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The Effect of *Naphtaleine Acetic Acid* and *Benzyl Amino Purine* on The Subculture of Kepok Banana Var. Unti Sayang

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Abstract
The Kepok Banana Unti Sayang variety is a superior type of banana that is resistant to Fusarium wilt. The high demand for Kepok Banana var. Unti Sayang needs to be balanced with good seed production. One of the methods used to multiply high-quality seeds of Kepok Banana Unti Sayang is through tissue culture. This research aims to determine the optimal concentration of Naphtalene Acetic Acid (NAA) in the subculture medium for promoting root growth in the Kepok Banana var. Unti Sayang. The second objective is to determine the optimal concentration of *Benzyl Amino Purine* (BAP) in the subculture medium for shoot number growth in Kepok banana var. Unti Sayang. Another goal is to identify the optimal treatment combination that yields the highest number of shoots in the Kepok Banana variety. Unti Sayang. The research was conducted at the Laboratory of Plant Physiology and Biotechnology at Sebelas Maret University from February to July 2025. The study was conducted with 4 levels of NAA treatment: without NAA, 0.5, 1, and 1.5 ppm. The BAP treatment was administered at four levels: without BAP, 1 ppm, 2 ppm, and 3 ppm. The variables observed in this study were the time of shoot emergence, number of shoots, the time of root emergence, root length, and plantlet height. Data from each variable were then analyzed using analysis of variance (ANOVA) with an F-test at a 95% significance level. Significantly different data were then analyzed using Duncan's Multiple Range Test (DMRT) at a 95% significance level. The significant combination of NAA and BAP on the observed variables was then reanalyzed using regression tests. The results showed that the application of various concentrations of NAA does not affect the growth of the number of roots of Kepok bananas var. Unti Sayang. The application of 2 ppm BAP is optimal for increasing the number of shoots in Kepok bananas var. Unti Sayang. The interaction between 1 ppm NAA and BAP at 1-2 ppm resulted in the most optimal average time to shoot emergence.

Keywords: Auxin; Cytokinin; In Vitro; Multiplication; *Musa paradisiaca*.

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Introduction

Bananas are one of the most popular fruits in Indonesia. According to data from the Food and Agriculture Organization, Indonesia was the third-largest banana producer in the world in 2022, after India and China, with a total production of 9.24 million tons, increasing to 9.33 million tons in 2023 (1). One of the most widely consumed banana varieties is the Kepok banana. The Kepok banana is a highly sought-after variety of banana. This fruit can be used to make various traditional dishes. The high demand for Kepok bananas in the market has led to an increase in production. Cultivating banana plants requires a lengthy process. Therefore, banana seedling propagation through

tissue culture has been developed (2). Tissue culture techniques on banana plants have been reported to be more effective than field germination.

The medium used in subculture contains high levels of nutrients and Growth Regulators (GRs) that support plantlet growth. The nutrients in the medium have a significant influence on plantlet growth and the number of plantlets. The addition of synthetic GRs is widely practiced to enhance nutrient content in the medium. The application of GRs, such as auxin and cytokinin, must be regulated according to specific requirements. Higher auxin levels promote the height and length of plantlet roots. Higher auxin levels promote the height and length of plantlet roots. This needs to be balanced because excessive auxin is detrimental to growth. High auxin concentrations inhibit plant growth (3).

One of the synthetic Plant Growth Regulators (PGRs) widely used in in vitro banana propagation is NAA (Naphthalene *Acetic Acid)* and BAP (*Benzylaminopurine).* NAA is one of the synthetic auxins commonly used in *in vitro* culture. BAP is one of the synthetic PGRs from the adenine-type cytokinin group that can help enhance cell division. The application of cytokinin PGRs must be balanced with auxin PGRs to optimize plant growth. Cytokinin in appropriate amounts can enhance shoot growth. Determining the optimal concentration is crucial for achieving maximum shoot multiplication in bananas (4).

This study aims to determine the optimal NAA concentration in the subculture medium for the root growth of Kepok Banana var. Unti Sayang. The second objective is to determine the optimal BAP concentration in subculture medium for the shoot growth of Banana Kepok var. Unti Sayang. Another aim was to choose the best treatment combination that can produce the highest number of shoots in Kepok Banana var. Unti Sayang.

Material and Methods

This study was conducted at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, from February to July 2025. The equipment used in this study included an autoclave, *magnetic stirrer*, and *laminar air flow* (LAF), with the primary material being fifth-generation subcultured Kepok Banana var. Unti Sayang planlets aged one month. The medium used was Murashige and Skoog (MS) medium with additional PRG in the form of NAA and BAP. This study was an experimental study using a completely randomized design (CRD) with two treatment factors. The first factor was BAP (without BAP; concentrations of 1, 2, and 3 ppm), and the second factor was NAA (without NAA; concentrations of 0.5, 1, and 1.5 ppm). Each treatment was repeated five times, resulting in 80 experimental units.

