Available online at http://proceeding.rsfpress.com/index.php/ess/index
LPPM UPN "Veteran" Yogyakarta Conference Series
Proceeding on Engineering and Science Series (ESS)
Volume 1 Number 1 (2020): 295-300

Induction Of Banana Roots In Various Media And *In Vitro* Growth Regulators

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Abstract

This research aims to determine the appropriate media and the best concentration of growth regulators. Furthermore, the method used was a completely randomized design with two factors, namely the type of media (Murashige & Skoog, Media B5, and ½ MS + vitamin B5) and NAA (0.5; 1.0; 1.5 ppm) as well as BA (1; 2; 3 ppm.). The data obtained were analyzed for diversity and further evaluation using Duncan Multiple Range Test (DMRT) at a 5% level. Furthermore, the result showed that Murashige & Skoog media interacts with NAA 1 ppm + BAP 2 ppm on root length parameters. Considering this result, media B5 is the best for plantlet roots number and dry weight parameters, while NAA 1.5 ppm and BA 3 ppm were the best plantlets dry weight concentrations.

Keywords: banana, media types, growth regulators, *in vitro*

I.INTRODUCTION

Indonesia has a high level of biodiversity. Therefore it is called a country with mega biodiversity. One of the plants with a high level of biodiversity is the banana, which is a tropical plant originating from Southeast Asia, including Indonesia (3). The Ministry of Agriculture stated that banana cultivation in Indonesia contributes to the state revenue. Furthermore, according to the Central Statistics Agency (BPS), banana production in 2018 was 30,373 tons, an increase of 67 percent compared to 2017 of 18,192 tons (4). Therefore, Indonesia is one of the banana producing countries with more than 200 types, one of which is the *Kepok Unti Sayang* banana.

Kepok Unti Sayang banana has a high economic value due to its good and sweet taste with a sugar content of 300 Brix (12), and it is cultivated in the South Sulawesi area. Based on the Decree of

Minister of Agriculture no. 2084/Kpts/SR.120/5/2010, *Kepok "Unti Sayang"* is a superior national banana originating from South Sulawesi.

The technique of conventional banana propagation is becoming a problem that farmers have to face. The propagation using a hump or plant saplings takes a relatively long time of about 10-18 with a limited amount of production, namely in 1 (one) banana clump. It only produces 5-10 plant seeds per year (14). Furthermore, the propagation method is considered inefficient because it is time-consuming, is not economical, and the resulting seeds are not free from pests and diseases (1). Therefore, to overcome this problem, It is necessary to develop *in vitro* banana propagation techniques, especially in providing banana seeds quickly.

II.LITERATURE REVIEW

Tissue culture is an appropriate alternative to conserve endangered plants or to reproduce plants in large numbers and in a short time. Through this method, the plant propagation is performed in bulk with a relatively short time, and the seeds produced are free from pests and diseases with the same properties as the parent(2).

MS, Gamborg, and VW media are basic media widely used in the propagation of *vitro* plants. (6) It was reported that the use of MS media supplemented with 1 mg IBA + 2 mg BAP supports the best growth of vanilla plantlets. (9) Meanwhile, the plantlets are obtained using a modified MS medium with the addition of 1.0 mg NAA + 2 mg BAP.

In MS media with vitamin B5 containing 10 times more thiamin than vitamins, the content of nicotinic acid and pyridoxine hydrochloride in vitamin B5 was also 2 times higher. Thiamin is an essential vitamin for almost all plant tissue cultures because it affects the growth and development of the cell.

The common form of auxin and cytokinin growth regulators used are NAA and BA. NAA is a ZPT of auxin group that functions to initiate root and stem at certain concentrations (11). BA is a ZPT of cytokines group that functions to stimulates cell division and shoot multiplication at certain concentrations (15). Therefore, the interaction of various media and ZPT in the form of NAA and BA is expected to support the growth and development of banana plantlets *in vitro.*

Therefore, the main problem that becomes the urgency (priority) of conducting this research is a complete study of various aspects of *the Kepok Unti Sayang* banana in vitro propagation technique that is capable of producing large amounts of plantlets. It is propagated in the laboratory with ZPT treatment on various media and needs to be acclimatized in future studies.

