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Callogenesis of Dayak Onion (*Eleutherine palmifolia*) Bulb in response of Picloram, 2,4-D, and Kinetin

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Abstract. Dayak onion contains bioactive compounds that are traditionally used for medicine. The production of bioactive compounds can be increased through callus culture. This study aims to analyze the effect of the combination of plant growth regulator on the growth of Dayak bulb callus. The design of this study used a completely randomized factorial design with 2 factors. The first factor is the concentration of auxin. While the second factor is the concentration of kinetin. The parameters observed include the percentage of callus, the time of callus formation, fresh weight, dry weight, and callus morphology. In this study, a callus growth curve was also made to determine the best harvest time. The results showed that highest callus fresh weight was obtained in the P_3K1 treatment. The percentage of callus formation in the picloram + kinetin treatment was higher than in the 2,4-D + kinetin treatment. The time of callus formation occurred in the combination of picloram + kinetin faster than the 2.4-D + kinetin treatment. The callus color is diverse, while the texture of the entire callus is compact. The best callus induction medium for Dayak onion bulbs is to use picloram 2-4 ppm + kinetin0.025-0.5 ppm. The results of this study provide optimal growth regulatory information for the induction of dayak onion callus that has never been reported before. This information can be the basis for the development of methods of production of bioactive compounds from dayak onions through callus culture.

Keywords: Dayak onion; callus; 2,4-D; picloram; kinetin

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INTRODUCTION

Eleutherine palmifolia (L.) Merr (Dayak onion) is an endemic plant of Kalimantan. Dayak people have used this plant as a medicine for various types of diseases.The results of research showed that Dayak onions contain 31-40 compounds depending on the growth place. The main compounds are isoliquiritigenin, trans-resveratrol, oxyresveratrol, eleuterol, eleuterin, eleuterinol, isoeleuterin, isoeleuterol, eleukanarol, and eleuterinol (Prayitno et al., 2018; Mutiah et al., 2019). Dayak onions have been scientifically proven to have anticancer bioactivity (Mutiah et al., 2019; Mutiah et al., 2020; Wijayanti et al., 2019), antimicrobial (Puspadewi et al., 2013), antioxidant (Arwati et al., 2018), antidiabetic (Ahmad et al., 2018; Dewi et al., 2016; Harahap et al., 2023), antifungal, anti-inflammatory (Arbain et al., 2022) and anticholesterol (Febrinda et al., 2014). The antidiabetic abilities of this plant are thought to be related to inhibiting alpha-glucosidase which can lower postprandial blood glucose levels, and the ability to repair pancreatic beta cell damage, thereby increasing insulin secretion directly (Febrinda et al., 2014). Dayak onion extract is recommended as a colon anticancer through increasing goblet cells, inducing apoptosis through increasing TNF-α, and decreasing TGF-β (Mutiat et al., 2020).

The importance of plant bioactive compounds in numerous aspects of human health, agriculture,

and industry cannot be overstated. For instance, plant bioactive compounds are used as medicines in many cultures' traditional healing systems. These age-old treatments frequently serve as the foundation for contemporary herbal medicine (Dincheva et al., 2023). The production of plant bioactive compounds can be increased by the callus culture method. Callus is the mass of cells that undergo dedifferentiation. Dedifferentiation is the transformation of cells from a differentiated state to a less differentiated state such as stem cells (Jiang et al., 2015). The return of cells from differentiated to dedifferentiated conditions can be caused by the addition of growth-regulating substances. Exogenous application of plant growth regulators can strongly alter the endogenous hormone levels during the in vitro culture and exert significant effects on morphogenetic responses. Plant growth regulators are involved in gene activity control at the transcription and transduction level through the selective activation and differential of genes. Plant growth regulator are involved in control of DNA methylation too, and the dedifferentiation process, most likely, is related to its action (Almeida et al., 2015).

The use of appropriate media in combination with appropriate plant growth regulators can be a decisive factor in the induction process of callus formation in the explant. The administration of balanced concentrations of auxins and cytokines will stimulate the explant to form undifferentiated cells (Hunaish & Almasoody, 2020). In some plant species, plant growth regulators including brassinosteroids and abscisic acid can cause the development of calluses and, in other situations, can take the place of auxin or cytokinin in the callus-forming process (Ikeuchi et al., 2013). However, plant growth regulator which has been frequently used in inducing plant calluses is a combination of auxin and cytokinin. The production of callus in plant explants requires the hormone auxin. It is essential for promoting cell elongation, division, and subsequent growth and development processes. (Rahayu et al., 2016) . The auxins that are widely used in callus induction are 2,4-D and picloram. Picloram is reported to be effective in inducing callus in *Stelechocarpus burahol* (Habibah et al., 2016; 2017), *Verbena bipinnatifida* (Ezzat, 2017; Aghaali et al., 2019), *Papaver rhoeas*, *Pogostemon cablin* (Wardani, 2020), and *Coryophantha macromeris* (Cabañas-García et al., 2021). The administration of 2,4-D (in combination with BAP) was reported to be effective in inducing patchouli leaf callus (Wardani, 2020), and *Dioscorea esculenta* (in combination with kinetin) (Habibah et al., 2021).

Research related to the bioactive activity of Dayak onions has been widely conducted, but it is hard to obtain information on Dayak onion callus culture because there have been no reports related to this information. In vitro cultures of Dayak onions that have been reported are related to the propagation of Dayak onion buds (Sari et al., 2018; Trisnawati et al., 2023). This research is needed to obtain the most optimal Dayak onion callus induction protocol. Dayak onion callus culture can be used for the production of various kinds of bioactive compounds contained in Dayak onion bulbs.

METHOD

Explant Sterilization

Sterilization of onion bulbs was carried out using running water, Dettol solution, bactericidal and fungicidal solution, and bleaching agent. Each was rinsed with sterile aquades three times for 3-5 minutes. Before planting into the media, the bulbs are peeled off 2-3 layers.

