BIOCHAR-SUPPLEMENTED IN VITRO CULTURE OF *MUSA ACUMINATA* VAR. *BARANGAN* TO CONTROL CONTAMINATION AND ENHANCE REGENERATION

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ABSTRACT

This study aimed to evaluate the effectiveness of coconut shell-derived biochar supplementation in Murashige and Skoog (MS) medium to suppress contamination and enhance regeneration efficiency. A Completely Randomized Design (CRD) with 11 biochar concentrations (0–5 g/L) was used, each with three replicates. Parameters observed included contamination and browning onset, browning intensity, propagule formation time, fresh weight, shoot number, and shoot height. Results showed that biochar significantly delayed contamination and browning. The 2 g/L treatment yielded the best results: 13.8 days after culture (DAC) for contamination and 12.67 DAC for browning. The lowest browning intensity (score 1.41) occurred at 5 g/L. Propagule formation was fastest at 3 g/L, while optimal shoot multiplication and height were observed at 2 g/L. These benefits are attributed to biochar's ability to enhance nutrient availability, adsorb phenolics, stabilize pH, and regulate endogenous hormones. However, concentrations ≥4 g/L reduced culture performance, likely due to osmotic stress and media imbalance.

Key words: biochar, tissue culture, contamination control, browning, micropropagation.

الزراعة النسيجية للموز *Musa acuminata* var. barangan المدعّمة بالفحم الحيوي للتحكم في التلوث وتعزيز التجديد

موستيكا توو فهر الدين إليس تمبارو نابيلة سيام نور فاضلة س. جاگا

المستخلص

هدفت هذه الدراسة إلى تقييم فعالية إضافة الفحم الحيوي المستخلص من قشور جوز الهند إلى وسط Murashige and Skoog (MS) للحد من التلوث وتعزيز كفاءة التجديد. تم استخدام تصميم عشوائي كامل (CRD) يشمل أحد عشر تركيزًا من الفحم الحيوي (من 0 إلى 5 غم/لتر)، مع ثلاث مكررات لكل تركيز. شملت المعايير التي تم رصدها وقت بدء التلوث والتلون، وشدة التلون، ومدة تكوين البراعم، والوزن الطازج، وعدد البراعم، وارتفاعها. أظهرت النتائج أن الفحم الحيوي ساهم بشكل كبير في تأخير التلوث والتلون. وقد أعطى التركيز 2 غم/لتر أفضل النتائج: 13.8 يومًا بعد الزراعة (DAC) لظهور التلوث، و12.67 يومًا للتلون. وسُجلت أدنى شدة للتلون (بقيمة 1.41) عند تركيز 5 غم/لتر. وحدث أسرع تكوين للبراعم عند تركيز 3 غم/لتر، بينما لوحظ أفضل تكاثر وارتفاع للبراعم عند تركيز 2 غم/لتر. تُعزى هذه الفوائد إلى قدرة الفحم الحيوي على تعزيز توفر المغذيات، وامتصاص المركبات الفينولية، وتثبيت الرقم الهيدروجي(pH)، وتنظيم الهرمونات الذاتية. ومع ذلك، فإن التراكيز التي تساوي أو تزيد عن 4 غم/لتر أدت إلى تراجع الأداء، ويرجّح أن يكون ذلك بسبب الإجهاد الاوزموزي أو عدم توازن الوسط الغذائي.

الكلمات المفتاحية :بايوشار، زراعة الأنسجة، مكافحة التلوث، التلون، الإكثار الدقيق.

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INTRODUCTION

Banana (*Musa acuminata* Colla.) is one of the most economically valuable horticultural commodities and plays a vital role in supporting food security across tropical countries, including Indonesia. Among the various cultivated banana varieties, *Musa acuminata* var. *barangan* (Barangan banana) is widely grown in Indonesia due to its high economic value, superior agronomic traits, and rich nutritional profile, including carbohydrates, folic acid, fiber, calcium, iron, vitamin B, and vitamin C (7, 46). Market demand for this cultivar continues to rise, with national production increasing from 9.24 million tons in 2022 to 9.69 million tons in 2024. However, in several regions such as South Sulawesi, productivity remains constrained by pathogen attacks, climate change, land degradation, and the limited availability of elite planting materials (33, 43). The mass propagation of high-quality seedlings within a short time is difficult to achieve through conventional vegetative methods. In vitro culture offers an effective approach to produce pathogen-free and genetically uniform plantlets. Nevertheless, the application of this technique is challenged by microbial contamination and tissue browning due to phenolic compound accumulation, both of which significantly reduce culture success rates (30). The success of this technique is highly influenced by the culture medium composition, which determines the direction of growth and explant differentiation (16, 35). Murashige and Skoog (MS) medium is the most widely used basal medium, yet it remains susceptible to microbial contamination that can inhibit explant regeneration. Contamination is a major challenge in culture, often caused by incomplete explant sterilization, non-sterile equipment, or non-aseptic environmental conditions (1, 24). In banana cultures, the high accumulation of phenolic compounds exacerbates contamination and causes media browning, which negatively affects explant growth. Phenolic compounds released by wounded or stressed explants can serve as a carbon source for pathogenic and opportunistic microorganisms, thereby accelerating contamination and reducing micropropagation success (28). To overcome these challenges, innovations in culture media formulation are needed particularly approaches that not only suppress contamination but also enhance explant growth and regeneration. One promising strategy is the incorporation of biochar as a culture medium additive. Biochar is a pyrolyzed biomass product with high adsorptive capacity, capable of absorbing toxic compounds, reducing pathogenic microbial populations, and improving medium quality by enhancing water retention and micronutrient availability (32). Furthermore, biochar has been reported to stimulate explant growth. Although several studies have demonstrated the potential of biochar in supporting tissue culture, its application in banana micropropagation systems remains limited, and its optimal concentration has yet to be clearly defined. Therefore, this study aims to evaluate the effectiveness of various biochar concentrations in suppressing contamination and improving in vitro regeneration efficiency of *Musa acuminata* var. *barangan*, as an initial step toward the production of high-quality, contaminant-free planting materials.

