



The Effect of Zn Nanoparticle Concentration and the Comparison of Growth Regulator Concentration on the Growth of Cavendish Banana Explants (*Musa acuminata*)

Sarah Nurmala Putri Rukmana¹, Pangesti Nugrahani^{2*}, Didik Utomo Pribadi³

¹²³ Department of Agrotechnology, Universitas Pembangunan Nasional Veteran Jawa Timur, Indonesia

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ABSTRACT

Cavendish banana (Musa acuminata L.) propagation through conventional methods requires a considerable amount of time. Tissue culture techniques offer a more efficient alternative, enabling the production of plantlets with superior quality and quantity in a shorter period. The application of Zn nanoparticles has been shown to reduce media contamination and act as a biostimulant, enhancing propagation efficiency in tissue culture. Auxins, such as Naphthalene Acetic Acid (NAA), facilitate cell development and root induction, while cytokinins, including 6-Benzyl Amino Purine (BAP), are effective in stimulating cell division, morphogenesis, and shoot formation with improved stability and resistance to oxidation. This study was conducted from February to June 2023 at the Biotechnology Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur. The research utilized a Completely Randomized Design (CRD) with two factors: the concentration of Zn nanoparticles and the ratio of ZPT concentrations. The results indicated that the best treatment for multiplication was obtained with 50 ppm Zn nanoparticles, resulting in the fastest shoot emergence at 24,63 days and the highest number of shoots per plantlet at 2,58 shoots. Furthermore, the treatment with a ZPT concentration ratio of NAA + BAP (0:0) produced the highest average number of plantlet leaves, with 5,78 leaves per plantlet.

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* Corresponding author: pangesti_n@upnjatim.ac.id

INTRODUCTION

Cavendish bananas (*Musa acuminata* L.) are renowned for their soft and sweet flesh, larger size, and clusters containing approximately 10 hands per bunch (Linda, 2018). The

conventional propagation of Cavendish banana plants requires a significant amount of time. Tissue culture techniques provide a solution to the challenges posed by conventional propagation methods, which are time-consuming, produce non-uniform results, and do not guarantee disease-free plants. Tissue culture techniques involve several stages, including initiation, explant culture, subculture, multiplication, rooting, and finally, plantlet acclimatization. Subculture in tissue culture refers to the process of transferring grown plantlets to a new medium to increase the number of seedlings within a specific period ([Avivi et al., 2013](#)). However, the success of this process largely depends on the growth medium used.

The growth medium must meet ideal standards, including macro- and micronutrients, vitamins, carbon sources, and Plant Growth Regulators (PGRs) from the auxin or cytokinin groups. Auxins, such as Naphthalene Acetic Acid (NAA), support cell development and root induction, while cytokinins, such as 6-Benzyl Amino Purine (BAP), are effective in stimulating cell division ([Sintha et al., 2017](#)). According to Sulistiani and Yani (2012), banana shoot subculture involves stimulating the proliferation of axillary shoots from the shoot corm, typically during the multiplication phase of banana plants. This process often employs a combination of cytokinin and auxin PGRs, with auxin concentrations lower than those of cytokinins.

The use of nanoparticles in tissue culture media has been increasingly developed. Nanoparticles, with their small size, large surface area, and reactive properties, can enhance plant growth responses ([Mandeh et al., 2012](#)). Specifically, Zn nanoparticles have been found to reduce media contamination, although their effects on growth require further investigation. According to Lala (2021), nanoparticles possess unique properties that enhance their biochemical reactivity, making them potential biostimulators for improving plant propagation or seedling production in tissue culture. This study modifies the in vitro culture media for Cavendish bananas using Murashige and Skoog (MS) medium supplemented with Zn nanoparticles and varying concentrations of auxin (NAA) and cytokinin (BAP). The objective of this research is to determine the optimal concentration of Zn nanoparticles and the appropriate ratio of Plant Growth Regulators (PGRs) to support Cavendish banana growth.

RESEARCH METHOD

1. Time and Place

The research was conducted from March 2023 to June 2023 at the Biotechnology Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur.

2. Equipment

The equipment used in the research included paper, an oven, Petri dishes, an analytical balance, beaker glasses, Erlenmeyer flasks, drop pipettes, a hot plate, a magnetic stirrer, a pH meter, a pot, a stove, measuring glasses, culture bottles, plastic wrap, aluminum foil, rubber bands, a refrigerator, an autoclave, a spray bottle, a Laminar Air Flow (LAF) cabinet, tweezers, scissors, scalpels, a Bunsen burner, matches, culture racks, and writing instruments.