III. RESEARCH METHODOLOGY

Materials and tools used include a 3-month-old plantlet of *Kepok "Unti Sayang"* banana, 96% alcohol, methylated spirits, aluminum foil, MS media, B5, thiamin, NAA, BA, analytical scales, LAF, tweezers, scalpel, camera, and label.

The research was conducted at Biotechnology Laboratory, UPN "Veteran" Yogyakarta, and the test was prepared using a completely randomized design with 2 factors, namely the type of media

(Murashige & Skoog, Media B5, and ½ MS + vitamin B5) and the concentration of NAA (0.5, 1.0, 1.5 ppm) as well as BA (1, 2, 3ppm). Furthermore, the medium was sterilized by autoclaving at a pressure of 20 psi with a temperature of 121^0C for 30 minutes. Each culture bottle was planted with one explant by removing a bunch of banana plantlets from the bottle, then separated one after another, and it was further planted on the media according to the treatment, as well as the plantlets were placed in the incubation room for 10 weeks at 22^0C.

IV.FINDING AND DISCUSSION

The treatment with NAA and BA concentration on various media showed an interaction with the root length parameter (cm). Furthermore, the combination of various media treatments with NAA and BA concentrations showed that M1Z2 treatment (MS medium and NAA 1 ppm + BA 2 ppm) had significantly longer roots compared to other treatments. These results showed that the combination of treatment is the most appropriate to stimulate root elongation, and there is a synergistic interaction between the MS medium and NAA as well as BA. MS medium (M1) has the highest nutritional content compared to B5 media and ½ MS medium + vitamin B5 and it also has the highest macro N (Nitrogen) nutrients content compared to others. N element plays an important role in supporting the vegetative growth of plants including in root growth and elongation. It is also one of the macronutrients that make up amino acids that is important in the metabolic process of plant growth. Therefore, MS media added with ZPT at the appropriate concentration facilitated root elongation.

Root formation is related to the endogenous auxin and cytokinin content in plant tissue, followed by the process of cell lengthening and enlargement. Besides the effects of endogenous auxins and cytokinins, the root formation is also influenced by light intensity. Meanwhile, low light intensity stimulates endogenous ZPT to work more actively in the process of root growth and development. According to (13), light conditions have a significant effect on improving the plantlet regeneration ability. Also, auxins in plant tissues work actively even in the dark. However, its synthesis occurs in light conditions. The increase in root length is due to the cell division process in the root tip meristem, followed by the process of cell elongation and enlargement. Therefore, auxin causes the expansion of the cortex, phloem, and cambium tissue thereby damaging sclerenchyma cells, spur the roots out and, cause root cells to elongate.

Table 1. The average length of banana roots in various media treatments and concentrations of NAA and BA (days)

Media Types	The Concentration 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)	
Media MS (M1)	9,07 b	10,52 a	8,76 bc	9,45
Media B5 (M2)	8,53 c	7,41 d	6,78 f	7,57
Media ½ MS+vit B5 (M3)	6,35 g	7,13 e	7,30 e	6,92
Average	7,98	8,35	6,52	(+)

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

The percentage parameter forming roots (%) showed no interaction, and there was no significant difference in the treatment of various media or the concentration of NAA and BA. This shows that the power of the banana plantlet has sufficient endogenous auxin in the media; therefore, the percentage of root formation remains high. All the concentrations of NAA and BA were not significantly different in affecting the percentage banana root formation, and it is assumed that the growth response of cultured explants depends on the interaction and balance between the endogenous growth regulators present in the explants and the exogenous added to the media. According to (7), an endogenous growth regulator is a factor that stimulates the growth process and morphogenesis of plants. Therefore, it is also inseparable from the nutrients available in the media needed by the explants to grow in sufficient and balanced conditions.

