E3S Web of Conferences **595**, 02012 (2024) https://doi.org/10.1051/e3sconf/202459502012
IConARD 2024

The Effect of Medium Substitution on Cavendish Banana Shoot Multiplication (*Musa acuminata* L.)

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Abstract. The Cavendish banana (*Musa acuminata* L.) is a highly significant cultivated plant for individuals living in tropical and subtropical regions. In vitro cultivation serves as a viable option for banana propagation, particularly for the Cavendish variety. The objective of this study is to identify the optimal medium for the in vitro multiplication of Cavendish banana shoots. This work used a method of research utilizing a single factor Completely Randomized Design, specifically involving a medium supplemented with cytokinin. The media were New Dogashima medium, Murashige and Skoog, Growmore Leaf Fertilizer, Sugihtani Liquid Organic Fertilizer, and DI Grow Liquid Organic Fertilizer. The treatment medium was supplemented with 3 mg/L of Benzyl Amino Purine, 0.1 mg/L of Naphthalene Acetic Acid, and 3 g/L of activated charcoal. Each treatment was replicated 3 times, with each replication comprising 3 samples, resulting in a total of 45 treatment units. The findings indicated that the Growmore leaf fertilizer medium, supplemented with 3 mg/L Benzyl Amino Purine and 0.1 mg/L Naphthalene Acetic Acid, together with 3 g/L activated charcoal, is the most effective medium for promoting banana shoot multiplication, thereby serving as a suitable alternative to synthetic medium.

1 Introduction

The Cavendish banana plant (*Musa acuminata* L.) belongs to the Musaceae family and originates from Southeast Asia. The advantages of Cavendish bananas include their larger fruit size and the presence of approximately 10 hands/bunches. This banana variety only has 2-3 shoots per parent plant, therefore requiring an appropriate alternative method to enhance its productivity [1]. The Cavendish banana plant (*Musa acuminata* L.) is one of the most crucial cultivated plants for communities residing in tropical and subtropical regions [2]. Cavendish is a highly sought-after type of banana that is currently in high demand in the international market. The characteristics of Cavendish bananas are defined by their

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attractive yellow skin, pale yellowish-white flesh, firm and sweet taste, as well as fine fruit fibers [3]. The Cavendish banana has the advantage of having a high economic value, especially as an export commodity. The high potential for Cavendish banana exports needs to be balanced with an increase in productivity [4]. The Cavendish banana is one of the most popular types of fruits worldwide. According to the Food and Agriculture Organization of the United Nations (FAO), the Cavendish banana is the most commercially grown in the world. It accounts for 47% of the total worldwide banana production, which is over 50 billion tons per year. The data indicates that the commodity of Cavendish bananas is highly sought for in the global market [5].

Conventional vegetative propagation techniques for banana plants possess intrinsic limitations, such as susceptibility to disease and the generation of a restricted quantity of suboptimal progeny. Every mature banana plant yields approximately 5-10 progeny. To address these vulnerabilities, it is necessary to find a solution to overcome them [6]. In vitro culture techniques, frequently recognized as tissue culture, can be used to overcome the limitations of traditional Cavendish banana propagation [7]. In vitro culture propagation provides a feasible method for the rapid bulk production of seedlings. Furthermore, tissue culture can preserve the exceptional traits of the original plant and generate disease-free seedlings [8].

Medium substitution using leaf fertilizer and liquid organic fertilizer has been carried out on Cattleya orchids [14], and Vanda tricolor [15], but has not been used for Cavendish banana shoot multiplication. Therefore, this study aims to determine the best substitution medium for Cavendish banana shoot multiplication.

2 Research method

This work employed an experimental approach utilizing a single-factor Completely Randomized Design, specifically a medium supplemented with cytokinins. The mediums utilized comprised New Dogashima medium (NDM), Murashige and Skoog (MS), Growmore Foliar Fertilizer, Sugihtani Liquid Organic Fertilizer, and DI Grow Liquid Organic Fertilizer, supplemented with BAP, NAA, and activated charcoal. Consequently, there are five treatment mediums. All treatment media contained 3 mg/L Benzyl Amino Purine (BAP), 0.1 mg/L Naphthalene Acetic Acid (NAA), and 3 g/L activated charcoal. Each treatment was conducted three times, with each repetition comprising three samples, resulting in a total of 45 treatment units.

2.1 Medium Preparation

The medium is prepared by dissolving the basic elements (NDM, MS, Growmore Leaf Fertilizer, Sugihtani Liquid Organic Fertilizer, DI Grow Liquid Organic Fertilizer) in sterile distilled water utilizing an Erlenmeyer flask. Add sugar, vitamins (if necessary), and activated charcoal, then stir until fully dissolved. All treatment media contain 3 mg/L BAP, 0.1 mg/L NAA, and 3 g/L activated charcoal. The pH of the media is adjusted to 6. If the pH is below 6, a few drops of 1 N NaOH are added, and conversely. If the pH exceeds 6, then many drops of 1 N HCl can be added. Agar is later added as a solidifying agent. The medium is transferred into a culture bottle and sealed with heat-resistant plastic. An autoclave is used to sterilize the medium for 20 minutes at 121 degrees Celsius and one atmosphere of pressure. The sterile medium is subsequently stored in the incubation room until required.