3. Materials

The materials used in the research included Murashige and Skoog (MS) medium with the composition detailed in Appendix 1 (Composition of Murashige and Skoog Medium),

6-Benzylaminopurine (BAP), Zn nanoparticles, sterile distilled water, NaOH, HCl, 70% alcohol, spiritus, Betadine, liquid soap, and 2-month-old Cavendish banana explants obtained from the SEAMEO BIOTROP Laboratory.

4. Research Methodology

This study employed a Factorial Experiment design arranged using a Completely Randomized Design (CRD) with two factors:

Zn Nanoparticle Concentration (N) with three levels of treatment:

N₀: No treatment (Control)

N₁: 50 ppm

N₂: 100 ppm

Ratio of Plant Growth Regulators (PGRs) Concentrations (NAA and BAP) (Z) with four levels of treatment:

Z₀: No treatment (Control)

Z₁: NAA + BAP (1:1)

Z₂: NAA + BAP (1:2)

Z₃: NAA + BAP (2:1)

This study involved 12 treatment combinations with 3 replications, each replication consisting of 3 samples. Each culture bottle contained one explant. In total, 108 experimental units were used. The research process began with the sterilization of equipment and materials, preparation of Zn nanoparticle stock solution, preparation of BAP stock solution, preparation of NAA stock solution, preparation of Murashige and Skoog (MS) medium, sterilization of the laminar air flow, subculture of explants, and maintenance and observation.

The parameters observed included the time of shoot emergence, time of leaf emergence, number of shoots, and number of leaves. The time for shoot and leaf emergence (in days) was recorded by counting the days until the first new shoot or leaf appeared on the explants, with observations made daily from planting until the first new shoot or leaf emerged. The number of shoots (in number) and leaves (in number of leaves) was determined by counting the shoots or leaves formed during destructive sampling. Data analysis was performed using Analysis of Variance (ANOVA), and if significant differences were found, further testing was carried out using the Honest Significant Difference (HSD) test at a significance level of $\alpha = 5\%$.

RESULT AND DISCUSSION

Time of Shoot and Leaf Emergence

Table 1. Average Time of Shoot Emergence Due to Zn Nanoparticle Application and PGR Ratio

Treatments	Time of Shoot Emergence (days)
Zn Nanoparticle Concentration	
0 ppm	32,17
50 ppm	24,63
100 ppm	27,00
BNJ 5%	tn
Ratio of PGRs Concentrations	
NAA + BAP (0:0)	28,67

NAA + BAP (1:1)	28,33
NAA + BAP (1:2)	25,17
NAA + BAP (2:1)	29,50
BNJ 5%	tn

Note: tn = no significant effect

Source: Processed Data, 2023

The analysis of variance (ANOVA) results showed that there was no significant interaction effect between the combination of Zn nanoparticle concentration and the ratio of PGRs concentrations on the time of shoot emergence in Cavendish bananas. Both the single treatment of Zn nanoparticle concentration and the single treatment of the PGRs concentration ratio also had no significant effect on shoot emergence time. The average time of shoot emergence due to the influence of Zn nanoparticle concentration and the ratio of PGRs concentrations is presented in Table 1.

Table 1 shows that the single treatment of various Zn nanoparticle concentrations did not significantly affect the shoot emergence time of Cavendish banana plantlets. The single treatment of Zn nanoparticles at concentration 0 ppm resulted in the longest average shoot emergence time of 32.17 days, while the 50 ppm concentration resulted in the quickest average shoot emergence time of 24.63 days. The single treatment of various PGRs concentration ratios also had no significant effect on shoot emergence time. Among the single treatments, the PGRs concentration ratio of NAA + BAP (1:2) resulted in the quickest average shoot emergence time of 25.17 days, while the NAA + BAP (2:1) ratio resulted in the longest average shoot emergence time of 29.50 days.

Zn nanoparticles play a complex role in plant tissue culture. According to [Lala \(2021\)](#), nanoparticles have several properties that enhance biochemical reactivity. Nanoparticles have the potential to act as biostimulants to improve the multiplication or provision of plantlets in tissue culture. The single treatment of Zn nanoparticles at concentration 50 ppm resulted in the highest number of plantlet shoots, with an average of 2,58 shoots. This positive outcome is also supported by the properties of nanoparticles, which can protect against bacterial and fungal contamination, promote differentiation and morphogenesis, induce somaclonal variation, stimulate secondary metabolite production, and facilitate genetic transformation ([Sharma et al., 2021](#)).