MATERIALS AND METHODS

Preparation of Biochar-Based Culture Media: The biochar used in this study was derived from coconut shells. The shells were thoroughly cleaned to remove residual fibers, then broken into smaller fragments and dried to reduce moisture content. Carbonization was conducted in a furnace under an inert gas atmosphere at 400 °C for two hours to eliminate volatile compounds. The resulting charcoal was cooled in a desiccator. Once cooled, the charcoal was ground and sieved through a 200-mesh screen to obtain fine carbon powder, which served as biochar (20). The prepared biochar was then incorporated into the culture media. Murashige and Skoog (MS) medium was used as the basal medium in this study. For the preparation of 1 liter of culture medium, 4.43 g of MS powder, 30 g of sucrose, 4 ppm of BAP, 2 ppm of IAA, and biochar at various concentrations (0; 0.5; 1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5; 5 g/L) were dissolved in distilled water to a final volume of 1 liter. The control medium was prepared without biochar. The solution was stirred until completely dissolved, and the pH was adjusted

to 5.8. Subsequently, 2 g of gellan gum powder was added. The media was heated with continuous stirring until fully homogenized, then sterilized in an autoclave at 121 °C and 1 atm for 15 minutes.

Explant Sterilization and Culture Initiation

Corms of Barangan banana plants were used as explants, initially washed under running water for 30 minutes. Surface sterilization was carried out using a fungicide solution followed by 5% sodium hypochlorite (NaOCl) containing two drops of Tween-80 for 20 minutes. The explants were then rinsed thoroughly with sterile distilled water. Further sterilization was conducted in a laminar airflow cabinet by agitating the explants in 0.5% NaOCl with Tween-80 for 10 minutes. The outer layers of the explants were then trimmed to a size of approximately 2 cm, rinsed three times with sterile distilled water, and placed on sterile filter paper (49). The explants were then inoculated onto MS medium supplemented with 4 ppm BAP, 2 ppm IAA, and biochar according to the treatment concentration. Culture bottles were placed on shelves in the incubation room. Once propagules formed, the explants were subcultured into fresh media at four propagules per bottle. Culture bottles were incubated under controlled conditions of 26 ± 2 °C, relative humidity of 55 ± 5%, and a 16-hour photoperiod.

Experimental Design: The experiment was arranged in a Completely Randomized Design (CRD) with a single treatment factor: biochar concentration in the culture medium. Eleven treatment levels (0; 0.5; 1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5; 5 g/L) were tested, each with three replicates. Each replicate consisted of five culture bottles, resulting in a total of 165 experimental units (11 treatments × 3 replicates × 5 bottles).

Observation and Data Analysis

Observations were carried out daily after planting. The parameters recorded included: time of contamination onset (days after culture; DAC), time of browning onset (DAC), browning intensity, time to propagule formation (DAC), fresh weight of propagules (g), number of shoots, and shoot height. Data were statistically analyzed following a Shapiro-Wilk test for normality and a Levene's test for homogeneity of variance. If the data met both assumptions, parametric analysis was conducted using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level. If the assumptions were not met, non-parametric analysis was applied using the Kruskal-Wallis test. When significant differences were found, pairwise comparisons were conducted using the Mann-Whitney U test at a 5% confidence level (3,50). Browning intensity was assessed using a semi-quantitative scoring system based on the estimated percentage of explant surface area showing browning symptoms. Observations were made visually, and scores were assigned on a scale from 1 to 5 to represent the severity of browning. The scoring criteria are presented in Table (1).