2.2 Explant Preparation and Inoculation

This study used sterile shoot explants of 4-month-old Cavendish bananas. Planting was done by preparing sterile distilled water with 3 drops of iodine in a petri dish. Then the shoot explants were taken from the culture bottle and soaked briefly in the iodine solution. The shoots were subsequently positioned on the lid of the petri dish, lined with filter paper. After that, the shoots were planted in each treatment medium as much as 1 shoot per bottle. The bottles that had been planted were then covered using aluminum foil tied using rubber and finally coated with plastic wrap. Each sample of the medium treatment was arranged on a culture bottle rack and sprayed with alcohol to prevent contamination and remain sterile.

2.3 Parameter Observed and Data Analysis

Data and observations were gathered from the time of planting until five weeks later. The observed parameters comprised the percentage of viable explants, the percentage of browning explants, the percentage of contaminated explants, shoot emergence, the number of shoots, shoot height, and the number of leaves. Data were examined by analysis of variance with a 5% significance level. If the analytical results were significant, the data were subsequently evaluated using the Duncan Multivariate Range Test with SAS software.

3 Results and discussion

3.1 Percentage of viable explants, contaminated explants and browning explants

The efficacy of in vitro cultivation is contingent upon the proportion of viable explants, the presence of contaminants, and the occurrence of browning. The results presented in Table 1 indicate that all treatment media resulted in a 100% survival rate of explants, except the DI Grow liquid organic fertilizer medium. Most treatments yielded a 100% survival rate for explants, as the explants utilized were sterile, specifically obtained from prior in vitro cultures. Furthermore, the explants were pre-treated with a povidone-iodine solution before planting.

Table 1. The effect of medium on the percentage of viable, contaminated and browning explants of Cavendish banana shoot at 5 weeks after planting

Medium Treatment	Percentage of viable explant (%)	Percentage of contaminated explant (%)	Percentage of browning explant (%)
New Dogashima	100 a	0 b	0 b
Murashige and Skoog	100 a	0 b	0 b
Growmore Foliar Fertilizer	100 a	0 b	0 b
Sugihtani Liquid Organic Fertilizer	100 a	0 b	0 b
DI Grow Liquid Organic Fertilizer	77.78 a	11.11 a	11.11 a

Povidone iodine is an antiseptic capable of removing any microbes responsible for nosocomial infections, including both gram-negative and gram-positive bacteria, in addition to antibiotic-resistant microorganisms [16]. Povidone iodine oxidizes bacterial respiratory enzymes, such as tyrosine, and inhibits microorganisms by iodinating amino acids, hence blocking protein synthesis and finally resulting in their mortality [17]. Table 1 indicates that the application of DI Grow liquid organic fertilizer medium resulted in a viable explant percentage below 100% due to browning and contamination of the explants. Browning in explants may arise from injuries sustained during the separation of shoot explants from their clusters. The wound situation causes the explants to emit phenolic compounds, which subsequently oxidize in the presence of oxygen, resulting in a brown discoloration of the explants. Contaminated explants, in addition to browning, contribute to an explant survival rate of less than 100%.

3.2 Time of shoot emergence

By including cytokinins in the medium, cell division in the explants will be stimulated, leading to their differentiation into shoots. Consequently, this will impact the development of shoots in each treatment. The results shown in Figure 1 demonstrate a considerable impact of different media types on the development of Cavendish banana shoots.

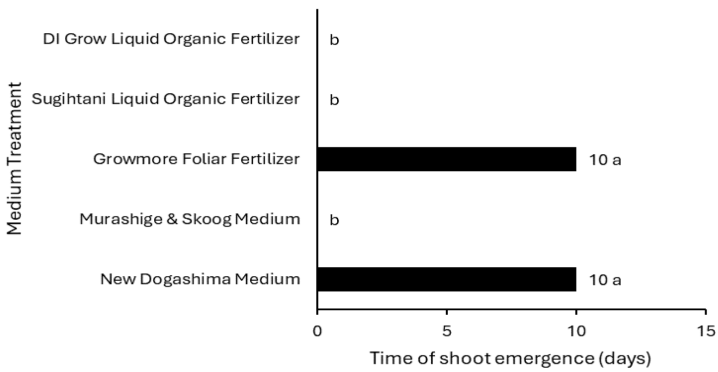

Fig. 1. The effect of medium on the time of shoot emergence of Cavendish banana shoots at 5 weeks after planting

The New Dogashima Medium treatment stimulated the growth of new shoots within 10 days of planting, which was comparable to the effects of the Growmore foliar fertilizer treatment. The nutrient composition of Growmore foliar fertilizer can effectively substitute the nutrients found in NDM. According to the findings of research [18], Growmore promotes the most rapid growth of shoots in sweet potato plants in vitro. As stated in reference [19], Growmore foliar fertilizer contains a comprehensive range of macro and micronutrients, including nitrogen, phosphorus, potassium, magnesium, and sulfur. The nitrogen concentration in Growmore media is 32%, which is sufficient to promote explant growth. According to [20], plants require nitrogen during the vegetative phase as it is essential for the development of cells, tissues, and plant organs. However, there has been a lack of new shoots formation in MS medium, as well as in Sugihtani and DI Grow liquid organic fertilizer media.

