AGROTECH–FOOD SCIENCE, TECHNOLOGY AND ENVIRONMENT VOL. 3 NO. 2 (2024) P.98-103

AGROTECH
ISSN: 2773-4870 **eISSN:** 2821-3106
DOI: https://doi.org/10.53797/agrotech.v3i2.10.2024

Optimization of Murashige and Skoog (MS) Medium with Coconut Shell Charcoal in the Subculture Phase of Raja Bulu Banana (*Musa* spp.)

Septiandar, Gracia Ayu, Nurhayati, Dewi Ratna & Saputro, Avisema Sigit*

Department of Agrotechnology, Faculty of Agriculture, Universitas Slamet Riyadi Jl. Sumpah Pemuda No. 18, Kadipiro, Banjarsari, Surakarta 57136, INDONESIA

*Corresponding author: avis_sigit@yahoo.com

Received 9 November 2024; Accepted 27 December 2024; Available online 29 December 2024

Abstract: Tissue culture is a rapid and effective method of propagating banana plants, especially for producing pest- and disease-free planting materials. This study aimed to: (1) optimize the Murashige and Skoog (MS) medium by supplementing it with coconut shell charcoal, (2) determine the most effective concentration of coconut shell charcoal for improving root quality and plantlet growth of Raja Bulu banana, and (3) evaluate the influence of charcoal addition during the subculture phase. The experiment was conducted at the Salaman Seed Garden for Food Crops and Horticulture, Magelang, Central Java, from February to March 2025. A Completely Randomized Design (CRD) was used with four treatments: MS only (A1), MS + 0.5 g/L charcoal (A2), MS + 1.0 g/L charcoal (A3), and MS + 1.5 g/L charcoal (A4). Parameters observed were root formation percentage, root mass, plantlet height, and stem diameter. The results showed that the addition of coconut shell charcoal significantly affected stem diameter. All treatments yielded homogeneous root formation, with 100% root formation in MS without charcoal and 83% for all other treatments. The best average root mass and stem diameter were obtained at 1.0 g/L concentration, suggesting a potential benefit of coconut shell charcoal at this level in promoting root growth and strengthening stem development.

Keywords: in vitro culture, banana plantlet, MS medium, coconut shell charcoal, root development

1. Introduction

Bananas (*Musa* spp.) are among the world's most important fruit crops, widely cultivated in tropical and subtropical regions due to their high nutritional value, versatility, and economic significance. As a staple fruit in many developing countries, bananas not only serve as a vital source of carbohydrates and micronutrients but also contribute substantially to rural livelihoods and food security (Heslop-Harrison & Schwarzacher, 2007). Among the various banana cultivars, the Raja Bulu variety is particularly valued in Indonesia for its distinct flavor, attractive fruit morphology, and adaptability to local agroecological conditions (Zulkifli & Sutriana, 2020).

Despite its importance, conventional propagation of bananas through suckers poses several limitations, including low multiplication rates, the spread of soil-borne diseases, and limited uniformity among planting materials. In response to these challenges, in vitro propagation, particularly through plant tissue culture techniques, has emerged as a reliable and efficient method for producing large quantities of disease-free, genetically uniform plantlets in a relatively short time (Yusnita, 2015; Tripathi et al., 2008). Tissue culture not only enables year-round production but also facilitates the conservation and rapid distribution of elite cultivars.

One critical stage in the tissue culture process is the subculture phase, during which explants are transferred to fresh medium to stimulate further growth and rooting. However, this phase is often hindered by issues such as oxidative browning caused by the release of phenolic compounds, poor root development, and physiological stress (Gaspar et al., 2002). To address these limitations, researchers have explored the use of activated charcoal as a media supplement due to its adsorptive capacity to remove inhibitory substances and improve nutrient uptake (Pan & van Staden, 1998; Thomas, 2008). Coconut shell charcoal, in particular, is an accessible and sustainable source of activated carbon that has been reported to enhance rooting, reduce tissue browning, and improve overall plantlet vigor (Lempang, 2015).