Table 2. Average Percentage of root formation, Total Roots and Dry Weight of Banana plantlets on various media treatments and concentrations of NAA and BA

Media Types	**The Concentration of NAA and BA**		
	Root Percentage	**Total Root**	**Dry Weight (g)**
Media MS (M1)	78,80 a	3,87 b	0,59 b
Media B5 (M2)	87,00 a	4,36 a	0,84 a
Media ½ MS+vit B5 (M3)	88,67 a	3,42 b	0,55 b
The Concentration of NAA and BAP			
NAA 0.5 ppm+BA 1 ppm(Z1)	87,40 p	3,84 p	0,64 q
NAA 1 ppm+BA 2 ppm(Z2)	86,37 p	3,76 p	0,60 q
NAA 1.5 ppm+BA 3 ppm(Z3)	86,60 p	4,04 p	0,83 p
Interaction	(-)	(-)	(-)

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

In the parameter of the number of roots, the treatment on MS medium (M1) was significantly different from that of B5 (M2) media and ½ MS medium + vitamin B5 (M3). Also, Banana plantlets cultured on MS medium had significantly more roots than ½ MS medium + vitamin B5. Vitamins have a catalytic function in the plant enzyme system and are required in small amounts. Furthermore, the vitamins contained in MS media consist of glycine (2.0 mg/L), niacin (0.5 mg/L), pyridoxine-HCl (0.5 mg/L), and thiamine-HCl (0.1 mg/L) (2). While in B5 media consist of niacin

(1.0 mg/L), pyridoxine-HCl (1.0 mg/L), and thiamine-HCl (10.0 mg/L). The composition of vitamins B5 and MS have similarities; however, the difference is the concentration, where vitamin B5 is higher than MS. Therefore, the provision of niacin (nicotinic acid) and pyridoxine (vitamin B6) increases the culture growth; in this case, it can also increase root growth in black orchids (8).

Treatment of NAA and BA concentrations showed no significant difference in the number of roots parameters. Auxin has a broad effect on growth since it stimulates and accelerates the root growth, as well as increases its quality and quantity. The more roots that are formed in the media added with NAA have shown that auxin activates the enzymes that play a role in cell components; therefore, when cell division begins, NAA stimulates the formation of cells quickly and subsequently initiates root formation. Apart from the auxins, growth regulators that are often used are the cytokinins, since it plays a role in increasing cell division and regulating the growth and development of plants. Therefore, explants with shoots will form roots because endogenous auxins are formed in plant shoots. In general, explants that have sprouted forms roots [5]

The dry weight parameter indicates no interaction. However, there was a significant difference between the treatment of media types ½ MS + vitamin B5 (M3) and in media MS (M1), and B5 (M2) was important. Furthermore, the dry weight of banana plantlets cultured on MS and B5 media was significantly higher than that of ½ MS media + vitamin B5. Dry weight is a description of the accumulation of organic matter and minerals that play an important role in plantlet growth. It is also the material weight after drying, as well as the accumulation of photosynthate (10). Therefore, the main factors affecting the total dry weight are the absorbed irradiation and the efficiency of energy utilization for CO2 fixation. Furthermore, it can also be caused by the ability of plants to bind energy through the photosynthetic process, therefore, increases the dry weight of plantlets.

V.CONCLUSION AND FURTHER RESEARCH

There is an interaction on Murashige & Skoog media with NAA 1 ppm + BAP 2 ppm in root length parameters. Therefore, media B5 is the best for the number of roots and dry weight of plantlets, while NAA 1.5 ppm and BA 3 ppm are the best concentrations in plantlet dry weight.

ACKNOWLEDGMENTS

The authors are grateful to the Ministry Research, Technology, and Higher Education for the assistance of Higher Education Fundamental Research Funds for this Research of UPN "Veteran" Yogyakarta.

VI. REFERENCES

Abebe, Z., A. Mengesha, A. Teressa & W. Tefera. 2009. Efficient *In Vitro* Multiplication Protocol for *Vanilla planifolia* Using Nodal Explants in Ethiopia. *African Journal of Biotechnology* 8 (24): 6817-6821.

