Research Article

Effect of Different Strengths of MS Media and BAP on Banana Plantlet Growth *in Vitro*

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Abstract | Banana tissue culture is commonly conducted using Murashiage and Skoog (MS) media supplemented with various types of growth regulators (PGRs). Cytokinin and Auxin are incorporated into MS media to promote shoot and root growth in banana explants. 6-benzyl amino purine (BAP), a form of cytokinin, plays a significant role in stimulating cell division and differentiation. This study aimed to assess the impact of BAP on various growth parameters, including shoot initiation, root initiation, leaf initiation, shoot and root length, plantlet weight, root number, leaf number, and shoot number. The study followed a completely randomized design with one factor, namely the composition of the planting medium, consisting of five treatments: full MS without BAP (MS0), ½ MS without BAP (½MS0), ¼ MS without BAP (¼ MS0), ½ MS supplemented with BAP at 3 mg L-1 (½ MSB), and ¼ MS supplemented with BAP at 3 mg L-1 (¼ MSB). The results revealed that the timing of shoot, root, and leaf initiation was not significantly altered by any of the treatments. However, shoot and root lengths were notably influenced by the treatment without BAP. Intriguingly, treatments without BAP consistently yielded superior growth outcomes, as evident from root growth and the number of leaves observed over 56 days after planting. The treatment of ¼ MS without BAP demonstrated the highest number of roots, with the ½ MS0 treatment displaying the highest number of leaves. In conclusion, utilizing MS growth media without BAP in banana tissue culture resulted in plantlets with robust growth and vigor, although the production of shoots was limited. The addition of 3 mgL-1 BAP at 1/2 MS gave the best number of shoots.

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Introduction

Banana stands as one of the highly sought-after tropical fruit commodities in Indonesia due to its taste, nutritional value, and relatively affordable price. The Cavendish banana, a cultivated banana variant, stands out for its tender, sweet pulp, and larger dimensions (Rapetti and Dorel, 2022). However, propagation technique of using suckers directly detached from a mother plant is limited by low multiplication rates and propensity of disseminating pests and diseases, which culminates in reduced banana productivity (Tumuhimbise and Talengera, 2018).

The constraints in conventional propagation of Cavendish banana plants can be surmount by utilizing in vitro culture techniques. Micropropagation has become a widely utilized technique for effectively multiplying banana plant seeds. Continuous investigations are being carried out in the field of micropropagation techniques for banana plants, with the goal of pushing forward technological capacities and improving crop production due to the numerous advantages offered by this commodity. This research also serves the dual purpose of safeguarding genetic resources and acquiring specific cultivated varieties. The integration of biotechnological approaches is increasingly vital to comprehending and mitigating environmental stressors, bolstering resistance to both biological and environmental pressures, boosting the market value, and formulating suitable propagation procedures for diverse banana cultivars (Justine *et al*., 2022).

The growth of plantlet shoots and roots are significantly influenced by the composition of the tissue culture growing media. The success of micropropagation for banana plants hinges on factors like media selection, nutrient composition, and mineral concentration (Suman, 2017). Several fundamental media types, such as Murashige and Skoog (MS), Schenk and Hildebrant (SH), Linsmaier and Skoog (LS), N6, and B5 media, have been employed in banana shoot propagation (Justine *et al*., 2022). However, among these options, MS media (with specific growth regulators) emerged as the most effective for shoot propagation (Hui *et al*., 2012; Hossain *et al*., 2016). Additionally, MS media, supplemented with foliar fertilizer and coconut water, was developed by Mardhikasari *et al*. (2020), revealing that full MS media with 50-100 ml L^{-1} of coconut water yielded the optimal outcomes. Damayanti *et al*. (2021) also modified MS media through the incorporation of foliar fertilizer. Furthermore, variations in MS media concentration were explored for banana micropropagation, as evidenced by Mardhikasari *et al*. (2020) who utilized both full MS and ½ MS to stimulate banana plantlet growth.