Table 1. Browning intensity assessment (2)

No.	Score Range	Description	Category
1	1-1.99	Score 1: 0–24% of the explant surface shows browning	Very Low
2	2-2.99	Score 2: 25–49% of the explant surface shows browning	Low
3	3-3.99	Score 3: 50–74% of the explant surface shows browning	Moderate
4	4-4.99	Score 4: 75–99% of the explant surface shows browning	High
5	> 5	Score 5: 100% of the explant surface shows browning	Very High

RESULTS AND DISCUSSION

The micropropagation of *Musa acuminata* var. *barangan* was carried out through several key stages, including explant sterilization, initiation on Murashige and Skoog (MS) medium supplemented with various concentrations of biochar, and the multiplication phase. Each stage was designed to comprehensively assess the explant's response to biochar treatments. Figure (1) presents the schematic workflow of the in vitro culture procedures conducted in this study. The effects of different biochar concentrations on the growth and regeneration parameters of *Musa acuminata* var. *barangan* are presented in Table (2).

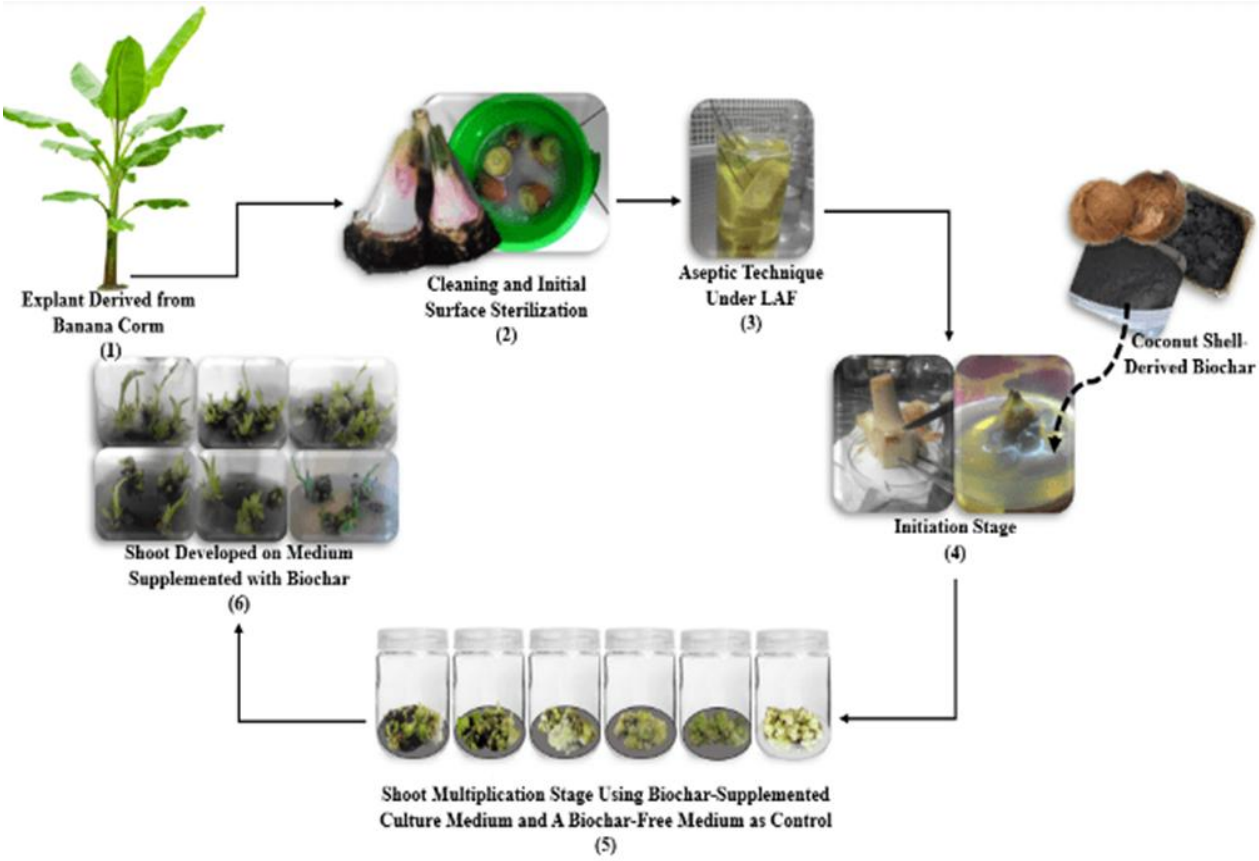

Figure 1. Schematic overview of the culture procedure of *Musa acuminata* var. *barangan* using biochar supplementation. The process begins with explant derived from banana corm (1), cleaning and sterilization (2), aseptic culturing under LAF (3), and biochar-supplemented MS medium application (0-5 g/L) during initiation (4), shoot multiplication stages (5), and shoots developed were compared to a biochar-free control (6)

Table 2. Effect of different biochar concentrations on in vitro growth and regeneration parameters of *Musa acuminata* var. *barangan*

