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Research Article

BANANA SPROUTS INDUCTION IN VARIOUS MEDIA AND *IN VITRO* GROWTH REGULATORS

RINA SRILESTARI* AND SUWARDI
Faculty of Agriculture, Universitas Pembangunan Nasional Veteran Yogyakarta, 55283, Indonesia
*Corresponding Author: Email - rinasriletariupnvy@gmail.com

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Abstract: Kepok bananas have high economic value because of their delicious taste and high sugar content. The success of banana propagation with *in vitro* method is strongly influenced by the growing medium and the addition of growth regulators. The purpose of this research is to determine the appropriate media and the best concentration of growth regulators. The method of this research uses 2 factors complete randomized design, namely the media type (Murashige & Skoog, B5 Media and ½ MS Media + vitamin B5) and ZPT (NAA 0.5; 1.5;2.0 ppm & BA 1;2;3 ppm). The data obtained is analyzed for their diversity at 5% level. Then, the researchers conduct further tests using the Duncan Multiple Range Test (DMRT) at 5% level. The result shows that there is an interaction in the treatment of Murashige & Skoog media and NAA 1.5 ppm + BAP 3 ppm on the parameters when sprout is growing. The researchers also find that Murashige, Skoog, and B5 media are the best media for the number of sprouts, number of leaves and fresh weight of plantlets.

Keywords: *Banana, Various Media, Growth Regulator, In vitro*

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Introduction

The unpredictable productivity of bananas causes fluctuation in the export and import value of bananas. In 2019, world gross exports records that Indonesia exports 23 thousand tons of bananas. It decreases from the amount in 2018 which reaches 30 thousand tons [1]. However, the declining in export value still remains a high value for Indonesia's banana exports to banana importing countries. It made bananas become one type of fruits that has high potency to be developed. On the other hand, the high volume of banana exports from Indonesia to importing countries does not mean the banana cultivation process not experience various problems in Indonesia [2].

The conventional banana plant propagation technique is a problem that faced by farmers. The propagation which uses tuberous or plant saplings takes long time (10-18 months) and the amount produced is limited, namely in 1 (one) banana clump only produces 5-10 plant seeds per year [3]. This propagation method is inefficient because takes long time, not economical, and the seeds are not free from pests and diseases [4]. To overcome this problem, especially in providing banana seeds quickly, it is necessary to develop *in vitro* banana propagation technique.

Tissue culture is an appropriate alternative to conserve endangered plants or to multiply plants in large numbers and in fast time. Through tissue culture technique, plant propagation can be done in bulk, short time, the seeds produced are free from pests and diseases, and have the same nature with the parent [5].

MS, Gamborg, and VW media are basic media that have been widely used in in-vitro plant propagation. FAO [6] reports that the use of MS media which added 1 mg IBA + 2 mg BAP is able to support the best growth of vanilla plantlets. [2] obtains Raja Bulu Banana plantlets using modified MS medium with the addition of 1.0 mg NAA + 2 mg BAP. MS media with vitamin B5 contains 10 times more thiamine than vitamins in MS media. The content of nicotinic acid and pyridoxine hydrochloride in vitamin B5 is also 2 times more than vitamins in MS media. Thiamine is an essential vitamin for almost all plant tissue cultures because thiamine affects cell growth and development.

Plant propagation using tissue culture techniques is divided into several stages, namely: explant culture initiation, multiplication, rooting, and acclimatization. Both exogenous and endogenous growth regulators are very influential at the multiplication stage. In the multiplication stage, exogenous growth regulator that is often added to the media is cytokinin and auxin groups [7]. Type of auxin and cytokinin growth regulators that commonly used is NAA and BA. NAA is ZPT in auxin group which at certain concentration has function to initiate plant roots and stems [8].

Benzyl Adenine (BA) is a cytokinin group that has function to increase cell division, sprout proliferation, and sprout morphogenesis [9]. The use of BA at high concentrations increases the proliferation of banana plants. While at low concentrations, the sprouts grow at the base of the leaves and the adventitious sprout is absence [10]. BA is ZPT in cytokinin group which at certain concentration stimulates cell division and sprout multiplication [11]. The interaction of various media and ZPT in the form of NAA and BA are expected to be able to support the growth and development of banana multiplication in vitro. Thus, the main problem that is being the urgency (priority) to conduct this research is a complete study of various aspects about “Unti Sayang” Kepok banana *in vitro* propagation technique which is capable to produce large amounts of plantlets and can be mass reproduced in the laboratory with ZPT treatment on various media. The researchers expects in further study that it can be acclimatized.

