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Shoot Induction of *Agathis borneensis* Warb in *in vitro* Culture

Irni Furnawanthi Hindaningrum[1], Andhika Rahmansani[2], Linda Novita[1], Roni Kartiman[1], Mardoni Elya[1], Restu Siwi Muharromah[1], Kassandra Budiarni[1], Yelnititis[1], Rendie Prasetyo[2], Sugiyono[2*]

[1] Applied Botany Research Center, Life Science and Environment Research Organization, National Research and Innovation Agency, Indonesia 16911;[2] Department of Botany, Faculty of Biology, Jenderal Soedirman University, Indonesia 53122

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Abstract

Agathis borneensis Warb., a member of the *Araucariaceae* Family, is listed as an Endangered *A4cd* plant. *A. borneensis* population in its natural habitat is decreasing due to habitat destruction and uncontrolled harvesting. *A. borneensis* propagation remains challenging due to slow growth, recalcitrant seed, low natural regeneration, and uneven germination, which can take months. Vegetative propagation through cutting is also difficult to root, and success depends on the age of the mother tree. To provide alternative *A. borneensis* ex-situ conservation and propagation, the plant's *in vitro* culture technique can be applied, as it produces many seedlings in a shorter time. This research aimed to evaluate the effects of cytokinins on A. borneensis shoot growth and to determine the optimal cytokinin and its concentration to stimulate *A. borneensis* shoot growth. A completely randomized split-plot design has been used in this study. The treatments included three types of cytokinin (BAP, Kinetin, and TDZ) and cytokinin concentrations at 0, 1, 2, 3, and 4 µM. The observed variable was *A. borneensis* shoot growth, with the parameters measured as shoot emergence, shoot and leaf numbers, and shoot length. The data gained were analyzed using DSAASTAT VER 1.514 software through an Analysis of Variance at 95% and 99% significance levels. The means were compared using the Duncan Multiple Range Test (DMRT) at a 95% confidence level. This study showed that MS media supplemented with 1 µM kinetin produced the fastest shoot emergence time. Meanwhile, *A. borneensis* shoots grown on MS media without cytokinin resulted in the formation of the most leaves.

Keywords: *Agathis borneensis*, 6-Benzylaminopurine, *in vitro*, Kinetin, Thidiazuron.

1. Introduction

Agathis borneensis Warb., a member of the Araucariaceae family, originated from the island of Borneo, Indonesia. *A. borneensis* can grow at low and high altitudes in tropical rainforests (Darma *et al.*, 2022; Farjon, 2017). *A. borneensis* trees can reach 50-55 m and up to 3.5 m in diameter at breast height. *A. borneensis* is considered the king of wood in Borneo, a highly valuable timber. Its large and smooth wood is popular for light construction, household items, panel boards, chopsticks, matches, veneers, fuel wood, and charcoal. The resin exudate from the stem bark is a widely traded Non-Timber Forest Product (NTFP) known as copal. The Dayak communities of highland Borneo traditionally used resin for lighting (Stalin & Franco, 2021). Essential oils from *Agathis borneensis* contain α-pinene, δ-limonene, β-pinene, terpinen-4-ol, and α-terpineol. Adam *et al*. (2017) identified 60 chemical compounds in the leaves and stem bark, including monoterpene hydrocarbons, oxygenated monoterpenes, oxygenated diterpenes, oxygenated sesquiterpenes, and sesquiterpene hydrocarbons. These compounds exhibit antitumor, anticancer, anti-inflammatory, antimalarial, antibacterial, and anesthetic properties (Stalin & Franco, 2021).

According to the IUCN Red List, *A. borneensis* is categorized as Endangered A4cd due to *overexploitation*, which has halved the total habitat area and is ongoing. These conditions are exacerbated by the decline of Agathis' natural habitat quality due to climate change and human activities (Adam *et al*., 2017; Farjon, 2013). Therefore, conservation efforts must be inclusive and comprehensive, using available approaches. However, its propagation remains challenging due to slow growth, seed dormancy issues, and habitat loss. Seeds are recalcitrant (losing viability quickly when dried) and have low natural regeneration due to irregular seed production and slow, uneven germination, which can take months. Cuttings are difficult to root, and success depends on the age of the mother tree.

In vitro culture methods can be applied to propagate rare plants such as *A. borneensis* (Widyatmoko, 2019) to obtain large numbers of contaminant-free seedlings. Ishii and Mohsin (1994) identified the shoot tip of Agathis as a suitable explant for tissue culture initiation. Multiple shoots were produced when Agathis borneensis shoot tips were cultured on half-strength Gamborg's medium (0.5 G) with 1 mg/L zeatin. When the shoot tip was subsequently cultured on 0.25 G with 1 mg/L IBA, shoot elongation occurred.

[*] Corresponding author. e-mail: sugiyono@unsoed.ac.id.

Plant growth and differentiation *in vitro* culture are influenced by several factors, including plant genotype, nutrients, growth environment, and growth regulators (PGRs) (Biradar, 2022; George *et al*., 2008; Park, 2021). Cytokinins are often used for shoot propagation in vitro. Cytokinins can induce shoot initiation and proliferation. Some commonly used cytokinins include BAP (6-Benzylaminopurine), Kinetin (6-furfurylaminopurine), and Thidiazuron (TDZ). BAP is the most frequently used cytokinin because it is effective for stimulating shoot formation, stable on heating, resistant to oxidation, and the cheapest among cytokinins (Agustina *et al*., 2019). Kinetin is stable and more resistant to high temperatures (Andriani *et al*., 2023; Putriana *et al*., 2019). Meanwhile, TDZ is a growth regulator that functions like auxin and cytokinin, producing responses such as callus induction, embryogenesis, and organogenesis (Taha *et al*., 2021).

Only a few reports on both *A. borneensis in vitro* culture and PGRs application have been published. Therefore, it is crucial to evaluate the effect of cytokinin application on *A.* borneensis shoots' growth in *in vitro* culture and determine the best cytokinin and its concentration to stimulate this growth. This research is expected to produce high-quality seedlings to support both seedling production and conservation efforts.