Biochar Concentration (g/L)	Contamination Time (DAC)	Browning Time (DAC)	Browning Intensity	Propagule Formation Time (DAC)	Fresh Weight of Propagule (g)	Number of Shoots (units)	Shoot Height (cm)
0	3.33 ± 0.62^{h}	3.27 ± 0.46^{h}	5 ± 0^{i}	36.73 ± 0.7^{h}	0.45 ± 0.05^{h}	3.07 ± 0.96^{g}	1.06 ± 0.53^{ef}
0.5	5.07 ± 0.8^{g}	7.4 ± 0.51^{f}	4.83 ± 0.28^{g}	33.8 ± 1.37^{f}	0.63 ± 0.02^{g}	4.6 ± 0.63^{f}	1.53 ± 0.61^{de}
1	6.2 ± 0.77^{e}	9.33 ± 0.49^{f}	4.14 ± 0.21^{e}	30.13 ± 1.46^{e}	1.05 ± 0.27^{d}	7.73 ± 1.1^{d}	2.09 ± 0.29^{cd}
1.5	8.27 ± 0.8^{d}	11.4 ± 0.51^{d}	3.9 ± 0.34^{c}	27.6 ± 1.3^{c}	1.22 ± 0.32^{c}	10.07 ± 0.8^{c}	3.2 ± 0.29^{b}
2	13.8 ± 0.77^{a}	12.67 ± 0.49^{a}	3.3 ± 0.25^{a}	23.33 ± 1.11^{a}	2.83 ± 0.31^{a}	15.07 ± 0.96^{a}	5.61 ± 0.37^{a}
2.5	12.33 ± 1.23^{b}	13.8 ± 0.77^{b}	3.1 ± 0.18^{b}	20.8 ± 1.08^{b}	2.27 ± 0.52^{b}	12.53 ± 2.17^{b}	4.81 ± 1.07^{ab}
3	10.33 ± 1.11^{c}	12.67 ± 0.49^{c}	2.96 ± 0.31^{b}	19.67 ± 0.49^{d}	0.89 ± 0.01^{d}	7.13 ± 1.06^{d}	3.01 ± 0.38^{bc}
3.5	8.6 ± 0.63^{d}	10.8 ± 0.41^{e}	2.57 ± 0.22^{d}	21.07 ± 0.96^{d}	0.82 ± 0.01^{e}	6.13 ± 0.74^{e}	2.81 ± 0.67^{bc}
4	7.6 ± 0.51^{f}	9.33 ± 0.49^{f}	2.16 ± 0.22^{f}	24.07 ± 1.28^{e}	0.71 ± 0.02^{f}	5.87 ± 0.64^{e}	2.17 ± 0.21^{cd}
4.5	6.4 ± 0.51^{e}	8.53 ± 0.52^{g}	1.95 ± 0.16^{h}	26.13 ± 0.74^{g}	0.62 ± 0.01^{g}	4.07 ± 0.7^{h}	1.19 ± 0.58^{ef}
5	5.4 ± 0.51^{g}	7.33 ± 0.49^{i}	1.41 ± 0.1^{j}	30.6 ± 1.5^{h}	0.5 ± 0.01^{i}	2.13 ± 0.83^{i}	0.87 ± 0.52^{f}

Note: Different letters (a, b, c, ...) indicate significant differences ($p < 0.05$) based on post hoc Mann–Whitney test. Identical letters denote no significant difference

Effect of Biochar on Contamination and Browning: The addition of biochar had a significant effect on the onset of contamination and browning in during propagation of *Musa acuminata* var. *barangan*. The control treatment without biochar (0 g/L) exhibited the earliest contamination, occurring at 3.33 ± 0.62 days after culture (DAC), which was significantly different from all other treatments. In contrast, supplementation with 2 g/L biochar significantly delayed contamination onset to 13.8 ± 0.77 DAC, followed by the 2.5 g/L and 3 g/L treatments, with contamination observed at 12.33 ± 1.23 DAC and 10.33 ± 1.11 DAC, respectively. This delay in contamination is presumed to result from the biochar's ability to adsorb microorganisms and organic compounds that