This study was conducted in several steps, including preparation of tools and materials, sterilization, medium preparation, planting of explants for multiplication, and maintenance and observation. The addition of PGRs in the form of NAA and BAP was made during medium preparation to ensure that both PGRs were thoroughly mixed in the medium and could be effectively absorbed by the plants. The variables observed were the time of shoot emergence, number of shoots, the time of root emergence, root length, and plantlet height. The number of shoots indicates the total number from the beginning to the end of the observation period. The time of shoot emergence and root emergence indicate how long the explants respond to the treatment medium provided. Root length and plantlet height were measured using a thread and ruler, starting from the base of the plantlet to the tip of the highest leaf and the longest root tip. The data obtained were analyzed using an analysis of variance (ANOVA) at a significance level of 95%. If there were significant effects, the study was continued with *Duncan's Multiple Range Test* (DMRT) at a 95% significance level. The crucial combinations of NAA and BAP on the observed variables were then analyzed again using regression analysis.

Results and Discussion

General conditions of the location

The study was conducted in the Physiology and Biotechnology Laboratory of the Faculty of Agriculture, Sebelas Maret University, Surakarta. The laboratory is located at Jalan Ir. Sutami 36 Kentingan, Jebres, Surakarta, Central Java, Indonesia 57126. Lighting in the laboratory was provided throughout the day using 12-watt LED lamps. The temperature in the laboratory storage room was 25°C. According to (5), high temperatures can increase metabolism, such as respiration and carbohydrate breakdown. High temperatures can inhibit photosynthesis in leaves before respiration, resulting in a decrease in carbohydrate availability in plant cells.

The study on the Kepok Banana variety, Unti Sayang, was conducted with 16 treatment combinations and 5 replications, resulting in 80 experimental units. The variables observed in this study were the time of shoot emergence, number of shoots, the time of root emergence, root length, and plantlet height. Data from each variable were analyzed using analysis of variance (ANOVA) with an F-test at the 5% level. Data that were significantly different were then subjected to Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 1. Summary of the Results of the 5% F-Test of the Response of NAA and BAP Application to the Growth of Kepok Banana var. Unti Sayang Subcultures

Observation Variables	NAA	BAP	NAA × BAP
Time of Shoot Emergence	*	tn	*
Time of Root Emergence	tn	*	tn
Number of Shoots	tn	*	tn
Root Length	tn	*	tn
Planlet Height	*	*	tn

Note: * = significantly different at the 5% F-test level tn = not significantly affected in the 5% F test

Table 1 shows that NAA treatment has a significant effect on the observed variables of bud emergence time and planlet height. BAP treatment is having a substantial impact on the observed variables of the number of buds, root emergence time, root length, and plantlet height. The combination of NAA and BAP has a significant effect on bud emergence time.

Time of Shoot Emergence

The combination of NAA and BAP has a significant effect on the observed variables of shoot emergence time.

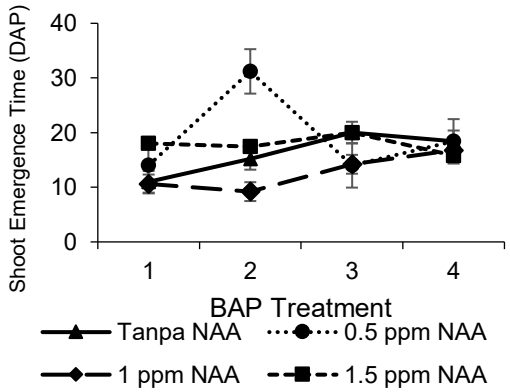

Figure 1. Regression of NAA and BAP on the time of shoot emergence in Kepok Banana var. Unti Sayang

The graph in Figure 1 shows the relationship between BAP treatment and bud emergence time in Kepok Banana var. Unti Sayang, influenced by variations in NAA concentration. Based on Figure 1, it can be seen that there is an interaction between NAA and BAP application on bud emergence time. The application of 1 ppm NAA resulted in the fastest bud emergence time, 10 Days After Planting (DAP). The interaction between 1 ppm NAA and BAP.

