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Callus Induction from Stem Explants of *Binahong* (*Anredera cordifolia (Ten.) Steenis*) with the Addition of Picloram and BAP

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Abstract

The manifold benefits of medicinal plants necessitate development techniques to produce high and diverse secondary metabolite content. Correspondingly, this study analyzed the effect of picloram and BAP concentrations to determine the optimal combination for inducing callus in *binahong*. The research employed a complete randomized design with two factors: the combination of picloram and BAP (0, 1, 2, and 3 ppm). Callus incubation occurred for 40 days after planting (DAP). Observed callus growth indicators included days to callus formation, percentage of callus explants, callus morphology (texture and color), and callus diameter. Days to callus formation and frequency of callus explants were analyzed utilizing the Kruskal-Wallis test and further tested by Dunn's test if a significant effect was observed. Results indicated that picloram significantly affected the parameters of days to callus formation and the percentage of callus explants. In contrast, BAP significantly affected the days to callus formation but not the percentage of callus explants. Specifically, picloram at concentrations of 1 ppm, 2 ppm, and 3 ppm significantly affected days to callus formation and the percentage of callus explants. Meanwhile, BAP at concentrations of 1 ppm, 2 ppm, and 3 ppm only significantly affected days to callus formation but not the percentage of callus explants. Additionally, the interaction between BAP and picloram significantly affected the days to callus formation and the percentage of callus explants. The resulting callus exhibited a compact texture with white, green, and brown colors. The most optimal concentration in all indicators was 0 ppm BAP + 1 ppm picloram, with callus formation occurring at 6 DAP, 100% explants forming callus, white overall callus with compact texture, and very high callus growth.

Keywords: callus, *binahong*, picloram, BAP

Introduction

Plants offer numerous benefits, particularly in the realm of medicine. An illustrative example of a medicinal plant is *binahong* (*Anredera cordifolia (Ten.) Steenis*), renowned for its potential in treating diseases such as typhoid, colitis, rheumatism, ulcers, gout, bruises, and hemorrhoids (Manoi & Balitro, 2009). *Binahong* harbors various secondary metabolite compounds, including saponins, triterpenoids, steroids, glycosides, and alkaloids, in its tubers, leaves, and stems (Astuti et al., 2011). Additionally, it contains flavonoids exhibiting antioxidant properties (Sukandar et al., 2013). Over time, there has been a burgeoning interest in utilizing natural materials as medicines, with the World Health Organization's Regional Office for South-East Asia (2020) reporting that 25% of contemporary pharmaceuticals originate from medicinal plants. Besides their therapeutic efficacy, medicinal plants

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offer economic advantages and safety, leading many to prefer herbal treatments over chemical drugs.

The diverse benefits of medicinal plants necessitate developing in vitro techniques for seedling propagation and producing callus enriched with secondary metabolites. Callus, comprising amorphous cells derived from dividing cells, consists predominantly of parenchyma cells (Slater et al., 2003). Callus culture techniques offer several advantages, including ease of morphological observation, time and labor efficiency, and enhanced production of secondary metabolites through manipulation of growth media (Stafford & Warren, 1991).

Successful implementation of in vitro culture techniques hinges on several factors, among which explant selection plays a crucial role. Optimal explants for callus culture are derived from young tissues exhibiting active division (Henuhili, 2013). Research by Damanik et al. (2017) demonstrated that stem explants yield the highest percentage of callus formation, prompting the selection of meristem tissue—specifically, shoots from *binahong* stems—as explants for this study.

In addition to explant selection, the choice of growth media, environmental conditions, and appropriate concentrations of growth regulators are paramount for optimal callus formation. Notably, auxins and cytokinins constitute crucial growth regulators in callus culture. Therefore, the present study employed picloram from the auxin group and BAP from the cytokinin group. Research by Gantait and Mahanta (2021) highlighted picloram's efficacy in inducing callus formation more rapidly than other auxins, such as 2,4-D and NAA. Picloram has been utilized in inducing callus in various plant species, including *Verbena bipinnatifida* (Ezzat, 2017), *Papaver rhoeas* (Aghaali et al., 2019), *Pogostemon cablin* (Wardani, 2020), and *Coryphantha macromeris* (Cabañas-García et al., 2021). Optimal concentrations of picloram for callus induction have been reported as 2 mg/L for *Verbena bipinnatifida* (Ezzat, 2017) and 1 mg/L for *Papaver rhoeas* (Aghaali et al., 2019).