2. Methods

2.1. Research design

This study was conducted in the Plant Tissue Culture Laboratory of the Applied Botany Research Centre at Indonesia's National Research and Innovation Agency. The study has been carried out experimentally using a completely randomized split-plot design. The main plot applied was cytokinin types, including BAP, TDZ, and Kinetin. The subplot was the cytokinin concentration, with four levels: 0, 1, 2, 3, and 4 µM. All treatment combinations were replicated 5 times, resulting in 45 experimental units. The variable observed was *A. borneensis* shoot growth, with the parameters measured including shoot emergence, shoot and leaf numbers, and shoot length.

2.2. Explant preparation and sterilization

The lateral shoots of *A. borneensis* were collected from a three-year-old mother plant of the Bogor Botanical Garden (collection number vak. XX.D.5.4 originated from Central Kalimantan (Central Borneo). The lateral shoot apexes with five leaves were collected, wrapped in cotton moistened with a vitamin B1 solution at the base of the cut, and placed in an airtight plastic bag to avoid stress and maintain explant freshness and vigor.

Upon arrival in the laboratory, the leaves were excised from the explants, and the shoots were gently cleaned with a cotton swab and detergent. The shoots were then rinsed in running water for 15-30 minutes. The clean shoots were then cut, and the second internode, measuring 3-4 cm, was used as an explant. Explants were soaked in sterile water supplemented with 3 drops of Tween 80 per 100 mL, then shaken for 30 minutes. The explants were subsequently rinsed for 10 seconds with sterile water, which was repeated 3 times. After rinsing, the explants were immersed in citric acid ($C_6H_8O_7$) solution for 30 minutes. Explants were transferred and soaked in bactericide and fungicide solutions without rinsing for 60 minutes. Explants were thoroughly rinsed with sterile water for 10 seconds and 3 times.

The next step was conducted in the Laminar Air Flow cabinet, in which explants were soaked for two minutes in a 70% ethanol solution with agitation, followed by a rinse in sterile water for three ten-second intervals. Explants were soaked in 0.1% HgCl2 for 2 x 7-minute intervals while shaking, then rinsed with sterile water for 3 x 10-second intervals. The use of 0.1% HgCl2 following 70% ethanol sterilization has also been reported by Chukwu *et al*. (2025), Justine *et al*. (2022), Kulkarni *et al*. (1996) and Yadav *et al*. (2021). Sterile explants were blotted on sterile filter paper. The tips of the explants in contact with the sterilizing agent were excised, resulting in an explant size of ± 2 cm. The explants were soaked again in sterile water and blotted on sterile filter paper.

2.3. Plant in vitro culture procedures

Preculture. The sterile explants were planted onto sterilized testing media (MS0) consisting of an MS medium supplemented with 3% sucrose, 100 mg/L myo-inositol, solidified with 0.7% Agar, and incubated for 7 days. This medium was used to test whether the explants were sterile.

Treatment application. The basic medium used is MS with 3% sucrose and 100 mg/L myo-inositol. A total of 750 mL of basic medium was prepared and divided into 15 flasks of 50 mL each. Cytokinins and their concentrations were then added according to the treatment combination. The media pH was measured and set to 5.8 before sterilization, and 0.7% Agar was added to each flask. The media were heated until agar was completely dissolved. Each treatment medium was divided into 5 test tubes and sterilized in an autoclave at 0.15 MPa and 121°C for 20 minutes. Sterile media were then cooled until use. The sterile explants from the preculture stage were planted onto the treatment medium and sealed tightly. They were incubated at 24 oC with continuous tube luminescent light for 16 weeks.

2.4. Data collection

Shoot emergence, shoot and leaf numbers, and shoot length were measured when shoot size was > 2 mm. Shoot emergence was observed and recorded at weekly intervals. Shoot and root numbers were counted at week 16. Shoot length was measured at week 16 by measuring the shoot from the shoot base until the tip of leaf number 1. The data were recorded, and the explant was photographed.

2.5. Data analysis

The data were analyzed using DSAASTAT VER 1.514 software to generate an Analysis of Variance (ANOVA) at 95% and 99% significance levels. The means were compared using a Duncan Multiple Range Test (DMRT) at a 95% confidence level.

3. Results and Discussion

At 16 weeks after planting (WAP), the *A. borneensis* Warb. explants exhibited growth, as shown by increases in shoot and leaf number and shoot length, even when grown in media without cytokinin. The increase in plant size, measured by organ size and height, is a sign of plant growth, while changes in the shape of stem organs, roots, leaves, and the appearance of flowers and fruits on plants are signs of plant development (Prasetyo *et al*., 2020; Sitompul & Guritno, 1995; Tamyiz *et al*., 2022). Figure 1 illustrates the growth of explants cultivated in media without any growth regulators after 16 weeks of culture. Ahmed & Mohamed (2022) stated that kauri or resin trees are perennial, long-lived plants with moderate growth rates. Moreover, Steward *et al*. (2014) indicated that kauri exhibits modest growth at its juvenile stage.

The research results (Table 1) revealed that the types of cytokinin used (BAP, Kinetin, and TDZ) have no significant effect on shoot emergence time, shoot number, shoot length, and leaf number ($p > 0.05$). Table 1 also showed that cytokinin concentrations controlled the leaf number parameter; meanwhile, the interaction between cytokinin types and concentrations strongly controlled shoot emergence time. Plant responses to plant growth regulators can differ depending on the type and concentration used. According to Ružić *et al*. (2016), the optimal type and concentration of cytokinin for woody plants may vary depending on species and genotype.