One of the methodologies developed in micropropagation research involves incorporating plant growth regulators (PGRs) into the growing media used for in vitro culture. PGRs like cytokinin and auxin, which belong to groups such as abscisic acid, auxin, cytokinin, and gibberellin, are introduced to stimulate the initiation of shoot and root growth. In general, cytokinins (CKs) are involved in regulating cell growth, differentiation, and various physiological processes in plants. CKs like BAP, zeatin, thidiazuron (TDZ), and kinetin (KN) are typically employed to facilitate axillary shoot growth and the multiplication of shoots. Meanwhile, auxins like indole acetic acid (IAA), indole butyric acid (IBA), and naphthalene acetic acid (NAA) are utilized to promote the development of roots (Justine *et al*., 2022). Among these compounds, BAP has gained significant usage in initiating shoot growth within banana plant culture due to its notable cytokinin activity, accessibility, and relatively affordable cost (Sugiyono *et al*., 2020). BAP is the most widely recognized artificial cytokinins (ArCKs). BAP is highly activity and has superior stability in aqueous solutions, as well as its effective uptake by plants compared to other cytokinin compounds.

The achievements of various research endeavors involving modified MS media enable the utilization of MS plant media without PGRs within the context of enhancing efficiency in banana plant micropropagation. This study aims to determine the appropriate composition of a PGRs growth medium for the initial development of banana plantlets. While BAP currently stands as the most cost-effective and extensively employed ArCKs in tissue culture-based micropropagation, its application is linked to several drawbacks, including inhibition of lateral root development, inconsistent growth patterns, challenges in acclimatizing plants to the greenhouse environment, and susceptibility to shoot tip necrosis (Podlesˇa´kova *et al*., 2012; Martins *et al*., 2022).

Based on the research results of Manurung *et al*. (2021), the best BAP concentration was 2.5 mg/L with an average number of 3.2 shoots/explant. Scalps were formed in the treatment of BAP 5 mg/L and

7 mg/L after four weeks of incubation and has the potential to become new shoots. It has even been mentioned previously by Jafari *et al*. (2011), that the application of BAP in the media needs to be carefully monitored in establishing optimized culture systems for in vitro propagation to obtain normal plantlets.

Utilization of BAP can be explored in this research through two concentrations to determine the optimal treatment for Cavendish banana plantlet growth in vitro. The application of more cost-effective substitute media can serve as a solution to reduce expenses in the production of in vitro culture media. The study aimed to assess the impact of these treatments on various growth parameters including shoot initiation, root initiation, leaf initiation, shoot and root length, plantlet weight, root number, leaf number and shoot number.

Materials and Methods

Plant materials and chemicals

The Cavendish variety of bananas (Musa acuminata) based on Ministry of Agriculture Decree No.702/Kpts/SR.120/5/2008 belongs to PT. Nusantara Tropical Fruit, located in Rajabasa Lama Village, Labuan Ratu Subdistrict, East Lampung Regency, Province of Lampung, Indonesia. The Cavendish banana plantlet was approximately 6 months old. MS medium was purchased from HiMedia (Pennsylvania, USA). Other chemicals employed in both the plant growth environment and analysis were all of the reagent grade from Merck (Darmstadt, Germany).

MS medium preparation

The MS medium was prepared according to the method reported by Murashige and Skoog (1962) with partial modification using various concentration of BAP. A stock solution of BAP was prepared at a concentration of 100 ppm in 100 ml of sterile distilled water. The stock solution was prepared by weighing 10 mg of BAP powder and adding it to a glass beaker. Then, 100 ml of sterile water was added, and the mixture was dissolved using a magnetic stirrer until homogenous. The prepared BAP stock solution was transferred to an Erlenmeyer flask, covered with aluminum foil or plastic wrap and secured with a rubber band, and then stored in a refrigerator. The base medium employed was MS planting medium supplemented with BAP-PGRs according to the treatment. Afterward, sterile distilled water was added to a volume of 1000 ml, and then homogenized on a hot plate stirrer. The pH of the medium was measured with the optimal pH range for the medium being 5.5 to 5.8. The MS medium was transferred to a container to be heated while stirring until it foams. The foamy MS medium was poured into culture bottles, approximately 25 ml per bottle, which were then sealed with plastic lids and secured with rubber bands. The MS medium-filled bottles were sterilized in an autoclave at 125°C and 1.5 atm pressure for 30-45 minutes. The sterilized MS medium was incubated for one week to observe any potential contamination.