3.3 The number of shoots

The success of multiplication is determined by the proliferation of splits that exceed one in number from the planted explants. Figure 2 data indicates that the medium treatment did not substantially impact the number of shoots generated by Cavendish banana explants 5 weeks after planting. The New Dogashima Medium treatment exhibits a considerably higher shoot production compared to other treatments, but it does not show a statistically significant difference when compared to the Growmore leaf fertilizer medium treatment or other treatments. This demonstrates that alternative forms of media have the potential to substitute both artificial media, specifically NDM and MS, particularly in the case of leaf fertilizer media. Growmore leaf fertilizer contains a comprehensive range of nutrients that can substitute for NDM medium in promoting shoot growth. The presence of cytokinins in the medium further enhances shoot production. These results are following research [21] which shows that Growmore medium can replace MS medium for subculture of Vanda tricolor orchids, namely encouraging the increase in the number of leaves.

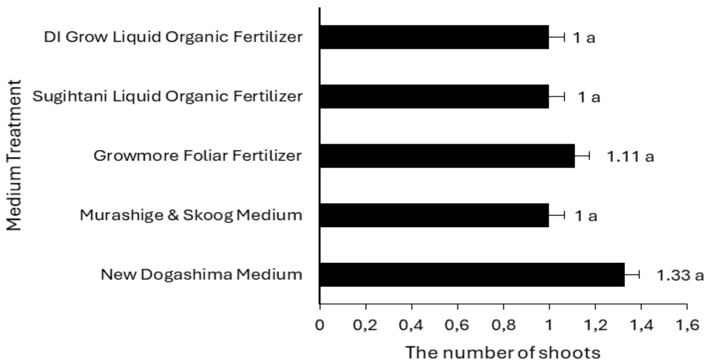

Fig. 2. The effect of medium on the number of shoots of Cavendish banana at 5 weeks after planting

3.4 The shoot height and the number of leaves

The height of a plant can increases because of cell division, leading to an increase in the number of cells. Additionally, the presence of growth regulators in the medium can stimulate cell elongation, further contributing to plant height. Table 2 demonstrates a considerable impact of the medium treatment on shoot height. In addition, subsequent testing revealed that the MS medium yielded the greatest shoot height (3.88 cm) in comparison to other treatments, although it did not exhibit a statistically significant difference from the NDM media (3.21 cm). However, the plant height achieved in the NDM medium did not show any significant variation compared to the treatment with Growmore leaf fertilizer and Sugihtani liquid organic fertilizer. Growmore fertilizer includes enough macronutrients, particularly nitrogen, which effectively promotes explant growth by increasing plant height. Growmore leaf fertilizer and Sugihtani liquid organic fertilizer can serve as alternatives to synthetic media, particularly NDM, to stimulate cell division and elongation, resulting in increased shoot height. This study demonstrates that Growmore leaf fertilizer and Sugihtani Liquid Organic Fertilizer has macro and micronutrients capable of substituting the nutrients found in NDM and MS media.

Table 2. The effect of medium on the shoot height and the number of leaves of Cavendish banana shoot at 5 weeks after planting

Treatment	The shoot height (cm)	The number of leaves
New Dogashima Medium	3.21 ab	2.56 a
Murashige and Skoog Medium	3.88 a	2.11 ab
Growmore Foliar Fertilizer Medium	2.08 bc	1.11 c
Sugihtani Liquid Organic Fertilizer Medium	2.48 bc	2.22 ab
DI Grow Liquid Organic Fertilizer Medium	1.69 c	1.33 bc

The use of leaf fertilizer to replace in vitro culture medium was also carried out by [14] on the *Cattleya pastoral Innocence* orchid plant, namely Hyponex leaf fertilizer was able to increase plant height and number of shoots. [22] also stated that the use of liquid organic fertilizer produced a better number of shoots and leaves on the protocorms of the *Vanda* *limbata* Blume X *Vanda tricolor* Lindl orchids, when compared to the Vacint and Went culture medium.

4 Conclusion and recommendation

The results of this study indicate that synthetic media can be substituted with alternative media derived from foliar fertilizer or liquid organic fertilizer to multiply Cavendish banana shoots. The optimal medium for promoting banana shoot multiplication and replacing synthetic media is the Growmore leaf fertilizer medium, supplemented with 3 mg/L BAP, 0.1 mg/L NAA, and 3 g/L activated charcoal. The usage of Growmore leaf fertilizer is highly recommended for the in vitro multiplication of Cavendish banana shoots.

Acknowledgment. The author expresses gratitude to the Research and Innovation Institute of Universitas Muhammadiyah Yogyakarta for their provision of research funding, as stated in decree number 19/LRI/II/2024. The author would like to express gratitude to Supriyatin Kartika Sari for assisting in carrying out this research.

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