Anisa, N., S. Reine, W. Asnawati. 2016. Pengaruh BAP Terhadap Multiplikasi Tunas Anggrek Hitam (*Coelogyne pandurata* Lindl.) Secara Kultur Jaringan. *Jurnal Hutan Lestari* 4(4): 591-595

Bella, D. R. S., Suminar, E., Nuraini, A., & Ismail, A. 2016. Pengujian Efektivitas Berbagai Jenis dan Konsentrasi Sitokinin terhadap Multiplikasi Tunas Mikro Pisang (*Musa paradisiaca* L.) secara *In Vitro*. *Jurnal Kultivasi,* 15(2), 74-80.

BPS. 2019. Statistik Konsumsi Pangan Tahun 2018. Pusat Data dan Sistem Informasi Pertanian. Sekretariat Jendral Kementerian Pertanian.

Elma,T.A. E.Suminar., & S.Mubarok, A.Nuraini. .2017. Multiplikasi tunas mikro pisang (*Musa paradisiaca* L*.)* 'Raja Bulu' secara *in vitro* pada berbagai jenis dan konsentrasi sitokinin. *Jurnal Kultivasi* 16 (3) .

Ferdous, M.H., A.A.M. Billah, H. Mehraj, T. Taufique, & AFMJ Uddin. 2015. BAP and IBA pulsing for in vitro multiplication of Banana cultivars through shoot-tip culture. *J.Bioscie*. *Agri. Research* 3(2): 87-95.

Indria, W., Mansyur, & A. Husni. 2016. Pengaruh Pemberian Zat Pengatur Tumbuh 2,4-Dikhlorofenoksiasetat (2,4-D) Terhadap Induksi Kalus dan Penambahan Zat Pengatur Tumbuh Benzyl Adenine (BA) Terhadap Induksi Kalus Embriogenik Rumput Gajah Varietas Hawaii (*Pennisetum purpureum* Cv. Hawaii) (*In Vitro*). *Jurnal Ilmu Ternak Universitas Padjadjaran* 18(1): 34-46.

Kartiman, R., S. Dewi, I. A. Syarifah, dan P. Agus. 2018. Multiplikasi *In Vitro* Anggrek Hitam (*Coelogyne pandurata* Lindl.) pada Perlakuan Kombinasi NAA dan BAP. *Jurnal Bioteknologi dan Biosains Indonesia* 30(5): 75-87.

Kasutjianingati, Poerwanto R, Widodo, Khumaida N & Efendi D. 2017. Pengaruh Media Induksi terhadap Multiplikasi Tunas dan Pertumbuhan Planlet Pisang Raja Bulu (AAB) dan Pisang Tanduk (AAB) pada Berbagai Media Multiplikasi. *J Agron Indonesia* 39 (3) : 180-187

Mengesha, A., A. Biruk, G. Elias, dan T. Tewedros. 2012. Micro-Propagation of *Vanilla planifolia* Using Enset (*Ensete ventricosum* (Welw, Cheesman)) Starch as a Gelling Agent. *Journal of Biological Sciences* 4(4): 519-525.

Pranata, M. G., Y. Ahmad, dan P. Bambang. 2015. Pengaruh Konsentrasi NAA dan Air Kelapa Terhadap Multiplikasi Temulawak (*Curcuma xanthorrizha* Roxb.) Secara *In Vitro. Journal of Sustainable Agriculture* 30(2): 62-68.

Poerba YS, Imelda M dan Martanti D .2012. Analisis Kestabilan Genetik Pisang Kepok 'Unti Sayang' hasil mikropropagasi dengan marka RAPD dan ISSR. *Berita Biologi*. 11: 275-282. doi: 10.14203/berita biologi.v11i2.497

Widiastoety, D. 2014. Pengaruh Auksin dan Sitokinin Terhadap Pertumbuhan Planlet Anggrek Mokara (Effect of Auxin and Cytokinin on the Growth of Mokara Orchid Plantlets). *J. Hort* 24(3):230-238

UNCTS (Uganda National Council for Science and Technology). 2007. The Biology of Bananas and Plantains. Uganda National Council for Science and Technology (UNCTS) with Program for Biosafety Systems (PBS

Yelnititis. 2014. Perbanyakan Tunas *Gyrinops versteegii* (Gilg.) Domke. *Jurnal Pemuliaan Tanaman Hutan* 8(2): 108-120.