Tissue culture growth conditions

This research was conducted at the Laboratory of Biotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional Veteran Jawa Timur, Surabaya, Indonesia, from May to September, 2022. The plant material as explants consisted of 1 cm shoots from Cavendish banana plantlet. The best growth condition of this research was arranged by the various composition of the planting medium, which were full MS without BAP (MS0), ½ MS without BAP (½MS0), ¼ MS without BAP (¼ MS0), ½ MS supplemented with BAP at 3 mg L^{-1} (½ MSB), and ¼ MS supplemented with BAP at 3 mg L^{-1} (¼ MSB). Each treatment was repeated three times, each consisting of three culture bottles containing one explant per culture bottle. The cultures were stored in the incubation room for 56 days, at 25°C and 24 hours a day with fluorescent light. The plantlets were placed on the culture rack in the incubation room for 2 months. The cleanliness of the culture environment was maintained to prevent contamination by spraying 70% alcohol around the bottles and culture rack, and periodic fumigation was conducted.

Parameter's evaluation and statistical analysis

Data collection were made every day including the time for shoots initiation, roots initiation, and leaves initiation. The data of the number of shoots, the number of leaves, and the number of roots were observed every week. At the end of the observation, on day 56 after planting, the root length, plantlet length and weight were observed. The collected data were processed using analysis of variance (ANOVA), followed by the least significant difference (LSD) test at the 5% level.

Results and Discussion

Time for shoots, roots, and leaves initiation

The results of data analysis showed that there was no significant effect on the concentration of MS media with the addition of BAP on the emergence of shoots, roots, and leaves of Cavendish banana explants. The average time of emergence of shoots, roots, and leaves is presented in Table 1.

Table 1. *Number of days required for shoot, root, and leaf initiation was observe across different strengths of MS basal medium without and supplemented with BAP.*

Treatment	Days to shoot initiation	Days to root initiation	Days to leaf initiation
MS 0	7.80	10.73	12.87
½ MS 0	8.80	8.67	16.33
¼ MS 0	10.40	12.83	18.40
½ MSB	6.87	8.53	10.53
¼ MSB	7.75	9.53	9.47
LSD 5%	ns	ns	ns

Note: ns = not significant

The treatment of MS basal medium supplemented with BAP did not significantly affect the time of shoot initiation. Table 1 shows that the average time for shoot initiation was faster in the ½ MSB treatment (½ MS + BAP 3 mg L^{-1}), which was 6.87 days after planting (DAP), compared to the slower time for shoot initiation in the ¼ MS 0 treatment (¼ MS without BAP), which was 10.40 days.

Similarly, the treatment of MS basal medium supplemented with BAP had no significant effect on the time of root initiation. Table 1 indicates that the average time for root initiation was faster in the ½ MSB treatment (½ MS + 3 mg L^{-1} BAP), at 8.53 days, while the slower time for root initiation was reported in the ¼ MS 0 treatment (¼ MS without BAP), which took 12.83 days.

Moreover, the treatment of MS basal medium supplemented with BAP did not significantly affect the time of leaf initiation. Table 1 shows that the average time for leaf initiation was faster in the ¼ MSB treatment (¼ MS + 3 mg L^{-1} BAP), at 9.47 days, while the slower time for leaf initiation was reported in the ¼ MS 0 treatment (¼ MS without BAP), at 18.40 days.