promote microbial growth. The porous structure and high surface area of biochar allow for the adsorption of toxic substances and excess nutrients, thereby inhibiting the proliferation of pathogenic microorganisms (21,27,31,39). The onset of browning was also influenced by biochar concentration. The longest delay in browning was observed at 2.5 g/L, occurring at 13.8 ± 0.77 DAC, followed by the 2 g/L and 3 g/L treatments with mean values of 12.67 ± 0.49 DAC. These were significantly different from the control (0 g/L), which showed browning as early as 3.27 ± 0.46 DAC. Browning in plant tissue culture is primarily triggered by the oxidation of phenolic compounds released from wounded or stressed explants, a process catalyzed by polyphenol oxidase (PPO) and peroxidase (POD) enzymes (13,52). Biochar exhibits a protective effect by adsorbing free phenolic compounds, thereby reducing their availability as substrates for oxidative reactions and inactivating browning-related enzymes (PPO and POD), which helps delay tissue browning (13,52). In this study, biochar treatment significantly reduced browning intensity, especially at higher concentrations (4–5 g/L). The lowest browning score was observed at 5 g/L (1.41 ± 0.10), which was significantly lower than the control (0 g/L) that recorded the highest score (5.00 ± 0.00). However, the time to browning onset decreased at the highest concentrations (4.5–5 g/L), with values of 8.53 ± 0.52 and 7.33 ± 0.49 DAC, respectively. These findings suggest that excessive biochar may induce stress—possibly due to ionic imbalance, osmotic pressure, or reduced aeration resulting from increased solid content in the medium (15). Dong et al. (19), demonstrated that biochar binds phenolic molecules through hydrophobic interactions and hydrogen bonding. In some cases, biochar may also reduce the activity of oxidative enzymes indirectly by altering the chemical microenvironment of the medium, such as changing redox potential or adsorbing metal ions required as enzymatic cofactors (12). Furthermore, biochar contains natural antioxidants—depending on the feedstock and pyrolysis temperature—such as stable aromatic compounds that can neutralize reactive oxygen species (ROS), thus mitigating stress responses in explants (5,10). At concentrations above 3 g/L, biochar was no longer effective, likely due to several factors including ionic and osmotic imbalance, over-adsorption of essential nutrients, and excessive pH increase. High levels of biochar may disrupt ionic equilibrium in the culture medium. Ions such as K^+, Ca^{2+}, Mg^{2+}, and P released from biochar can exceed optimal thresholds for tissue growth and induce osmotic or ionic stress.

Table 3. Application of anti-browning agents used banana in vitro culture (2009-2024)

Browning Inhibitor	Applied Concentration	Explant Material	References
Ascorbic Acid; Melatonin	100-200 mg/L; 10-14 mg/L	Flowers of banana *Musa paradisiaca* var. Raja	(38)
Activate Charcoal	0.1 g/L	Protocorm of *Vanda tessellata* (Roxb.) Hook. ex G.Don	(5)
Ascorbic Acid	15 mg/L	*Musa paradisiaca* cv. Tanduk	(37)
Citric acid + Ascorbic acid	50 mg/L + 50 mg/L	Sucker of banana var. nanjangudu rasabale *Musa* spp. AAB	(9)
Vitamin C	20 g/L	Planlet pisang mas kirana *musa acuminata* C.	(11)
Activated Charcoal + Ascorbic Acid + Citric Acid	1.5 g/L + 150 mg/L + 150 mg/L	Meristematic tips (sucker) of *Musa* spp.cv. Grand Naine	(45)
Ascorbic acid	1.2 g/L during explants preparation; 100 mg/L in growth medium	Shoot tip of *Musa* spp. Cv. Mzuzu	(40)
Potassium citrate + Citrate (K-C: C)	0.1-0.5 mg/mL	Meristem buds of *Musa paradisiaca*	(42)
Ascorbic acid	0.005%	Corm-derived plantlets of *Musa acuminata* cv. Formosana	(29)

Biochar may also adsorb essential compounds in the medium—including vitamins, hormones, and nitrogen—leading to nutrient limitation and physiological stress in explants (47). Excessive increases in pH due to high biochar concentrations may interfere with enzyme activity or alter the availability of macro- and micronutrients, negatively affecting plant tissue (22). Over the past decade, various anti-browning strategies have been developed and tested across different plant species. Table (3) summarizes the use of commonly applied agents such as activated charcoal, ascorbic acid, citric acid, and polyvinylpyrrolidone (PVP), highlighting their concentrations, target species, and relevant studies. These compounds exhibit varying degrees of effectiveness in maintaining tissue viability and enhancing the success of in vitro regeneration.

Effect of Biochar on Propagule Initiation.