The balanced concentration of auxin and cytokinin influences callus induction efficacy. A higher auxin concentration relative to cytokinin promotes callus and root formation, while a higher cytokinin concentration stimulates bud formation on explants. BAP, a cytokinin hormone, offers advantages such as economic viability, stability, and oxidation resistance (Maninggolang, 2018). BAP has been previously employed in callus induction of *Anredera cordifolia* (Sugiyarto & Kuswadi, 2014), *Piper betle L. var. Nigra* (Junairiah et al., 2019), and *Artemisia annua* (Purnamaningsih & Ashrina, 2011). Research by Damanik et al. (2017) indicated that concentrations of 0.5 mg/L and 1 mg/L of BAP, combined with auxin, effectively induce callus formation at high percentages.

Each plant species exhibits distinct nutritional requirements for optimal growth, necessitating appropriate growth regulators for explant cultivation and subsequent callus formation. Given the background above, it is evident that growth regulators play a pivotal role in callus induction in plants, including *binahong*. Hence, research on callus induction of *binahong* stem explants employing various concentrations of growth regulators picloram and BAP is warranted.

Research Methods

Plant

The research was conducted at the Laboratory of Plant Tissue Culture, Department of Biology, Universitas

Negeri Semarang, from December 2022 to April 2023. This study utilized *binahong* stems obtained from the Greenhouse of the Biology Laboratory at Universitas Negeri Semarang as explants. The stems selected were those located between the third and fifth leaves from the shoot. The independent variables in this study consisted of growth regulators of picloram and BAP at concentrations of 0 ppm, 1 ppm, 2 ppm, and 3 ppm. The dependent variables included days to callus formation, callus growth percentage, callus morphology, and callus diameter. Control variables included MS media, a temperature range of approximately 20-25°C, light intensity of 2,000 lux, and explants in the form of stem shoots.

Explant Sterilization

Stem shoot explants were first washed with running water, followed by a 10-minute wash with 2g/L detergent and rinsed with sterile distilled water. Subsequently, the explants underwent a 30-minute wash with 2g/L fungicide and were again rinsed with sterile distilled water. The stems were then sterilized in a Laminar Air Flow cabinet using a 10% sodium hypochlorite solution (*Bayclin*) for 15 minutes. Finally, the explants were rinsed once more with sterile distilled water.

Callus Induction

MS media containing a combination of picloram and BAP growth regulators was used to induce callus formation in this study. The sterilized explants were then cut into 1 cm segments and placed on the prepared media. Culture bottles containing the explants were covered with plastic wrap and stored in an incubation room at approximately 20-25°C. The explants were left to incubate for 40 days after planting.

Data Analysis and Statistical Test

The researchers recorded research data, including days to callus formation, callus induction frequency, callus morphology, and callus growth. Quantitative data such as days to callus formation and percentage of callus growth were analyzed non-parametrically using the Kruskal-Wallis test. Meanwhile, qualitative data such as morphology and callus diameter were analyzed descriptively.

Research Results and Discussion

Data on the days to callus formation were observed daily to determine when the callus began to form, represented as days after planting (DAP). A high percentage of callus formation characterizes successful callus growth. In this regard, the percentage of callus explants was observed at 40 DAP and calculated by dividing the number of formed explants by the number of planted explants, then multiplied by 100%. Callus morphology, including color and texture, was also observed at 40 DAP, with color data recorded based on the Munsell Color Chart and callus texture depicted descriptively. Detailed data on the average time to callus formation, percentage of callus explants, and callus morphology are presented in Table 1.