Plantlet length, root length, and plantlet weight

The results of the data analysis showed that there was a significant effect on the concentration of MS basal medium supplemented with BAP on plantlet length and root length and weight of plantlet of Cavendish banana plantlets is presented in Table 2.

Table 2. *The plantlet length, root length, and plantlet weight of Cavendish bananas plantlet on different strengths of MS basal medium without and supplemented with BAP.*

Treatment	The length of the shoot (cm)	The length of the root (cm)	Weight of Plantlet (g)
MS 0	8.00 a	8.70 a	2.84
½ MS 0	7.80 a	10.50 a	2.42
¼ MS 0	5.30 b	9.20a	2.08
½ MSB	2.20 c	2.50 b	1.37
¼ MSB	0.90 c	1.50 b	0.40
LSD 5%	3.58	3.58	ns

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

The treatment of MS basal medium without BAP shows a significant effect on length of the shoot. The results indicate that the longest shoot was reported in MS 0 treatment, measuring 8.00 cm, followed by ½ MS 0 treatment, which measured 7.08 cm. On the other hand, MS basal medium supplemented with BAP shows the shortest shoot length as reported in ¼ MSB treatment, which measured 0.90 cm, followed by ½ MSB treatment, which measured 2.20 cm.

The treatment of MS basal medium without BAP shows a significant effect on length of the root. The results indicate that the longest root was reported in ½ MS 0 treatment, measuring 10.50 cm, followed by the ¼ MS 0 and MS 0 treatments, which measured 9.20 and 8.70 cm, respectively. While, MS basal medium supplemented with BAP shows the shortest roots length as reported in ¼ MSB treatment, measuring 1.50 cm, followed by ½ MSB treatment, measuring 2.50 cm. Treatment of MS basal medium supplemented with BAP had no significant effect on the weight of Cavendish banana plantlets. Table 2 shows that the treatment that result in the largest plantlet weight was the MS 0 treatment, measuring 2.84 g, while the treatment yielding the smallest plantlet weight was the ¼ MSB (¼ MS + BAP 3 mg L^{-1}) treatment, measuring 0.40 g.

The number of roots, leaves, and shoots

The results of data analysis showed that there was a significant effect of MS basal medium supplemented with BAP on the number of Cavendish banana roots at 7 DAPs until 56 DAPs observations. The results of the average number of Cavendish banana roots are presented in Figure 1 and Table 3.

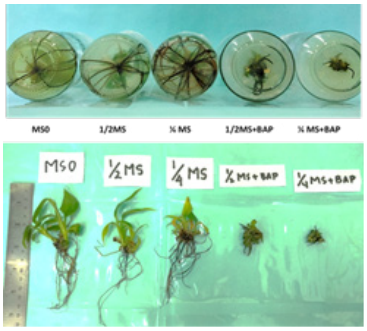

Figure 1. *The root of Cavendish bananas plantlet on different strengths of MS basal medium without and supplemented with BAP at 56 DAPs.*

Figure 1 shows a visual representation of banana culture root growth of at 56 days after planting (DAP). When examining the treatments, it becomes evident that the use of media without BAP supplementation, encompassing full MS (MS0), ½ MS, and ¼ MS, resulted in superior growth (Figure 2). In contrast, the ½ MSB treatment (½ MS + 3 mg L^{-1} BAP) and ¼ MSB treatment (¼ MS + 3 mg L^{-1} BAP) showed slower growth in comparison to the other treatments. Notably, in the ¼ MS0 treatment without BAP supplementation showed the highest number of roots at 14.57 (Figure 2 and Table 3), This result, while notable, did not significantly different from the number of roots in MS0 treatment (MS without BAP) and ½ MS0 treatment (½ MS without BAP).