Statistical analysis revealed that propagule formation was significantly influenced by biochar supplementation, as shown in Figure (2). The treatment with 3 g/L biochar resulted in the earliest propagule initiation, occurring at 19.67 ± 0.49 days after culture (DAC), while the control (0 g/L) exhibited the slowest response at 36.73 ± 0.70 DAC. Similarly, 0.5 and 1 g/L treatments showed delayed propagule formation, whereas concentrations ranging from 2 to 3.5 g/L accelerated the process. The time of propagule initiation reflects how quickly explants respond to the culture medium by forming new organs through adventitious organogenesis or shoot development. This process is highly sensitive to the chemical and physical conditions of the medium, including the presence of inhibitory compounds, nutrient availability, and hormonal balance. The accelerated propagule formation observed at biochar concentrations of 2–3.5 g/L is likely due to enhanced availability of micronutrients, detoxification of inhibitory compounds, and stabilization of the culture environment. Biochar has been shown to improve the bioavailability of essential micronutrients such as Fe, Zn, Mn, and Cu, which are critical for enzymatic activities and cell division, particularly those involved in morphogenesis and differentiation. Micronutrients such as Fe and Zn serve as important cofactors for enzymes involved in DNA and protein biosynthesis, supporting mitotic activity in meristematic tissues (23,51). The addition of biochar also enhances nutrient retention in the medium through increased cation exchange capacity (CEC), thereby optimizing the nutritional environment for explant regeneration (17,31). During the early stages of culture, explants commonly release phenolic compounds and other secondary metabolites that can inhibit growth and slow morphogenesis. Biochar, particularly that derived from lignocellulosic materials such as coconut shells, has a high capacity to adsorb phenolic compounds, thereby reducing oxidative stress and preventing cellular damage (25). A cleaner medium environment, free from toxic compounds, allows faster and more efficient cell differentiation and proliferation. Biochar also functions as a biological and chemical buffer capable of stabilizing medium pH and adsorbing toxic ions, such as excessive NH_4^+, which can inhibit organ initiation. A stable pH environment supports the optimal activity of plant hormones such as cytokinins and auxins, which are crucial for propagule formation. Additionally, biochar may adsorb certain endogenous plant hormones, thereby contributing to a more balanced hormonal environment within explant tissues (18). At higher concentrations (≥ 4 g/L), propagule formation was notably delayed, particularly at 5 g/L, where initiation occurred at 30.6 ± 1.5 DAC. This delay is likely attributable to osmotic stress and hormonal imbalances caused by ion excess or hormone adsorption. High levels of biochar can increase the total dissolved solids (TDS) in the medium, creating high osmotic pressure that hinders water and nutrient uptake by explants. In addition, excessive biochar may adsorb essential nutrients or plant growth regulators such as auxins and cytokinins from the culture medium, thereby disrupting morphogenic signalling pathways. This phenomenon aligns with the principle of the dose–response curve, which posits that the beneficial effects of a treatment are observed only within an optimal concentration range; beyond this range, efficacy declines or adverse effects may occur (34).

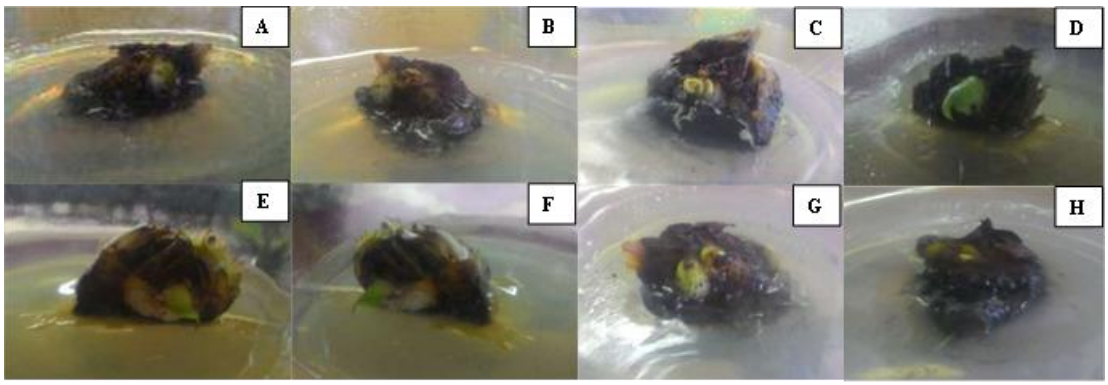

Figure 2. Early propagule emergence of *Musa acuminata* var. *barangan* on MS medium supplemented with biochar: (a) 0 g/L, (b) 1 g/L, (c) 1.5 g/L, (d) 2 g/L, (e) 2.5 g/L, (f) 3 g/L, (g) 4 g/L, (h) 5 g/L

Effect of Biochar on Propagule Biomass and Shoot Multiplication: The highest fresh weight of propagules was recorded at a biochar concentration of 2 g/L, reaching 2.83 ± 0.31 g, followed by 2.5 g/L with 2.27 ± 0.52 g (Figure 3). The increased fresh weight at 2–2.5 g/L biochar is likely attributed to improved water retention and aeration in the medium, enhanced nutrient bioavailability, and reduced toxic compound accumulation. Biochar enhances the physical structure of the culture medium by increasing porosity, thereby balancing water-holding capacity and drainage. This condition promotes stable water absorption by explant tissues and prevents localized desiccation stress. Chang et al. (14), reported that biochar improves medium structure and supports vegetative tissue and root growth through enhanced micro-moisture conditions. Biochar increases the availability of micronutrients such as K, Ca, Mg, Zn, and Fe through its high cation exchange capacity (CEC), allowing these nutrients to be gradually released and available over a longer period (31). These micronutrients are essential for cell wall protein synthesis, mitotic activity, and vegetative structure formation. Additionally, biochar has been reported to adsorb toxic molecules and heavy metal ions in diverse environments (26,36). It is therefore possible that, when applied in plant tissues culture media, this adsorptive properly also extends to phenolic compounds released by stressed explants, thereby reducing physiological stress and promoting more stable biomass accumulation. However, higher concentrations (≥ 3 g/L) resulted in a significant decline in biomass, likely due to ionic imbalance in the medium (e.g., excess K^+ or Ca^{2+}), excessive pH elevation, which may interfere with Zn, Fe, and Mn uptake, and a reduction in the effective medium space. Excessive biochar may absorb water and nutrients, making them unavailable to the explants (8, 41).