Normality and homogeneity tests were conducted on the data for days to callus formation and callus induction frequency. The significance levels were less than 0.05, so the data were analyzed non-parametrically utilizing the Kruskal-Wallis test. The results of the Kruskal-Wallis test for the combination of picloram and BAP indicated a significance value of 0.00 < 0.05, suggesting that adding this combination of growth regulators affected the days to callus

formation. Further analysis using Dunn's test revealed that treatments with 0 ppm BAP + 1 ppm picloram, 0 ppm BAP + 2 ppm picloram, and 0 ppm BAP + 3 ppm picloram exhibited the fastest average days to callus formation.

Both BAP and picloram treatments were subjected to Kruskal-Wallis test and Dunn's test to ascertain the significant effect of growth regulators on the parameter of days to callus formation. The results indicated a significance value of 0.00 < 0.05 for both BAP and picloram treatments, demonstrating their significant effects. Dunn's test results further revealed that concentrations of 1 ppm, 2 ppm, and 3 ppm for both BAP and picloram significantly differed from 0 ppm, indicating their efficacy in inducing callus formation on *binahong*.

Table 1
Average days to callus formation (days), callus induction frequency (%), texture, and color of callus

Growth Regulator (ppm)		Average Days to Callus Formation (Days)	Average Callus Induction Frequency (%)	Texture	Color
BAP	Picloram				
0	0	13.33 ± 7.10^{d}	30 ± 27.39^{c}	Compact	Green 5GY 8/4
	1	6.00 ± 0.00^{a}	100 ± 0.00^{a}	Compact	White N9
	2	6.00 ± 0.00^{a}	100 ± 0.00^{a}	Compact	Brown 10YR 7/4
	3	6.00 ± 0.00^{a}	100 ± 0.000^{a}	Compact	White N9
1	0	10.33 ± 2.89^{c}	60 ± 54.78^{b}	Compact	Green 7.5GY 9/6
	1	6.80 ± 0.45^{a}	100 ± 0.00^{a}	Compact	Brown 5YR 4/3
	2	6.80 ± 0.45^{a}	100 ± 0.00^{a}	Compact	Brown 5YR 7/2
	3	7.00 ± 0.00^{a}	100 ± 0.00^{a}	Compact	White N9
2	0	9.50 ± 2.89^{bc}	80 ± 44.72^{ab}	Compact	Green 5GY 9/2
	1	6.80 ± 0.45^{a}	100 ± 0.000^{a}	Compact	White N9
	2	7.00 ± 0.00^{a}	100 ± 0.000^{a}	Compact	White N9
	3	6.80 ± 0.45^{a}	100 ± 0.000^{a}	Compact	White N9
3	0	7.50 ± 0.58^{a}	80 ± 44.72^{ab}	Compact	Green 5GY 9/6
	1	6.60 ± 0.55^{a}	100 ± 0.000^{a}	Compact	White N9
	2	6.80 ± 0.45^{a}	100 ± 0.000^{a}	Compact	Brown 7.5YR 6/3
	3	6.60 ± 0.55^{a}	100 ± 0.000^{a}	Compact	White N9

Notes: superscript letters indicate no significant difference if the notation is the same, while different letters indicate a significant difference.

The Kruskal-Wallis test results for the combination of picloram and BAP demonstrated a significant effect with a significance value of 0.00 < 0.05. Further analysis indicated an interaction between these two growth regulators, causing a significant effect with a significance value of 0.00. The treatment with a 100% percentage of callus explants was identified as the most effective in terms of callus explant percentage.