Between 1 and 2 ppm, the most optimal average time of shoot emergence was observed. BAP applications above 3 ppm resulted in increasingly longer times of shoot emergence. In line with (6), who found that the optimal BAP concentration was approximately 5 ppm in modern banana stems, this reduced the bud emergence time to 7.5 days. This may occur because the explants used already contained adequate endogenous hormones, allowing them to induce budding effectively even without exogenous hormone supplementation. According to (7), the emergence time of shoots and plant growth are influenced by BAP concentration to stimulate shoot growth. High concentrations of cytokinin can cause slow shoot growth because endogenous cytokinin levels are already sufficient. According to Arsa et al. (2020), the application of exogenous hormones does not always have a positive effect, as these hormones can influence unrelated growth processes or disrupt the balance of endogenous hormones in plants. This is because concentration is related to the growth response that occurs. The hormone requirements of each plant species vary, so the application of exogenous hormones must consider the concentration of hormones administered.

High endogenous hormone content can occur due to stress on the explants caused by frequent subculturing. Subculturing requires the explants to be cut and transferred to a new medium so that the explants can respond by altering their own production and metabolism. According to (8), subculture frequency can lead to undesirable variations, such as the emergence of somaclonal variants with different metabolite profiles and phenotypes compared to normal plants.

Number of Shoots

The application of BAP influences the growth of shoots in Kepok Banana var. Unti Sayang.

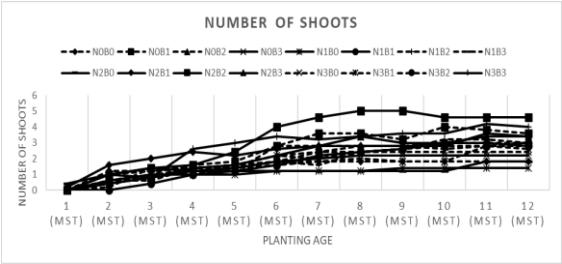

Figure 2. Growth curve of the number of shoots of Kepok Banana var. Unti Sayang 12 WAP (Weeks After Planting)

Figure 2 shows the growth of Kepok Banana var. Unti Sayang shoots up to 12 WAP. The number of shoots increased from week 1 to around week 10. The growth of the number of shoots began to stabilize after week 10. This indicates that active shoot growth occurred up to 10 WAP and then slowed down or stagnated. This finding is consistent with (9), who reported that banana explants exhibited a significant increase in the number of shoots until the 8th to 10th week, after which growth slowed due to the limited space and nutrients in the medium. At this age, nutrient content in the medium had begun to decline, and hormone levels had decreased, thereby reducing the growth potential of the explants.

Table 2. Number of Shoots of Banana Plants (*Musa paradisiaca*) var. Unti Sayang with Different Concentrations of BAP

BAP (ppm)	Number of Shoots
0	$1{,}90^{a}$
1	$2{,}90^{ab}$
2	$3{,}45^{b}$
3	$3{,}10^{b}$

Note: Numbers followed by the same letter in the same column are not significantly different at the 5% DMRT level.

From Table 2, it can be seen that the number of shoots produced with a 2 ppm BAP treatment averaged 3.45 shoots, which was significantly different from the treatment without BAP but not substantially different from the 1 ppm and 3 ppm BAP treatments. This indicates that the application of 2 ppm BAP is already optimal for shoot number growth. This suggests that exogenously added cytokinin, with or without auxin, in the medium can support callus formation. This could be caused by the function of cytokinins, which help shoot growth in plantlets. According to (10), a balanced ratio of exogenous and endogenous hormones will stimulate the emergence of new shoots and produce a large number of shoots. However, without additional BAP, planlet growth will also be poor. This is consistent with (11), who stated that treatments without BAP yielded the lowest observation results. Treatment without BAP supplementation resulted in the lowest average number of shoots because the endogenous hormones in the Pisang Kepok var. Unti Sayang explants were not yet optimal for shoot number growth.

Time of Root Emergence and Root Length

The faster the time of root emergence, the broader the nutrient absorption with the help of the already grown roots. The quicker the roots emerge, the better the physiological and hormonal conditions of the planlets. Planlets that have already emerged with roots will be more optimal in absorbing nutrients from the medium.

Table 3. The Time of Root Emergence and Root Length of Kepok Banana Unti Sayang with Various Concentrations of BAP

BAP (ppm)	Time of Root Emergence (DAP)	Root Length (cm)
0	$22{,}10^{a}$	$22{,}08^{b}$
1	$33{,}10^{b}$	$11{,}72^{a}$
2	$36{,}10^{b}$	$12{,}36^{a}$
3	$39{,}35^{b}$	$12{,}88^{a}$

Note: Numbers followed by the same letter in the same column are not significantly different at the 5% DMRT level.