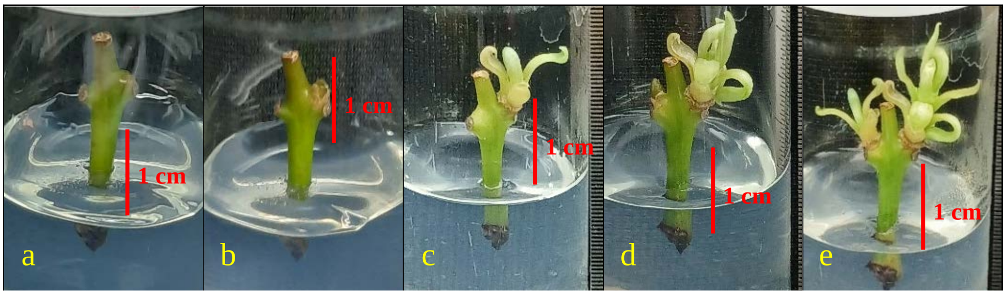

Figure 1 *A. borneensis* shoots appearance on media without cytokinin addition. (a) At age 0 weeks after planting; (b) 4 weeks after planting; (c) 8 weeks after planting; (d) 12 weeks after planting; (e) 16 weeks after planting.

Table 1. Summary of ANOVA results on the effects cytokinin of types and concentrations on A. borneensis shoots 16 weeks after planting.

Source of Variety	Significance (p-value)			
	Shoot Emergence	Shoot Number	Shoot Length	Leaf Number
p - Types of cytokinin	0,301	0,103	0,208	0,104
p - Concentrations of Cytokinin	0,055	0,093	0,215	0,017*
p - Interaction between cytokinin type and concentration	0,001**	0,306	0,472	0,113

Notes: Numbers followed by ** indicate highly significant differences in the F-test with 99% confidence level; * indicates significant differences in the F-test with 95% confidence level.

Shoot emergence is the time when a plant organ begins to grow. This growth is closely related to the development of meristematic tissues and to growth regulators that act in plants (Fajar *et al*., 2018). The results of DMRT (Table 2) revealed that *A. borneensis* explants cultured on media added with 1 µM Kinetin produced the fastest shoot emergence with an average time of 37.00±11.79 days after planting (dap). However, no significant differences were found among those cultured on BAP 1 µM, BAP 2 µM, Kin 3 µM, Kin 4 µM, TDZ 0 µM, and TDZ 4 µM. The longest shoot emergence was observed on explant culture on TDZ 3 µM (92.00±0.00). These non-significant differences in some treatments indicate that all cytokinins can stimulate shoot emergence at each optimal concentration. Kher *et al*. (2014) stated that bud formation on plant nodes varies with the type and concentration of growth regulators used.

Cytokinin influences shoot formation, cell division, and shoot proliferation in plants. Adding cytokinins to the media is crucial for inducing shoot formation and proliferation in node explants (Bala et al., 2018; J. Zhao et al., 2024). Cytokinins can regulate gene expression related to the cell division cycle. It suggests that growth regulators promote shoot growth by affecting the cell division cycle (Zhao *et al*., 2020). Kinetin is a type of cytokinin effective in shoot formation and rejuvenation. Kinetin can stimulate the growth of lateral buds and break apical (Fathy *et al*., 2022; Thorat *et al*., 2022; Zhao *et al*., 2024).

Table 2. The DMRT results of the average of A. borneensis shoot emergence time in response to the interaction between cytokinin types and concentrations at 16 wap. Note: means followed by the same letter indicate no significant difference at 95% DMRT level of confidence.

Treatments	Shoot emergence time (day)
BAP 0 µM	$71.00±32.14^{abcd}$
BAP 1 µM	$54.00±4.00^{de}$
BAP 2 µM	$52.67±12.22^{de}$
BAP 3 µM	$76.50±3.50^{abcd}$
BAP 4 µM	$89.50±16.50^{ab}$
Kin 0 µM	$78.00±28.00^{abcd}$
Kin 1 µM	$37.00±11.79^{e}$
Kin 2 µM	$90.33±2.89^{ab}$
Kin 3 µM	$54.00±4.00^{de}$
Kin 4 µM	$59.67±24.01^{cde}$
TDZ 0 µM	$62.33±21.36^{bcde}$
TDZ 1 µM	$76.50±3.50^{abcd}$
TDZ 2 µM	$87.00±7.81^{abc}$
TDZ 3 µM	$92.00±0.00^{a}$
TDZ 4 µM	$58.00±8.00^{de}$

According to Sarmast *et al*. (2012), cytokinins at low concentrations are more effective in inducing shoots in *A. excelsa* R. Br. In addition, Kinetin produced better shoot proliferation and height than other cytokinins. Additionally, Gogoi *et al*. (2017) observed that shoot initiation of *Morus indica* L. performed optimally on Murashige and Skoog media supplemented with Kinetin at different concentrations. Kinetin is needed in the late stages of the G2 phase of the cell division cycle. Cells that did not have enough Kinetin will be stuck in the G2 phase. Kinetin stimulates phosphate release, thereby activating enzymes, which then trigger the synchronous and rapid entry of cells into the mitotic phase (Barciszewski *et al*., 2007).

Several studies have reported that BAP and TDZ were more effective than Kinetin for shoot induction. BAP was more effective in axillary bud formation of *Balanites aegyptiaca*, *Citrus limon*, and *Syzygium cuminii* (Rathore *et al.*, 2004). BAP promotes shoot formation by releasing axillary buds from the dormancy phase (Li *et al*., 2021; Neogy *et al*., 2020). Thidiazuron was more effective for shoot formation in node explants of three *Vitex* species (*Vitex trifolia*, *Vitex negundo,* and *Vitex doniana*) as well as *Syzygium cuminii* and did not show any abnormal morphology (Ahmad & Faisal, 2018; Naaz *et al*., 2021). TDZ promotes shoot formation by inducing cell division and proliferation and rearranging meristematic regions to differentiate axillary buds (Ram *et al*., 2022).