Activated charcoal's effectiveness is primarily attributed to its high surface area and porosity, which allow it to adsorb exudates such as polyphenols and ethylene that can be detrimental to in vitro plant development. Additionally,

*Corresponding author: avis_sigit@yahoo.com

charcoal can serve as a source of essential microelements including potassium (K), calcium (Ca), phosphorus (P), and magnesium (Mg), which play important roles in root morphogenesis and structural integrity (Pratama, 2023; Chawla, 2009). However, excessive concentrations of charcoal may lead to undesirable adsorption of growth regulators and nutrients, potentially inhibiting plant development (George et al., 2008). Therefore, determining the optimal concentration of charcoal in the culture medium is critical for maximizing its benefits while minimizing adverse effects.

This study aims to optimize the Murashige and Skoog (MS) medium supplemented with coconut shell charcoal to improve the growth performance of Raja Bulu banana plantlets during the subculture phase. Specifically, it seeks to determine the most effective concentration of charcoal for promoting root development, plantlet height, and stem diameter, thereby contributing to the production of vigorous and high-quality planting materials. The outcomes of this research are expected to inform practical recommendations for commercial-scale banana micropropagation systems, particularly in resource-limited settings where cost-effective and sustainable media additives are essential.

2. Materials and Methods

This study was conducted from February to March 2025 at the Salaman Seed Garden for Food Crops and Horticulture, located in Magelang, Central Java, Indonesia, at an elevation of approximately 270 meters above sea level. The experiment was designed to evaluate the effect of coconut shell charcoal on the growth performance of Raja Bulu banana (*Musa* spp.) plantlets during the subculture phase. A Completely Randomized Design (CRD) was employed, consisting of a single factor: the concentration of coconut shell charcoal supplemented into the Murashige and Skoog (MS) medium. Four treatments were tested: MS medium without charcoal as the control (A1), MS supplemented with 0.5 g/L charcoal (A2), 1.0 g/L charcoal (A3), and 1.5 g/L charcoal (A4). Each treatment was replicated to ensure the reliability of the results.

The MS medium used in all treatments was prepared according to standard protocols and sterilized prior to use. Coconut shell charcoal was ground into a fine powder, sterilized, and incorporated into the medium at the specified concentrations. Banana plantlets that had reached the subculture stage were selected for uniformity and transferred into culture vessels containing the respective treatment media. Cultures were maintained under controlled laboratory conditions conducive to plantlet development, including a temperature of approximately 25 ± 2°C, photoperiod of 16 hours light and 8 hours dark, and light intensity of 2000–3000 lux.

Several parameters were evaluated to assess the effect of charcoal supplementation. These included the percentage of plantlets that formed roots, the fresh weight of roots (expressed in grams), plantlet height (measured in centimeters from base to apex), and stem diameter (measured in millimeters using a digital caliper). Root formation percentage was calculated as the number of plantlets that developed roots relative to the total number of explants in each treatment. Root mass was determined by carefully removing the plantlets and weighing the roots using an analytical balance. Plantlet height and stem diameter were measured after the completion of the subculture period to determine vegetative growth performance.

All collected data were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) to determine the significance of treatment effects. In cases where the assumption of homogeneity of variance was met, a Bonferroni post hoc test was applied to compare means. If the assumption was violated, a Games-Howell test was used instead. Statistical significance was considered at $p < 0.05$, and the results were presented as means with standard deviations.

3. Results and Discussion

3.1 Root Formation Percentage

The results demonstrated that the percentage of root formation was highest in the control treatment (MS medium without charcoal), which exhibited a 100% rooting rate, while all other treatments supplemented with coconut shell charcoal (0.5 g/L, 1.0 g/L, and 1.5 g/L) showed a slightly reduced rooting rate of 83% (Figure 1). These findings suggest that the standard MS medium provides an adequate hormonal and nutritional environment for root induction in *Musa* spp. plantlets. The slight decline in rooting observed with charcoal addition may be attributed to the adsorptive nature of charcoal, which could sequester growth-promoting substances such as auxins (Thomas, 2008; Pan & van Staden, 1998). Although charcoal is generally used to absorb phenolic compounds and reduce tissue browning, its excessive use or high adsorption efficiency might limit the bioavailability of essential phytohormones required for root initiation (George et al., 2008). Nevertheless, the 83% rooting rate in charcoal-amended treatments remains acceptable and demonstrates that coconut shell charcoal at moderate concentrations does not inhibit root formation entirely.