Table 2. Average Time of Leaf Emergence Due to Zn Nanoparticle Application and PGRs Ratio

Time of Leaf Emergence (days)	Ratio			
Treatments	Ratio of PGRs Concentrations			
Zn Nanoparticle Concentration	0:0	1:1	1:2	2:1
0 ppm	4,00 a	17,33 ab	22,33 ab	20,33 ab
50 ppm	18,33 ab	19,00 ab	16,00 ab	27,67 ab
100 ppm	6,00 a	20,00 ab	18,67 ab	29,00 ab
BNJ 5%	30.76			

Note: Numbers followed by the same letter indicate no significant difference based on the HSD test at 5% level.

Source: Processed Data, 2023



Figure 1. First Shoot Emergence on Cavendish Banana Explants



Figure 2. First Leaf Emergence on Cavendish Banana Explants

The analysis of variance (ANOVA) results showed a highly significant interaction effect between the combination of Zn nanoparticle concentration and the ratio of PGR concentrations on the time of leaf emergence in Cavendish bananas. The single treatment of Zn nanoparticle concentration had no significant effect, while the single treatment of the PGR concentration ratio had a significant effect on the time of leaf emergence. The average time of leaf emergence due to the influence of the combination of Zn nanoparticle concentration and the PGR concentration ratio is presented in Table 2. Table 2 shows that the treatment without the combination of nanoparticles and without PGR treatment had the fastest average leaf emergence time of 4.00 days. The combination treatment of 100 ppm Zn nanoparticles with NAA + BAP (2:1) resulted in the longest average leaf emergence time of 29.00 days.

The time of leaf emergence in the combination treatment showed that the best result was achieved with 0 ppm Zn nanoparticles + NAA: BAP (0:0), which had the fastest average leaf emergence time of 4.00 days. Meanwhile, the combination treatment of 100 ppm Zn nanoparticles + NAA: BAP (2:1) resulted in the longest average leaf emergence time of 29.00 days. According to Nisak (2012), exogenous auxin at relatively low concentrations can stimulate cell division and morphogenesis. Based on the results obtained, the combination of low PGRs concentrations and low Zn nanoparticle concentrations had a relatively faster leaf emergence time compared to combinations with higher concentrations. This is also suspected to be due to high auxin concentrations, which can induce the synthesis of other PGRs that inhibit growth. Additionally, the high concentration of nanoparticles may affect the metabolic processes. Too high a concentration can be toxic to the explants due to saturation, which inhibits the uptake of nutrients from the medium.

The results indicated that the interaction of both factors did not show optimal results. In contrast, the control treatment demonstrated better outcomes. According to Basha and Selvaraju (2015), the toxicity effects of zinc on plant growth and development include a reduction in growth inhibition and plant biomass. The study showed that Zn concentrations higher than 50 mg/L led to a decrease in morphophysiological parameters in treated plants compared to the control plants. The addition of zinc to the plants caused an increase in the Zn content in the plants.

Number of Shoots and Leaves

Table 3. Average Number of Shoots and Leaves Due to the Combination of Zn Nanoparticle Concentration and PGR Concentration Ratio

Treatments		Number of Shoots (Shoots)	Number of Leaves (Leaves)
Zn Concentration	Nanoparticle Concentration		
0 ppm		2,08	3,50
50 ppm		2,58	3,00
100 ppm		1,58	3,83
BNJ 5%		tn	tn
Ratio of Concentrations	PGRs	Number of Shoots (Shoots)	Number of Leaves (Leaves)
0:0			
1: 1		1,33	5,78 b
1: 2		2,78	4,56 b
2:1		2,33	2,11 a
BNJ 5		1,89	1,33 a
		tn	2,32

Note: The average values followed by the same letter within the same treatment indicate no significant difference at the 5% level of the Honest Significant Difference (HSD) test; tn = no significant effect

Source: Processed Data, 2023

The analysis of variance (ANOVA) results showed no significant interaction effect between the combination of Zn nanoparticle concentration and the ratio of PGRs concentrations on the number of Cavendish banana plantlet shoots. The single treatments of Zn nanoparticle concentration and PGR concentration ratio both resulted in no significant effect. The average number of plantlet shoots due to the influence of Zn nanoparticle concentration and PGR concentration ratio is presented in Table 3.

