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Research Article

Callus formation response from immature male flower explant of plantain banana (*Musa acuminata* x *Musa balbisiana* cv. Kepok) treated by 2,4-D and BAP

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ABSTRACT

Callus induction is an important step in indirect somatic embryogenesis. The aim of this study was to obtain an optimal medium for the callus formation of plantain bananas (cv. Kepok). The research was conducted from May to August 2022 at the Tissue Culture Laboratory of PT. ITCI Kartika Utama, Penajam Paser Utara District, East Kalimantan. The explant used an immature male flower of plantain banana (cv. Kepok). The experiment used a completely randomized design with two factors concentration of 2,4-D (1,2 and 4 ppm) and BAP (5, 10, and 15 ppm). The appearance of callus was monitored daily until it developed explants. On the final observation day, the percentage of explants with callus, as well as the color and texture of the callus, were assessed. The research findings suggest that the optimal medium for inducing callus in male banana flower explants was MS medium supplemented with 2 ppm 2,4-D and 5 ppm BAP. This medium resulted in calluses that exhibited a yellowish-white color and a compact texture.

Keywords: callus morphology; genetic improvement; plant growth regulator; tissue culture; triploid banana

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INTRODUCTION

Banana is an important fruit plant in the world. More than 400 million people in tropical and subtropical countries consume bananas as a staple food (Kennedy et al., 2019). Banana production in Indonesia is increasing every year. In 2020, banana production in Indonesia increased by 12.39% from 2019, reaching 8.18 tons (BPS, 2021). Bananas have a good taste and are affordable; the nutritional content of bananas also varies, including complex carbohydrates, fiber, protein, vitamins (A, C, and B6), iron, folate, magnesium, potassium, as well as various types of antioxidants such as flavonoids, saponins, lutein, and beta-carotene (USDA, 2018; Cafasso, 2020). The benefits of consuming bananas are to improve the digestive system, maintain heart health, prevent anemia, control blood sugar, counteract the effects of free radicals, and overcome nausea and vomiting in pregnant women, obstetrics complex carbohydrates are also beneficial for weight loss (Ratih & Qomariah, 2017; Costa et al., 2019; Oguntibeju et al., 2019). The various nutritional content and benefits of bananas make this plant have great potential to continue to be developed, including to maintain national food security and as an export fruit product from Indonesia.

Genetic improvement in cultivated bananas with a triploid genome, such as the kepok banana, is almost impossible to do conventionally due to pollen sterility and parthenocarpic fruit constraints. Conventional propagation can only produce a small number of seeds and a high chance of transmitting pathogens from the parent tree. The biotechnology approach through tissue culture can solve several problems of conventional banana breeding, such as sterility, long regeneration period, and limited genetic diversity (Ortiz & Swennen, 2014). Here are several ways in which tissue culture propagated materials might eliminate issues associated with conventional propagation: rapid multiplication, production of disease-free plants throughout the year, genetic uniformity, customization, and genetic modification. Tissue culture propagation methods on bananas have been widely used, such as for rapid multiplication (Fitramala et al., 2016; Imelda et al., 2018; Rai et al., 2019; Sadat et al., 2018), embryogenesis somatic (Natarajan et al., 2020), and anther culture (Gogoi et al., 2020; Kumar et al., 2017).

Plant regeneration through somatic embryogenesis is useful in genetically manipulating plants derived from somatic embryos. Plants propagated by somatic embryogenesis show a high level of genetic uniformity in several research results on various plants based on molecular markers, for example, in banana plants (Nandhakumar et al., 2018). Research on plant development through somatic embryos has been carried out on several types of banana explants, such as shoots, zygotic embryos, meristem organs, and female and male flowers. Of the several explants, the male flower obtained from the banana bud was the most responsive in regenerating somatic embryos, especially at the callus induction stage (Dwiyani et al., 2018; Morais-Lino et al., 2016; Natarajan et al., 2020).

