CALLUS INDUCTION OF ANREDERA CORDIFOLIA L WITH VARIOUS CONCENTRATION OF BENZYL AMINO PURIN (BAP)

Junairiah, Fatimah[1], Tri Nurhariyati[2], Nabilah Istighfari Zuraidassanaaz[3]

Article History: **Received:** 17.02.2023 **Revised:** 04.04.2023 **Accepted:** 18.05.2023

Abstract

Anredera cordifolia is a member of the Basellaceae family with great potential to be developed. Basellaceae contains antimicrobial and antioxidant properties that have been reported the secondary metabolites present in it include terpenoids, steroids, saponins, alkaloids, flavonoids, and tannins which make them useful for medicinal purposes. This study aimed to induce callus of A. cordifolia stems with growth regulator Benzyl Amino Purine (BAP) on Murashige and Skoog (MS) medium. The concentration of BAP used was 0 mg/L; 0.5 mg/L; 1.0 mg/L and 3.0 mg/L. The results obtained were able to produce callus formation. However, in control treatment, apart from forming callus, also forms leaves and roots. On 0.5 mg/L treatment, callus and leaves were formed. Callus was induced in the second week after planting. Callus textured crumbs and white. The highest average fresh and dry weight was the concentration of 1.0 mg/L BAP treatment.

Keywords: Callus, BAP, *Anredera cordifolia*

[1,2,3]Department of Biology, Faculty of Science and Technology, Universitas Airlangga

Email: junairiah@fst.unair.ac.id

DOI: 10.31838/ecb/2023.12.1.319

1. Introduction

Indonesia is country with tropical climate, so tropical diseases are easy to develop (Suwarto, 2014; Alamoudi, 2016). Tropical diseases are caused by several microorganisms, bacteria, fungi and viruses. Several diseases caused by these microorganisms are still a national health problem (Pujara et al., 2016). Recently, the prevention of tropical diseases has been using antibiotics, but this has caused various impacts, such as impaired immunity and microbial resistance (Alavijeh et al., 2012; Cai et al., 2017). Recently, attention has also been paid to antioxidants along with the COVID-19 pandemic. This is because antioxidants can act as an antidote to free radicals that enter and weaken the body's immune system, improve health, and produce anticancer activity. The use of synthetic antioxidants raises concerns about side effects (Sajid et al., 2012; Ramdani et al., 2018). Therefore, an effort to overcome this will explore plants as natural medicines (Burlec et al., 2017; Haque et al., 2012; Sulsen et al., 2017). Basellaceae contains secondary metabolites of terpenoids, steroids, saponins, alkaloids, flavonoids, tannins which have potential as antimicrobial and antioxidant ingredients (Singh et al., 2016; Harianja et al., 2020; Kumar et al., 2020; Alakinde et al., 2019). *Anredera cordifolia* is a member of Basellaceae family with great potential to be developed. There is not much information that reveals bioactive compounds and secondary metabolites that are responsible as antimicrobials and antioxidants. Likewise, efforts to produce compounds and secondary metabolites using callus culture methods have not been carried out.

Callus culture is one of the first steps in effective biotechnology due to suppress exploitation of plants from their natural habitats and also includes appropriate methods for large-scale biomass production and accumulation of secondary metabolite components (Sharif et al., 2016; Uyur et al., 2019). Based on the foregoing, research was conducted to produce antimicrobial and antioxidant material products from callus cultures of *Anredera cordifolia* as an effort to control tropical diseases in Indonesia.

2. Methods

This research was conducted at Plant Physiology Laboratory in the Department of Biology, Faculty of Science and Technology, Universitas Airlangga. The plant material used was the stem of *Anredera cordifolia*. Plant material was obtained from Pacar Kembang, Surabaya, Indonesia. The stem explants were washed with liquid detergent solution for five minutes and afterward rinsed three times under running tap water. After that, a sodium hypochlorite/clorox solution with a concentration of 10% was used for sterilization in Laminar Air Flow Cabinet (LAFC) for five minutes and then washed three times using sterile distilled water. Furthermore, the explants were washed with 70% alcohol solution and 0.01% Tween 80 for five minutes, then washed with sterile distilled water three times. The explants were then planted on Murashige and Skoog (MS) medium with growth regulator BAP with a concentration of 0 mg/L; 0.5 mg/L; 1.0 mg/L; and 3.0 mg/L, three replicates each. The explant planting was done in Laminar Air Flow Cabinet. The harvest process finished after four weeks of planting. The parameters observed were duration for formation of calluses, percentage of explants forming callus, morphology, and color.