Media Preparation

Preparation of treatment media using protocols from MS media from PhytoTech Labs. callus induction media is prepared from 4.43 grams of MS media, 25 grams of sucrose, 0.1 grams of Myo-inositol (PhytoTech LABS), 8 grams of agarose and dissolved in 1 liter of aquades. Then, the pH measurement was carried out using a pH meter and made the pH of the media at a position of 5.8-6. 2,4D, Pichloram, and kinetin were then added according to the predetermined treatment. The media is stirred until all dissolved materials are completely dissolved. The media is then heated for 20 minutes to make it homogeneous. After that, the media was put into sterilized culture bottles of 25-30 ml and then closed again with a bottle cap, and then sterilized in an autoclave at 121^{o} C for 20 minutes.

Callus Induction

The medium used for dayak onion bulb explant culture was Murashige and Skoog (MS) with a combination of plant growth regulator 2,4-D (0 ppm, 1 ppm, 2 ppm, 3 ppm) + kinetin (0 ppm; 0.25 ppm; 0.5 ppm) and picloram (0 ppm, 1 ppm, 2 ppm, 3 ppm) + kinetin (0 ppm; 0.25 ppm; 0.5 ppm). Explants were incubated for 40 days. The culture room is set to ±20-25^{o}C, and the irradiation of fluorescent (neon) lamps with a light intensity

of 2000 lux is also arranged. The growth parameters observed include the percentage of callus formation, the time of callus formation, the fresh weight of the callus, the dry weight of the callus, and the callus morphology.

Callus growth curves

The best growth medium is used to make the growth curve. A total of 1.0 grams of callus was grown in the best medium and every 5 days callus harvesting was carried out until it reached stationary weight.

Data Analysis

The fresh and dry weight parameters of the callus were analyzed with Two-way ANOVA, the percentage of callus formation and the time of callus formation were analyzed using the non-parametric *Kruskall Wallis* test, and the callus morphology was analyzed descriptively.

RESULTS AND DISCUSSION

Plant growth regulators 2,4-D and picloram combined with kinetin exert different effects on callus growth depending on the concentration applied. The growth parameters observed are the time of callus formation, percentage of callus formation, fresh weight, dry weight, and callus morphology. The average time of callus formation varied greatly in both 2,4-D and picloram treatments (Figure 1). In general, picloram 2-4 ppm combined with kinetin 0.25-0.5 ppm is more effective in inducing callus from Dayak onion bulbs compared to 2,4-D with the same concentration. At low auxin concentrations (1 ppm) in both 2,4-D and picloram treatments, it was unable to induce a dedifferentiation process so that calluses were not formed. Picloram and 2,4-D have almost the same way of working because they are included in the auxin group. However, the response of plants to the type of auxin varies. Although auxins play a major role in callus formation, some plant species also require a combination of auxins and cytokines in the growing medium for cell proliferation (Hardjo, 2018). The use of a combination of Kinetin and 2,4-D increases the percentage of callus formation from the endosperm of mountain papaya *(Vasconcella pubescens A. DC*) (Zuhro et al., 2021).

The added MS medium of 2 mg/L kinetin and 0.5 mg/L 2,4-D resulted in the best callus percentage on *Phaseolus vulgaris* L callus induction (Küçükrecep & Tekdal, 2021). This is also seen in the results of this study, especially in the combination treatment with picloram. In general, higher kinetin concentrations induce faster callus formation. The role of kinetin in callus formation is related to 3 proteins that will bind to kinetin, namely cytokinin Arabidopsis histidine kinase-2 (AHK2), AHK3, and cytokinin response 1 (CRE1) /AHK4 protein receptors in cell membranes, which then activate multistep autophosphorylation of histidine amino acids.

All three proteins have their Cyclases/Histidine Kinases Associated Sensing Extracellular (CHASE), which bind to extracellular cytokines (Antoniadi et al., 2022). Through a series of reactions, cytokines accumulate in the nucleus and activate the transcription of functional genes that play a role in the growth response and defense against stress.

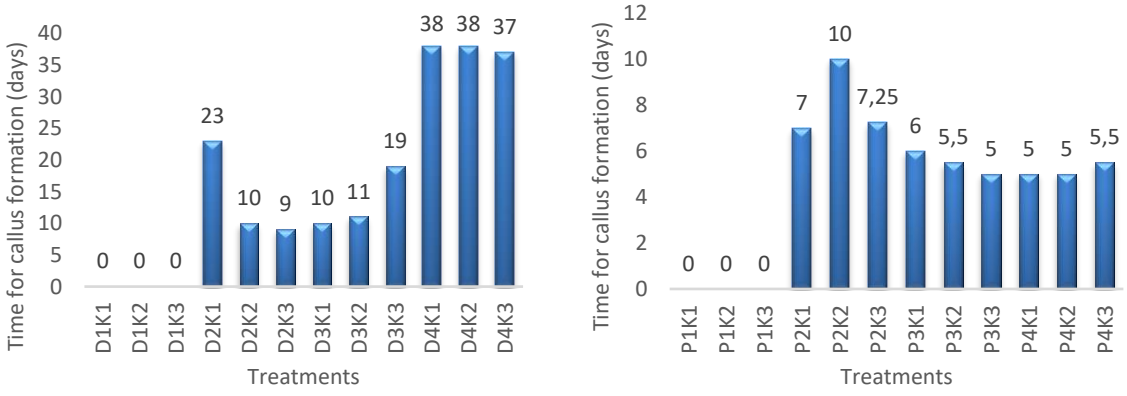

Figure 1. The mean time of *E. palmifolia* callus formation on 2,4-D and kinetin combination and the combination of picloram and kinetin treatment media.

The appearance of dayak onion callus is characterized by the formation of a mass of

yellowish-white cells at the base of dayak onion bulbs. The callus grows from the part of the explant that is injured and then grows continuously. The formation of this callus is closely related to the process of cell division, enlargement, and elongation. This is influenced by the addition of plant growth regulators in the culture media. The addition of plant growth regulators into the culture medium will stimulate cell division and enlargement in the explant so that it can spur the formation and growth of calluses and increase bioactive chemical compounds.

