The Effect Of Plant Growth Regulators On Callus Induction Of Mangosteen (Garcinia mangostana L.)

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Abstract. Induction callus is one of the tissue culture techniques. Callus formation can be driven by the addition of Plant Growth Regulator such as 2,4-dichlorophenoxy acetic acid (2,4-D) and coconut water on the media to promote callus formation. The purpose of this research was to obtain the best medium formulation for induction and growth callus from an explant of stem mangosteen (Garcinia mangostana L.). The research design was used completely randomized design with two factors: the concentration of 2,4-D (0, 1, 2, and 3 ppm) and coconut water (0, 15, and 30%) with 12 treatments and 3 replications. Parameters observed in this study were the time of formation callus (weeks), callus fresh weight (g) and callus morphology (colour and texture). The result after 12 weeks observation showed that the addition of 2,4-D and coconut water on the media can induced callus formation from an explant of stem mangosteen. The treatment combination of 2 ppm 2,4-D and 15% coconut water was the best combination for the formation of mangosteen callus and produced yellow and compact callus. This concentration was recommended to grow the mangosteen (Garcinia mangostana L.) callus, might be useful for the production of disease free and healthy plant materials and also it would be useful for genetic transformation and secondary metabolite production of mangosteen (Garcinia mangostana L.) using biotechnological approach.

1. Introduction

Garcinia is a plant of the family Gusiaceae native to Asia, Australia, tropical and southern Africa and Polynesia. The genus Garcinia has more than fifty species [1]. Mangosteen (Garcinia mangostana L.) has been hailed as the “Queen” of tropical fruits with promising economic value [1–4]. In addition, the mangosteen has long been used as medicine among them are as anti-inflammatory [5], antibacterial [6], and for treatment of infections and wounds. The antioxidant ingredient in pericarp such as xanthone, alpha and beta mangosteen is utilized as anticancer agents [7,8]. Callus culture can facilitate optimization of the antioxidant ingredient production and subsequent isolation. Tissue grown as callus masses can sometimes yield high amount of secondary metabolites [9].

Therefore, research development is required in search of effective and efficient method to meet the supply of useful secondary metabolites from mangosteen. Plant tissue culture methods or in vitro culture can be used as alternative solutions to increase crop productivity, such as producing secondary metabolites in medicinal plants, propagation, and plant breeding in a relatively short time, take place continuously, and result in more consistent and controlled quality as well as higher content levels than direct harvesting [10,11,12].
The success of callus culture depends on the use of the basic media, a combination of plant growth regulators [13,14,15], appropriate environmental factors, and development of explants [16,17]. The most influential factors of in vitro plant growth are the interaction and balance between the plant growth regulators endogenously and exogenously by cultured cells [18].

Plant growth regulators are synthetic compounds that act like natural plant hormones which are often manipulated in tissue culture works [19]. There are five main classes of naturally occurring plant hormones namely, auxins, abscisic acid, cytokinins, ethylene and gibberellins. Auxins, cytokinins and auxin-cytokinin interactions are usually considered to be the most important and generally required to regulate growth and organize development in plant tissue and organ cultures [20,21]. The purpose of this research was to obtain the best medium formulation for induction and growth callus from an explant of stem mangosteen (*Garcinia mangostana* L.).

2. Method

The explants used mangosteen (*Garcinia mangostana* L.) stem obtained from the in vitro plantlets of mangosteen collected on the plant tissue culture laboratory YAHDI in Indonesian. Mangosteen stem was cut to a size of approximately 0.5 cm and then planted in a culture bottle on a solid MS medium [22] according to treatment combined concentration of plant growth regulators (2,4-D; coconut water; TDZ 0,1 ppm; and Kinetin 1,5 ppm). This experimental used completely randomized design (CRD) factorial with two factors. The first factor is the concentration of 2,4-D (D), which was 0; 1; 2; 3 ppm combined with the second factor using coconut water concentration (C), which was 0; 15; 30%. The experimental treatment of each treatment and each treatment was repeated 3 times. The total of experimental units was 36 culture bottles. Before it was used, the medium was sterilized with autoclave for 15 minutes, 15 psi and at 121° C; then it was incubated for 1 week. After then, the explants were planted on MS sterile with a treatment combined concentration of plant growth regulators. Observations were made for 12 weeks. Data were collected for time of formation callus (weeks), callus fresh weight (g) and callus morphology (color and texture). These data were analyzed descriptively. The effect of treatment in inducing callus is analyzed using the Statistical Analysis System (SAS) Version Program version 9.1. Mean number of callus fresh weight (g) were compared using the Duncan's Multiple Range Test (DMRT) at a significant level 0.05.

