

Application of Benzyl Adenine and IAA on The Growth of Banana Barangan Subculture (*Musa Acuminata* L.)

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Abstract

Barangan banana is a banana variety with high economic value, but its production is still carried out conventionally, resulting in limited availability of quality planting material on the market. To overcome this limitation, the use of tissue culture methods can quickly produce high-quality, disease-free plants regardless of seasonal conditions. This research aims to determine the effect of providing the best PGR IAA and BA for the growth of the Barangan banana plantlet. The study was conducted from August to October 2023 at the Biotechnology and Plant Physiology Laboratory, Sebelas Maret University. This research used a two-factor Completely Randomized Design (CRD) with 6 replications. The first factor was the use of PGR Indole Acetic Acid (concentrations 0, 0.5, 1, and 1.5 ppm and the second factor was the use of PGR Benzyl Adenine (0, 0.5, 1, 1.5 ppm). The variables observed were the number of roots, root length, the number of leaves, and shoot height. The observed data were analyzed using Analysis of Variance (ANOVA) with a 5% significance level. If a significant difference was found, Duncan's Multiple Range Test (DMRT) was performed at the 5% significance level. The results showed that giving 0.5 ppm IAA increased root length, indicating that 0.5 ppm IAA was optimal for root formation. Additionally, 1 ppm IAA increased the shoot height of the Barangan banana plantlet. Giving BA 0.5 ppm root number; BA 1, 5 ppm produces the highest number of leaves; BA 1 ppm increases the shoot height of barangan banana plantlets.

Keywords: Auxin; Horticulture; Cytokinin; Musaceae; In Vitro.

Cite this as: Setyawati, A., Samanhudi, Purwanto, E., Arniputri, R.M., Setyawan, K.P., Cahyadi, M.A. (2024). Application of Benzyl Adenine and IAA on The Growth of Banana Barangan Subculture (*Musa Acuminata* L.). Journal of Biodiversity and Biotechnology. 4(2), 75–80. doi: <http://dx.doi.org/10.20961/jbb.v4i2.108497>

Introduction

Banana (*Musa paradisiaca* L) is one of the horticultural commodity plants originating from Southeast Asia, including Indonesia. According to (1), bananas are one of the germplasm that are widely distributed in the territory of Indonesia. Data from (2) shows that banana production in Indonesia is substantial, reaching 8,741,147 tonnes. The centre of banana production is located on the island of Java, with a total output of 4,570,695 tonnes, accounting for approximately 53% of the country's total banana production. One of the banana varieties that has the potential to be

cultivated in Indonesia is the barangan banana. Barangan banana is one of the essential commodities that plays a crucial role in supporting the local economy in banana-producing centres in North Sumatra. Barangan banana is one of the banana varieties that has high nutritional and economic value, so its consumers continue to grow.

Banana cultivation is generally carried out conventionally through the process of planting saplings or cleavage. The result of this cultivation is a limited number of seedlings, so that the fulfilment of the need for banana plants is not optimal. According to (3), one of the

problems in conventional banana cultivation is the limited number of seedlings in large quantities, uniform, and disease-free. Additionally, banana seedlings derived from conventional cultivation have the potential to harbor pathogenic germs that can cause various diseases.

One way to produce quality banana seedlings is through tissue culture techniques (in vitro). According to (4), the tissue culture technique is a vegetative plant propagation. Tissue culture involves planting cells, tissues, and plant organs on a growing medium that contains vitamins, sugars, amino acids, growth regulators, water, organic salts, and agar as a solidifying agent. (5) states that tissue culture can produce pathogen-free planting material because it produces a larger number of seedlings in a relatively short time, does not depend on weather and season, is free from disease attacks, produces healthy plants, inherits the good properties of the parent, does not require large areas of land for breeding, and can reproduce certain plants that are difficult if propagated conventionally.