In this study, explants cultured in media supplemented with TDZ showed browning at the base of the explants (Figure 2). In some cases, TDZ has serious adverse effects on in vitro growth. Although TDZ has high cytokinin activity, vitrified shoots and the return of shoots to callus or tissue necrosis can occur due to prolonged exposure to TDZ (Vinoth & Ravindhran, 2018). The ability of thidiazuron to both inhibit cytokinin oxidase enzyme and trigger cytokinin biosynthesis will lead to cytokinin accumulation in plant cells. Excessive cytokinin accumulation during prolonged TDZ exposure can lead to necrosis or cell death (Nisler, 2018; Pai & Desai, 2018).

The increased number of shoots indicates *in vitro* plant propagation success (Akbar *et al*., 2017). ANOVA results (Table 1) showed no significant difference in shoot number and length. Nevertheless, adding 6-Benzylaminopurine at 0 and 2 μM; Kinetin at 0, 1, 2, and 4 μM gave rise to the highest axillary bud growth percentage (100%) (Figure 3). In comparison, the supplementation of thidiazuron at 2 μM produced the smallest percentage of axillary bud appearance (33.3%). According to Pallardy & Kozlowski (2008) and Sarmast *et al*. (2012), most conifers produce few or no axillary buds. Members of the *Araucariaceae* family, such as Araucaria, Agathis, and Wollemia, have unique axillary buds with undifferentiated meristems that lack leaf primordia and contain vascular tissue. In *pines*, *cypresses*, oaks, and hickories, shoots originate from the expansion of apical buds of the main stem and branches. There is a period of dormancy after the apical bud extends until a new terminal bud is formed and developed. Due to strong apical dominance, the apical bud extends more yearly than the lateral buds below it. Removal of the apical bud, which then removes apical dominance in conifers, can significantly affect the growth of axillary buds (Pallardy & Kozlowski, 2008).

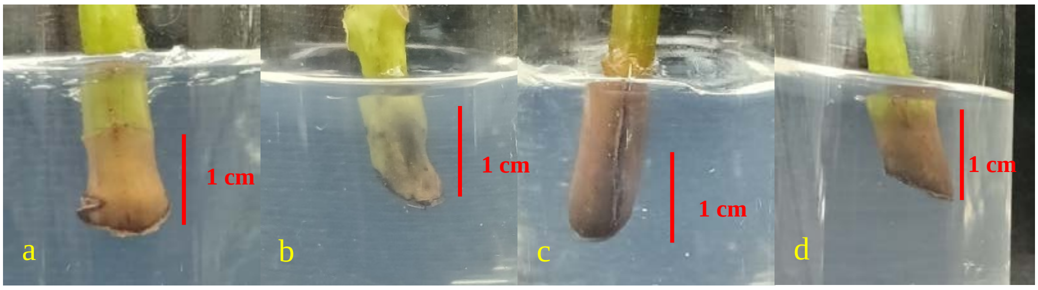

Figure 2. Condition of *A. borneensis* explants on media with the addition of TDZ at the age of 16 wap: (a) 1 μM TDZ; (b) 2 μM TDZ; (c) 3 μM TDZ; (d) 4 μM TDZ.

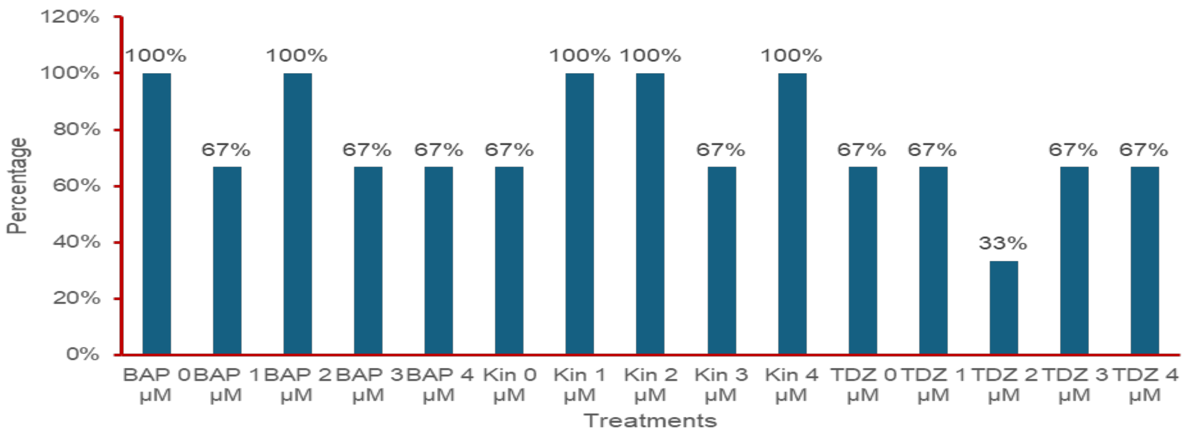

Figure 3. Percentage of *A. borneensis* sprouting explants in response to cytokinin types and concentrations at 16 wap.

Leaf formation is a form of vegetative growth in plants. Leaf formation in plants is susceptible to environmental changes and growth regulators, both endogenous and exogenous (Magdhalena *et al*., 2021). The ANOVA results

(Table 1) showed that *A. borneensis* Warb leaf formation was controlled by cytokinin concentration. Moreover, DMRT results (Figure 4) showed that explants of *A. borneensis* cultured on media without cytokinin produced the largest number of leaves (2.74±0.31 leaves/explant), which was not significantly different to those cultured on media supplemented with cytokinin at 1 µM and 4 µM, which produces 2.53±1.50 and 1.98±1.10 leaves/explant, respectively. Figure 4 also revealed that increasing the cytokinin concentration to 3 µM significantly reduced leaf number. Shoot's response to 3 types of cytokinin at 4 different concentrations is shown in Figure 5. According to Sarmast *et al*. (2012), axillary buds can be produced from *Araucaria excelsa* R.Br. orthotropic stems, even when growth regulators were absent. In other studies, cytokinin-free MS media also increased plant height with more leaves and shoots in *Guazuma ulmifolia* (Hernandez-Santana *et al*., 2021), *Cedrela fissilis* (Bonfá *et al*., 2021), *Citrus hystrix* (Handayani *et al*., 2024), and *Morus nigra* (Phillips & Garda, 2019) cultures because *in vitro* culture media contain sufficient nutrients for plant development.