3. Result and Discussion

The data consisted of recording various aspects including the duration for formation of calluses, percentage of explants forming callus, fresh weight, dry weight, and morphology of callus. Induction time and percentage of explants forming callus were shown in Table 1.

Induction Time and Percentage of Explants Forming Callus

Based on observations, results showed that all treatments including control could induce callus formation, so that the explants in all treatments were 100% forming callus. The callus formation usually begins with explant swelling on the wound accompanied by the appearance of white patches. Callus induction was initiated by thickening of explant on cut and in injured area. The thickening is result of interaction between explants and growth media so that the explants increase in size (Yelnitis, 2012). All of treatments showed that callus formation occurred on fourteenth day. Callus can be formed according to totipotency theory which states that each cell has ability to grow into a new individual in the right environment. Growth regulator BAP was used for callus induction in root, shoot, leaf and cotyledon explants in *Trachyspermum ammi* (Nasab, 2018). Callus induction from *Alstroemeria* stem explants using BAP at a concentration of 0.5 mg/L (Seyyedyousefi et al., 2013).

Table 1. Induction time and percentage of explants forming callus

No	Treatment	Induction time (day)	Percentage of callus (%)
1	Control	14	100
2	BAP 0.5 mg/L	14	100

3	BAP 1.0 mg/L	14	100
4	BAP 3.0 mg/L	14	100

Fresh Weight and Dry Weight from Callus

Callus is a group of parenchyma cells that are irregular in shape. This callus is formed on the cut wounds of plant organs. Callus usually comes from different plant organs, such as leaves, stems, nodes, internodes, roots, etc (Hartmann et al., 2002). The difference in callus growth rate is not only influenced by increasing in dividing rate of cell due to the influence of hormone administration but is also influenced by genetic conditions, tissue age, plant species and environmental factors including light, O_2 content, temperature, and humidity as well as the ability of tissue to absorb nutrients (Mahadi et al., 2016). The highest fresh and dry weight were found in 1.0 mg/L BAP treatment, which were 53.6 mg and 5.7 mg, respectively. (Table 2). Optimal cell division will lead to optimal callus growth so that it increases callus fresh weight, especially in the treatment given. Cytokinins are growth regulators derived from adenine that stimulate cell proliferation (Yang et al., 2021).

Table 2. Average Fresh Weight and Dry Weight of Callus

No	Treatment	Fresh weight (mg)	Dry weight (mg)
1	Control	36.4	3.8
2	BAP 0.5 mg/L	22.7	2.6
3	BAP 1.0 mg/L	53.6	5.7
4	BAP 3.0 mg/L	48.7	5.0

Callus Morphology

Plant tissue culture is one of biotechnology that plays an important role in propagation of various types of plants. This method has various advantages, namely obtaining plants in large quantities and free of pathogens. Plants from tissue culture have same characteristics as their parents, the time is relatively short and does not depend on the season. In addition, tissue culture biotechnology can be used as a method to produce secondary metabolites (Almemary, 2020). Based on observations on control treatment, callus differentiated to form leaves and roots, this was due to presence of endogenous hormones contained in callus (Table 3 and Figure 1). In the 0.5 mg/L BAP treatment, callus formed leaves (Table 3 and Figure 2). Administration of thidiazuron cytokinin at a concentration of 0.1; 0.5 and 1.0 mg/L can induce shoot formation in *Dendrobium lenale* explants (Hosen and Sukamto, 2008). In MS medium, the addition of BAP at a concentration of 2 mg/L and NAA at a concentration of 0.5 mg/L can initiate the formation of shoots on callus *Vigna radiata* (Rao and Patil, 2005).Various aspects determine the success of plant tissue culture such as composition of media culture, plant growth regulators, organic matter added according to growth and development phase, types of explants, etc. Murashige and Skoog medium contains mineral salts with relatively high concentrations which encourage organogenesis. Growth regulators are added to the culture medium to regulate the growth and development of explants. The comparison between growth regulators from outside and hormones produced by plants (endogenous) will determine the direction of culture development and the type of organ formation. The addition of growth regulators from outside will change level of endogenous growth regulators so that new levels of growth regulators will become a trigger factor for growth process and explant morphogenesis (Murashige, 1975; Gunawan, 1988; George, 1993).In all treatments, the resulting callus texture was crumb, and generally white (Table 3 and Figure 3). Callus crumb texture was also found in the callus of *Boerhaavia paniculata* grown on MS medium with addition of coconut water, glucose and 2,4D (Souza et al., 2014). Callus texture consists of two kinds, namely compact and crumb. While callus is compact, it is more difficult to separate because of its strong texture. Callus color is affected by light, pigmentation and explants. The green color indicates chlorophyll from callus. Generally, callus is green in color along with high cytokinin content. Cytokinins are able to activate protein synthesis and metabolism (Hendaryono and Wijayani, 1994; Wattimena et al., 1992).