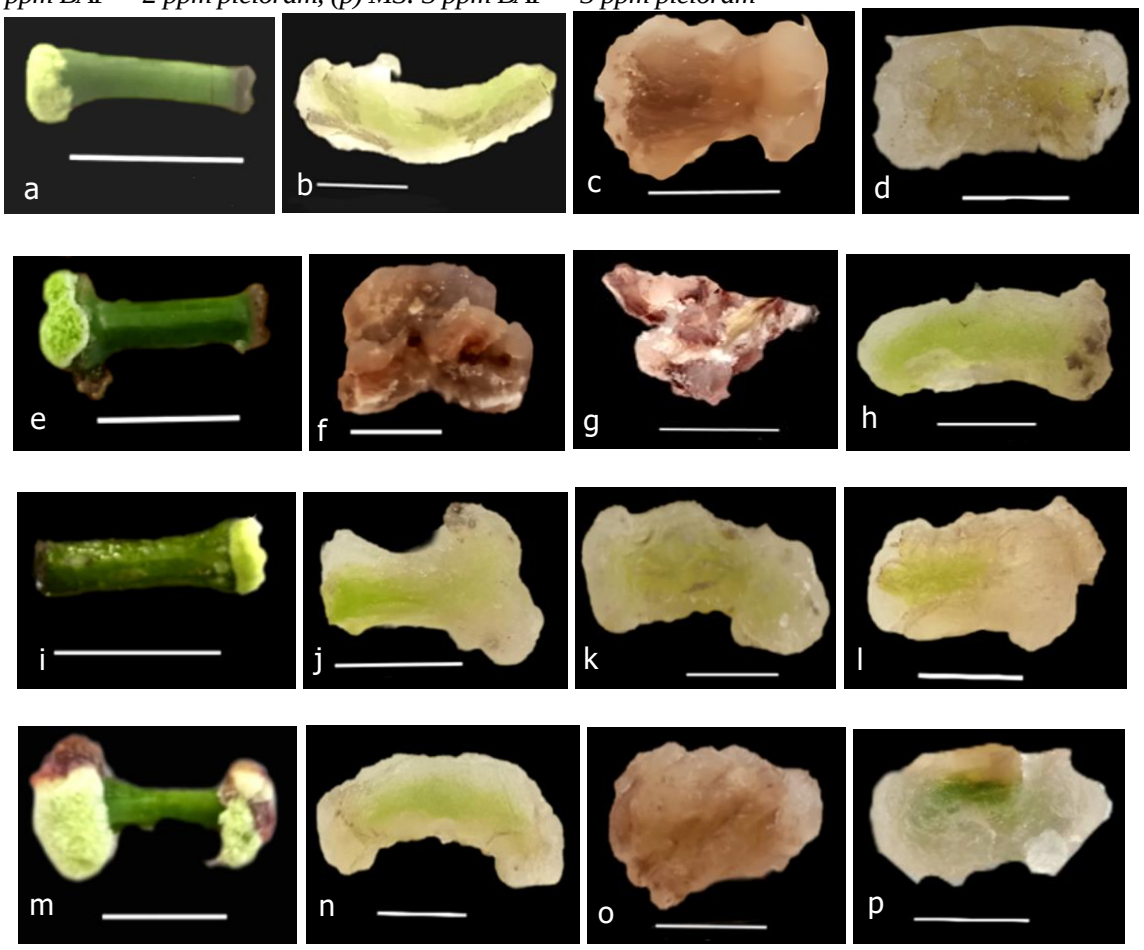
Both BAP and picloram treatments were subjected to Kruskal-Wallis and Dunn's tests. In the Kruskal-Wallis test, the BAP treatment showed a significance value of 0.187 > 0.05,

suggesting no significant effect on inducing *binahong* callus. Conversely, the picloram treatment yielded a significance value of 0.00 < 0.05, indicating a significant effect in inducing callus. Furthermore, Dunn's test results confirmed the efficacy of picloram treatments at concentrations of 1 ppm, 2 ppm, and 3 ppm with a significance value of 0.00 < 0.05.

Callus morphology, including texture and color, was visually observed at 40 DAP. Figure 1 below illustrates the morphology of the *binahong* callus after 40 days of incubation with the addition of BAP and picloram growth regulators.