MS medium is commonly used in *in vitro* plant propagation. MS media contains high nitrogen content (nitrate and ammonium), suitable for plant regeneration (Phillips & Garda, 2019). MS medium's macro-element composition (K+, Mg2+, N, NO3-, and NH4+) may balance nitrogen uptake, osmotic regulation, pH, and cation-anion balance in plant growth. At high nitrate levels, *Eucalyptus* hybrid cultures showed average shoot growth with green leaves, no hyperhydration, and produced an average shoot length of more than 1.4 cm (Máximo *et al*., 2015).

The growth of *A. borneensis* on MS media without additional growth regulators could also be attributed to endogenous growth regulators in *the A. borneensis* shoot. According to Li *et al.* (2021) and Rai *et al*. (2022), success or failure in in vitro culture can result from variations in endogenous growth regulator levels in plants. In plants, endogenous cytokinin levels are well-regulated in response to growth events, such as axillary bud formation, and to environmental factors, such as light and nutrients (Bredmose & Costes, 2017). Regulation of growth and organogenesis is associated with balancing endogenous and exogenous growth regulators (Aremu *et al*., 2014; Boston *et al*., 2013; George, 1993; Park, 2021).

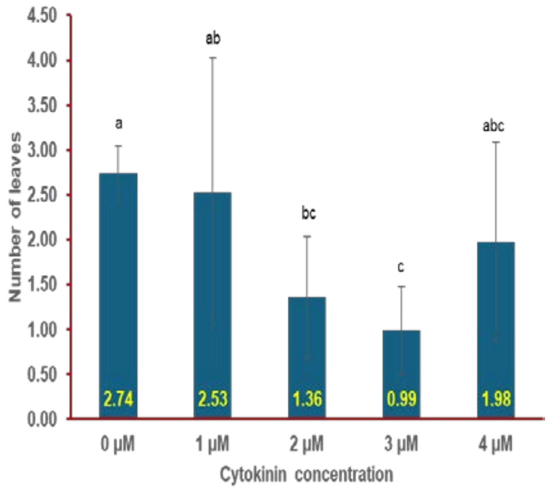

Figure 4 DMRT results of the average number of *A. borneensis* shoots at different cytokinin concentrations at 16 wap. Note: Means followed by the same letter indicate no significant difference at 95% DMRT level of confidence.

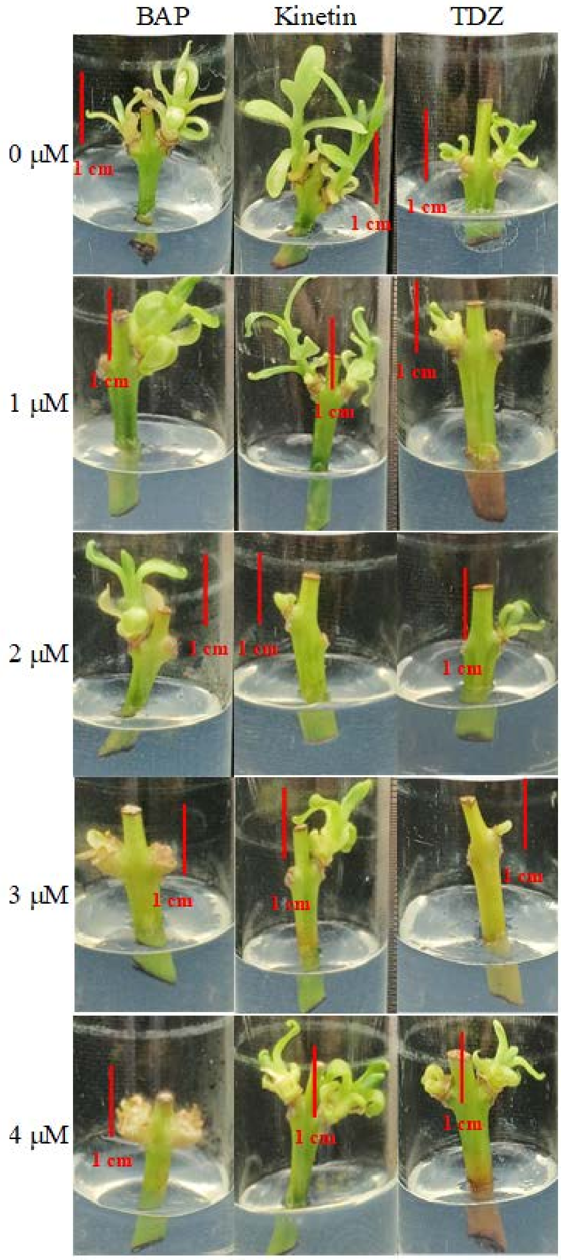

Figure 5. Conditions of *A. borneensis* shoots grown in 3 types of cytokinins on 5 concentrations at 16 wap.

In *in vitro* culture, the need to modify nutrient media composition, types and concentrations of cytokinins depends on plant species, genotype, explant type, ontogeny phase, and stage of the micropropagation process (Iliev, 2017). In this study, *A. borneensis* explants that grew in MS media without growth regulators suggest that the nutrient content, vitamins, organic components, and carbon sources were sufficient to support *A. borneensis* growth and differentiation. Kinetin at 1 μM induced the fastest shoot emergence. It is suggested that the application of kinetin interacts with endogenous growth regulators in *the A. borneensis* shoot. According to Bredmose & Costes (2017), increased growth of axillary buds results from the decrease of auxin and increase of cytokinin concentrations, thus increasing the ratio of cytokinin to auxin. Therefore, it is suggested that 1 μM kinetin could accelerate the production of *A. borneensis* seedlings.