Table 3. Callus morphology

No	Treatment	Texture	Callus color	Description
1	Control	Crumb	Yellowish white	Formed leaves and roots
2	BAP 0.5 mg/L	Crumb	White	Formed leaves
3	BAP 1.0 mg/L	Crumb	White	-
4	BAP 3.0 mg/L	Crumb	White	-

Figure 1. Callus morphology of Anredera cordifolia stem explants in the control treatment.

Figure 2. Callus morphology of *Anredera cordifolia* stem explants at 0.5 mg/L. BAP treatment.

Figure 3. Callus morphology of *Anredera cordifolia* stem explants at 1.0 mg/L. BAP treatment.

4. Conclusion

Based on the results of the research can be concluded that the treatment with growth regulator BAP 1.0 mg/L gave the best response by producing highest fresh weight and dry weight, which were 53.6 mg and 5.7 mg, respectively. The results of this study can be further developed to produce callus as secondary metabolite producer.

Acknowledgement
All of researchers would like to thank the Chancellor of Universitas Airlangga who has provided research funding assistance through the implementation of Universitas Airlangga internal research program (Faculty Excellence Research) in 2022, with contract number 1434/UN3.1.8/PT/2022 and Rector's Decree Number 251/UN3/ 2022.

5. References

Alakinde, T. A. A., & Ojo, M. F. (2019). Phytochemical and Antioxidant Properties of Forms of *Basella*. *International Journal of Vegetable Science. Vol. 25. Issue 5: 431-440.*

Alamoudi, M. Y. (2016). Pentingnya Eradikasi Penyakit Tropis di Indonesia. *International Journal of Tropical Medicine 11(4): 80-83.*

Alavijeh, D. K., Alavijeh P.K., & Shara, D. (2012). A Study of Antrimicrobial Activity of few Medicinal Herbs. *Asian Journal of plant Science and Research 2(4): 496-502*.

Almemary, A. M. S. (2020). Callus Induction and Differentiation. *The Future Journal of Agriculture*, *3*(1), 5-9.

Burlec, A. F., Arsene, C., Gille, E., Hăncianu, M., & Cioancă, O. (2017). Ornamental Asteraceae species as new sources of secondary metabolites. *Indian J Pharma Educ Res*, *51*, S425-S428.

Cai, Y., Zhang, Q., Fu, Y., Li, L., Zhao, N., Lu, A., ... & Jiang, M. (2017). Effectiveness of Chinese herbal medicine combined with antibiotics for extensively drug-resistant enterobacteria and nonfermentative bacteria infection: real-life experience in a

retrospective cohort. *BioMed research international*, *2017*.

Fazeli-Nasab, B. (2018). The effect of explant, BAP and 2, 4-D on callus induction of Trachyspermum ammi. *Potravinarstvo*.

George, E. F. (1993). *Plant propagation by tissue culture. Part 1: The technology* (No. Ed. 2). Exegetics limited.

Gunawan, L. W. (1988). *Technique of Tissue Culture*. Bogor Agricultural University.

Haque, M. A., Hassan, M. M., Das, A., Begum, B., Ali, M. Y., & Morshed, H. (2012). Phytochemical investigation of Vernonia cinerea (family: Asteraceae). *Journal of Applied Pharmaceutical Science*, (Issue), 79-83.