Based on Table 3, the treatment without BAP yielded the best average of 22.10 Days After Planting (DAP), which was significantly different from the other treatments. The 3 ppm BAP treatment yielded an average of 39 DAP. This treatment did not differ significantly from the 1 ppm BAP and 2 ppm BAP treatments. This indicates that the application of BAP in the medium can inhibit root growth in the Kepok Banana variety. Unti Sayang planlets. This finding is consistent with (12), who stated that excessive BAP content can inhibit root growth, as BAP is one of the cytokinin hormones that function to inhibit root growth. According to (13), to promote root growth, a combination of BAP hormone at low concentrations is still required to avoid inhibiting the performance of auxin hormones. Cytokinin hormones promote the formation of plantlet buds, while higher auxin concentrations stimulate the height and length of plantlet roots. (14) think that explants cultured on media without added BAP already contain endogenous auxin to support plant root growth. According to (15), low auxin concentrations increase adventitious root formation, while high auxin concentrations stimulate callus formation and inhibit morphogenesis.

In this study, NAA treatment did not have a significant effect on the number of roots, unlike the results of (16), which stated that the addition of NAA significantly stimulates rooting, confirming previous findings that auxin generally promotes rooting. This can occur because there is no balance between endogenous auxin and the addition of NAA. According to (17), some genotypes show root/differentiation capability without the addition of auxin because the endogenous levels are sufficient, so exogenous addition does not provide significant improvement in some cases.

Table 3 also shows that the treatment without BAP yielded the best results with an average of 22.08 cm, which was significantly different from the other treatments. BAP, as a cytokinin, inhibits root elongation when applied at high concentrations. High concentrations of BAP disrupt the endogenous hormone balance within the plantlets themselves. This aligns with (18), who stated that high levels of cytokinins, such as BAP, are known to promote shoot proliferation but can also negatively impact root development by disrupting the auxin-cytokinin balance, which is crucial for root initiation. According to (19), when using BAP at 3 mg/L in conjunction with kinetin, hyperhydricity (excessive growth of liquid) increased by 10%, damaging the structure and inhibiting root growth. Although rooting was eventually achieved, this abnormal condition highlights the negative impact of high BAP levels on root development.

Planlet Height

The higher the plantlet height, the better the growth and development of the plantlets. The effect of plantlet height on plantlet growth is crucial in in vitro culture, as plantlet height reflects the physiological condition of the plantlets.

Table 4. Plant Height of Kepok Banana var. Unti Sayang plantlets with various concentrations of NAA and BAP.

Treatment	Concentration (ppm)	Height (cm)
NAA	0	$10{,}05^{ab}$
	0.5	$11{,}7^{b}$
	1	$7{,}74^{a}$
	1.5	$9{,}990^{ab}$
BAP	0	$14{,}45^{b}$
	1	$8{,}34^{a}$
	2	$7{,}83^{a}$
	3	$8{,}38^{a}$

Note: Numbers followed by the same letter in the same column are not significantly different at the 5% DMRT level.

Table 4 shows that the application of 0.5 ppm NAA resulted in an average height of 11.7 cm, which was not significantly different from the heights achieved with the treatments without NAA and 1.5 ppm NAA. The treatment with 1 ppm NAA resulted in an average height of 7.74 cm, which was not significantly different from the treatment without NAA and 1.5 ppm NAA. The treatment with 0.5 ppm NAA resulted in a significantly different height compared to the treatment with 1 ppm NAA, where 1 ppm NAA yielded less optimal results than 0.5 ppm NAA. This is consistent with (20), who stated that the application of 1 mg/L NAA combined with 10% coconut water resulted in the lowest plant height. This is likely due to the sufficient endogenous auxin and cytokinin content in the plantlets, which stimulates shoot elongation.

Treatment without BAP resulted in an average of 14.45 cm, which was significantly different from other BAP treatments. Treatment with 2 ppm BAP, with an average of 7.83 cm, was not significantly different from treatment with 1 ppm BAP and 3 ppm BAP. BAP is a cytokinin that stimulates cell division, thereby playing a crucial role in shoot growth. According to (21), BAP increases the number of banana shoots, but does not affect shoot height. A higher number of shoots reduces the effectiveness of BAP in promoting shoot height growth. The treatment without BAP yielded the highest average shoot height but had the fewest number of shoots. According to (22), a higher number of shoots leads to lower shoot height growth because the nutrients required for shoot height growth are used for the formation of new shoots. According to (23), high concentrations of BAP inhibit auxin activity and root growth. High concentrations of cytokinin with low auxin cause numerous plantlets to form, but result in suboptimal plantlet height growth because energy for elongation is utilized for new shoot formation.

Conclusion

The application of various concentrations of NAA does not affect the growth of root numbers in Pisang Kepok var. Unti Sayang. The application of 2 ppm BAP is optimal for increasing the number of shoots of Pisang Kepok var. Unti Sayang. The interaction between 1 ppm NAA and BAP at

concentrations between 1 and 2 ppm results in the optimal average time for shoot emergence.

Conflict of Interest

All authors declare no conflicts of interest.

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