Several factors affect the success of plant propagation in tissue culture, including the source of explants, growth media, growth regulators (PGR), and the culture growth environment. The medium commonly used in banana subculture is Murashige and Skoog medium, also known as MS medium, which serves as the basic medium and is typically supplemented with PGR. Growth regulators are compounds that play an essential role in directing plant cell growth (6). In tissue culture, two classes of growth regulators are fundamental: cytokinins and auxins. Benzyl Adenine (BA) is one of the growth regulators included in the cytokinin group. According to (7), benzyladenine is a synthetic cytokinin hormone, one of whose functions is to break the seed rest period (seed dormancy), stimulate embryo growth, and promote organ formation. One type of PGR that is included in the auxin group is Indole Acetic Acid (IAA). According to (8), auxin plays a primary role in supporting root growth and stimulating cell elongation. This research aimed to determine the effect of BA and IAA addition on the growth of banana barangan.

Material and Methods

The research was conducted at the Laboratory of Biotechnology and Plant Physiology, Faculty of Agriculture, UNS Surakarta, from August to October 2023. The

materials used in this research were barangan banana plantlets, PGR BA, PGR IAA, MS macro stock solution, MS micro stock solution, Fe-EDTA, MS vitamin solution, NaOH solution, HCL solution, agar, sugar, and distilled water. The tools used were a LAF, autoclave, Bunsen burner, tweezers, scalpel blade, petri dish, sterile opaque paper, sterile tissue, hot plate stirrer, magnetic stirrer, and culture bottles. This study used a completely randomised design (CRD) with two treatment factors. The first factor is the concentration of PGR BA, and the second factor is the concentration of IAA; each factor consists of 4 levels, ie, 0, 0.5, 1, and 1.5 ppm. Each treatment was repeated 6 times, resulting in 96 experimental units.

The research stages consisted of preparing tools and materials, sterilization, media preparation, explant planting, and maintenance and observation. Variables observed included the number of roots, root length, shoot height, and number of leaves. The data obtained were analysed using analysis of variance. If the results of the study of variance showed a significant effect on the 5% F-table test, then further tests were carried out using Duncan's Multiple Range Test (DMRT) at the 5% level.

Results and Discussion

Root length

Roots are one of the essential organs in plants. Roots have a function to absorb nutrients, water, and minerals, as well as provide support to the plant body. Observation of root length was taken at the end of the study. BA alone did not affect the growth of root length of banana plantlets, while IAA alone increased the growth of root length of banana plantlets. The combination of BA and IAA did not affect the root length of banana plantlets.

Table 1. Effect of IAA on root length of barangan banana plantlets.

IAA (ppm)	Root length (cm)
0	12,88±2,6b
0,5	15,32±2,6a
1	13,14±2,0b
1,5	14,16±2,0a

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



Figure 1. Root length of barangan banana plantlets

Based on Table 1, it is found that the provision of IAA at 0.5 ppm is the optimal concentration for increasing the root length of banana plantlets. According to (9), the increase in root length of plants can be attributed to the plant adapting to its new medium, which can facilitate organ growth and increase cell division. Roots that continue to grow and form lateral roots are a plant's response to adaptation to environmental conditions. During the process of root formation, according to (10), auxin plays a significant role in initiating root development.

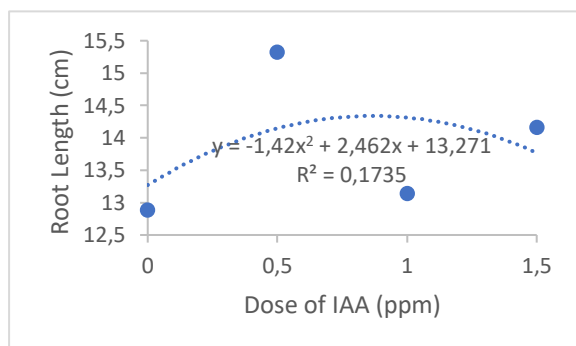


Figure 2. Regression of root length on IAA

Figure 2 shows the results of regression analysis between the variable of banana root length and the environment. Based on graphic 2, it is known that the application of IAA has a fluctuating effect on the growth of banana root length. The regression graph above shows that the application of IAA at a dose of 0.5 ppm is the optimal dose, while a dose of 1 ppm has the lowest effect. IAA plays a role in stimulating plant root growth. IAA is one of the hormones that has a significant impact on plant root growth. (11) stated that auxin given at the beginning of planting can induce rapid root growth. (12) stated that the provision of IAA as a PGR resulted in an induction response (the process of stimulating or growing roots in plants in vitro) and good root elongation. According to (13), IAA has a role in cell enlargement and influences root development and elongation.