In the auxin-induced callus formation, the auxin signal is transduced through Auxin Response Factor (ARF) transcription factor, especially ARF7, and ARF19, to activate the expression of the transcription factor of the Lateral Organ Boundaries Domain (LBD). LBD induces E2 Promoter Binding Factor a (E2Fa) and E2Fa then forms dimerization with protein dimerization partner (DP), and encourages transcription of genes needed for DNA replication. Cytokines also play a role in callus induction. Cytokinin signals are transduced through 2 pathway components set to activate the ARRtype-B transcription factor. The expression of D-Type Cyclins (CYCD)3 is highly regulated by cytokines.

The AP2/ERF transcription factor of estrogen receptor 1 (ESR1 also known as ER) is also regulated by cytokines. ESR1 and ESR2 promote cell cycle reactivation because ESR2 induces CYCD1 gene expression. ESR2 also induces odorant-binding proteins (OBP1). OBP1 promotes cell cycle activation by inducing gene expression of CYCD3 and several other cell cycle regulators (Ikeuchi et al., 2013). Auxins added to planting media play a role in regulating cell proliferation, namely in the preparation stage of replication from the G1 to S phase.

In the G1 phase, auxin induces expression of the cyclin-D genes namely cycD 3;1 and CDKA;1, and functions in the assembly of cyclin-dependent kinase A (CDKA)/CYCD complexes. With the addition of auxin, it causes a decrease in the regulation of Kip-Related Proteins (KRP) transcripts, namely KRP1 and KRP2 which are inhibitors of CDK, so that the CDKA/CYCD phosphorylated complex is maintained. Activation of the CDKA/CYCD complex further spurs phosphorylation of Repressor Retinoblastoma-Related (RBR) protein, releasing Adenovirus E2 Promoter-binding Factor A/B (E2FA/B) target, and Dimerization Partner A (DPA) complex. Through the post-transcription regulation, auxin will play a role in the stimulation of S Phase Kinase-Associated Protein 2A (SKP2A) F-box degradation by Skp, Cullin, and F-box complex (SCF) ubiquitin ligase E3 complex, which indirectly causes E2 Promoter-Binding Factor C (E2FC) and Dimerization Partner B (DPB) complex to stabilize, and proceed to the gene expression suppression stage in phase S. However, most data indicate that auxin acts as a permissive signal when entering the DNA synthesis stage (G1/S transition) and also in the G2/M transition to complete the synthesis process (Wang & Ruan, 2013).

The mechanism of cytokinin hormone is initiated in the transition process between G1S and G2M phases. Cytokinin activates RNA synthesis and will encourage the process of protein synthesis and subsequently activate enzymes involved in cell division powered by the Cyclin-Dependent Kinase (CDK) Enzyme. In cell division, CDK affects the phase transition from G1 to S and from G2 to M. There are several types of CDK in the cycle of cell division and cooperation between cytokines. The G1S phase is controlled by CYCD. The action of CYCD is influenced by external factors such as hormones and sucrose that will form the active complexes of CYCD and CDKA. This complex activates the E2F promoter and activates the transcription genes involved in synthesis phase. Acceleration in this phase will affect the process of division and differentiation in cell growth (Dewitte & Murray, 2003)

Callus formation, in addition to being induced by auxins and cytokines, can also occur due to injury. The overall cutting of the hypocotyl in Arabidopsis induces the expression of the Wound Induced Dedifferentiation (WIND) genes at the site of injury, which then induces a cytokinin response to induce callus formation. When the rod is partially cut, auxin is transported from the apex of the shoot and accumulates in the injured part, which then induces NAC domain containing protein 71 (ANAC071) gene expression. Auxin also induces the RAP2 gene. Both responses are necessary for the local activation of cell proliferation to close the wound (Ikeuchi et al., 2013). In this study, an incision was made on the bulb to stimulate the formation of a callus through the wounding process.

The other growth parameter observed is the percentage of callus formation which is to see what percentage of explants can form a callus in each treatment. The growth response of callus percentage for each treatment can be seen in Figure 2.

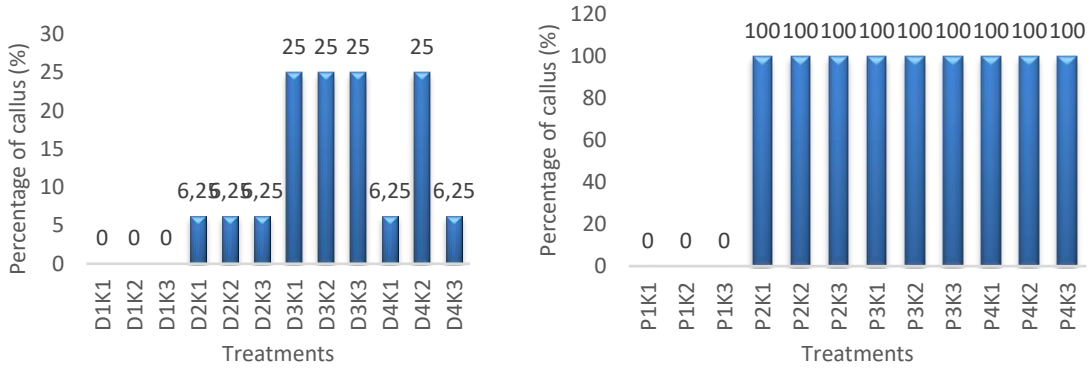

Figure 2. The average percentage of explants with callus from *E. palmifolia* in 2,4-D + kinetin and picloram + kinetin treatment media

Based on Figure 2, it can be seen that a picloram concentration of 2-4 ppm with a kinetin combination is more effective in inducing callus compared to 2.4D at the same concentration. The treatment of picloram with a concentration of 2-4 ppm induced all explants in the callus-producing treatment despite varying time and weight. Both in 2.4D and picloram, a concentration of 1 ppm with a kinetin combination of 0-0.5 ppm cannot induce callus formation. This shows that the auxin concentration of 1 ppm is not strong enough to encourage the dedifferentiation process of Dayak onion bulbs so the callus formation process does not occur.