Plant growth regulators (PGR) are factors supporting success in tissue culture. PGRs are essential tools in tissue culture as they enable the manipulation of plant growth and development, leading to successful propagation and regeneration of plants in a controlled environment. The nutritional needs of plant tissue in vitro culture have been fulfilled with basic media, but the addition of PGR can affect the further development of explants (Philips & Garda, 2019; Rademacher, 2015). PGR types and concentrations in tissue culture media must be appropriate to the desired direction of plant development. The most common PGRs added to media for embryogenic callus induction are 2,4-dichloro phenoxy acetic acid (2,4-D) and Benzyl Amino Purine (BAP). Based on Dwiyani et al. (2018), adding 2 ppm of 2,4-D to MS media induced callus from banana flower explants at 4.83 days after planting, with a percentage of explants forming callus of 67%. Callus induction from banana leaf explants has also been reported by Latunra et al. (2017), the results of this study showed that the best medium for callus induction on Barangan Merah banana leaves was MS which was added 2,4-D 2 ppm and BAP 3 ppm with a green callus color and a compact texture. Research on callus formation response of male kepok flower explants to growth regulators 2,4-D and BAP has never been reported. The selection of banana male flowers as explants in tissue culture is a strategic choice that combines the embryogenic potential of the floral meristem, high multiplication rates, genetic uniformity, disease-free propagation, ease of handling, and suitability for large-scale commercial production. This study aimed to study the response of banana callus formation on callus induction media to the addition of 2,4-D and BAP. It was designed to obtain optimal growth media information for callus induction from male flowers of kepok bananas.

MATERIALS AND METHODS

This research was conducted from May 2022 to August 2022 at the Tissue Culture Laboratory of PT. ITCI Kartika Utama, Penajam Paser Utara, East Kalimantan. The explants used male flowers of yellow kepok banana. The medium used Murashige and Skoog (MS) with 2,4-*dichloro phenoxy acetic acid* (2,4-D) and *benzyl amino purine* (BAP) addition.

Collecting, sterilization, and explants planting

The male banana bud flower was collected from the parent tree, which is about one year aged and bears fruit at the site of PT. ITCI Kartika Utama. Flowers were separated from the banana bunch, washed with detergent, and rinsed with water. Sheath of five male buds was removed in a laminar airflow cabinet then sprayed with 96% alcohol and sterilized with a bunsen burner. The flower was released from the petals, and then

carefully separated one by one. Incised ovaries sized ±8 mm were prepared as explants, and planted in culture bottles containing MS medium with plant growth regulator (PGR) according to treatment for callus induction.

The experiment used a completely randomized design with two PGR factors concentration of 2,4-dichloro phenoxy acetic acid (2,4-D) and benzyl amino purine (BAP). The 2,4-D consisted of three concentration levels (1, 2, and 4 ppm), and the BAP consisted of three concentration levels (5, 10, and 15 ppm). Thus, there were nine treatment combinations plus control media without PGR. Each treatment consisted of 4 bottles, each containing 3 explants per bottle. Explants observed in each treatment were 12 explants. Observations were made for 30 days after planting. The time callus appeared was observed every day until the explants had a callus. The percentage of explants with callus, as well as color and texture of callus, were observed on the last day of observation.

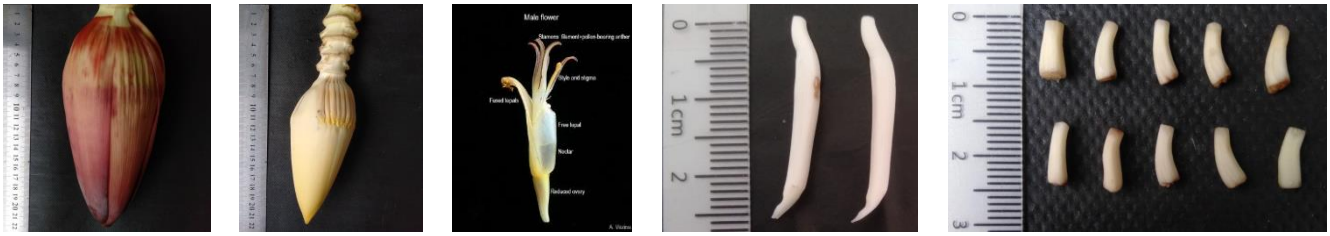
Data analysis

Qualitative data (callus color and texture) were presented as a description, while the quantitative data (time of callus appearance and percentage of explant with callus) were subjected to analysis of variance (ANOVA). If the results of the ANOVA show that the treatment has a significant effect, the differences between treatment means were studied using Tukey's honestly significant difference (HSD) test at the 5% level.