Number of Roots

The number of plant roots is crucial because the root system plays a vital role in

supporting plant life. The more roots a plant has, the greater the plant's ability to absorb water, nutrients, and minerals from the soil. The application of BA and IAA alone can influence the growth of the number of roots of barangan banana plantlets. The combination of BA and IAA did not affect the number of roots of banana plantlets. The effects of BA and IAA are shown in the table below.

Table 2. Effect of BA and IAA on the number of roots of banana barangan plantlets

IAA (ppm)	Number of roots
0	8,08±1,19b
0,5	9,33±1,24a
1	9,33±1,24a
1,5	9,00±1,24a
BA (ppm)	Number of roots
0	9,17±1,44a
0,5	9,42±0,88a
1	8,08±1,37b
1,5	9,08±1,12a

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

Based on the table above, the provision of IAA influences the variable number of roots of barangan banana plantlets. Administering IAA at 0.5 ppm is the optimal concentration for promoting root growth. IAA is a phytohormone from the auxin group that plays a role in regulating various developmental processes, including tissue swelling, cell division, and root formation. According to (11), plant growth and development can be influenced by phytohormones that affect the initiation of cell division. This is in accordance with (14), which states the effectiveness of the addition of PGR in stimulating the vegetative growth of plants propagated using tissue culture technology.

For the administration of BA, 0.5 ppm is the optimal dose for promoting root growth. According to (15), BA is a cytokinin that has a role in the process of cell division. This is in accordance with (16) BA, which encourages cell division, having a positive impact on vegetative passivity and plant root growth. Exogenous administration of Benzyladenine can increase the concentration of endogenous cytokinins, thereby enhancing cell division activity.

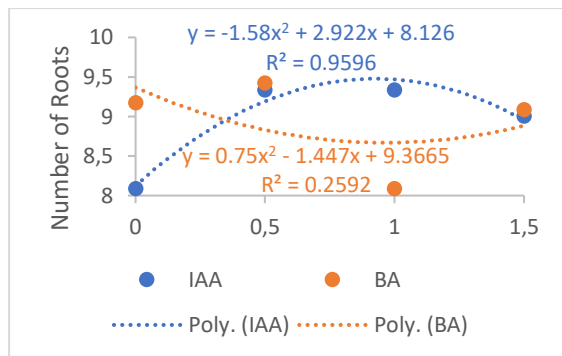


Figure 3. Regression of root number on BA and IAA

Based on Figure 3, it is evident that the administration of IAA has an optimal effect at a dose of 0.5 ppm, while administering IAA at a dose of 1 ppm has a lesser influence on the formation of roots in banana plantlets. BA at a dose of 0.5-1 ppm gives an optimal effect on the formation of the number of roots of pisang barangan. (17) stated that the provision of IAA in rooting media can spur cell differentiation towards root formation.

Number of Leaves

Leaves are one of the vital organs in plants that play a role in photosynthesis. An increase in the number of leaves will enable the plant to carry out photosynthesis optimally, supporting its transition to the reproductive phase. According to (18), the greater the number of leaves, the higher the photosynthetic activity and the amount of photosynthate produced. BA alone can increase the number of leaves on barangan banana plantlets, while the administration of IAA has no effect. The combination of BA and IAA does not affect the number of leaves on banana plantlets. The impact of BA is evident in the table below.

Table 3. Effect of BA on the Number of Leaves of Banana Barangan Planlets.

BA (ppm)	Number of leaves
0	4,58±1,08b
0,5	4,08±1,08b
1	4,17±0,83b
1,5	5,67±1,15a

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

Based on the research conducted, the provision of BA itself affects the number of leaves. Based on the table above, it is known that giving BA as much as 1.5 ppm yields the highest number of leaves, namely 5.67 leaves. The provision of BA at 1.5 ppm was able to increase the number of leaves 23,79% more than without the use of PGR. The provision of

BA is one of the efforts considered effective in reproducing the growth of various banana types in vitro. Benzyl Adenine can function to encourage cell division and organogenesis processes in micropropagation. According to (19), the provision of BA can influence the number of leaves during the multiplication of the Cavendish banana. The BA application is one of the efforts considered effective in promoting the growth of various banana types in vitro. According to (20), during the vegetative stage of plants in tissue culture conditions, the presence of cytokinins can stimulate the formation of plant organs.