Fresh and dry weight are among the growth parameters that are quite important to observe. In this study, fresh and dry weight showed variations between auxin-type treatments (2,4-D and picloram), as well as the concentration used. Data on the fresh and dry weight of the callus can be seen in Figure 3.

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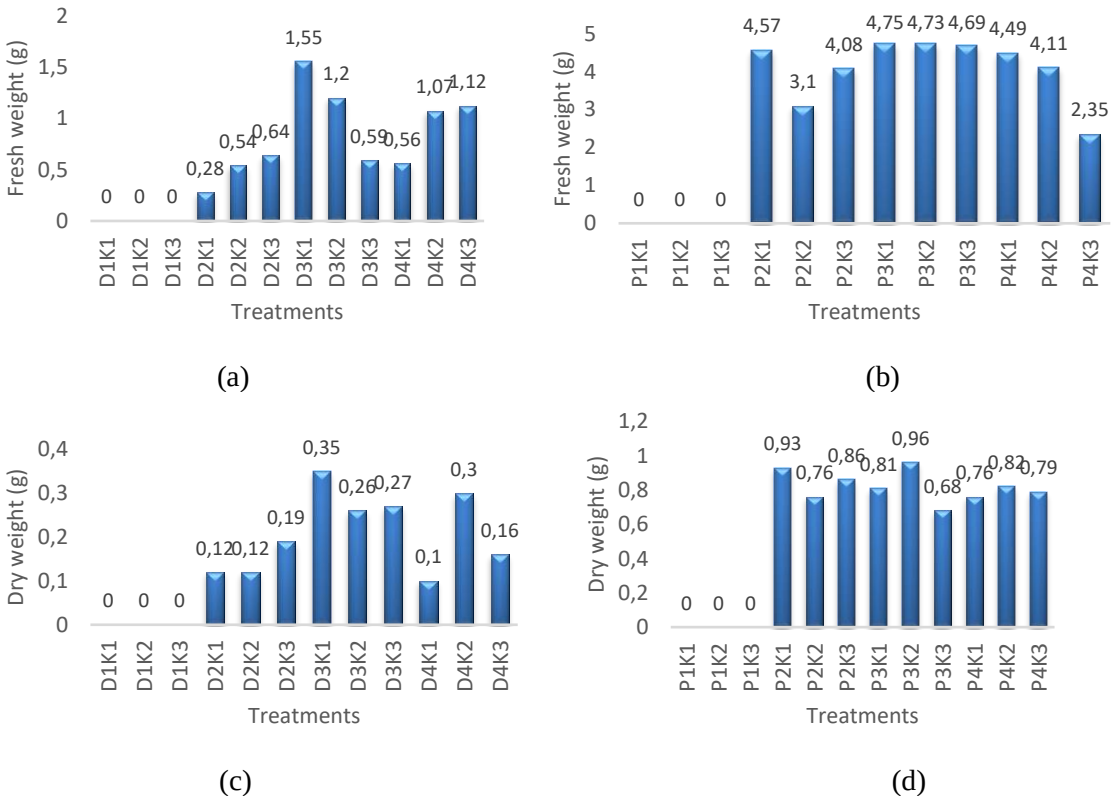

Figure 3. Average fresh weight of *E. palmifolia* callus (a) on medium 2,4-D + kinetin (b) on medium picloram + kinetin; average dry weight of Eleutherine palmifolia callus (c) on medium 2,4-D + kinetin (d) on medium picloram + kinetin

Table 1. Morphology of *E. palmifolia*'s callus

No	Plant Growth Regulator	Callus Morphology		Plant Growth Regulator	Callus Morphology	
		Color	Texture		Color	Texture
1	D1K1	-	-	P1K1	-	-
2	D1K2	-	-	P1K2	-	-
3	D1K3	-	-	P1K3	-	-
4	D2K1	Yellow	Compact	P2K1	Yellow	Compact
5	D2K2	Yellow	Compact	P2K2	Yellowish white	Compact
6	D2K3	Yellow	Compact	P2K3	Brownish white	Compact
7	D3K1	Yellow	Compact	P3K1	Yellowish white	Compact
8	D3K2	Yellow	Compact	P3K2	Reddish yellow	Compact
9	D3K3	Yellow	Compact	P3K3	Yellowish white	Compact
10	D4K1	Brownish white	Compact	P4K1	Yellow-brown	Compact
11	D4K2	Yellow	Compact	P4K2	Yellow-brown	Compact
12	D4K3	Yellow-brown	Compact	P4K3	Reddish white	Compact

The morphology of dayak onion callus in the treatment media can be seen in Table 1, Figure 4, and Figure 5. Calluses formed in the 2,4-D + kinetin and picloram + kinetin combination treatment showed the same texture, namely compact. The compact callus is a callus with tight spaces between cells so that the bonds between cells are difficult to separate.

The color of the callus is lighter when it first grows, then becomes darker with age of the callus. With time, the callus will turn brownish, due to an increase in the content of phenolic compounds or other secondary metabolites in the callus. Phenolic compounds react with oxygen with the enzyme polyphenol oxidase assisted to produce highly reactive ortho-diquinones. Ortho-diquinones react with proteins and other cellular components spontaneously to form melanin (dark pigment) (Tang & Newton, 2004). The color of the callus indicates the growth phase of the callus. The color of dayak onion callus varies from yellowish white to brownish yellow. The white-to-yellow color indicates that the callus cells are actively dividing, while the reddish-to-brownish color indicates that the callus is mature (Rahayu & Suharyanto, 2020). In the treatment of 2.4-D 3 ppm + kinetin 0 ppm (D4K1) and 2.4-D 3 ppm + kinetin 0.5 ppm (D4K3), there were parts of the callus that were dark to blackish brown. This shows the accumulation and oxidation of phenolic compounds in the callus (Liu et al., 2021).