3. Results and Discussions

The ability of callus formation depends on explant source and influenced by type of growth regulator in their concentrations and combinations in the growth medium. Stem explants were cultured on MS medium with different combination of plant growth regulators (2,4-D and coconut water) for callus induction. The addition of plant growth regulators on tissue culture medium influenced the growth rate of the explant cultured cells. Out of twelve hormonal concentrations, the treatment of 2 ppm 2,4-D and 15% coconut water were best result for formation time and fresh weight of stem callus mangosteen (*Garcinia mangostana* L.). Based on the observations, the formation of callus using stem as explants started from week 4,33 to week 7 after planting. The most rapid formation of callus occurred in the combination treatment of 2,4-D 2 ppm and coconut water 15 % on the 4,33 weeks after planting (Figure-1). Callus obtained from stem were yellow and compact (Figure-2). The maximum growth in terms of fresh weight of stem callus were 0,4 g on medium 2 ppm 2,4-D and 15% coconut water after 12 weeks of inoculation (Table-1).
Callus Formation

![Bar chart showing time of formation callus](chart.png)

**Figure 1.** Value of average time of formation callus mangosteen (*Garcinia mangostana* L.)

**Table 1.** Role of Growth Regulators on Callus Induction Stem of Mangosteen (*Garcinia mangostana* L.)

| Treatment | Fresh Weight (g) | Color   | Texture |
|-----------|------------------|---------|---------|
| D0C0      | 0.13cde          | Brown   | Compact |
| D1C0      | 0.21bed          | White   | Compact |
| D2C0      | 0.06de           | White   | Compact |
| D3C0      | 0.14cde          | Brown   | Compact |
| D0C15     | 0.20bcd          | Green   | Compact |
| D1C15     | 0.31ab           | Yellow  | Compact |
| D2C15     | 0.40a            | Yellow  | Compact |
| D3C15     | 0.08cde          | Yellow  | Compact |
| D0C30     | 0.14cde          | Yellow  | Compact |
| D1C30     | 0.22bc           | Yellow  | Compact |
| D2C30     | 0.16cde          | Yellow  | Compact |
| D3C30     | 0.05e            | Yellow  | Compact |

Figures followed by same alphabet along the columns are not significantly different at 0.05 probability level Using Duncan’s Multiple Range Test.

**Figure 2.** The color of Callus induction from stem of mangosteen (*Garcinia mangostana* L.); (a) Brown; (b) White; (c) Green; (d) Yellow
3.1 Time of Callus Formation
Parameter of callus formation time was intended to determine whether the concentration of growth regulators was faster in inducing callus with the use of plant growth regulators (2,4-D and coconut water). The addition of plant growth regulators on tissue culture medium influenced the growth rate of the explant cultured cells. Based on the observations, the formation of callus started from week 4,33 to week 7 after planting. The most rapid formation of callus occurred in the combination treatment of 2,4-D 2 ppm and coconut water 15 % on the 4,33th week after planting.

The 2,4-D demonstrated stronger and more optimal activity as compared to other auxins due to the separation of carboxyl groups by carbon or carbon and oxygen [23]. 2,4-D is a plant growth regulator mostly used on callus culture due to its strong activities to stimulate cell dedifferentiation process, press organogenesis, and maintain callus growth [24]. Callus formation is marked by the emergence of cell clumps of yellowish green or light green on the wound. Furthermore, these clots will form a mass of cells called callus. Correspondingly, state that cell division that leads to the formation of callus on their response to cuts and supply of endogenous or exogenous hormones into explants [25]. Closure of callus tissue is derived from parenchymal cells. The fastest growth of callus formation occurs in the peripheral areas of the region due to the availability of nutrients and better oxygen. The percentage of callus formation indicates the response level of the explants on the use of tested plant growth regulators.