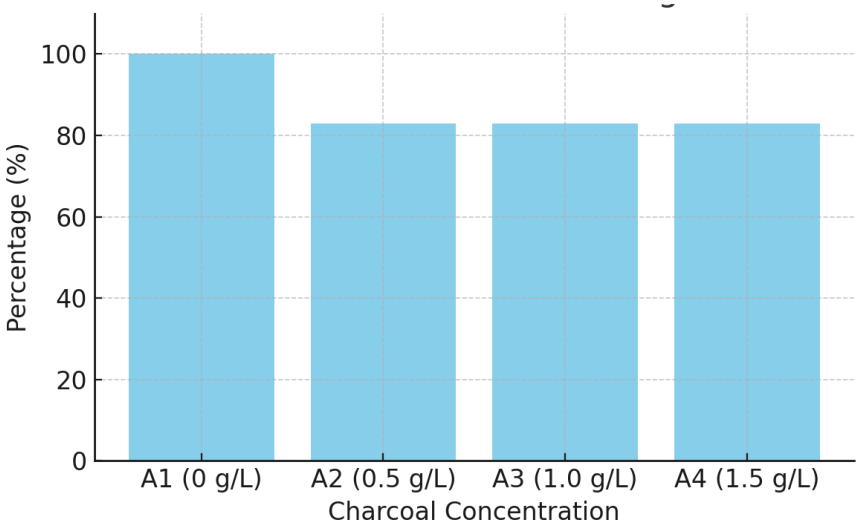

Fig. 1: Effect of Coconut Shell Charcoal Supplementation on Root Formation Percentage of Raja Bulu Banana Plantlets during the Subculture Phase

3.2 Root Mass

The average root mass varied significantly across treatments, with the highest recorded in the 1.0 g/L charcoal treatment (A3), yielding an average of 0.50 g per plantlet (Figure 2). In contrast, the lowest root mass was observed at the highest charcoal concentration of 1.5 g/L (A4), which produced an average of 0.28 g. These results highlight the importance of optimizing the concentration of charcoal in the culture medium. Moderate charcoal supplementation appears to enhance root biomass, possibly due to its role in adsorbing toxic exudates and providing a more stable medium pH (Lempang, 2015). Furthermore, coconut shell charcoal contains micronutrients such as calcium, magnesium, and potassium, which have been shown to positively influence root development (Chawla, 2009; Pratama, 2023). The reduced root mass at higher charcoal concentrations could result from over-adsorption of essential nutrients and growth regulators, which has been previously reported to suppress morphogenesis (George et al., 2008). Therefore, a balance must be maintained to harness the benefits of charcoal without compromising nutrient availability.