Harianja, S. H., Salni., & Subandrate. (2020). Cytotoxic, Antiproliferation, and Necrosis Effects of the n-Hexane Fraction Extract of Gendola Leaf (*Basella rubra* Linn.). *Journal of Scientific and Applied Chemistry 23(1): 8-13.*

Hartmann H. T., Kester, D. E., Davies, F. T., & Geneve, R. L. (2002). *Plant Propagation Principles and Practices 7 th ed. Perntice Hall Inc*. New Jersey. USA.

Hendaryono, D. P. S., & Wijayani, A. (1994). *Technique of Tissue Culture and Introduction of Plant Propagation. Kanisius*. Yogyakarta.

Hoesen, D. S. H., Witjaksono, W., & Sukamto, L. A. (2008). Induksi kalus dan organogenesis kultur in vitro Dendrobium lineale Rolfe. *Berita Biologi*, *9*(3), 333-341.

Kumar, S. S., Arya, M., Mahadevappa, P., & Giridhar, P. (2020). Influence of photoperiod on growth, bioactive compounds and antioxidant activity in callus cultures of Basella rubra L. *Journal of Photochemistry and Photobiology B: Biology*, *209*, 111937.

Murashige, T. (1975). Plant Propagation through Tissue Culture. *Ann rev Plant Physiol 25*: 135-166.

Pujara, P., Parmar, M., Rupakar, P., Asawa, K., & Patel, S. (2016). An introduction to Tropical Disease: A review article. *Int. J. Med. Microbiol. Trop. Dis*, *2*, 81-83.

Ramadani, A. P., Paloque, L., Belda, H., Tamhid, H. A., Augereau, J. M., Valentin, A., ... & Benoit-Vical, F. (2018). Antiprotozoal properties of Indonesian medicinal plant extracts. *Journal of Herbal Medicine*, *11*, 46-52.

Rao, S., & Patil, P. (2012). In vitro selection of salt tolerant calli lines and regeneration of salt tolerant Plantlets in Mung Bean (Vigna radiata L. Wilczek). *Biotechnology-Molecular Studies and Novel Applications for improved quality of human life*, 198-206.

Sajid, Z. I., Anwar, F., Shabir, G., Rasul, G., Alkharfy, K. M., & Gilani, A. H. (2012). Antioxidant, antimicrobial properties and phenolics of different solvent extracts from bark, leaves and seeds of Pongamia pinnata (L.) Pierre. *Molecules*, *17*(4), 3917-3932.

Seyyedyousefi, S. R., Kaviani, B., Dehkaei, N. P., & Salehzadeh, A. (2013). Callus induction in Alstroemeria using NAA and BAP. *European Journal of Experimental Biology*, *3*(5), 137-140.

Sharif, S., Kuitaz A., & Al Kayali, R. (2016). TLC Screening and Evaluation of antioxidant, antibacterial Activity of *Onopordon macrocephalum* by Bioautography Method. *Iranian journal of Pharmaceutical Sciences 12(2): 1-8.*

Singh, M., Kumari, R., Nandini, D., & Kotecha, M. (2016). Preliminary Phytochemical Screening of *Basella rubra* Linn. *Journnal of Pharmacognosy and Phytochemistry 5(4): 224-226.*

Souza, J. M. M., Berkov S., & Santos, A. S. (2013). Improvement of Friable Callus Production of *Boerhaavia paniculata* Rich and the Investigation of its Lipid Profile by GCMS. *Annals of Brazilian of Sciences 86 (3): 1015-1017.*

Sülsen, V. P., Lizarraga, E., Mamadalieva, N. Z., & Lago, J. H. G. (2017). Potential of terpenoids and flavonoids from Asteraceae as anti-inflammatory, antitumor, and antiparasitic agents. *Evidence-based complementary and alternative medicine*, *2017*.

Suwarto, S. (2014). Penyakit Tropik dan Infeksi pada Abad 21: Apakah Masih Relevan?. *Jurnal Penyakit Dalam Indonesia*, *1*(2), 77-78.

Wattimena, G. A., Gunawan L., Mattjik N.A., Syamsudin E., Wiendi, N. M. A., & Ernawati A. (1992). *Plant Biotechnology. Laboratory of Plant Tissue Culture*. Bogor agricultural University.

Yang, W., Cortijo, S., Korsbo, N., Roszak, P., Schiessl, K., Gurzadyan, A., ... & Meyerowitz, E. (2021). Molecular mechanism of cytokinin-activated cell division in Arabidopsis. *Science*, *371*(6536), 1350-1355.