Table 3. *The number of roots, leave and shoot of Cavendish bananas plantlet on different strengths of MS basal medium without and supplemented with BAP.*

Treatment	No. of roots	No. of leave	No. of shoot
MS0	10.20 a	6.60 a	4.90
½ MS0	11.33 a	7.70 a	3.73
¼ MS0	14.57 a	6.27 a	4.47
½ MSB	1.40 b	5.20 ab	7.10
¼ MSB	0.93 b	1.80 b	3.47
LSD 5%	6.68	4.26	ns

Note: Numbers followed by the same letter in the same columns and rows, show no significant at 5% LSD, ns = not significant.

Table 3 shows that the treatment of MS basal medium without BAP significantly affected the number of Cavendish banana roots in the observations at 56 DAPs. Additionally, the Table 3 shows that the highest average number of roots was found in the 56 DAP observations with ¼ MS0 treatment (¼ MS without BAP) with 14.57 roots. This was followed by ½ MS0 treatment (½ MS without BAP) with 11.33 roots, and MS0 treatment, which yielded 10.20 roots. Notably, these exhibited a gradual increase in root production on a weekly basis. Conversely, the treatment of MS basal medium supplemented with BAP, which was the ¼ MSB showed the lowest number of roots with 0.93 followed by the ½ MSB treatment with 1.40 roots at 56 DAPs.

The result for number of leave indicates that MS basal medium without BAP resulted in the highest number of leave in the ½ MS0 with 7.70 leaves. This was followed by the MS0 and ¼ MS0 treatment, which exhibited number of leaves 6.60 and 6.27, respectively. The lowest number of leaves was observed in the ¼ MSB treatment, which exhibited number of leaves 1.80 leaves.

Table 3 also shows that the treatment of MS basal medium without and supplemented with BAP did not significantly affect the number of shoots of Cavendish bananas during observations at 56 DAPs. Among the treatments, ½ MSB treatment (½ MS + 3 ppm BAP) exhibited the highest yield of 7.10 shoots, though this result did not show a significantly different from the MS 0 treatment, which produced 4.90 shoots.

Visual Observations of Cavendish bananas plantlet

The results of the data analysis showed that there was a significant effect on the concentration of MS media with the addition of BAP on shoot and root length. The average length of shoot, root, and weight of Cavendish banana plantlets is presented in Table 2.

It is evident that the plantlets under the treatment of the MS basal medium without BAP exhibit larger sizes as shown in Figure 2. They have a greater number of leaves, roots, and shoots compared to the plantlets treated with the MS basal medium supplemented with BAP. Especially notable is the fact that the plantlets in the ½ MS basal medium without BAP treatment are the largest in size. Conversely, plantlets in the ¼ MS basal medium supplemented with BAP

treatment are the smallest among all the treatments.

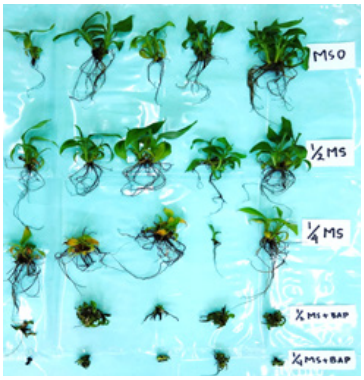

Figure 2. *Growth of Cavendish bananas plantlet under different treatments was evaluated at 56 days after planting (DAPs) using varying strengths of MS basal medium without and supplemented with BAP.*

Discussion

This study used MS planting media, which according to several research results is stated to be the most appropriate medium for banana micropropagation (Nur'riyani, 2021; Justine *et al*., 2022). MS media is a planting medium that contains macronutrients, micronutrients, vitamins, carbon sources and organic compounds. The results of this study indicated that MS medium, encompassing full MS, ½ MS, and ¼ MS, without the addition of BAP, exhibited superior initial growth compare to the treatment on ½ and ¼ MS media supplemented with BAP. Furthermore, the results also showed that MS0 media (MS medium without BAP) could effectively support the growth of banana plantlets, even at ½ MS and ¼ MS (Table 2 and Figure 2).

