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OPTIMIZATION OF IBA AND ACTIVATED CHARCOAL ON IN VITRO ROOTING OF KOPYOR COCONUT

Amanda Putri Viony*, Susiyanti, Sulastri Isminingsih, Zahratul Millah, Kartina AM

Department of Agroecotechnology, Faculty of Agriculture, Sultan Ageng Tirtayasa University, Jl. Raya Palka Km 3 Sindangsari, Pabuaran, Serang Regency, Banten.

*Correspondence: amandaviony27@gmail.com

ABSTRACT

Kopyor coconut (*Cocos nucifera* L.) is a high-value commodity, but its conventional propagation is constrained by abnormal endosperm, resulting in poor germination. In vitro embryo culture on Y3 medium offers an alternative to improve propagation efficiency. This study evaluated the effects of Indole-3-Butyric Acid (IBA) and activated charcoal on in vitro growth of kopyor coconut embryos cultured on Y3 medium. The experiment used a factorial Randomized Block Design (RBD) with two factors, IBA concentrations (0, 1, 2, and 3 mg L^{-1}) and activated charcoal (0, 1, and 2 g L^{-1}), with four replications. Observed parameters included time to root emergence, explant height, and survival percentage. Data were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level. Results showed that activated charcoal significantly affected time to root emergence and explant height, while IBA and its interaction with activated charcoal were not significant for all parameters. The application of 1 g L^{-1} activated charcoal produced the fastest root emergence at 9.25 weeks after culture and achieved 100% explant survival. In conclusion, activated charcoal at 1 g L^{-1} effectively enhances early in vitro development of kopyor coconut embryos by accelerating root emergence and improving survival, while IBA up to 3 mg L^{-1} does not significantly affect early growth responses.

Key words: Activated charcoal, IBA, In vitro, Kopyor coconut, Y3 Medium.

INTRODUCTION

Kopyor coconut (*Cocos nucifera* L.) is a unique tropical germplasm with high economic value due to its soft and detached endosperm structure. This abnormal endosperm formation causes low natural germination, making conventional propagation inefficient for large-scale production and conservation of superior genetic resources. Embryo-based in vitro culture is widely recognized as an important strategy for coconut germplasm conservation and propagation. Recent studies confirm that embryo culture plays a key role in overcoming germination limitations in coconut species (Liu *et al.*, 2024).

In vitro embryo culture using Y3 medium has been widely applied as an effective system for coconut propagation under controlled conditions. The medium provides balanced macro and micronutrients that support embryo germination and early seedling establishment. The Y3 formulation was specifically developed for coconut tissue culture and remains the most widely used basal medium for coconut embryo development and regeneration studies. This medium

is recognized for its ability to support consistent embryo growth and plantlet formation in coconut biotechnology systems (Kalaipandian *et al*., 2021).

Plant growth regulators such as Indole-3-Butyric Acid (IBA) are commonly used to stimulate root induction in in vitro culture systems due to their role in promoting cell elongation and differentiation. Activated charcoal is widely used in plant tissue culture to adsorb phenolic compounds and reduce toxic accumulation in the culture medium. Studies show that activated charcoal improves morphogenesis by reducing oxidative stress and stabilizing in vitro physiological conditions. Its role in controlling phenolic browning and enhancing regeneration has been widely documented in tissue culture systems (Danova *et al*., 2023).

However, studies on the combined effects of IBA and activated charcoal on kopyor coconut embryo culture using Y3 medium are still limited, particularly in early growth performance responses. Therefore, this study aimed to evaluate the effects of different concentrations of IBA and activated charcoal on the in vitro growth performance of kopyor coconut embryos cultured on Y3 medium. Understanding these interactions is important to improve root emergence and seedling development efficiency. Phenolic browning control and medium optimization are critical factors in successful coconut embryo culture systems (Abohatem *et al*., 2025).

MATERIALS AND METHODS

This study was conducted from January to April 2026 at the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, Sultan Ageng Tirtayasa University. It aimed to evaluate the effects of Indole-3-Butyric Acid (IBA) and activated charcoal on the in vitro growth of kopyor coconut (Cocos nucifera L.) embryos using Y3 medium. The experiment was arranged in a factorial Randomized Block Design (RBD) consisting of two factors: IBA concentration (0, 1, 2, and 3 mg L^{-1}) and activated charcoal concentration (0, 1, and 2 g L^{-1}), with four replications, resulting in 48 experimental units.