RESULTS AND DISCUSSION

Morphology of flower

Banana flowers shape inflorescence that is commonly known as banana heart or 'jantung pisang' in Indonesia; male flower is located at the upper part and lower part known as fingers of ovaries (Jones & Daniells, 2015; Wenas, 2017). This study used unopen male flowers which were located on the inside of the banana heart and still covered by bracts (Figures 1a and 1b). Each hand of the banana kepok consisted of 18-20 fingers. Figure 1c shows a finger with opened flower attached, and Figure 1d fingers with closed flower. According to Vezina et al. (2020), banana flower organs consist of stamens, flower petals, stylus and stigma, nectar, and reduced ovary. The explant used in this study was the reduced ovary which had been cut off at the base of the fruit as high as ± 8 mm (Figure 1e). Based on several studies that have been conducted, banana flower organs obtained from banana heart are responsive explants in callus induction (Dwiyani et al., 2018; Marlin et al., 2012; Morais-Lino et al., 2016).



(a) (b) (c) (d) (e)

Figure 1. Morphology of flower in Kepok banana. (a) banana bud covered by bracts, (b) banana bud when bracts are opened, (c) banana male flower organs (Vezina et al., 2020), (d) finger-shaped male flowers, and (e) reduced ovary which had been cut off at the base of the fruit as high as ± 8 mm.

Callus formation by 2,4-D and BAP

Figure 2 shows the effect of PGR addition to induction media on forming the explant callus of male banana flowers explant. Callus did not form on media without PGR, whereas on media added with 2,4-D and BAP, explants were able to form calluses 14 days after induction.

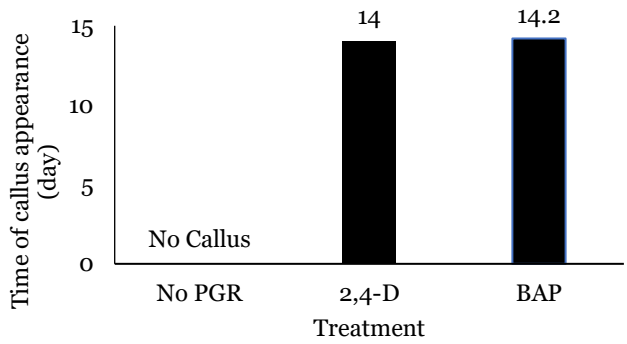
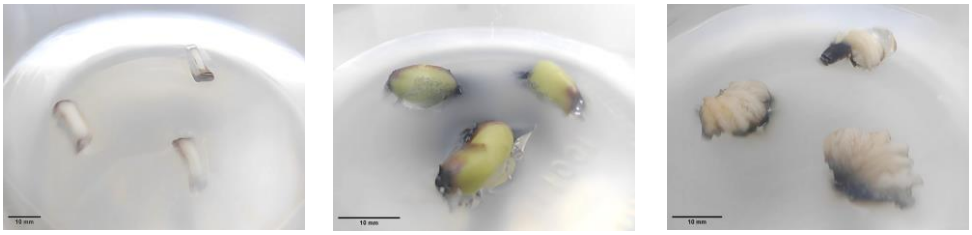


Figure 2. Time of callus formation of kepok banana from different PGR treatments.

The emergence of the callus of banana kepok flower explants in this study was initiated by the swelling and discoloration of the explants. At the beginning of planting, the explants were white (Figure 3a), and then at the first WAP (weeks after planting), the explants began to swell, and the color changed to green (Figure 3b). Subsequently, the explants started to develop to form a callus at 2 until 8 weeks after planting (Figure 3c). Callus formation consists of several stages, including induction, cell division, and differentiation. Waryastuti et al. (2017) revealed that callus induction is characterized by swelling or the appearance of clear white tissue-like water spots on the incision marks on the surface and then developing into small spheres and fine aggregates.



(a) (b) (c)

Figure 3. Growth and development of callus. (a) at the day of planting (bar: 10 mm), (b) 1 week after planting (bar: 10 mm), (c) 2 week after planting (bar: 10 mm).

The addition of 2,4-D combined with BAP in the induction medium affected the formation of the banana male flower explant callus. The results of ANOVA showed that the combination treatment of 2,4-D and BAP added to the media did not affect the time callus was formed but the percentage of explants with callus (Table 1). The explants started to form callus as soon as 12 days after induction on MS medium which was added 2 ppm 2,4-D and 10 ppm BAP. The highest percentage of explants with callus was produced on MS medium with the addition of 2 ppm 2,4-D and 5 ppm BAP, 79.17% of the explants formed callus. The use of a combination of auxin and cytokinin hormones can have a good effect on the growth and development of callus from banana flower explants. The research results of Dwiyani et al. (2018) revealed that MS media without 2,4-D could not induce callus from banana flower explants, while the addition of 2 ppm 2,4- D to MS media could induce banana flower callus at 4.83 days with a percentage of explants forming calluses by 67%.