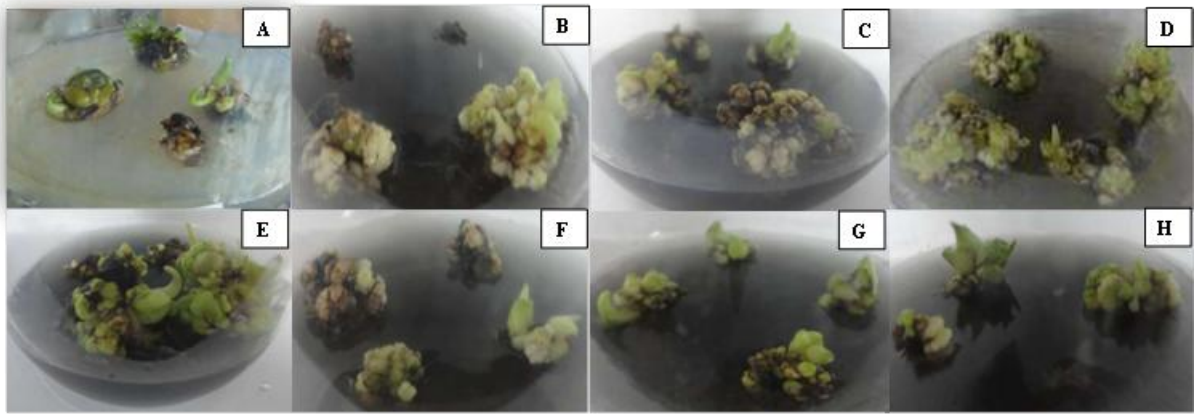

Figure 3. Propagules of *Musa acuminata* var. *barangan* formed on MS medium supplemented with biochar at different concentrations: (a) 0 g/L, (b) 1 g/L, (c) 1.5 g/L, (d) 2 g/L, (e) 2.5 g/L, (f) 3 g/L, (g) 4 g/L, (h) 5 g/L

In terms of shoot multiplication, the 2 g/L biochar treatment produced the highest number of shoots (15.07 ± 0.96), followed by 2.5 g/L (12.53 ± 2.17), as shown in Figure 4. The 1.5 g/L and 1 g/L treatments produced 10.07 ± 0.8 and 7.73 ± 1.1 shoots, respectively. In contrast, higher concentrations (3–4 g/L) resulted in a sharp decline in shoot number to around 7.13–5.87 shoots. The control (0 g/L) produced only 3.07 ± 0.96 shoots, even lower than some high biochar treatments such as 4.5 g/L (4.07 ± 0.7) and 0.5 g/L (4.6 ± 0.63). The lowest number of shoots was observed at 5 g/L, producing only 2.13 ± 0.83 shoots. These findings suggest that the presence of biochar, even at low concentrations, is more beneficial than its complete absence. Shoot multiplication in vitro depends strongly on hormonal regulation—particularly cytokinin efficiency—as well as nutrient availability and the physiological environment of the medium. Biochar influences both factors by stimulating cytokinin responses and providing nutritional support for shoot formation (34). At optimal concentrations, biochar helps regulate the balance between endogenous and exogenous hormones, especially by adsorbing excess auxins that can suppress shoot formation when dominant. Biochar also creates favorable physiological conditions (pH, redox potential, moisture) that enhance cytokinin signaling for shoot induction (48). Increased availability of Zn, Cu, and B due to biochar supplementation plays a key role in meristem differentiation and the activation of shoot morphogenesis-related genes (6). Shoot height followed a similar pattern. The highest shoot elongation was observed at 2 g/L (5.61 ± 0.37 cm), followed by 2.5 g/L (4.81 ± 1.07 cm), both of which were statistically high. The 2–2.5 g/L range appeared to be optimal for supporting shoot elongation. Lower concentrations such as 1.5 g/L (3.2 ± 0.29 cm) and higher concentrations such as 3 g/L (3.01 ± 0.38 cm) and 3.5 g/L (2.81 ± 0.67 cm) showed significantly reduced shoot height. Treatments with 1 g/L and 4 g/L also resulted in reduced shoot height at 2.09 ± 0.61 cm and 2.17 ± 0.21 cm, respectively, indicating that both insufficient and excessive biochar concentrations limit shoot growth. The lowest shoot heights were observed in the control (0 g/L) and high-dose treatments of 4.5–5 g/L, yielding 1.06 ± 0.53 cm, 1.19 ± 0.58 cm, and 0.87 ± 0.52 cm, respectively. Shoot height reflects the success of continuous vegetative tissue growth and is influenced by nutrient accessibility, osmotic pressure of the medium, water availability, and light exposure. The 2–2.5 g/L concentration range supports these processes by enhancing water retention, buffering pH, adsorbing phenolics and toxic ions that inhibit elongation, and stimulating apical tissue growth. Conversely, high concentrations (≥ 4 g/L) may inhibit elongation due to osmotic stress, nutrient and hormone over-sorption, and physical disruption of the substrate structure, such as reduced aeration and pore saturation.