Table 3 shows that the single treatment of several concentrations of Zn nanoparticles did not have a significant effect on the number of Cavendish banana plantlet leaves. The single treatment of Zn nanoparticles at a concentration of 100 ppm resulted in the highest average number of living plantlet leaves, which was 3.83 leaves, while the concentration of 50 ppm resulted in the lowest average number of leaves, which was 3.00 leaves. On the other hand, the single treatments with different ratios of PGR concentrations showed significantly different results on the number of plantlet leaves. The treatment with a concentration ratio of NAA + BAP (0:0) resulted in the highest average number of plantlet leaves, which was 5.78 leaves, while the concentration ratio of NAA + BAP (2:1) resulted in the lowest average number of plantlet leaves, which was 1.33 leaves.

According to [El-Mahdy et al. \(2019\)](#), nanoparticles used at an optimum concentration have a significant effect on the number of banana leaves. Nanoparticles have the ability to support the growth of explants. However, in media with excessively high concentrations, saturation can occur. This is due to the instability of the culture medium (delayed subculture). As mineral ions are absorbed from the medium, the pH of the medium will continue to increase, making it no longer suitable for the growth requirements of the explants.

The addition of Zn nanoparticles needs to be carefully considered in terms of concentration, as it can affect plant growth. This is similar to the study by [Ahmad et al. \(2015\)](#), which showed that both optimal and excessive concentrations of Zn can play a role in

stimulating or inhibiting growth. If the concentration is not applied correctly, it can affect growth, physiology, and biochemistry at various concentrations of Zn during different stages of plant growth and development. Zinc is a component of many different enzymes with various functions, including alcohol dehydrogenase, carbonic anhydrase, superoxide dismutase, RNA polymerase, and others.

Cytokinins have an active role in shoot formation and plant morphogenesis. The single treatment with different PGR concentration ratios showed a positive response to the growth and development of Cavendish banana plantlets (*Musa acuminata*) in several parameters. This is evident from the analysis of variance (ANOVA) results, which showed a significant effect of the single treatment with PGR concentration ratios on the time of leaf emergence and the number of leaves.

According to [Paserang and Riska \(2022\)](#), banana shoot subculture is done by stimulating the increase in the proliferation of axillary shoots from the corm part. Usually, in the multiplication stage of banana plants, cytokinin is combined with auxin, with a lower concentration of auxin than cytokinin. Additionally, high concentrations of auxin can inhibit shoot growth. High levels of cytokinin can disrupt the growth and development of cells in the plantlet. Explants may not form shoots when exposed to high concentrations of PGRs.

As stated by [Nadaek et al. \(2012\)](#), each plant requires a specific amount of PGRs to ensure that their concentrations are sufficient to stimulate growth. Another factor is that the addition of cytokinin to the medium, followed by the addition of auxin, will inhibit shoot initiation. Explants already contain endogenous auxin. Physiologically, the addition of exogenous auxin will inhibit the release of endogenous cytokinin from the explant. When exogenous auxin continues to be added, no matter how much cytokinin is added, it will not be sufficient to stimulate shoot initiation in the explants in vitro ([Trinopsagiarti and Seprido, 2021](#)).



Figure 3. The Highest Number of Shoots in Cavendish Banana Explants (*Musa acuminata*)



Figure 4. The Highest Number of Leaves in Cavendish Banana Explants (*Musa acuminata*)



Figure 5. The Lowest Number of Shoots in Cavendish Banana Explants (*Musa acuminata*)



Figure 6. The Lowest Number of Leaves in Cavendish Banana Explants (*Musa acuminata*)

CONCLUSION

The conclusions from this study indicate an interaction between Zn concentration and ZPT ratio in the parameter of leaf emergence time. However, the best results were obtained in the control treatment, without the addition of Zn nanoparticles and ZPT ratio, with a leaf emergence time of (4 days). Additionally, the single treatment of Zn nanoparticles at a concentration of 50 ppm produced an average of (2.58 shoots) and the fastest shoot emergence time of (24.63 days). The treatment with an NAA + BAP ratio of (1:1) resulted in the highest average number of shoots, totaling (2.78 shoots). Furthermore, the treatment with a ZPT concentration ratio of NAA + BAP (0:0) yielded the highest average number of plantlet leaves, at (5.78 leaves) per plantlet.

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