The auxin group growth regulator often used to stimulate callus formation is 2,4-D. Generally, auxin plays a role in increasing cell division, cell elongation, and the formation of adventitious roots; adding auxin to the culture medium is also needed to increase somatic embryogenesis in cell suspension cultures (Marlin et al., 2012). Auxin 2,4-dichloro phenoxy acid (2,4-D) is often used in in vitro cultures because it is stable and not easily damaged by light or heat during sterilization (Indria et al. 2017). The addition of 2,4-D in the growth medium plays a significant role in inducing callus. Meneses et al. (2005) revealed that 2,4-D has a role in stimulating DNA hypermethylation which can

keep cells always in the mitotic phase; this causes callus formation to be optimal because cells are actively dividing.

Table 1. Combination effect of 2,4-D and BAP on kepok banana callus formation

Treatments		Time of callus appearance (days)	Percentage of explant with callus (%)
2,4-D (ppm)	BAP (ppm)		
	5	15.0	33.33e
1	10	15.0	69.17b
	15	17.5	56.94cd
	5	13.0	79.17a
2	10	12.0	71.67ab
	15	17.5	70.83ab
	5	14.0	66.67b
4	10	14.0	54.17d
	15	14.0	65.15bc
2,4-D x BAP		ns	*
CV (%)		19.91	21.11

Note: Numbers followed by the same letter are not significantly different based on the HSD at the level of α=5%.

BAP is a hormone from the cytokinin group which is relatively stable and plays an active role in cell division. The cytokinin hormone BAP plays a role in spurring cell division, cells which divide will then form a mass of unspecialized cells called calluses (Fitri & Armaini, 2019). Syahid et al. (2010) revealed that the addition of cytokinins could increase cell division in the process of cytokinesis, especially during RNA synthesis, so that the protein will stimulate auxin activity in cell division and form callus.

Dwiyani (2015) stated that callus is formed in culture media containing auxin and cytokinins in the same ratio or media containing 2,4-D. According to Waryastuti et al. (2017), embryogenic callus requires high auxin and low cytokinin concentrations for its growth. Research by Latunra et al. (2017) obtained a percentage of explants with callus of 77% on MS induction media added 2 ppm 2,4-D and 3 ppm BAP. The percentage of callus formation is influenced by many factors, including the type and condition of the explants, endogenous hormones in the explants, and the content of exogenous hormones or PGR added to the media (Mellisa & Febliza, 2018). Meanwhile, the inhibition of the process of callus formation from explants can be affected by an imbalance in the PGR concentration given, where the PGR concentration is too low and unable to induce callus, whereas if the concentration is too high, it will be toxic to explants (Khairan et al., 2021; Khaniyah et al., 2012).

Callus morphology

The results of the observation of callus color, seen in Figure 4, show that the callus color obtained in various treatments in this study was generally dominated by yellowish white. It indicates that the resulting callus has embryogenic properties. However, other indicators determine embryogenic properties, namely callus texture. The color and texture of the callus can determine the quality of the callus produced in response to the treatment used. Types of calluses based on their ability to regenerate are divided into embryogenic and non-embryogenic calluses. Embryogenic callus has a good ability to regenerate. An embryogenic callus is characterized by white or yellowish color, crumbly texture, and nodular (Xu et al., 2008). The color of the callus formed can indicate the level of development in the cell growth phase and determines the presence of cells that are still actively dividing or have died (Fauzy et al., 2016; Lizawati, 2012). The color of the callus can indicate whether or not the growth of the callus is good; a callus with white and yellow pigments indicates that the growth is good (Mahadi et al., 2014). Embryogenic callus can be seen from its color; a white or yellowish callus can potentially become a somatic embryo because it is still actively dividing (Sorentina et al., 2013).

The texture of callus is divided into three types: friable, compact (non-friable), and intermediate (Mahadi et al., 2016). The observation results can be seen in Figure 4; the callus texture formed in all treatments generally has a compact texture. Somatic embryo regeneration requires embryogenic callus, so the desired type of callus was not obtained

in this study. Callus texture that tends to be compact does not characterize embryogenic properties, whereas the characteristic of embryogenic callus has a friable texture.