Figure 1

Morphology of binahong *callus with the addition of growth regulators of picloram and BAP at 40 DAP: (a) MS: 0 ppm BAP + 0 ppm picloram, (b) MS: 0 ppm BAP + 1 ppm picloram, (c) MS: 0 ppm BAP + 2 ppm picloram, (d) MS: 0 ppm BAP + 3 ppm picloram, (e) MS: 1 ppm BAP + 0 ppm picloram, (f) MS: 1 ppm BAP + 1 ppm picloram, (g) MS: 1 ppm BAP + 2 ppm picloram, (h) MS: 1 ppm BAP + 3 ppm picloram, (m) MS: 3 ppm BAP + 1 ppm picloram, (n) MS: 3 ppm BAP + 1 ppm picloram, (o) MS: 3 ppm BAP + 2 ppm picloram, (p) MS: 3 ppm BAP + 3 ppm picloram*



Notes: Scale bar (—) = 1 cm

Observations of callus morphology revealed that all textures produced were compact. In this regard, compact-textured callus has a dense structure and contains a significant amount of water (Manuhara, 2001). It generally exhibits small cell size with dense cytoplasm, large nucleus, and a

high content of wheat starch (carbohydrate) (Dodd, 1993). According to Indah and Ermavitalini (2013), compact callus texture can accumulate more secondary metabolites. Research by Latif et al. (2019) produced predominantly compact-textured callus using a combination of picloram and BAP growth regulators on durian explants.

The color of the callus produced in the present study ranged from green to white and brown. The presence of cytokinin (BAP) in the culture medium, as noted by Marthani et al. (2016), induces green callus formation due to chloroplast development and gene expression stimulated by cytokinin under bright light conditions (Chen, 1989). Cytokinin, in addition to the media, can activate metabolic processes and protein synthesis, inhibiting the breakdown of chlorophyll grains (Wattimena, 1991). The green color observed in treatments with MS: 1 ppm BAP + 0 ppm picloram, MS: 2 ppm BAP + 0 ppm picloram, and MS: 3 ppm BAP + 0 ppm picloram, along with the white callus color in other treatments suggested that cytokinin facilitated chlorophyll formation, while auxin suppressed it. The decrease in chlorophyll content is attributed to disturbances in carbohydrate metabolism, the primary substance for its synthesis (Audus, 1963).

Brown callus formation results from the browning process, involving the oxidation of phenolic and toxic compounds in plant tissues, leading to callus death (Kiong et al., 2008). Browning is suspected to occur due to decreased sucrose levels in the media, which activates phenol oxidase enzymes upon picloram addition. Khosroushahi et al. (2011) identified polyphenol oxidase and peroxidase enzymes converting phenolics into harmful compounds. Browning can be mitigated by thorough rinsing after sterilization to remove phenolic compounds, incubating in the dark, or adding activated charcoal to the media (Hartman et al., 2002; Kiong et al., 2007).

Table 2
Growth of binahong *callus at 40 DAP*

Growth Regulator (ppm)		Callus Diameter	Media Color
BAP	**Picloram**		
0	0	+	White
	1	++++	White
	2	+++	White
	3	+++	White
1	0	+	White
	1	+++	Pink
	2	++	Pink
	3	++++	White
2	0	+	White
	1	++++	Pink
	2	++++	Pink
	3	++++	White
3	0	+	Pink
	1	++++	White
	2	+++	White
	3	++++	White

Notes: ++++ : callus diameter of 1.6 – 2.0 cm; +++ : callus diameter of 1.1 – 1.5 cm; ++ : callus diameter of 0.6 – 1.0 cm; + : callus diameter of 0.1 – 0.5 cm

Observations regarding the days to callus formation parameter indicated that treatments with 0 ppm BAP + 1 ppm picloram, 0 ppm BAP + 2 ppm picloram, and 0 ppm BAP + 3 ppm picloram exhibited the fastest callus emergence at 6 days after treatment (DAT). Without growth regulators, control media stimulated callus formation, taking the longest average time of 13.33 DAT. This finding aligns with the research by Sugiyarto and Kuswandi (2014) on callus induction in *binahong*, where control media also induced callus formation. Furthermore, treatments with MS: 1 ppm BAP + 0 picloram, MS: 2 ppm BAP + 0 picloram, and MS: 3 ppm BAP + 0 picloram stimulated callus growth, with average formation times successively at 10.33 days, 9.5 days, and 7.5 days.