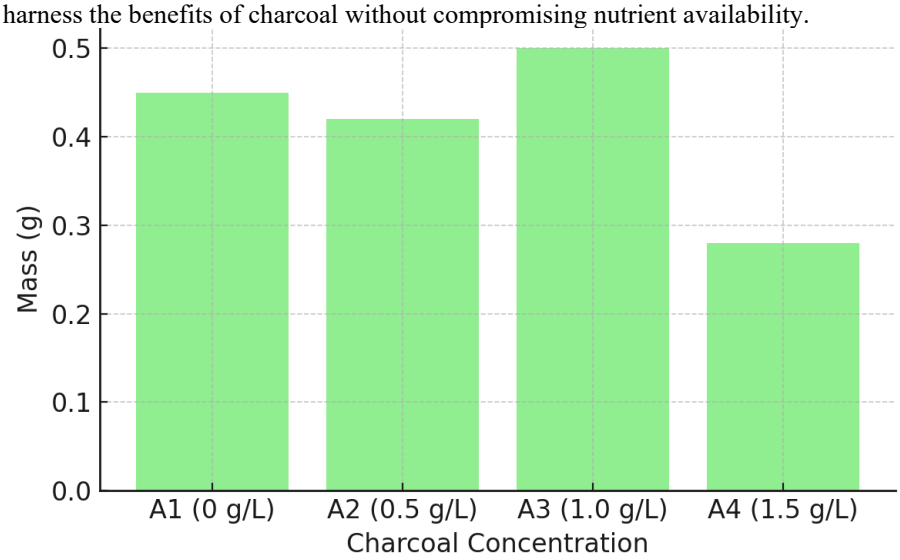

Fig 2: Average Root Mass of Raja Bulu Banana Plantlets Cultured on MS Medium with Varying Concentrations of Coconut Shell Charcoal

3.3 Plantlet Height

Plantlet height was positively influenced by charcoal supplementation, with the tallest average height (5.22 cm) observed in treatment A3 (1.0 g/L), followed by A2 (0.5 g/L), and the shortest plantlets found in the control and A4 (Figure 3). Although the differences were not statistically significant, the observed trend suggests that moderate levels of charcoal may promote cell elongation and vertical growth. Potassium, commonly found in charcoal, plays a pivotal role in osmotic regulation and turgor pressure maintenance, both of which are essential for cell expansion (Taiz & Zeiger, 2010). Additionally, the reduction of oxidative stress through phenolic adsorption may create a more favorable

microenvironment for shoot development. Previous studies in bananas and orchids have also reported enhanced shoot elongation with low to moderate concentrations of activated charcoal in the medium (Khalil et al., 2021; Pratama, 2023). However, the lower height at 1.5 g/L may reflect nutrient adsorption or potential phytotoxicity at higher concentrations.

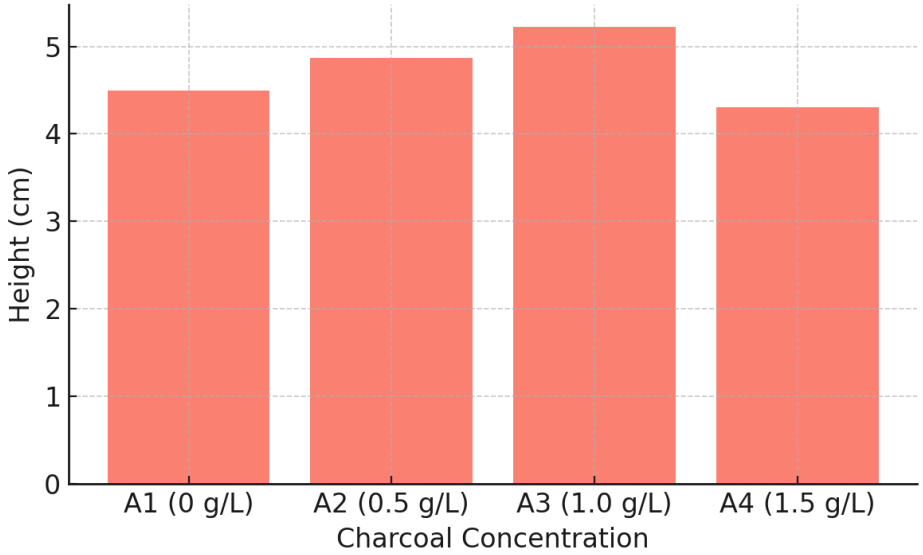

Fig. 3: Influence of Coconut Shell Charcoal Concentration on Plantlet Height of Raja Bulu Banana in Subculture Stage