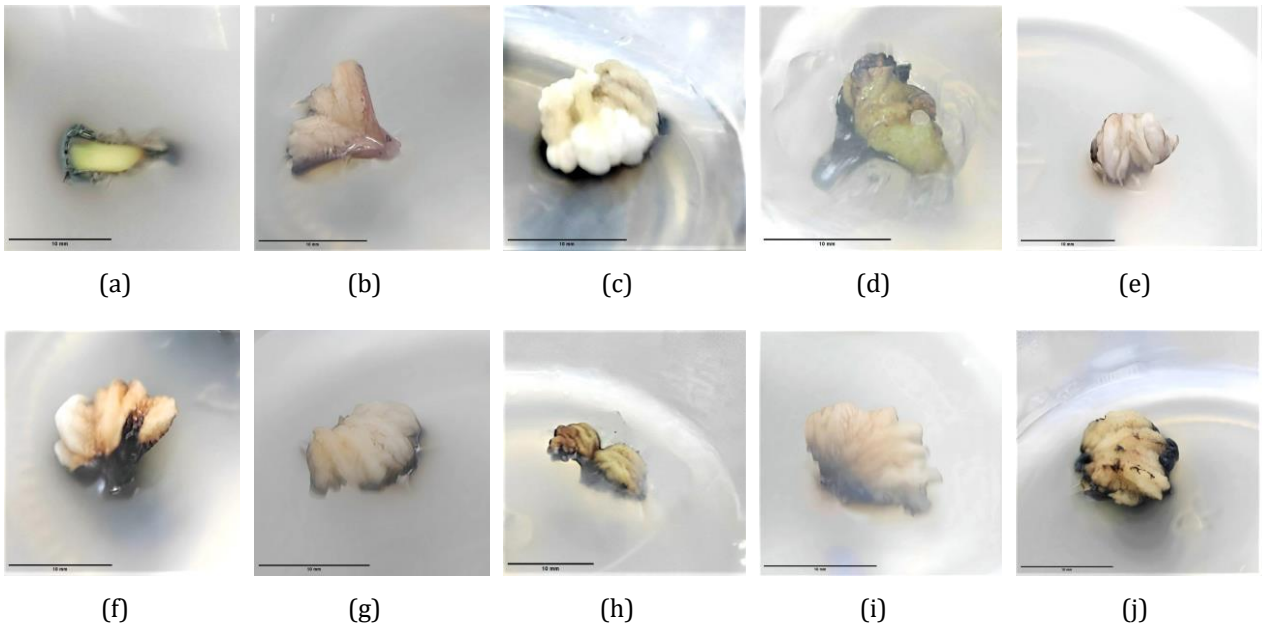


Figure 4. Callus texture and color from kepok banana. (a) MS without PGR, does not form callus; (b) MS + 1 ppm 2.4 + 5 ppm BAP, yellowish-white compact callus; (c) MS + 2 ppm 2.4 + 5 ppm BAP, yellowish-white compact callus; (d) MS + 4 ppm 2.4 + 5 ppm BAP, yellowish-white compact callus rather watery; (e) MS + 1 ppm 2.4 + 10 ppm BAP, yellowish-white compact callus; (f) MS + 2ppm 2.4 + 10 ppm BAP, yellowish-white compact callus; (g) MS + 4 ppm 2.4 + 10 ppm BAP, yellowish-white compact callus; (h) MS + 1 ppm 2.4 + 15 ppm BAP, yellowish-white compact callus; (i) MS + 2 ppm 2.4 + 15 ppm BAP, yellowish-white compact callus; (j) MS + 4 ppm 2.4 + 15 ppm BAP, yellowish-white compact callus; (bar: 10 mm)

The compact callus has a dense texture, non-friable, and has the potential to grow into organs such as roots or shoots (Wahyuni et al., 2014). In addition, the compact callus texture, which is hard and dense, is also considered suitable for secondary metabolite accumulation (Indah & Ermavitalini, 2013). Crumb callus has a lot of water content and is easily separated into small parts, so it is suitable for tissue augmentation and can potentially develop into somatic embryos (Sitorus et al., 2011). Mahadi et al. (2016) revealed that the formation of compact callus was caused by lignified callus, so it has a hard texture influenced by the use of cytokinin hormones which play a role in nutrient transport. In this study, a high concentration of the BAP cytokinin hormone (5-15 ppm) was used, so this might have resulted in the callus that formed tending to have a compact texture. In their research, Maulana et al. (2019) revealed that media added to high concentrations of auxin 2,4-D (2-3 ppm) could produce crumb-textured callus. Callus formation is an important step in banana tissue culture propagation, providing a foundation for the subsequent development of shoots, roots, and whole plants. It enables the efficient multiplication of plants, ensures genetic uniformity, and contributes to the production of healthy, disease-free banana plants for commercial cultivation.

