination. Four out of five tested bamboo species were able to form new shoots in 7 to 13% efficiency rate (Table 2, Figure 7). Most of the inoculated explants were turning brown after inducting in the culture medium for 2 weeks. In this case, in vitro shoots culture needs to be subcultured into a new medium within 2 weeks (Figure 8). The shoots were able to grow on the hormone free MS medium.

![Figure 6](image)

**Figure 6.** The three months old propagated bamboo shoot grown on the ex vitro planting medium. A cluster of (a) Bambusa sp, (b) O. abyssinica and (c) D. asper bamboo shoots.

![Figure 7](image)

**Figure 7.** Aseptic stem shoot bamboo explants planted in an MS medium without growth regulators (MS0) for shoot induction.

| Types of bamboo | Origin of collection | Percentage of explants forming shoots (%) | Time of shoots emerging (day after inoculation) |
|-----------------|----------------------|------------------------------------------|-----------------------------------------------|
| *D. asper*      | Bogor                | 7.47                                     | 10-12                                         |
| *Bambusa sp*    | Bogor                | 13.33                                    | 3-5                                           |
| *O. abyssinica* | Bogor                | 12.5                                     | 4-5                                           |
| *G. robusta*    | Aek Nauli            | 11.53                                    | 5-7                                           |

### 3.3. Callus induction

Callus induction was performed on different types of explant from two species, i.e. *O. abyssinica* and *G. robusta* (Table 3). In general, callus formation showed slow response. They took 1.5 to 3 months to produce initial callus form. However, nodal segment from both species showed strong callus response when inducted on the IBA and kinetin containing medium (Figure 9). Callus from *G. robusta* nodal segment regenerated in 13.3% efficiency rate, while callus from *O. abyssinica* nodal segment regenerated in 20% rate (Table 3). Callus induction from other type of explant, i.e., leaves disc and shoots tip were failed to produce callus (Table 3).
Table 3. Callus formation response of G. robusta and O. abyssinica various types of explant on different concentrations and combinations of growth hormone-MS medium.

| Medium/Growth regulators                      | Callus formation from types of bamboo and explants (%) |
|-----------------------------------------------|---------------------------------------------------------|
|                                               | G. robusta | O. abyssinica |
|                                               | leaves disk | shoot tip | nodal segment | leaves disk | shoot tip | nodal segment |
| M1 (MS + 3mg/L 2,4 D + 2 mg/L kinetin)         | -          | -         | -             | -          | -         | 13.33         |
| M2 (MS + 4 mg/L 2,4D + 0.5 mg/L IBA)          | -          | -         | 6.67          | -          | -         | 20            |
| M3 (MS + 8 mg/L 2,4D + 0.5 mg/L IBA)          | -          | -         | -             | -          | -         | -             |
| M4 (MS + 8 mg/L 2,4 D + 0.5 mg/L NAA)         | -          | -         | -             | -          | -         | -             |
| M5 (MS + 4 mg/L 2,4 D + 0.5 mg/L NAA)         | -          | -         | -             | -          | -         | -             |
| M6 (MS + 4 mg/L kinetin)                       | -          | -         | 13.33         | -          | -         | -             |

Figure 8. The two weeks old bamboo shoots on hormone free MS medium. Bamboo species (a) O. abyssinica, (b) Bambusa sp, (c) G. robusta.

Figure 9. Initial stage of callus formation of (a) O. abyssinica on M1 and M2 medium and (b) G. robusta on M6 medium documented using LEICA EZ4HD under 40X magnification.

4. Discussion
The preliminary study reported that there were several species of bamboo that have potential to be propagated as a raw material for charcoal and/or activated carbon [31]. Two of the highly nominated species were Betung Bamboo (D. asper) and Mayan Bamboo (G. robusta). This research finding showed that D. asper could be propagated from the shoots cutting by both ex vitro and in vitro.
methods (Table 1 and Table 2), in contrast to the G. robusta species which could not be propagated from the shoots cutting by the ex vitro method (Table 1). As the growth rate of bamboo depends on the species type and the method used in the plantation [28], proper ex vitro method of G. robusta is critical. Using different type of planting materials can be an effective alternative in developing the method. Other factor that can influence successful rate of bamboo growth is the environmental condition [28].

G. robusta species used in this current research was originated from the nursery at the Aek Nauli, North Sumatera. Aek Nauli is an area of about 1,000 - 1,750 meters above sea level with the average temperature of 18°C [35]. G. robusta seedlings (culm cuttings) were then acclimatized in Bogor, West Java. Bogor is an area of about 139 meters above sea level with the average temperature of 26°C [36]. Differences of environmental condition, such as temperature, may have influence the growth ability of G. robusta shoots in this research experiment. Bogor area has relatively higher temperature than the Aek Nauli area. Proper cultivation of G. robusta seedlings in a controlled nursery house or glasshouse room may be needed for future reference, especially when the tissue culture laboratory is not available on sites.

Although G. robusta seedling was not successfully propagated by the ex vitro technique in this present research, it was able to regenerate by the in vitro method. The young shoots from G. robusta could be induced and propagated as shown in Table 2. In fact, G. robusta shoot induction on free hormone MS medium performed faster, i.e., 5 – 7 days, compared to the D. asper shoot induction, i.e. 10-12 days (Table 2). The tested in vitro shoots from the other two species in this study, i.e., Bambusa sp and O. abyssinica, could also be induced in the hormone-free MS medium.

Callus induction from nodal segments of G. robusta and O. abyssinica was successfully obtained on a medium containing cytokinin, i.e. IBA (Table 3). This finding is in line with the in vitro propagation of Bambusa vulgaris that used cytokinin, such as BAP and auxin (IBA), in their induction medium to increase shoots and roots number [37]. Browning condition found on some tested nodal explants (Figure 9). This condition was similarly found in Phyllostachys nigra bamboo shoots induction [38]. Beside conducting regular explant subculture, applying additional antioxidant such as PVP with various concentration in culture medium may prevent explant from browning.

Optimization of explant material and application of plant growth regulators are essential for callus induction. Callus could be induced from various bamboo tissues using medium containing growth regulators, i.e., auxin such as 2,4-D, NAA, IBA [29, 39, 40]. Previous study reported combination application of auxin and cytokinin, such as kinetin, for high rate callus formation. Response in higher number of callus formation from O. abyssinica nodal segment was obtained in medium containing 4 mg/L 2,4 D and 0.5 mg/L IBA. This present result was in line with previous study that reported high number of callus induced from nodule of the young shoot of Dendrocalamus latiflorus Munro Bamboo [30]. Both ex vitro and in vitro methods of bamboo propagation using stem node segment were potential to induce new shoots of various bamboo species.

5. Conclusion
This research initiates an in vitro propagation protocol for G. robusta and O. abyssinica bamboo. An appropriate surface sterilisation method for the tested bamboo explants has also developed. Explants from nodal segment of G. robusta and O. abyssinica show a positive response, 6% and 20% rates consecutively, in the Murashige & Skoog (MS) medium containing 4 mg/L of 2,4 D and 0.5 mg/L of IBA. The nodal segment explant of G. robusta is also able to form callus (13% rate) on the MS medium containing 6 mg/L of kinetin. A similar efficiency percentage was also obtained from O. abyssinica nodal segment explant on the MS medium containing 3 mg/L of 2,4 D and 2 mg/L of kinetin. This research opens a possibility of cultivating bamboo by the in vitro propagation and generating new variety bamboo with desirable characteristics.

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