Material and Methods

Materials and tools are 3 months old of "Unti Sayang" Kepok banana plantlet, 96% alcohol, rubbing alcohol, aluminium foil, MS media, B5 media and ½ MS media + vitamin B5, thiamine, NAA (Nafthalene Acetic Acid), BA (Benzyl Adenine), analytical scales, LAF, tweezers, scalpel, camera, and label. The research conducted at the Biotechnology Laboratory, UPN "Veteran" Yogyakarta. The laboratory research used completely randomized design with 2 factors, namely various media (Murashige & Skoog, B5 Media and ½ MS + vitamin B5) and the concentration of ZPT (NAA (0.5;1.0;1.5 ppm), BA (1; 2; 3 ppm).

Table-1 *Average banana sprout growth on various media treatments and concentrations of NAA and BA (days)*

Media	Concentrations of NAA and BA			Average
	NAA 0,5+BA 1 ppm (Z1)	NAA 1+BA 2 ppm (Z2)	NAA 1,5+BA 3 ppm (Z3)	
MS Media (M1)	12,07f	11,33g	10,33h	11,24
B5 Media (M2)	14,33cd	13,67d	12,67e	13,55
½ MS Media +vit B5 (M3)	15,33b	15,00bc	16,00a	15,44
Average	13,91	13,33	13	(+)

Description: The average value followed by same letter notation shows no significant difference at the 5% level of DMRT. (+) indicates an interaction.

Table-2 *Average Percentage of Life, Number of sprouts, Number of Leaves and Fresh Weight Banana plantlets on various media treatments and concentrations of NAA and BA (days)*

Media	Concentrations of NAA and BA			
	Percentage of Life	Number of sprouts	Number of Leaves	Fresh Weight (g)
MS Media (M1)	70,80a	3,42a	4,73a	2,52a
B5 Media (M2)	80,00a	3,09a	4,73a	2,40a
½ MS Media +vit B5 (M3)	86,67a	2,20b	3,87b	1,84b
Concentrations of NAA and BA				
NAA 0.5 ppm+BA 1 ppm(Z1)	82,40p	2,93p	4,44p	2,15p
NAA 1 ppm+BA 2 ppm(Z2)	81,37p	2,98p	4,49p	2,28p
NAA 1.5 ppm+BA 3 ppm(Z3)	86,70p	2,20p	4,58p	2,33p
Interaction	(-)	(-)	(-)	(-)

Description: The average value followed by the same letter notation shows no significant difference at the 5% level of DMRT. (-) indicates no interaction.

The medium was sterilized by autoclaving at a pressure of 20 psi with temperature of 121°C for 30 minutes. Each culture bottle was planted with one explant by removing a bunch of Kepok banana plantlets from the bottle, separated one by one, then planted on the media according to the treatment. The plantlets were placed in the incubation room for 10 weeks at 22°C.

Results and Discussion

Treatment of various media with concentrations of NAA and BA shows an interaction on the parameters during sprout growth. The combination of MS medium treatment and NAA 1.5 + BA 3 ppm makes sprouts grow faster than the other treatments. The interaction between the treatment of various media and the concentration of NAA and BA treatment indicates that there is a synergistic relationship between the two treatments. This can be explained that the high content of macro nutrients in MS media, namely ammonium, nitrate, and potassium, interacts with NAA 1.5 + BA 3 ppm in producing the fastest sprout. The content of NAA 1.5 and BA 3 ppm compensates the hormone content in the explants. The growth rate that occurs is due to the proper interaction between the endogenous hormone explants and the addition of exogenous hormones. The balance of auxin and cytokinin concentrations added to this media result in physiological processes in explants that effective to spur early sprout growth.

MS media is media with the most complete nutritional content compared to B5 media and ½ MS medium + vitamin B5. Complete content of nutrients such as macro nutrients, micro nutrients, and vitamins better support the growth of banana plantlets, including spurring the emergence of sprout. In addition, the synergistic relationship between NAA (auxin) and BA (cytokinin) is able to prevent apical dominance in cultured plantlets during the early stages of growth and development [12]. Cytokinins and auxins act antagonistically in regulating axillary sprout growth. Cytokinin enters the roots into the plant canopy system and works together with auxin, by indicating axillary sprout to start growing. So, the ratio of cytokinins and auxins is a critical factor in controlling axillary sprout growth. Through the diversity analysis results in this research, it can be seen that the treatment of MS and NAA 1.5 + BA 3 ppm media is the best treatment for growing banana plantlet sprout compared to other treatments.

It can be seen in the parameter of plantlet life percentage (%), there is no interaction and no significant difference in the treatment of various media or the concentration of NAA and BA treatments. This shows that the banana plantlets have sufficient nutrients in the media so that they maintain the survival of the plantlets. All NAA and BA concentrations are not significantly different in affecting the percentage of banana life. It is assumed that the growth response of cultured explants depends on the interaction and balance between the endogenous growth regulators in the explants and the exogenous growth regulators which is added to the media. According to [13], endogenous growth regulator is a factor to promote the growth process and explant morphogenesis.