CONCLUSION

Application of 2,4-D and BAP had an effect on the formation of callus of kepok banana flowers. Callus morphology showed yellowish-white in color and compact in texture. The optimal medium for callus induction of banana flower explants was MS supplemented with 2 ppm 2,4-D and 5 ppm BAP, with callus appearing time 13 days after induction and callus percentage 79.17%.

REFERENCES

BPS. (2021). *Production of fruits, 2018-2020.* BPS-Statistics Indonesia. https://www.bps.go.id/en/statistics-table/2/NjIjMg==/produksi-tanaman-buah-buahan.html

Costa, E. S., Franca, C. N., Fonseca, F. A. H., Kato, J. T., Bianco, H. T., Freitas, T. T., Fonseca, H. A. R., Neto, A. M. F., & Izar, M. C. (2019). Beneficial effect of green banana biomass consumption in patients with pre-diabetes and type 2 diabetes: a randomised controlled trial. *British Journal of Nutrition, 121*(12), 1365-1375. https://doi.org/10.1017/S0007114519000576

Dwiyani, R. (2015). *Plant tissue culture.* (In Indonesian.). Pelawa Sari

Dwiyani, R., Yuswati, H., & Utami. (2018). Callus induction on banana flower's explant using 2,4 dichlorophenoxyacetic acid (2,4-D). (In Indonesian.). *Jurnal Biologi Udayana, 22*(2), 66-70. https://doi.org/10.24843/JBIOUNUD.2018.v22.i02.p03

Fauzy, E., Mansyur, & Husni, A. (2016). The effect of using murashige and skoog medium (MS) and vitamin to callus regeneration of napier grass (*Pennisetum purpureum*) cv. Hawaii post on gamma radiation LD50 doses (in vitro). (In Indonesian.). *Students e-Journal UNPAD, 5,* 1-22.

Fitramala, E., Khaerunisa, E., Djuita, N. R., Sunarso, H., & Ratnadewi, D. (2016). In vitro culture of banana (*Musa paradisiaca* L.) cv. 'Kepok Merah' for rapid micropropagation. (In Indonesian.). *Menara Perkebunan, 84*(2), 69-75. https://doi.org/10.22302/iribb.jur.mp.v84i2.221

Fitri, M. N., & Armaini. (2019). The application of BAP (benzyl amino purin) on the mini grafting in durian (*Durio zibethinus* L.) nursery. *JOM Faperta Universitas Riau, 6*(2), 1-11.

Gogoi, M. B., Chetia, I., Sarmah, B. K., Nath, T., Bhowal, S., Dey, K., & Bhorali, P. (2020). Study of androgenesis in (*Musa balbisiana*) cv. bhimkol banana and in vitro regeneration of haploids using isolated microspore culture. *International Journal of Current Microbiology and Applied Sciences, 9*(9), 2555-2565. https://doi.org/10.20546/ijcmas.2020.909.320

Cafasso J. (2020). *Plantains: nutrition facts and health benefits.* Healthline. https://www.healthline.com/health/food-nutrition/plantain-nutrition-benefits

Imelda, M., Wulansari, A., & Sari, L. (2018). In vitro propagation of kepok banana var. unti sayang resistant to blood disease through shoot proliferation. (In Indonesian.). *Jurnal Bioteknologi & Biosains Indonesia (JBBI), 5*(1), 36-43. https://doi.org/10.29122/jbbi.v5i1.2626

Indah, P. N., & Ermavitalini, D. (2013). Callus induction of nyamplung (*Calophyllum inophyllum* Linn.) leaf on various combinations of 6-benzylaminopurine (BAP) and 2,4-dichlorophenoxyacetic acid (2,4-D). (In Indonesian.). *Jurnal Sains dan Seni Pomits, 2*(1), 2337-3520.

Indria, W., Mansyur, & Husni, A. (2017). The effect of plant growth regulators 2,4- dichlorofenoxyacetic (2,4-D) to callus induction and enrich of plant growth regulators benzyl adenine (BA) to embryogenic callus induction elephant grass hawaii varieties (*Pennisetum purpureum* cv. Hawaii) (in vitro). (In Indonesian.). *Students e-Journal UNPAD, 6*(1), 1-12.

Jones, D. R., & Daniells, J. W. (2015). Introduction to banana, abaca, and enset. In D. R. Jones (Ed), *Handbook of diseases of banana, abaca and enset* (pp. 1-40). CABI Oxfordshire.