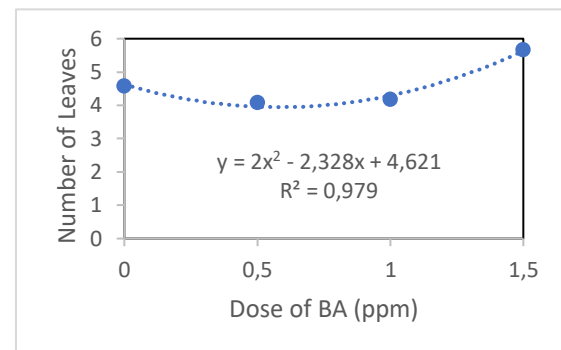


Figure 4. Regression of banana barangan leaf number against BA.

Figure 4 shows the results of regression analysis between the variable number of leaves of pisang barangan and the environment. Based on Graph 4, it is evident that administering BA at doses between 0.5 and 1 ppm has a less pronounced effect, whereas administering 1.5 ppm has an optimal impact on the formation of banana leaves. Based on the regression test, the coefficient of determination is 97%. According to (16), BA can affect leaf formation because BA can induce cell division in plant organs. According to (21), the cytokinin content in BA can trigger plant growth, leaf formation, and cell differentiation.

Shoot Height

Explant height is one of the critical indicators in measuring plant growth, as measured by the increase in shoot height. The higher the shoot height increases, the clearer it is that the explants experience growth and development. The administration of BA and IAA alone can affect the growth of the shoot height of barangan banana plantlets. The combination of BA and IAA did not affect the shoot height of banana plantlets. The effects of BA and IAA are shown in the table below.

Table 4. Effect of IAA and BA on the Shoot Height of Banana Barangan Planlets.

IAA (ppm)	Shoot height (cm)
0	12,13±2,53a
0,5	9,96±2,45b
1	12,60±2,74a
1,5	11,66±2,52b
BA (ppm)	Shoot height (cm)
0	10,09±1,40b
0,5	11,82±3,05a
1	13,11±3,69a
1,5	11,33±1,49a

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



Figure 5. Shoot height of banana barangan plantlets.

Based on the results of further tests, it is known that the provision of BA and IAA has a significant effect on the shoot height of banana plantlets. The provision of IAA 1 ppm is the optimal treatment for the growth of banana plantlets. Treatment of 1 ppm is not significantly different from the treatment of 0 ppm, but substantially different from the treatment of 0,5 and 1,5 ppm. (22) stated that the addition of IAA can affect various stages of plant growth, one of which is shoot height growth. According to (23), auxin has the function of encouraging cell elongation through two stages. The first stage is cell division, which results in cells experiencing stretching and thickening. The second stage of growth in the tip meristem produces new cells, increasing the height or length of the shoot.

The application of 1 ppm BA produced the best shoot height compared to other treatments. (24) In their research, they said that BA is included in the cytokinin PGR group, which has a role in spurring the growth of plant shoots grown on in vitro media. According to (25), the addition of cytokinins can support the formation of ribonucleic acid (RNA), proteins,

and enzymes in cells, thereby encouraging the process of cell division.

Conclusion

The results showed that the administration of Benzyl Adenine (BA) alone had different effects according to the concentration used. The administration of BA at a concentration of 0.5 ppm was able to increase the number of roots. In comparison, at a concentration of 1.5 ppm, it played a greater role in increasing the number of leaves, and at a concentration of 1 ppm, it was proven effective in improving the growth of shoots in barangan banana plantlets. Meanwhile, the single application of Indole Acetic Acid (IAA) also showed a specific effect, namely, at a concentration of 0.5 ppm, it increased root length and the number of roots. In comparison, at a concentration of 1 ppm, it increased shoot height growth. However, the combination of BA and IAA treatments did not show any significant interaction on barangan banana subcultures.

Acknowledgments

This research was funded by the RAKT Universitas Sebelas Maret fiscal year 2025 through the Riset Group Capacity Strengthening Research (PKGR-UNS), A scheme with Research Assignment Agreement Number: 371/UN27.22/PT.001.03/2025

Conflict of Interest

All authors declare no conflicts of interest.

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