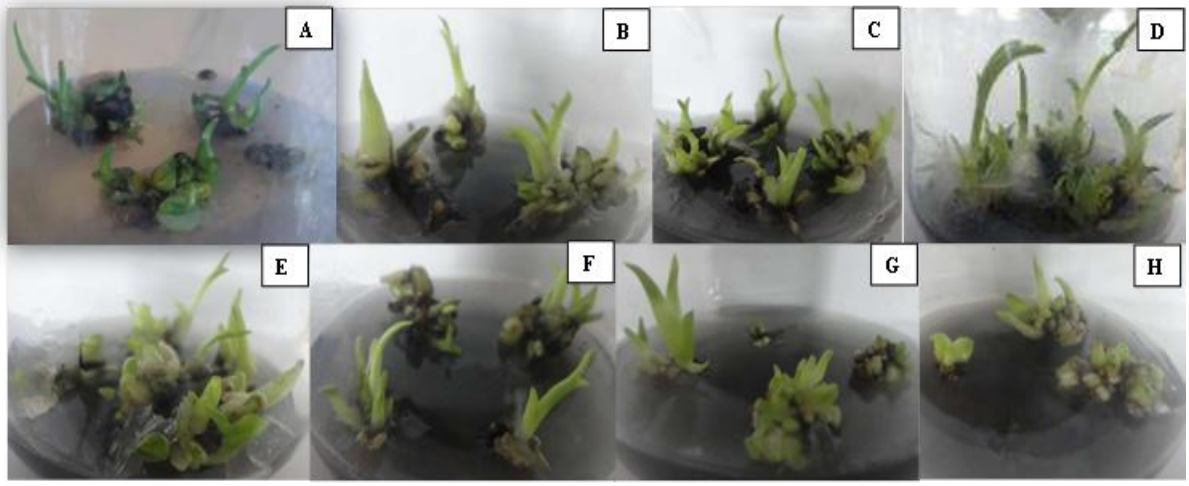

Figure 4. Shoots of *Musa acuminata* var. *barangan* formed on MS medium supplemented with biochar at different concentrations: (a) 0 g/L, (b) 1 g/L, (c) 1.5 g/L, (d) 2 g/L, (e) 2.5 g/L, (f) 3 g/L, (g) 4 g/L, (h) 5 g/L

The effectiveness of biochar in supporting the in vitro culture of *Musa acuminata* var. *barangan* can be attributed to the specific characteristics of its feedstock, namely, coconut shell (*Cocos nucifera* L.). Coconut shell-derived biochar is known for its high fixed carbon content—typical of lignocellulosic materials—well-developed microporous and mesoporous structure, and high chemical stability (4,50). These properties make biochar an efficient adsorbent for toxic compounds, particularly phenolic compounds released by explants during the early culture phase. This adsorption capability effectively reduces oxidative stress and inhibits tissue browning. Additionally, coconut shell biochar typically possesses a neutral to slightly alkaline pH and a high cation exchange capacity (CEC), which enhances the availability of essential micronutrients such as K, Ca, Mg, Zn, and Mn in the culture medium (28,29). These elements are vital for supporting metabolic activity in meristematic cells and stimulating morphogenetic processes, including propagule initiation and shoot formation. The large number of active pores in coconut shell biochar also contributes to improved water-holding capacity and promotes a balanced aeration-moisture environment, which is ideal for explant growth. Furthermore, the use of coconut shell as a biochar source offers ecological and economic benefits. As an abundant agricultural byproduct, coconut shell holds great potential as a sustainable and eco-friendly additive in plant tissue culture media.

CONCLUSION

The results of this study demonstrate that the supplementation of biochar in Murashige and Skoog (MS) medium exhibited the most optimal performance at a concentration of 2 g/L. At this concentration, biochar effectively suppressed microbial contamination and tissue browning, while simultaneously supporting propagule initiation and shoot development. These findings indicate that 2 g/L biochar represents an effective formulation for enhancing the efficiency of in vitro culture of *Musa acuminata* var. *barangan*.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have received a fund.

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