Kopyor coconut embryos were used as explants and cultured under aseptic conditions in a plant tissue culture laboratory. Cultures were maintained on Y3 basal medium under controlled environmental conditions at 20–25°C with continuous fluorescent light during incubation. All procedures were conducted under sterile conditions to prevent contamination.

The Y3 medium was used as the basal culture medium and supplemented with different concentrations of IBA and activated charcoal according to the treatment combinations. The media were prepared under sterile conditions and adjusted prior to autoclaving. Each treatment was applied to a single explant per culture vessel.

The observed parameters included time to root emergence, explant height, and explant survival percentage. Time to root emergence was recorded as the number of weeks after culture initiation when the first root was observed. Explant height were measured at the end of the observation period, while survival percentage was calculated based on the number of living explants.

Data were analyzed using analysis of variance (ANOVA) to determine the effect of treatments. When significant differences were detected, Duncan's Multiple Range Test (DMRT) at a 5% significance level was applied to compare treatment means.

RESULTS AND DISCUSSION

The results showed that activated charcoal significantly affected early in vitro growth of kopyor coconut embryos, including time to root emergence, explant height, and explant survival. In contrast, IBA and its interaction with activated charcoal were not significant for all parameters. These findings indicate that activated charcoal plays a key role in improving the physiological conditions of Y3 medium and supporting early embryo development.

Time to root emergence

Time to root emergence was significantly influenced by activated charcoal, while IBA showed no significant effect. The fastest root emergence was observed at 1 g L^{-1} activated charcoal with 9.25 weeks after culture initiation (WAC). Activated charcoal enhances root initiation by adsorbing phenolic compounds released from wounded explants. These phenolic compounds are rapidly oxidized into quinones by polyphenol oxidase, which are toxic and inhibit cell division and meristem activity. Phenolic browning has been widely identified as a major constraint in plant tissue culture because it reduces regeneration efficiency and explant viability (Ginting *et al*., 2023).

However, excessive concentrations of activated charcoal may reduce root initiation efficiency due to its non-selective adsorption properties. Essential nutrients and growth regulators can also be absorbed, reducing their availability to the explant. This condition leads to slower metabolic activity and delayed root emergence. Activated charcoal can adsorb not only toxic compounds but also beneficial medium components, which may limit plant growth responses when used excessively (George *et al*., 2015).

Explant height

Explant height was significantly affected by activated charcoal, particularly at 1 g L^{-1} concentration. The increase in shoot elongation indicates that medium detoxification strongly supports cellular expansion in early embryo development. IBA did not significantly influence this parameter, showing limited role in shoot elongation regulation. Growth regulation in monocot embryos is strongly dependent on internal physiological balance (Lestari, 2011).

Activated charcoal enhances shoot elongation by reducing oxidative stress caused by phenolic accumulation. Phenolic compounds released from injured tissues can form toxic quinones that damage cellular membranes. By adsorbing these compounds, activated charcoal maintains cellular integrity and supports continuous elongation. Control of oxidative stress is essential for successful in vitro morphogenesis (Amente & Chimdessa, 2024).

However, higher concentrations of activated charcoal may reduce growth due to excessive adsorption of nutrients and regulatory compounds. This reduces the availability of essential elements required for cell elongation. The imbalance in nutrient and hormone availability

leads to reduced metabolic activity. Excessive adsorption of medium components can negatively affect morphogenesis and growth responses in plant tissue culture systems (Danova *et al*., 2023).

Explant survival percentage

Explant survival reached up to 100% in treatments containing 1 g L^{-1} activated charcoal. This demonstrates that activated charcoal plays a crucial role in maintaining tissue viability during early culture stages. High survival rates indicate stable physiological conditions in the culture medium. Phenolic oxidation is a major cause of explant mortality in plant tissue culture. Wounded plant tissues release phenolic compounds that are converted into toxic quinones. These compounds induce oxidative stress, membrane damage, and eventual cell death. Phenolic-induced toxicity is a well-documented limitation in plant tissue culture systems (Ginting *et al*., 2023).