Plant growth regulators affect many processes and conditions, including enzymatic activity, membrane permeability, cell wall relaxation, cell division and elongation, and tissue and organ senescence. Many pieces of evidence suggest that plant growth and differentiation are controlled more by interactions between plant growth regulators (Pallardy & Kozlowski, 2008).

4. Conclusion

The lateral shoot apex of *A. borneensis* collected from the Bogor Botanical Garden was sterilized and cultured on MS medium, supplemented with 3% sucrose, 100 mg/L myo-inositol, and solidified with 0.7% agar. Different types and concentrations of cytokinin were tested to induce shoot formation. It was found that cytokinin type and concentration controlled *the time of A. borneensis* shoot emergence in *in vitro* culture. *A. borneensis* explants cultured on MS media supplemented with 1μM kinetin produced the fastest shoot emergence. Meanwhile, *A. borneensis* shoots grown on MS media without cytokinin yielded the highest leaf number. Shoot multiplication and subsequent plantlet formation must be done to produce a large quantity of *A. borneensis* seedlings.

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References

Adam, A. Z., Juiling, S., Lee, S. Y., Jumaat, S. R., & Mohamed, R. (2017). Phytochemical Composition of Agathis borneensis (Araucariaceae) and Their Biological Activities. *Mal. For.*, **80(2)**, 169–177.

Agustina, D., Tarwotjo, U., Rahadian, R., & Article, H. (2019). The Effectiveness of Plastic Mulch for Maintaining the Potato Farmland in Dieng Plateau Using Soil Biological Quality Index. *Biosaintifika*, **11(1)**, 125–131.

Ahmad, N., & Faisal, M. (2018). Thidiazuron: From urea derivative to plant growth regulator. In **Thidiazuron: From Urea Derivative to Plant Growth Regulator.** https://doi.org/10.1007/978-981-10-8004-3

Ahmed, A. H., & Mohamed, S. A. (2022). Triterpenoids from Agathis robusta Aerial Parts and Their Hepatoprotective Activity. *Phcog J*, **14(4)**, 362–366. https://doi.org/10.5530/pj.2022.14.108

Akbar, A. M., Faridah, E., Indrioko, S., & Herawan, T. (2017). Induksi Tunas, Multiplikasi dan Perakaran Gyrinops versteegii (Gilg.) Domke Secara In Vitro. *JPTH*, **11(1)**, 1–13.

Andriani, C., Kadapi, M., Suminar, E., & Nuraini, A. (2023). Perbandingan Efek BAP dan Kinetin Terhadap Laju Multiplikasi Stroberi Kultivar Sweet Charlie. *JA*, **14(1)**, 13. https://doi.org/10.24014/ja.v14i1.18824

Aremu, A. O., Plačková, L., Bairu, M. W., Novák, O., Plíhalová, L., Doležal, K., Finnie, J. F., & Van Staden, J. (2014). How Does Exogenously Applied Cytokinin Type Affect Growth and Endogenous Cytokinins in Micropropagated Merwilla plumbea? *PCTOC*, **118(2)**, 245–256. https://doi.org/10.1007/s11240-014-0477-5

Bala, R., Laura, J. S., & Beniwal, V. S. (2018). Efficient In vitro Direct Regeneration From Nodal Explants of Simmondsia chinensis (Link) Schneider-A Potential Multipurpose Plant. *PCBMB*, **19(7–8)**, 256–265. https://ikprress.org/index.php/PCBMB/article/view/1358

Barciszewski, J., Massino, F., & Clark, B. F. C. (2007). Kinetin-A multiactive molecule. *Int. J. Biol. Macromol.*, **40(3)**, 182–192. https://doi.org/10.1016/j.ijbiomac.2006.06.024

Biradar, S. R. (2022). **An Introduction of Plant Tissue Culture** (5th ed.). RUT printer and publisher. https://www.researchgate.net/publication/361304964

Bonfá, Y. S., De Laia Nascimento, V., & Terra Werner, E. (2021). In Vitro Multiplication of Cedrela fissilis Vell.: A Threatened Brazilian Hardwood Forest Tree. *PCCM*, **17**, 1–11. https://doi.org/10.46526/pccm.2021.v17.165

Boston, A. •, Heidelberg, •, London, •, San, P. •, San, D., Singapore, F. •, Tokyo, S. •, & Smith, R. H. (2013). **Plant Tissue Culture Techniques and Experiments**, Third Edition. Elsevier. www.elsevierdirect.com/rights

Bredmose, N., & Costes, E. (2017). Axillary Bud Growth. In **Reference Module in Life Sciences** (p. B9780128096338051000). Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.05056-1

Chukwu, S. C., Awala, · S K, Angombe, · S, Valombola, · J S, Nanhapo, · P I, Mberama, · C, Mohd, ·, Rafii, Y., Oladosu, Y., Thomas, · B, Okporie, E. O., & Musa, I. (2025). Recent progress in tissue culture techniques and biotechnological innovations for banana production (Musa spp.): a review. *Discov. Plant., 2025* **2(1)**, 1–24. https://doi.org/10.1007/S44372-025-00099-2

Darma, I. D. P., Iryadi, R., Rahayu, A., Hanum, S. F., & Sutomo, S. (2022). Keragaman Jenis Agathis di Dunia dan Riap Tahunan Agathis dammara (Lamb.) Poir. dan Agathis borneensis Warb. di Kebun Raya Eka Karya, Bali. *BKR*, **25(1),** 34–43.

Fajar, D., Cahyanto, T., & Fadillah, A. (2018). Waktu Tumbuh Mata Tunas Daun Mangifera indica L. Pada Berbagai Tingkatan. *Edubiotik* **3(01)**, 19–25. https://doi.org/10.33503/ebio.v3i01.73

Farjon, A. (2013). Agathis borneensis. **IUCN Red List of Threatened Species**. https://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T202905A2757743.EN

Farjon, A. (2017). **A Handbook of the World's Conifers** (rerevised Brill, Vol. 1).