Regarding the percentage of callus explants, all treatments with picloram concentrations of 1 ppm, 2 ppm, and 3 ppm produced callus with a maximum percentage of 100%. It demonstrated that appropriate growth regulators significantly affected callus induction. The appearing callus initially emerged at the tip of the explant and gradually covered its entire surface. According to Doods and Roberts (1995), initial callus characteristics include swelling of the explant followed by a roughened structure, which becomes shiny upon exposure to light, indicating changes in the explant's surface. The interaction between the explant and growth regulator facilitates nutrient absorption, resulting in explant swelling.

The variation in growth rates among treatments depended on the tissue's ability to absorb available nutrients. Auxin and cytokinin, at appropriate concentrations, promoted cell enlargement and division. The explants' physiological conditions also affected the callus growth rate disparity. Auxin plays multiple roles, such as increasing osmotic pressure and cell permeability to water, reducing pressure on the cell wall, promoting protein synthesis processes, and facilitating cell wall development and plasticity. Hence, the release of H^+ ions toward the cell wall by auxin enhances cell wall plasticity, increasing cell size (Wardani et al., 2004; Merthaningsih et al., 2018). Meanwhile, cytokinin is essential for stimulating plant tissue division (Taiz & Zeiger, 2010).

Auxin added to the growth media regulated cell proliferation, particularly in transitioning from the G1 to the S phase. During the G1 phase, auxin induced the expression of cyclin-D genes, such as cycD 3;1 and CDKA;1, facilitating the assembly of the CDKA/CYCD complex. Auxin addition suppressed KRP (Kip-Related Proteins) transcripts, namely KRP1 and KRP2, which inhibited CDK activity, thus maintaining the phosphorylated CDKA/CYCD complex. This activation further phosphorylated the RBR (Retinoblastoma-Related Repressor) protein, releasing the Adenovirus target E2FA/B (E2 Promoter-binding Factor A/B) and the DPA (Dimerization Partener A) complex. Through post-transcriptional regulation, auxin stimulated the degradation of F-box SKP2A (S Phase Kinase-Associated Protein 2A) by the E3 ubiquitin ligase complex SCF (Skp, Cullin, and F-box complex), indirectly stabilizing the E2FC (E2 Promoter-Binding Factor C) and DPB (Dimerization Partner B) complex, leading to gene expression suppression in the S phase. However, most data suggested that auxin acted as a permissive signal during DNA synthesis (G1/S transition) and in the G2/M transition to complete the synthesis process (Wang & Ruan, 2013).

In addition to auxin, cytokinin added to the culture medium affected callus induction by actively promoting cell division through CDK (Cyclin-

dependent kinase) protein kinases from G1 to S and G2 to M phases. CDK protein kinases included the cell division cycle of cdc2, cdk4, and cdk6. Cytokinin, along with several types of cyclin, activated CDK through phosphorylation. While cdk4 and cdk6 were catalyzed by Cyclin Activating Kinase (CAK), cdc2 required additional phosphorylation by protein kinase wee1. At the end of the G2 phase, cdc2 was bound with cyclin B to activate CDK. Upon entering the G1 phase, cytokinin and Cyclin Activating Kinase (CAK) induced CycD3 (D-type cyclin) formation, influenced by external factors such as sucrose and hormones, forming an active complex of CycD3 and CDKA. In this context, cytokinin in the growth medium enhances the transcription of cdc2 and CycD3, leading to increased cell proliferation (D'Agostino & Kieber, 1999).

Conclusion

Picloram significantly affected the parameters of days to callus formation and callus induction frequency. BAP significantly affected the parameter of days to callus formation, while it did not significantly affect the percentage of callus explants. Picloram concentrations of 1 ppm, 2 ppm, and 3 ppm significantly affected days to callus formation and the percentage of callus explants. Meanwhile, BAP concentrations of 1 ppm, 2 ppm, and 3 ppm significantly affected days to callus formation but did not significantly affect the percentage of explants forming *binahong* callus. The interaction between picloram and BAP significantly affected the parameters of days to callus formation, ranging from 6 to 6.8 days, and the 100% callus formation percentage in all combination treatments. In this study, an optimal concentration was observed: the MS treatment of 0 ppm BAP + 1 ppm picloram resulted in the fastest average days to callus formation of 6 days, with 100% callus formation characterized by white color and compact texture, indicating robust callus growth.