3.4 Stem Diameter

Among all the parameters measured, stem diameter showed the most statistically significant variation in response to charcoal concentration (Figure 4). The thickest stems were recorded in the 1.0 g/L charcoal treatment (A3), averaging 3.77 mm, while the thinnest were found in the control group (2.37 mm). The increased stem girth observed in A3 suggests that moderate supplementation of coconut shell charcoal can promote structural development and mechanical strength in plantlets. This could be linked to the presence of calcium, a key element in cell wall fortification and cell division (Marschner, 2012). A robust stem is a desirable trait for tissue-cultured plantlets, as it enhances survival rates during acclimatization and transplanting. These results are consistent with earlier studies by Thomas (2008), who reported that charcoal can improve explant vigor and stem thickness when appropriately dosed. The decline in stem diameter at the highest charcoal concentration (1.5 g/L) further confirms the threshold beyond which charcoal may hinder plant development.

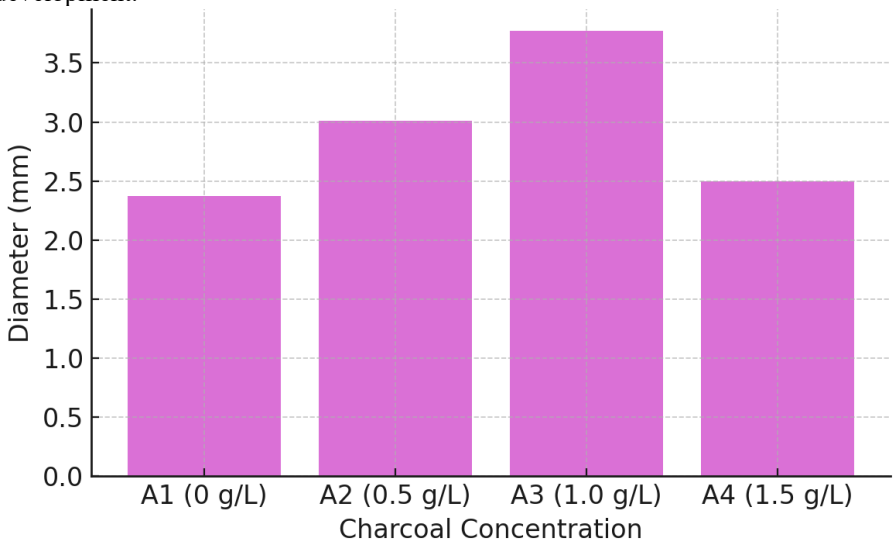

Fig. 4: Stem Diameter of Raja Bulu Banana Plantlets Grown on MS Medium with Different Levels of Coconut Shell Charcoal

4. Conclusion

This study demonstrates that supplementation of Murashige and Skoog (MS) medium with coconut shell charcoal influences the growth performance of Raja Bulu banana (*Musa* spp.) plantlets during the subculture phase. While the control treatment (MS without charcoal) yielded the highest root formation percentage (100%), the addition of charcoal, particularly at 1.0 g/L, significantly improved other key growth parameters including root mass, plantlet height, and stem diameter. The optimal concentration of 1.0 g/L provided a favorable balance between nutrient availability and toxin adsorption, resulting in plantlets with greater vigor and stronger morphological characteristics. However, higher concentrations such as 1.5 g/L appeared to reduce growth performance, likely due to the excessive adsorption of essential growth substances.

Based on these findings, it is recommended that a concentration of 1.0 g/L coconut shell charcoal be incorporated into MS medium to enhance the quality of plantlets produced during in vitro subculture of Raja Bulu banana. This practice can be adopted in commercial micropropagation protocols to improve rooting efficiency and structural integrity, thereby increasing survival rates during acclimatization. Future research should explore the long-term effects of charcoal supplementation during the acclimatization and transplanting phases, and investigate the interaction of charcoal with specific plant growth regulators to further optimize tissue culture protocols for banana and other tropical fruit crops.

Acknowledgement

The authors would like to express their gratitude to the Universitas Slamet Riyadi for their support in providing both facilities and financial assistance for this research.

Conflict of Interest

The authors declare no conflicts of interest.

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