This is also inseparable from the availability of nutrients in the media needed by the explants to grow in sufficient and balanced conditions. The parameter number of sprouts (fruit) shows that the treatment of ½ MS media + vitamin B5 is significantly different from the treatment of MS media and B5. The plantlets cultured on MS media and B5 media have significantly more sprouts compared to those cultured on ½ MS media + vitamin B5. Sprout growth is greatly influenced by the completeness of the elements contained in the media, especially Nitrogen (N). Other elements that needed to grow new sprouts are potassium (K), sulfur (S), iron (Fe), and zinc (Zn). The fulfilment of the N, K, S, Fe, and Zn elements in the explants will support explant sprout growth properly. From this research results, it can be concluded that the three types of media used have a good response in sprout growth, while MS and B5 media give better results.

All treatment concentrations of NAA and BA shows no significant difference in the number of sprouts parameters. This indicates that the increase in the number of sprouts is not influenced by the concentration of NAA and BA growth regulators. This result is caused by the auxin produced endogenously at the sprout of the plant which will be distributed in a polar manner inhibit the growth of lateral sprouts.

Apical dominance is caused by auxin which diffuses the sprout downward (polar) and is deposited on the lateral sprouts, this will inhibit the growth of lateral sprouts because the concentration is too high. High auxin concentrations will inhibit the growth of lateral sprouts close to the sprouts. According to [14], Benzyladenin (BA) is a synthetic growth regulator whose stimulatory power is not easily broken down by enzyme systems in plants. BA stimulates root formation and sprout formation.

In the leaf number parameter, the combination of various media treatments and the concentration of NAA and BA show no interaction. The leaves are the place where photosynthesis takes place, namely the formation of carbohydrates. [15] argue that leaf observation is needed as a growth indicator so that it explains the growth processes that occur such as in the formation of plant biomass. The more leaves that appear on the explants, the better the explant growth. The number of leaves in the growth of a plant plays a very important role, this is related to the ability of plants to conduct photosynthesis and other metabolic processes. A large number of leaves will produce a lot of photosynthate so that plant growth will be better.

The treatment of various media shows a significant difference in the treatment of MS media and B5 with ½ MS media + vitamin B5 in the number of leaves parameters. However, the NAA and BA concentrations are not significantly different from the number of leaves. It is suspected that in leaf formation, the addition of exogenous cytokinins will interact with endogenous auxins contained in the explants. This proves that plant growth *in vitro* is controlled by the balance and interaction between growth regulators, both those contained in the explants themselves (endogenous) and those absorbed from the media (exogenous). Besides being influenced by the presence of growth regulators, element contained

in the media such as N element plays an important role in the formation of leaves. N element forms fat, protein, and other organic compounds. The formation of many proteins is found in living cells, namely those that are actively growing. The elements needed to increase the number of leaves include calcium, phosphorus, iron, vitamin B1, vitamin B2, vitamin C, and niacin [16].

The use of media with auxin-cytokinin composition presumes at such concentrations, there has been a balance between cytokinins and auxins so that cell division occurs and stimulates leaf formation. This is in accordance with the opinion [17], that cell division is influenced by the ratio of cytokinins and auxins added to the media. In addition, the use of media with this concentration causes an interaction between NAA and BA as the exogenous hormones used with the phytohormones (endogenous hormones) contained in plants in order to obtain an appropriate amount for banana plantlet organogenesis in spurring leaf formation.

In the fresh weight parameter of the plantlets, there is a significant difference in the treatment of MS media (M1) with the treatment of various types of B5 media (M2) and ½ MS media + vitamin B5 (M3). Banana plantlet cultured on MS media has a significantly weighter than those cultured on B5 media and ½ MS media + vitamin B5. Fresh weight of plantlets occurs due to the accumulation of water absorbed which causes the cells in the explants become larger. This is related to the organic content and water absorbed in the tissue so it affects the fresh weight due to explant growth [18]. Fresh weight is the accumulated weight of water from respiration and metabolism of cells, especially protein, and the accumulation of photosynthetic products, in this case, is only obtained in the media through diffusion and contact between the media and the root surface [19]. The addition of NAA and BA growth regulators will stimulate cell division which ultimately affect the fresh weight of plantlets.

In terms of fresh weight, the treatment of various media has significant difference, this is caused by the formation of organs in banana plantlets. Almost every parameter of the Murashige & Skoog media gives the best response compared to other media. According to [20], the formation of new organs such as sprouts, roots, or leaves cannot be separated from cell division, enlargement, and elongation. In the process of cell division, the size, shape and volume of the explants will larger and affects the fresh weight of the explants [21,22].

Conclusion

There is an interaction on Murashige & Skoog media with NAA 1.5 ppm + BAP 3 ppm on the parameters when growing sprout. Murashige & Skoog media and B5 media are the best media for the number of sprouts, number of leaves and fresh weight of plantlets.

Application of research: For *in vitro* propagation of Kepok banana plants

Research Category: Plant Tissue Culture

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**Principal Investigator or Chairperson of research: Rina Srilestari
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