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References

Aghaali, Z., Hoshino, Y., Monfared, S. R., & Moeini, A. (2019). Regulation of Dedifferentiation and Differentiation in Different Explants of *Papaver rhoeas* L. by One-Step Culture. *Scientia Horticulturae*, *246*, 366–370.

Astuti, S. M., Sakinah A.M, M., Andayani B.M, R., & Risch, A. (2011). Determination of Saponin Compound from *Anredera cordifolia* (Ten) Steenis Plant (Binahong) to Potential Treatment for Several Diseases. *Journal of Agricultural Science*, *3*(4), 224–232.

Audus, L. J. (1963). *Plant Growth Substance*. New York: CRC Press, Inc.

Britannica, T. Editors of Encyclopaedia (2021). *Flavonoid*. *Encyclopedia Britannica*. https://www.britannica.com/science/flavonoid diakses pada 29 Januari 2023 00:01.

Cabañas-García, E., Areche, C., Gómez-Aguirre, Y. A., Borquez, J., Muñoz, R., Cruz-Sosa, F., & Balch, E. P. M. (2021). Biomass Production and Secondary Metabolite Identification in Callus Cultures of *Coryphantha macromeris* (Engelm.) Britton & Rose (Cactaceae), A Traditional Medicinal Plant. *South African Journal of Botany*, 137, 1–9.

Chen, C. M. (1989). *Cytokinin-Modulated Macromolecular Synthesis and Gene Expression*. *In* S Kung, CJ Arntzen

(Eds), *Plant Biotechnology* (pp. 245-256). London: Butterworths.

D'Agostino, I. B., & Kieber, J. J. (1999). Molecular Mechanisms of Cytokinin Action. *Current Opinion in Plant Biology*, 2(5), 359–364. https://doi.org/10.1016/S1369-5266(99)00005-9.

Damanik, I. T. S., Rosmayati, & Siregar, L. A. M. (2017). Effect of Explant Type and Growth Regulator Composition on Callus Induction in Binahong Plants (*Anredera cordifolia* (Ten.) Steenis). *Jurnal Agroekoteknologi FP USU.*, *5*(3), 532–536.

Dodd, B. (1993). *Plant Tissue Culture for Horticulture.* Schol of Life Science. Queensland University of Technology.

Doods, J. H. & Roberts, L. W. (1995). *Experiments in Plant Tissue Culture*: Melbourne: Press Syndicate of Universe of Cambridge.

Ezzat, G. (2017). Influence of 2,4-D and Picloram on *In Vitro* Callus Induction from *Verbena bipinnatifida* Nutt. and Evaluation of *In Vivo* Anti-Inflammatory Activity of Callus Extract. *Australian Journal of Basic and Applied Sciences*, *11*, 146–150.

Gantait, S., & Mahanta, M. (2021). Picloram-Induced Enhanced Callus-Mediated Regeneration, Acclimatization, and Genetic Clonality Assessment of Gerbera. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 1–8.

Indah, P. N. & Dini, E. (2013). Nyamplung (*Calophyllum inophyllum* Lin) Leaf Callus Induction at Several Combinations of 6-*Benzylaminopurine* (BAP) and 2,4-*Dichlorophenoxyacetic Acid* (2,4-D) Concentrations.*Jurnal Sains dan Seni POMITS*, *2*(1), 2337-2343.

Khosroushahi, A. Y., Naderi-Manesh, H., & Simonsen, H. T. (2011). Effect of Antioxidants and Carbohydrates in Callus Cultures of *Taxus brevifolia:* Evaluation of Browning, Callus Growth, Total Phenolics and Paclitaxel Production. *BioImpacts*, *1*(1), 37–45.