Callus texture can vary from friable to compact depending on the type of explant, basic medium, growth regulating agent, and biotic and abiotic supplements added in the medium (Sugiyarto & Kuswandi, 2014; Wahyuni et al., 2020). The friable callus formed in the explant has the characteristics of loose intercellular bonds, making it easy to separate. Calluses with a compact texture have a strong cell bond character, are difficult to separate, and tend to form solid clumps (Yusna et al., 2018). In this study, the Dayak onion callus has a compact texture. The compact callus texture of the Dayak onion has a tight cell arrangement and forms a dense bulge. A compact callus structure was also reported in begonia callus that grown in 2,4-D + kinetin medium (Hendriyani et al., 2020).

In this study, a callus growth analysis was also carried out to obtain the growth curve of Dayak onion callus on the best growth medium. The medium used for growth curve was a medium with the addition of 2 ppm picoram combined with 0 ppm kinetin.

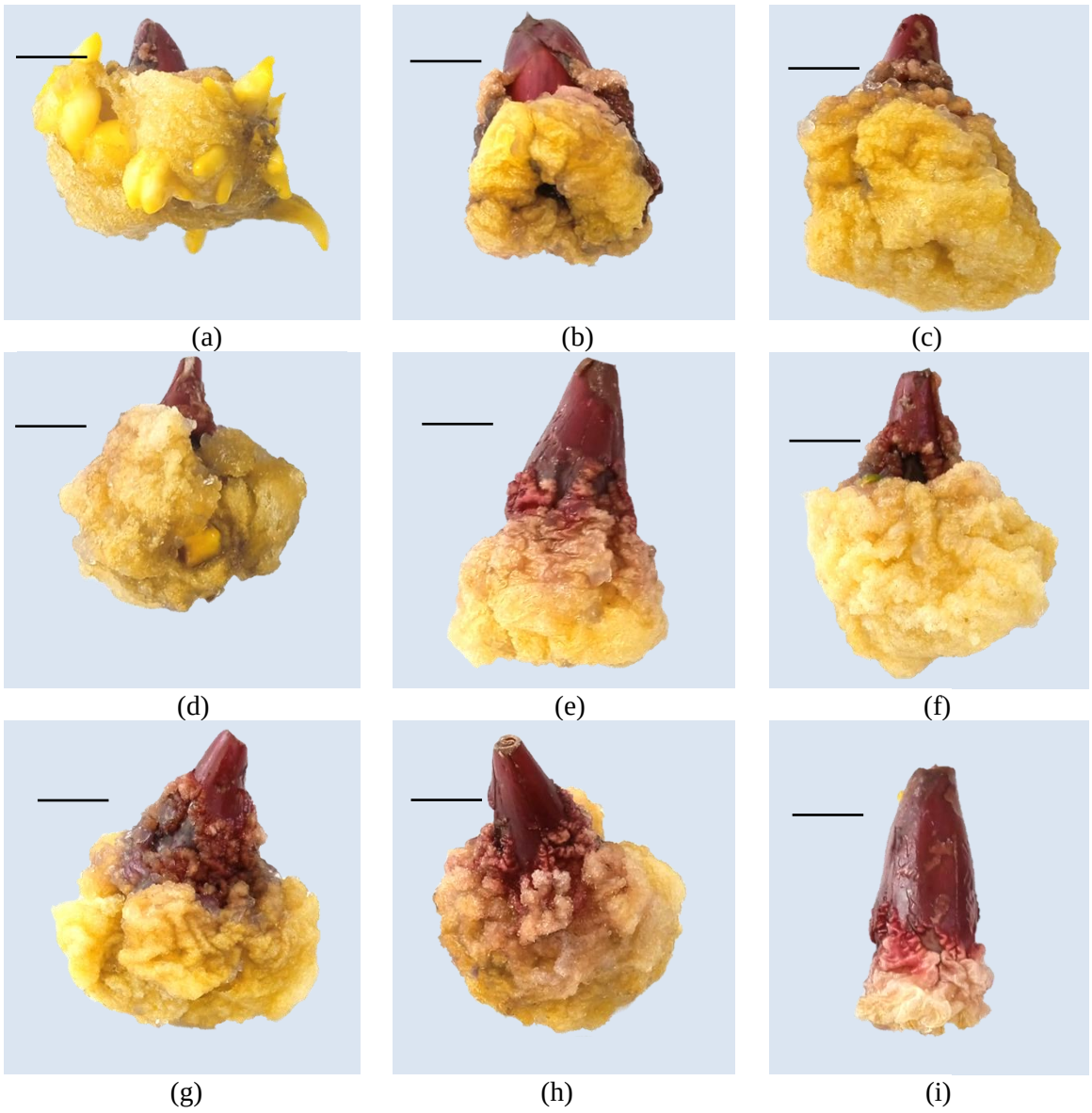

Figure 4. Dayak onion callus in MS medium with the addition of picloram and kinetin. (a) picloram 2 ppm + kinetin 0 ppm (b) picloram 2 ppm + kinetin 0.25 ppm (c) picloram 2 ppm + kinetin 0.5 ppm (d) picloram 3 ppm + kinetin 0 ppm (e) picloram 3 ppm + kinetin 0.25 ppm (f) picloram 3 ppm + kinetin 0.5 ppm (g) picloram 4 ppm + kinetin 0 ppm (h) picloram 4 ppm + kinetin 0.25 ppm (i) picloram 4 ppm + kinetin 0.5 ppm. Bar = 1 cm

The growth curve analysis is carried out by weighing the callus every 5 days until it reaches the stationary phase. In this study, the growth was carried out until the callus was 40 days from planting (Figure 6). The entire cycle of callus culture is divided into 6 phases, namely lag phase, exponential phase, linear phase, deceleration phase, stationary phase, and recession phase. At the lag stage, fresh and dry weight slowly increase, which indicates that the callus is adapting to the environment. At the exponential stage, the growth rate of the callus increases and reaches its maximum.