Kennedy, G., Raneri, J. E., Stoian, D., Attwood, S., Burgos, G., Ceballos, H., Ekesa, B., Johnson, V., Low, J. W., & Talsma, E. F. (2019). Roots, tubers and bananas: contributions to food security. *Encyclopedia of Food Security and Sustainability, 3,* 231-256. https://doi.org/10.1016/B978-0-08-100596-5.21537-0

Khairan, K., Bustam, B. M., Yunita, Meilinda, R., & Muna, R. (2021). Effect of 2,4-D and BAP composition on callus formation of patchouli shoot explants (*Pogostemon cablin* Benth.) in vitro with horizontal and vertical cutting. (In Indonesian.). *BIOTROPIC The Journal of Tropical Biology, 5*(1), 9-20. https://doi.org/10.29080/biotropic.2021.5.1.9-20

Khaniyah, S., Habibah, N. A., & Sumadi. (2012). In vitro growth of god leaf callus (*Gynura procumbens* (Lour) Merr.) with a combination of 2,4-dichlorophenoyacetic acid and kinetin. (In Indonesian.). *Biosaintifika, 4*(2), 98-105.

Kumar, K. G., Krishna, V., Venkatesh, Shashikumar, R., & Arunodaya, H. S. (2017). Production of haploids plant from anther culture of *Musa paradisiaca* cv. 'Puttabale'. *International Journal of Research and Scientific Innovation, 4*(3), 1-5.

Latunra, A. I., Masniawati, A., Baharuddin, Aspiani, W., & Tuwo, M. (2017). Callus induction of red barangan banana (*Musa acuminata* Colla.) with 2,4-D and BAP combination by in vitro. (In Indonesian.). *Jurnal Ilmu Alam dan Lingkungan, 8*(15), 53-61.

Lizawati. (2012). The Use of 2,4-D and TDZ to induction embryogenic callus from apical bud explant of physic nut (*Jatropha curcas* L.). (In Indonesian). *Bioplantae, 1*(2), 75-87.

Mahadi, I., Wulandari, S., & Omar, A. (2014). Callus formation of rosella (*Hibiscus sabdariffa*) with addition of naftalen acetyl acid (NAA) and benzyl amino purin (BAP). (In Indonesian.). *Jurnal Biogenesis, 11*(1), 1-7.

Mahadi, I., Syafi'I, W., & Sari, Y. (2016). Callus induction of calamansi (*Citrus microcarpa*) using 2,4-D and BAP hormones by in vitro methods. (In Indonesian.). *Jurnal Ilmu Pertanian Indonesia, 21*(2), 84-89. https://doi.org/10.18343/jipi.21.2.84

Marlin, Yulian, & Hermansyah. (2012). Initiation of embryogenic callus formation of banana 'Curup' male bud culture supplemented with sucrose, BAP, and 2,4-D. (In Indonesian.). *Jurnal Agrivigor, 11*(2), 276-284.

Maulana, M. R., Restanto, D. P., & Slameto. (2019). Effect of 2,4-dichloropheoxyacetic acid (2,4-D) consentration on induction of sorghum plant callus (*Sorghum bicolor* (L.) Moench). (In Indonesian.). *Jurnal Bioindustri, 1*(2), 138-148.

Mellisa, & Febliza, A. (2018). The effect of NAA and IBA hormones on nibung explant (*O. tigillarium*) to the callus development duration (days). (In Indonesian). *Jurnal Bioterdidik, 6*(5), 1-6.

Meneses, A., Flores, D., Munoz, M., Arrieta, G., & Espinoza, A. M. (2005). Effect of 2,4-D, hydric stress and light on indica rice (*Oryza sativa*) somatic embryogenesis. *Revista de Biología Tropical, 53,* 361-368.

Morais-Lino, L. S., Santos-Serejo, J. A., Amorim, E. P., de Santana, J. R. F., Pasqual, M., & Silva, S. D. O. E. (2016). Somatic embryogenesis, cell suspension, and genetic stability of banana cultivars. *In Vitro Cellular & Developmental Biology, 52,* 99-106. https://doi.org/10.1007/s11627-015-9729-2

Nandhakumar, N., Kumar, K., Sudhakar, D., & Soorianathasundaram, K. (2018). Plant regeneration, developmental pattern and genetic fidelity of somatic embryogenesis derived *Musa spp*. *Journal of Genetic Engineering and Biotechnology, 16*(2), 587-598. https://doi.org/10.1016/j.jgeb.2018.10.001