Excessive adsorption may also remove essential nutrients required for growth. Nutrient depletion due to over-adsorption can negatively affect explant viability. This may reduce metabolic activity and stress tolerance of cultured tissues. Overuse of activated charcoal can disturb nutrient and hormone balance in plant tissue culture systems (George *et al*., 2015).

CONCLUSION

Activated charcoal significantly improved in vitro growth of kopyor coconut embryos, particularly in accelerating root emergence, increasing explant height, and enhancing survival rate. The optimal concentration was 1 g L^{-1}, which consistently produced the best overall responses. In contrast, IBA showed no significant effect on all observed parameters. Excess activated charcoal may reduce growth performance due to nutrient and regulator adsorption.

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Table 1. Mean time of root emergence (WAC) of kopyor coconut explants under different concentrations of IBA and activated charcoal.

IBA Concentration (i)	Activated Charcoal Concentration (a)			Mean
	a_0	a_1	a_2	
		WAC		
i_1	10.50	9.50	8.50	9.50
i_2	12.00	10.50	9.00	10.50
i_3	12.00	9.00	11.50	10.83
i_4	12.00	8.00	12.00	10.67
Mean	11.62a	9.25b	10.25ab	

Table 2. Effect of IBA and Activated Charcoal Concentrations on Explant Height (cm) at 12 WAC.

IBA Concentration (i)	Activated Charcoal Concentration (a)			Mean
	a_0	a_1	a_2	
		cm		
i_1	0.9	1.82	1.82	1.51
i_2	0.72	1.3	3.22	1.75
i_3	0.7	1.87	1.45	1.34
i_4	0.62	2.72	0.77	1.37
Mean	0.73b	1.93a	1.81a	

Note: The post hoc test was performed using transformed data with the formula √(x + 0.5).

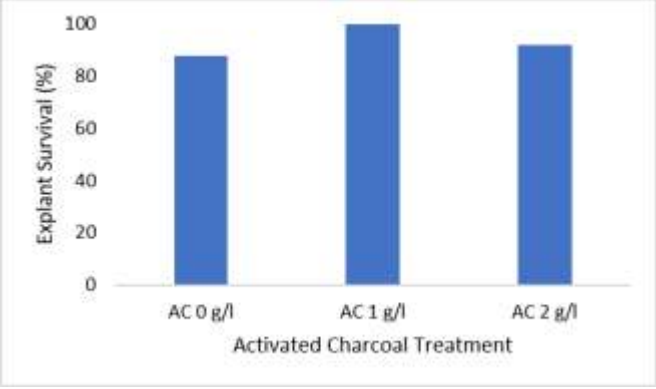
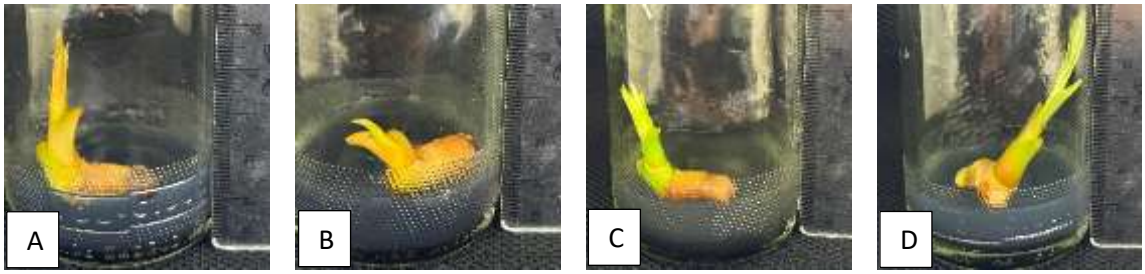


Figure 1. Effect of activated charcoal on explant survival percentage in kopyor coconut embryo culture



Notes:
A. i_1a_1 (0 mg L^{-1} IBA + 1 g L^{-1} AC)
B. i_2a_1 (1 mg L^{-1} IBA + 1 g L^{-1} AC)
C. i_3a_1 (1 mg L^{-1} IBA + 1 g L^{-1} AC)
D. i_4a_1 (1 mg L^{-1} IBA + 1 g L^{-1} AC)