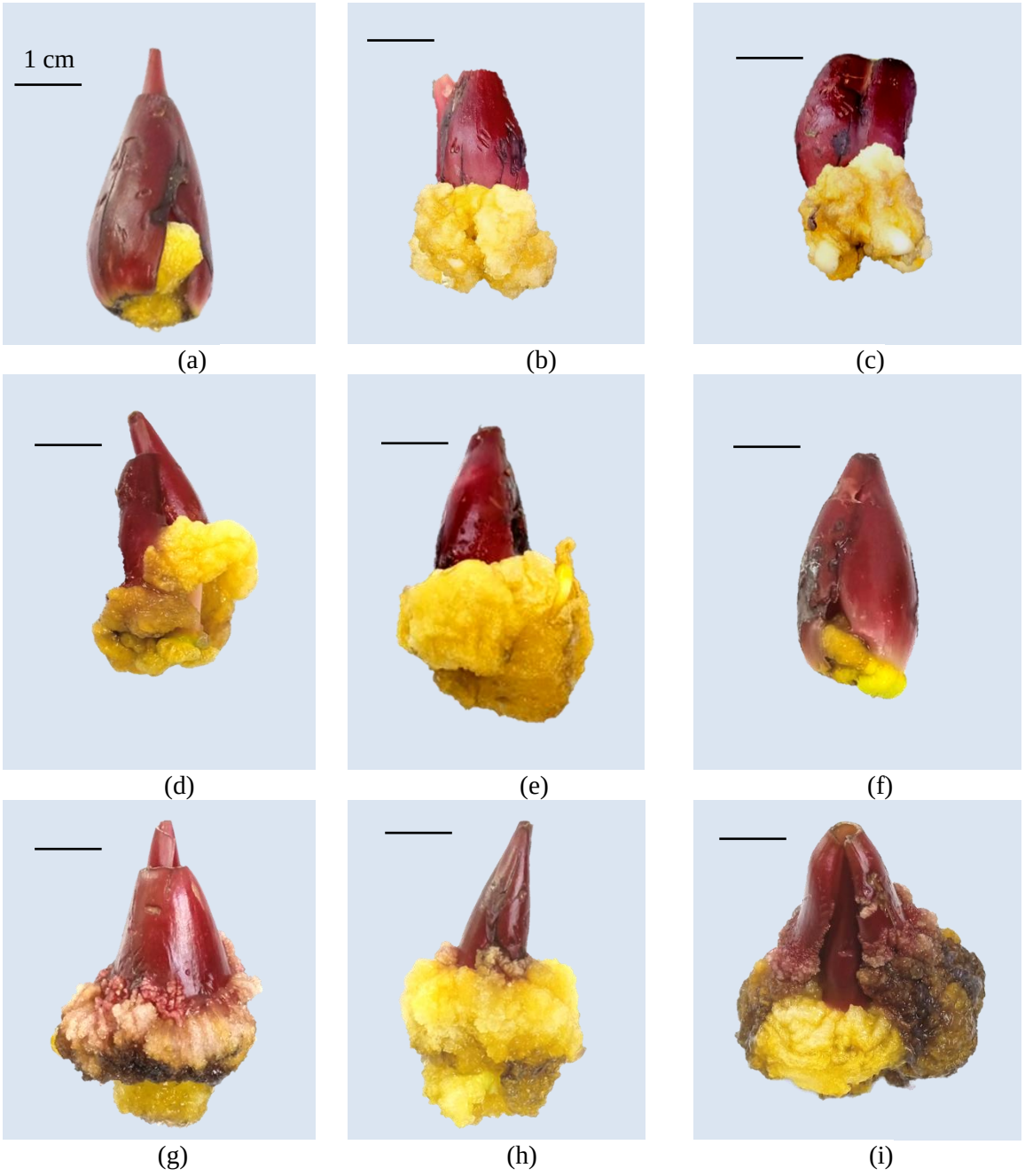

Figure 5. Dayak onion callus in MS medium with the addition of 2.4 D and kinetin. (a) 2.4-D 2 ppm + kinetin 0 ppm (b) 2.4-D 2 ppm + kinetin 0 ppm (c) 2.4-D 2 ppm + kinetin 0.25 ppm (d) 2.4-D 3 ppm + kinetin 0.5 ppm (e) 2.4-D 3 ppm + kinetin 0 ppm (f) 2.4-D 3 ppm + kinetin 0.25 ppm (g) 2.4-D 4 ppm + kinetin 0 ppm, h) 2.4-D 4 ppm + kinetin 0.25 ppm, g) 2.4-D 4 ppm + kinetin 0.5 ppm.

The growth rate began to stabilize in the linear phase and the growth rate began to decrease slowly in the deceleration phase. In the stationary phase, there is no significant change in fresh and dry weight. In the final phase, fresh and dry weight will begin to decrease (Pan et al., 2020). Based on the fresh weight, the lag phase of Dayak onion callus culture lasts from 0-5 days, followed by the exponential phase at 5-10 days, the linear phase followed by deceleration at 10-15 days, the stationary phase at 15-30 days and the recession phase at 30-40 days.