Natarajan, N., Sundararajan, S., Ramalingam, S., & Chellakan, P. S. (2020). Efficient and rapid in-vitro plantlet regeneration via somatic embryogenesis in ornamental bananas (*Musa spp.*). *Biologia, 75,* 317-326. https://doi.org/10.2478/s11756-019-00358-0

Oguntibeju, O. O. (2019). Antidiabetic, anti-inflammatory, antibacterial, antihelminthic, antioxidant, and nutritional potensial of *Musa paradisiaca*. *Asian Journal of Pharmaceutical and Clinical Research. 12*(10), 9-13. https://doi.org/10.22159/ajpcr.2019.v12i10.34239

Ortiz, R., & Swennen, R. (2014). From crossbreeding to biotechnology-facilitated improvement of banana and plantain. *Biotechnology Advances, 32*(1), 158-169. https://doi.org/10.1016/j.biotechadv.2013.09.010

Philips, G. C., & Garda, M. (2019). Plant tissue culture media and practices: an overview. *In Vitro Cell.Dev.Biol.-Plant., 55,* 242–257. https://doi.org/10.1007/s11627-019-09983-5

Rai, I. N., Sudana, I. M., Astawa, I. N. G., Dwiyani, R., & Fitriani, Y. (2019). Direct organogenesis in vitro propagation of local Balinese banana with thidiazuron. *International Journal of Life Sciences, 3*(3), 32-40. https://doi.org/10.29332/ijls.v3n3.360

Ratih, R. & Qomariah, S. (2017). The vitamin B6 content of kapok banana an alternative to overcome nausea for pregnant women. (In Indonesian). *Jurnal Kesehatan Komunitas, 3*(5), 193-195.

Rademacher, W. (2015). Plant growth regulators: backgrounds and uses in plant production. *Journal of Plant Growth Regulation, 34,* 845–872. https://doi.org/10.1007/s00344-015-9541-6

Sadat, M. S., Siregar, L. A. M., & Setiado, H. (2018). Effect of IAA and BAP on micro shoot induction of banana shoot (*Musa paradisiaca* L.). (In Indonesian). *Jurnal Online Agroekoteknologi, 6*(1), 107-112.

Sitorus, E. N., Hastuti, E. D., & Setiari, N. (2011). Callus induction of Binahong (*Basella rubra* L.) by in vitro methods on different concentrations of MS Medium. (In Indonesian.). *Bioma, 13*(1), 1-7.

Sorentina, M. S. M., Haliani, Muslimin, & Suwastika, I. N. (2013). Callus induction of shallot (*Allium ascalonicum* L.) cv. Local Palu on MS medium with addition of 2,4-D and coconut water. (In Indonesian). *Online Jurnal of Natural Science, 2*(2), 55-63.

Syahid, S. F., Kristina, N. N., & Seswita, D. (2010). Effect of medium composition on calli growth and tannin content from leaves of west indian elm (*Guazuma ulmifolia* Lamk.) through in vitro culture. (In Indonesian.). *Jurnal Littri, 16*(1), 1-5.

USDA. (2018). *Food data central: plantains, green, raw.* https://fdc.nal.usda.gov/fdc-app.html#/food-details/168215/nutrients

Vezina, A., Gibbs, J., & Turner, D. W. (2020). *Banana flowers.* Musapedia, the banana knowledge compedium. https://www.promusa.org/Banana+flowers

Wahyuni, D. K., Prasetyo, D., & Hariyanto, S. (2014). The leaf culture development of *Aglaonema sp*. treated by combination of NAA, 2,4-D and BAP as growth regulators. (In Indonesian.). *Jurnal Bioslogos, 4*(1), 9-16. https://doi.org/10.35799/jbl.4.1.2014.4837

Waryastuti, D. E., Setyobudi, L., & Wardiyati, T. (2017). Effects of 2,4-D and BAP concentration levels on MS media for embryogenic callus induction of Java turmeric (*Curcuma xanthorrhiza* Roxb.). (In Indonesian.). *Jurnal Produksi Tanaman, 5*(1), 140-149.

Wenas, D. M. (2017). A review of pharmacology effect of the ambon and kepok banana waste. (In Indonesian.). *Sainstech Farma, 10*(1), 30-36.

Xu, C., Zou, R., Pan, X., & Chen, H. (2008). Somatic embryogenesis in banana (*Musa spp.*). *International Journal of Plant Developmental Biology, 2*(1), 52-58.