Kiong, A. L. P., Nee, T. C., & Hussein, S. (2007). Callus Induction from Leaf Explants of *Ficus deltoidea* Jack. *International Journal of Agricultural Research*, *2*(5), 468–475.

Kiong, A. L. P., Yeo, S. T., Gansau, J. A., & Hussein, S. (2008). Induction and Multiplication of Callus from Endosperm of *Cycas revoluta*. *African Journal of Biotechnology*, *7*(23), 4279–4284.

Mahadi, I., Wan, S., Yeni, S. (2016). Callus Induction of Calamansi (*Citrus microcarpa*) Using 2,4-D and BAP Hormones by in vitro Methods. *Jurnal Ilmu Pertanian Indonesia*, *21*(2), 84–89.

Manuhara, Y. S. W. (2001). Regeneration of Mustard Plants (*Brassica juncea* L. var Morakot) Through Tissue Culture Techniques. *Jurnal MIPA*, *6*(2), 127-130.

Marthani, Q. K., Anggraito, Y. U., & Rahayu, E. S. (2016). Calogenesis of Koro Benguk (*Mucuna puriens* L.) Half-Seed Explants *In Vitro* Using BAP and NAA. *Life Science*, *5*(1), 72–78

Manoi, F. & Balitro (2009). *Binahong (Anredera cordifolia) Sebagai Obat*. Bogor: Pusat Penelitian dan Pengembangan Perkebunan.

Merthaningsih, N. P., Yuswanti, H., & Astiningsih, A. A. M. (2018). Callus Induction in Phalaenopsis Pollen Culture Using 2,4-*Dichlorophenoxyacetic Acid*. *Agrotrop*, *8*(1), 47–55.

Rahayu, T., & Mardini, U. (2015). The Response of Node and Leaf Explant of Binahong (*Anredera cordifolia* L.) on MS Media with Variation of BAP Concentration. *Proceeding Biology Education Conference: Biology,*

Science, Enviromental, and Learning, *12*(1), 657–661.

Slater, A., N. Scott, & M. Fowler. (2003). *Plant Biotechnology: The Genetic Manipulation of Plants*. Oxford: Oxford University Press.

Stafford, A., & G. Warren. (1991). *Plant Cell and Tissue Culture*. Milton Keynes: Open University Press.

Sugiyarto, L., & Kuswadi, P. C. (2014). Effect of 2,4-*Dichlorophenoxyacetate* (2,4-D) and *Benzyl Aminopurin* (BAP) on Binahong (*Anredera cordifolia* L.) Leaf Callus Growth and Total Flavonoid Content Analysis. *Jurnal Penelitian Saintek*, *19*(1), 56–60.

Sukandar, E. Y., Sigit, J. I., & Adiwibowo, L. F. (2013). Study of Kidney Repair Mechanisms of Corn Silk (*Zea mays* L. Hair)-Binahong (*Anredera cordifolia* (Ten.) Steenis) Leaves Combination in Rat Model of Kidney Failure. *International Journal of Pharmacology*, *9*(1), 12–23.

Taiz L. & Zeiger, E. (2010). *Plant Physiology 5th Edition*. Sunderland: Sinauer Associates Inc.

Wang, L., & Ruan, Y. L. (2013). Regulation of Cell Division and Expansion by Sugar and Auxin Signaling. *Frontiers in Plant Science*, *4*(163), 1–9.

Wardani, D. K. (2020). Induction of Patchouli Plant Callus (*Pogostemon Cablin* Benth) by Giving Concentration of Auxin Type 2,4-D (*Dichlorophenoxyacetic Acid*) and Picloram. *Jurnal Indonesia Sosial Sains*, *1*(5), 402–414.

Wattimena, G. A. (1991). *Zat Pengatur Tumbuh*. Bogor: PAU Bioteknologi IPB.

World Health Organization, Regional Office for South-East Asia. (2020). *Traditional Medicine in the WHO South-East Asia Region; Review of Progress 2014-2019*. New Delhi: SEARO Publication