Fathy, M., Saad Eldin, S. M., Naseem, M., Dandekar, T., & Othman, E. M. (2022). Cytokinins: Wide-Spread Signaling Hormones from Plants to Humans with High Medical Potential. *Nutrients*, **14(7)**, 1495. https://doi.org/10.3390/nu14071495

George, E. F. (1993). **Plant propagation by Tissue Culture Part I. The Technology Exegetics**.

George, E. F., Hall, M. A., & Klerk, G.-J. de. (2008). ***Plant Propagation by Tissue Culture*** (3rd ed). Springer.

Gogoi, G., Borua, P. K., & Al-Khayri, J. M. (2017). Improved Micropropagation and In Vitro Fruiting of Morus indica L. (K-2 cultivar). *J. Genet. Eng. Biotechnol.*, **15(1)**, 249–256. https://doi.org/10.1016/j.jgeb.2017.02.005

Handayani, R. S., Nilahayati, N., Ismadi, I., Akmal, A., Aprilia, D., & Sevia, E. D. (2024). Growth Response of Sweet Kaffir Lime (Citrus hystrix) Seeds Due to Kinetin and Coconut Water Application Using Tissue Culture Technique. *IOP Conf. Ser. Earth Environ. Sci.*, **1297(12–15)**, 1–7. https://doi.org/10.1088/1755-1315/1297/1/012015

Hernandez-Santana, V., Perez-Arcoiza, A., Gomez-Jimenez, M. C., & Diaz-Espejo, A. (2021). Disentangling the link between leaf photosynthesis and turgor in fruit growth. *Plant J.*, **107(6),** 1788–1801. https://doi.org/10.1111/tpj.15418

Iliev, I. A. (2017). Factors Affecting The Axillary and Adventitious Shoot Formation in Woody Plants In Vitro. *Acta Hortic.*, **1155(2)**, 15–28. https://doi.org/10.17660/ActaHortic.2017.1155.2

Ishii, K., & Mohsin, R. B. H. (1994). Tissue Culture of Some Dipterocarps and Agathis in Brunei. *Bull. Forestry Forest Prod. Res. Inst.*, **366**, 115–127.

Justine, A. K., Kaur, N., Savita, & Pati, P. K. (2022). Biotechnological interventions in banana: current knowledge and future prospects. *Heliyon*, **8(11),** 1–14. https://doi.org/10.1016/j.heliyon.2022.e11636

Kher, M. M., Joshi, D., Nekkala, S., Nataraj, M., & P. Raykundaliya, D. (2014). Micropropagation of Pluchea lanceolata (Oliver & Hiern.) Using Nodal Explant. *J. Hort. Res.*, **22(1)**, 35–39. https://doi.org/10.2478/johr-2014-0004

Kulkarni, A. A., Thengane, S. R., & Krishnamurthy, K. V. (1996). Direct in vitro regeneration of leaf explants of Withania somnifera (L.) Dunal. *Plant Sci.*, **119(1–2)**, 163–168. https://doi.org/10.1016/0168-9452(96)04462-7

Li, S. M., Zheng, H. X., Zhang, X. S., & Sui, N. (2021). Cytokinins as central regulators during plant growth and stress response. *Plant Cell Rep.*, **40(2)**, 271–282. https://doi.org/10.1007/s00299-020-02612-1

Magdhalena, A. P., Asmanto, B. P., Sulandjari, S., & Yunus, A. (2021). The Effect of Concentration and Time Interval of Kinetin Application on the Growth of Daun Duduk (Desmodium triquetrum L.) Seeds. *JBB*, **1(1)**, 29. https://doi.org/10.20961/jbb.v1i1.50418

Máximo, W. P. F., Santos, P. A. A., Mendonça, E. G., Santos, B. R., & Paiva, L. V. (2015). Nitrate (NO3-) and Ammonium (NH4+) Ratios for Propagation of Eucalyptus Hybrid in Two Different In Vitro Cultivation Systems. *AJCS*, **9(12)**, 1242–11248.

Naaz, A., Siddique, I., & Ahmad, A. (2021). TDZ-Induced Efficient Micropropagation from Juvenile Nodal Segment of Syzygium cumini (Skill): A Recalcitrant Tree. In I. Siddique (Ed.), **Propagation and Genetic Manipulation of Plants**. Springer Singapore. https://doi.org/10.1007/978-981-15-7736-9

Neogy, A., Singh, Z., Mushahary, K. K. K., & Yadav, S. R. (2020). Dynamic cytokinin signaling and function of auxin in cytokinin-responsive domains during rice crown root development. *Plant Cell Rep.*, **1(0123456789)**. https://doi.org/10.1007/s00299-020-02618-9

Nisler, J. (2018). TDZ: Mode of action, use, and potential in agriculture. In **Thidiazuron: From Urea Derivative to Plant Growth Regulator** (pp. 37–59). Springer Singapore. https://doi.org/10.1007/978-981-10-8004-3_2

Pai, S. R., & Desai, N. S. (2018). Effect of TDZ on Various Plant Cultures. In N. Ahmad & M. Faisal (Eds.), **Thidiazuron: From Urea Derivative to Plant Growth Regulator** (pp. 439–454). Springer Singapore. https://doi.org/10.1007/978-981-10-8004-3_25

Pallardy, S. G., & Kozlowski, T. T. (2008). **Physiology of Woody Plants** (3rd ed). Elsevier.

Park, S. (2021). ***Plant Tissue Culture Techniques and Experiments*** (Fourth Edition). Academic Press Elsevier.

Phillips, G. C., & Garda, M. (2019). Plant Tissue Culture Media and Practices: An Overview. *In vitro Cell Dev Biol Plant*, **55(3)**, 242–257. https://doi.org/10.1007/s11627-019-09983-5

Prasetyo, R., Sugiyono, & Prayoga, L. (2020). INDUKSI TUNAS MIKRO PISANG KULTIVAR AMBON NANGKA (Musa sp .) SECARA IN VITRO. *Vigor*, **5(2)**, 45–50.