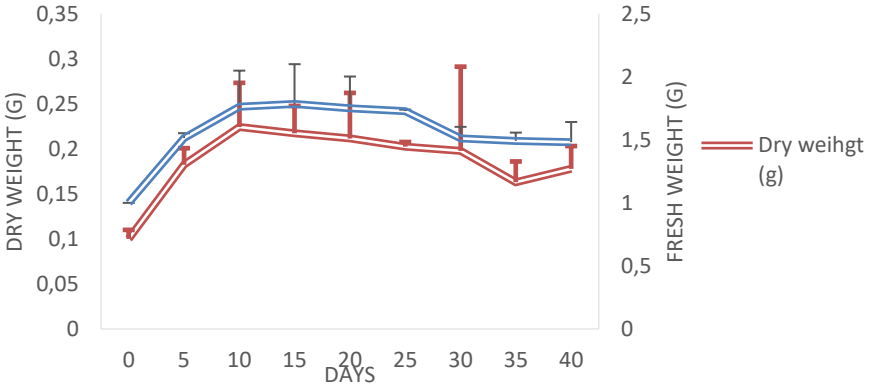

Figure 6. The growth curve of *E. palmifolia* callus culture

Bletilla striata callus suspension culture reached its maximum between days 13 and 14, and after 39 days, growth of callus did not show a significant decline in the functional model, but actual data and observation of the state of the cultured calluses showed that the cultured calluses obvious browning, indicating callus growth entered the recession stage (Pan et al., 2020). Callus culture of *Heliotropium indicum* showed maximum callus growth rate between the 20th and 30th day in various plant growth regulators in different concentrations either alone or in combination (Kumar et al., 2014). Based on these data, it can be seen that dayak onion calluses grown on MS medium with the addition of pichloram and kinetin achieve maximum growth faster than *Bletilla striata* and *Heliotropium indicum* callus. In linear phase, there is a decrease in the amount of nutrients so that callus growth will decrease. In the stationary stage, the nutrients in the medium are exhausted so that callus mass growth does not occur. In the recession stage, some cells will die so that the callus mass will decrease.

Based on the results of this study, information can be obtained that dayak onion callus can be grown well on MS medium with the addition of picloram and kinetin growth regulators. Information on the optimal growth medium and regulators for dayak onion callus growth is important information for the development of secondary metabolite production protocols through callus culture. The callus growth pattern is also important to know to determine the best incubation time. Further research is needed to analyze the production of bioactive compounds in callus cultures that have been successfully induced.

CONCLUSION

Dayak onion callus grown in the combination medium of picloram and kinetin showed better growth than the combination of 2,4-D and kinetin. The best callus induction medium for Dayak onion bulbs is to use picloram 2-4 ppm + kinetin0.025-0.5 ppm. Induction of onion callus Dayal onion should be done on MS medium with the addition of picloram 2-4 ppm + kinetin0.025-0.5 ppm to get the callus with the best 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. https://doi.org/10.1016/j.scienta.2018.11.009

Ahmad, I., Ambarwati, NS., Indriyanti, N., Sastyarina, Y., Rijai, L., & Mun 'im, A. (2018). Oral Glucose Tolerance Activity of Dayak Onion (*Eleutherine palmifolia* L. Merr.) Bulbs Extract Based on the Use of different Extraction Methods. *Pharmacogn J.; 10*(1): 49-54

Almeida, M., Graner, E.M., Brondani, G.E., Oliveira, L.S., Artioli, FA, et al. (2015). Plant morphogenesis: theorical bases. *Adv. For Sci., Cuiabá*, v.2, n.1, p.13-22

Arbain, D., Sriwahyuni, K., Susanti, D., & Taher, M. (2022). Genus eleutherine: A review

of its distribution, traditional uses, phytochemistry, biological activities and its interchangeable common names. South African Journal of Botany, 150, 731–743. https://doi.org/10.1016/j.sajb.2022.08.022

Arwati, N., Wijatmadi, B., Adriani, M., Meilanani, S., Winarni, D., & Hartingsih, S. (2018). The Effect of Dayak Onion Bulb-Stem (Eleutherine Palmifolia (L.,) Merr.) Extract on Blood Glucose Levels of Mouse Suffered Diabetes Mellitus. *Health Notions, 2* (3)

Antoniadi, I., O Novák, Z Gelová, A Johnson, O Plíhal, R Simerský, V Mik, T Vain, E Mateo-Bonmatí, M Karady, M Pernisová, L Plačková, K Opassathian, J Hejátko, S Robert, J Friml, K Doležal, K Ljung and C Turnbull. 2022 Cell-surface receptors enable perception of extracellular cytokines. *Nat Commun*. *11*, 4284. https://doi.org/10.1038/s41467-020-17700-9

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. https://doi.org/10.1016/j.sajb.2020.10.002

Dewi, N. P., Allia, R., & Sabang, S. M. (2016). Eleutherine Bulbosa (MILL.) Antidiabetic Effectiveness Test Urb. Against the Decreased Blood Glucose Levels of Obesity Rats. In *Proceedings of Mulawarman Pharmaceuticals Conferences (Proc. Mul. Pharm. Conf.)* 3:51-63

Dewitte, W., & Murray, J. A. H. (2003). The Plant Cell Cycle. *Annual Review of Plant Biology*, *54*, 235-264. https://doi.org/10.1146/annurev.arplant.54.031902.134836

Dincheva, I., Badjakov, I., & Galunska, B. (2023). New insights into the research of bioactive compounds from plant origins with nutraceutical and pharmaceutical potential. Plants, 12(2), 258. https://doi.org/10.3390/plants12020258

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

Febrinda, A. E., Yuliana, N. D., Ridwan, E., Wresdiyati, T. and 1 Astawan, M. (2014). Hyperglycemic control and diabetes complication preventive activities of Bawang Dayak (Eleutherine palmifolia L. Merr.) bulbs extracts in alloxan-diabetic rats. International Food Research Journal 21(4): 1405-1411

Habibah, n.a., Moeljopawiro, S., Dewi, K., & Indrianto, A. (2016). Flavonoid Production in Callus Cultures from Mesocarp of *Stelechocarpus burahol*. *Bioscientific, 8*(2), 214-221.

Habibah, n.a., Moeljopawiro, S., Dewi, K., & Indrianto, A. (2017). Flavonoid production, growth and differentiation of *Stelechocarpus burahol* (Bl.) hook. f. and th. cell suspension culture. *Mr. J Biol Sci*. *20*, 197-203. https://doi.org/10.3923/pjbs.2017,197,203

Habibah, n.a., S. Safitri, Y. R. Pratiwi, N. Wijawati, F Musafa, ADS Puspitasari and A Yuniastuti. (2021). Callus induction from tuber of lesser yam (*Dioscorea esculenta*) on MS media supplemented by 2,4-D and kinetin. *J Phys Conf Ser*. *1918*, 052029 https://doi.org/10.1088/1742-6596/1918/5/052029

Harahap, H. Y., Futri, C. L., Aminah, D., & Tri, W. (2023). Dayak Onion Tuber (Eleutherine Bulbosa (Mill) (Urb) Extract Ethanol Test on The Histopathological Description of Pancreas Wistar Rat Inducted Streptozotocin. *KnE Social Sciences*, 772-780.