The initial growth of banana plantlets on ½ MS and ¼ MS media up to 56 days after planting, showed better plantlet length, root length, and root weight compare to treatments with the addition of BAP. Similarly, this trend was observed with respect to number of leaves, number of roots, and number of shoots (Table 3 and Figure 2). It is presumed that the nutrient content in the MS medium, which was reduced to half and to a quarter was sufficient to support the initial growth of banana plantlets. Previous research (Mardhikasari *et al*., 2020; Chaidir *et al*., 2021) also found that MS media without PGRs could support the initial growth of the banana culture. In Mekonen *et al*. (2021) study, half strength MS medium augmented with 2.0 mgL^{-1} of IBA showed good rooting performance and was found to be the most effective for in vitro rooting of banana. Research conducted by Domez *et al*., (2022) showed that 1/2 and 1/4 MS strengths could be used instead of full MS in in vitro rooting studies of spathiphyllum.

In vitro culture of plantains can be carried out on MS or B5 basic media. Suseno (2017) also stated that MS media added with BAP and NAA were equally good for banana micropropagation in vitro. Several other studies state that MS media can be replaced with alternative media, for example, several types of foliar fertilizers, in the context of cost efficiency (Rosmaina *et al*., 2021). However, many other studies indicated that MS media is the most appropriate planting medium for banana plant culture (Nur'riyani, 2021)

The addition of BAP to support shoot growth has been proven by many other studies. The addition of BAP up to 4 mg L^{-1} can increase the number of banana shoots that are propagated by micropropagation (Karmina *et al*., 2022). An increase in the number of shoots resulting from the addition of BAP is possible when applied to full MS media. However, the results of this study, the addition of PGRs in the form of BAP, on ½ MS and 1/4 MS media, did not yield better plantlet growth compare to the treatment MS media without BAP.

BAP as one of the PGRs of the cytokinin group is a growth regulator that plays a role in the process of cell division, organ formation, and the formation of plant buds. BAP plays a greater role in stimulating cell division and differentiation toward the formation of shoots, but does not affect shoot elongation (Mayerni *et al*., 2020). These processes can occur if supported by an adequate supply of nutrients. Growth and bud initiation of a plantlet grown in vitro requires sufficient nutrients. Thus, for the cytokinin in BAP to work as expected, adequate nutrient support is needed. The addition of plant growth regulator BAP was effective for shoot initiation when added to full strength MS media. The use of BAP with IAA was found to enhance number of shoots, leaves, roots and fresh weight of different banana varieties (Khan *et al*., 2021). In some genotypes, the presence of BAP with IAA in the culture media led to more effective shoot multiplication than BAP alone. This impact was genotype dependent (Qamar *et al*.. 2015).

Treatment of MS media without plant growth regulators (PGRs) proves to be more efficient when banana

tissue culture is not intended for micropropagation purpose. Utilizing MS growth media without PGRs in banana tissue culture was able to produce plantlets with superior growth and vigor, but not many shoots were produced. According to Abdalla *et al*., (2022), given the relatively expensive nature of in vitro plant propagation, the pursuit of an efficient in vitro micropropagation protocol remains crucial and should persist for as long as feasible.

Conclusions and Recommendations

In conclusion, it can be concluded that among several strengths of MS media, both full MS and 1/2 MS have a good growth effect on banana plantlets. The addition of 3 mgL^{-1} BAP at 1/2 MS gave the best number of shoots.

The growth of shoots and roots of Banana plantlets is sufficiently supported by the nutrients in MS basic media, without the addition of growth regulating substances. Reducing the strength of the MS media to half still be done to get good plantlet growth. BAP is needed to increase shoot number.

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Novelty Statement

For banana micropropagation efficiency, it is enough to use MS media at half strength.

Author's Contribution

Pangesti Nugrahani: Conceptualization, Writing – original draft, Funding acquisition.
Hery Purnobasuki: Supervision.
Arif Nur Muhammad Ansori: Validation.
Anugerah Dany Priyanto: Statistical analysis.
Jatuporn Anuchai: Writing – review and editing

Conflict of interest
The authors have declared no conflict of interest.

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