Putriana, Gusmiati, Restu, M., Musriati, & Aida, N. (2019). Respon Kinetin dan Tipe Eksplan Jabon Merah (Antocephalus macrophyllus (Roxb.) Havil) Secara In Vitro. *BIOMA*, **4(1)**, 48–57.

Rai, A. C., Kumar, A., Modi, A., & Singh, M. (2022). **Advances in Plant Tissue Culture**. Elsevier.

Ram, K., Patel, A. K., Choudhary, S. K., & Shekhawat, N. S. (2022). Synergetic Effects of TDZ With Various Phytohormones on High-Frequency Plant Regeneration From Mature Nodal Explants of Capparis decidua and Their Ex vivo Implications. *PCTOC*, **149(3)**, 621–633. https://doi.org/10.1007/s11240-022-02234-3

Rathore, J. S., Rathore, V., Shekhawat, N. S., Singh, R. P., Liler, G., Phulwaria, M., & Dagla, H. R. (2004). Micropropagation of Woody Plants. In **Plant Biotechnology and Molecular Markers**. Springer. https://link.springer.com/chapter/10.1007/1-4020-3213-7_13

Ružić, Đ., Vujović, T., & Cerović, R. (2016). In Vitro Multiplication of Semi-Dwarfing Pear Rootstock 'Pyrodwarf' in Relation to Cytokinin Types. *Acta Hortic.*, **1139**, 279–284. https://doi.org/10.17660/ActaHortic.2016.1139.49

Sarmast, M. K., Salehi, H., & Khosh-Khui, M. (2012). Micropropagation of Araucaria excelsa R. Br. var. glauca Carrière from Orthotropic Stem Explants. *Physiol Mol Biol Plants*, **18(3)**, 265–271. https://doi.org/10.1007/s12298-012-0115-9

Sitompul, S. M., & Guritno, B. (1995). **Analysis of Plant Growth**. Universitas Gadjah Mada Press.

Stalin, A. N., & Franco, F. M. (2021). Agathis borneensis Warb. Araucariaceae. In F. M. Franco (Ed.), **Ethnobotany of the Mountain Regions of Southeast Asia** (pp. 65–71). Springer International Publishing. https://doi.org/10.1007/978-3-030-38389-3_163

Steward, G. A., Kimberley, M. O., Mason, E. G., & Dungey, H. S. (2014). Growth and Productivity of New Zealand kauri (Agathis australis (D.Don) Lindl.) in Planted Forests. *NZJFS*, **44(1)**, 27. https://doi.org/10.1186/s40490-014-0027-2

Taha, R. A., Allam, M. A., Hassan, S. A. M., Bakr, B. M. M., & Hassan, M. M. (2021). Thidiazuron-induced direct organogenesis from immature inflorescence of three date palm cultivars. *J. Genet. Eng. Biotechnol.*, **19(1)**, 14. https://doi.org/10.1186/S43141-021-00115-4

Tamyiz, M., Prayoga, L., Prasetyo, R., Murchie, E. H., & Sugiyono. (2022). Improving Agarwood (Aquilaria malaccensis Lamk.) Plantlet Formation Using Various Types and Concentrations of Auxins. *Caraka Tani J. Sustain. Agric.*, **37(1)**, 142–151. https://doi.org/10.20961/carakatani.v37i1.58370

Thorat, S. A., Kaniyassery, A., Poojari, P., Rangel, M., Tantry, S., Kiran, K. R., Joshi, M. B., Rai, P. S., Botha, A.-M., & Muthusamy, A. (2022). Differential Gene Expression and Withanolides Biosynthesis During In Vitro and Ex Vitro Growth of Withania somnifera (L.) Dunal. *Front. Plant Sci.*, **13**, 917770. https://doi.org/10.3389/fpls.2022.917770

Vinoth, A., & Ravindhran, R. (2018). In Vitro Morphogenesis of Woody Plants Using Thidiazuron. In N. Ahmad & M. Faisal (Eds.), **Thidiazuron: From Urea Derivative to Plant Growth Regulator** (pp. 211–229). Springer Singapore. https://doi.org/10.1007/978-981-10-8004-3_10

Widyatmoko, D. D. (2019). Strategi Dan Inovasi Konservasi Tumbuhan Indonesia Untuk Pemanfaatan Secara Berkelanjutan. *Prosiding Seminar Nasional Pendidikan Biologi dan Saintek (SNPBS)*, **4**, 1–22.

Yadav, A., Prasad, Y., Kumar, M., Pandey, S., Maurya, R., & Pandey, P. (2021). Effects of Mercuric chloride and Ethanol for surface sterilization under in vitro plant growth in banana (Musa paradisiaca L.) variety "Udhayam." *J. Pharmacogn. Phytochem.*, **10(1)**, 2281–2283. https://www.phytojournal.com/archives/2021.v10.i1.13693/effects-of-mercuric-chloride-and-ethanol-for-surface-sterilization-under-in-vitro-plant-growth-in-banana-musa-paradisiaca-l-variety-8220udhayam8221

Zhao, J., Wang, J., Liu, J., Zhang, P., Kudoyarova, G., Liu, C.-J., & Zhang, K. (2024). Spatially Distributed Cytokinins: Metabolism, Signaling, and Transport. *Plant Commun.*, **5(100936)**, 1–16. https://doi.org/10.1016/j.xplc.2024.100936

Zhao, X., Han, X., Wang, Q., Wang, X., Chen, X., Li, L., Fu, X., & Gao, D. (2020). Early Bud Break 1 Triggers Bud Break in Peach Trees by Regulating Hormone Metabolism, The Cell Cycle, and Cell Wall Codifications. *J. Exp. Bot.*, **71(12)**, 3512–3523. https://doi.org/10.1093/jxb/eraa119