Hardjo, P.H. (2018). *Orchid Tissue Culture; Vanda tricolor Lindl somatic embryogenesis.) var. Pallida.* Yogyakarta: Graha Science.

Hendriyani E., Warseno T. & Undaharta N.K.E. (2020). The Influence of Explanation Type and Combination of Growth Regulating Substances (ZPT) on Induction of Callus Begonia bimaensis Undaharta & Ardaka in Vitro. *Bultein Botanical Garden, 23(1)*: 82-90

Hunaish, AA, & Almasoody, M. M. (2020). Induction of Callus on Various Explants of Arugula Plant (*Eruca sativa* Mill.) using The Growth Regulators (2,4-D and Kinetin). *Plant Archives , 20* (2), 1654-1660

Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant Callus: Mechanisms of Induction and Repression. *The Plant Cell , 25* (9),3159-3173

Küçükrecep A. & Tekdal D. (2021). Callus Induction from Leaf and Stem Explants on Selected Turkish Genotyoe of Common Bean. *Acta Scientific Agriculture*, 5(11): 02-05

Kumar MS, Chaudhury S, Balachandran S. In vitro callus culture of Heliotropium indicum Linn. for assessment of total phenolic and flavonoid content and antioxidant activity. (2014). *Appl Biochem Biotechnol.* 174(8):2897-909. doi: 10.1007/s12010-014-1235-1.

Liu, J., Yang, J., Jiang, C., Zhou, J., Zhao, Y., &

Huang, L. (2021). Volatile Organic Compound and Endogenous Phytohormone Characteristics During Callus Browning in *Aquilaria sinensis*. *Industrial Crops and Products , 168*

Mutiah, R., Bhagawan, W.S., Arifin, B.M.Z.A., Rahmandika, J.M.S. (2019). Metabolite Fingerprinting *Eleutherine palmifolia* (L.) Merr. Using UPLC-QTOF-MS/Ms. 2019. *Traditional Medicine Journal*, 24(3) :139-159

Mutiah, R., Sari, RA., Firsyaradha, WY., Listiyana, A., Indrawijaya, YA., Wafi, A., Suryadinata, A., Susilowati, R., & Rahmawati, A. (2021). Activity and Toxicity of *Eleutherine palmifolia* (L.) Merr. Extract on BALB/c MiceColitis-Associated Colon Cancer Model. *Asian Pacific Journal of Cancer Prevention, Vol 21 : 3579 – 3586*

Pan Y, Li L, Xiao S, Chen Z, Sarsaiya S, Zhang S, et al. (2020) Callus growth kinetics and accumulation of secondary metabolites of Bletilla striata Rchb.f. using a callus suspension culture. *PLOS ONE 15(2)*: e0220084. https:// doi.org/ 10.1371/journal.pone.0220084

Prayitno, B., Mukti, B. H., & Lagiono. (2018). Optimization of the Potency of Dayak Onions *(*Eleutherine sp.) as Alternative Medicinal Ingredients. *Journal of Life Education , 4* (3), 149-158.

Puspadewi, R., Adirestuti, P., & Menawati, R. (2013). Efficacy of Dayak Onion Bulbs (Eleutherine Palmifolia (L.) Merr.) As a Skin Antimicrobial Herb. *Cartika Journal of Pharmaceutical Sciences, 1 (1)*, 31-37

Rahayu, S., Roostika, I., & Bermawie, N. (2016). The effect of types and concentrations of auxins on callus induction of Centella Asiatica. Nusantara Bioscience, 8(2), 283–287. https:// doi.org/10.13057/nusbiosci/n080224

Sugiyarto, L., & Kuswandi, P. C. (2014). Induction of Binahong Leaf Callus *(Anredera cordifolia* L.) in Medicinal Plant Development Efforts. *Journal of Basic Sciences*, 56-60

Tang, W. and Newton, R.J. (2004). Increase of Polyphenol Oxidase and Decrease of Polyamines Correlate with Tissue Browning in Virginia Pine (*Pine virginiana* Mill.). *Plant Sci.* 167: 621-628

Wardani, DK (2020). Induction of Patchouli Plant Callus (*Pogostemon cablin* Benth) with Administration of Auxin Concentration Type 2,4-D (Dichloropheoxyacetic acid) and Picloram. *Indonesian Journal of Social Science , 1* (5), 396-401

Wijayanti, SD., & Hasyati, N. (2018). Potency Of Dayak Onion *(Eleutherine palmifolia* (L.) Merr.) Bulbs Extract In Preventing Ulcerative Colitis In DSS (Dextran Sulphate Sodium) Iinduced Mice. Journal of Food Science and Agricultural Products 2 (1)

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. https://doi.org/10.3389/fpls.2013.00163.

Yusna A, Harahap F & Edi S. (2018). Effect of Plant Growth Regulator on In Vitro Callus Induction of Shoot Explant Mangosteen (*Garcinia mangostana* L.). *International Journal of Advanced Research (IJAR)*, 6(11): 123-129.

Zuhro R.K., Dewanto H. A., Suryadi A., Pribadi T., Hadjoeningtijas O. D., Santosa A. P. (2021). Callus Induction of Montain Papaya Endosperm *(Vasconcellea pubescens A. DC*) with Different Combinations of2,4-Dichlorophenoxyacetic Acid and Kinetin. *3rd International Conference on Agriculture and Bio-industry (ICAGRI 2021).* doi:10.1088/ 1755-1315/951/1/012072