Observations on the administration of plant growth regulator (2,4-D and coconut water) on mangosteen (Garcinia mangostana L.) stem explants showed that all treatments of explants formed callus. The results of this study are similar to a previous study conducted which callus grows in all combinations of concentrations of plant growth regulators 2,4-D [26]. The composition of the combination of concentrated growth regulators used in this study has proven to be able to induce callus and not to hamper growth. This is consistent with the previous study using 2,4-D and BAP at various concentrations ranging from 0.5 to 10 mg/L on Nerium odorum leaf explants [27]. In addition, the differences in the results in callus formation from each treatment medium were also influenced by the presence of coconut water in the medium. Medium added with coconut water will produce very fast callus while medium without coconut water will produce a very long callus. The components contained in coconut water can interact with endogenous hormones that are owned by each explant so that it can stimulate cell division [28]. Coconut water contains diphenyl urea which has activities such as cytokines, which have cell division activity [29].

The use of coconut water in this study was able to encourage callus induction and function as a buffer or buffer solution. The content of other organic compounds found in coconut water can help in the development and growth of callus. This is supported by previous study that the use of growth regulators (2,4-D and coconut water) in culture media can increase callus growth on explants used [11,30,31].

3.2 Fresh Weight of Callus
Fresh weight of callus is an increasing number of cells (cell division) and the increase in cell size (cell enlargement). Fresh weight physiologically consists of two contents, namely water and carbohydrates. The fresh weight of callus is caused by the high content of water [32]. The amount of fresh weight produced highly depends on how fast these cells divide, multiply, and then grow callus. Results of ANOVA statistical tests (α = 0.05) showed that plant growth regulators (2,4-D and coconut water) concentration significantly affected the fresh weight of callus from mangostostana stem explants. In general, the combination of 2,4-D 2 ppm and coconut water 15% had higher fresh weight of callus as compared to the other combination.

3.3 Callus Morphology
Morphological observation on callus with a combination of 2,4-D and coconut water concentration showed that in general the colour change began with a callus turning from yellowish green to yellowish white, then at the end it varied from green, white, yellow, and brown, however, there was also a white callus that remained unchanged until the end of the observation. The texture of the callus on a all treatment combination of 2,4-D and coconut water showed callus with compact texture.
Colour changes in callus that occurred during the 12-week observation indicated a change in the growth phase and the regeneration power of the cells. The colour of green, white, yellow and brown showed that cells were still actively dividing (cleavage stage), while the colour of brown, yellow, black or brown showed symptoms of aging cells. States the colour of callus describes its visual appearance that indicates the activity level of a cell division. The callus colour change is caused by the synthesis of phenolic substances on cells (callus) [31]. Phenol compounds appear to be toxic to cells when in excessive concentrations, which will inhibit growth [33].

The texture of the callus is a marker used to determine the quality of callus produced by explant. Friable callus grows separately into smaller parts, cuts off easily, and contains a lot of water [12]. On the contra, compact type callus has a texture that is not easily cut off and looks solid [34]. The good quality of callus texture depends on its purpose. Callus texture may vary from compact to friable depending on the type of plants used, the composition of the nutrient media, the growth regulators, and the environmental conditions of the culture. In general, callus with friable texture is more embryonic than callus with solid texture [23].

Based on the observation of overall callus induction from mangosteen stem explants treated with plant growth regulators of 2,4-D and coconut water, the majority of parameter analysis showed that the optimal treatment is 2 ppm 2,4-D and 15% coconut water. The success of callus culture depends on the use of basic media, the combination of plant growth regulators, and appropriate environmental factors [25]. In addition, the ability of each plant and plant tissue to form callus is not the same [35]. Plant growth regulator widely used for callus induction is the combination of auxin and cytokinin [36], 3 mg/L 2,4-D + 1 mg/L TDZ optimum for callus induction of Carica pubescens [37], 2.5 mg/L 2,4-D and 0.5 mg/L Kinetin optimum for callus induction of Datura metel [38]. The findings are consistent with studies conducted by [39] showing that the results of callus induction by the use of a combination of auxin and cytokinin for growth regulators are more effective than a single substance. The finding of optimum hormone concentration in this study is useful for the production of the next callus. The obtained callus will be optimized for secondary metabolite production using callus culture or cell suspension culture.

4. Conclusions
This research concluded that various concentration of plant growth regulators (2,4-D, IBA and coconut water) affected callus induction of mangostana stem explants. The most optimal treatment combination of concentration of plant growth regulators in inducing callus from stem explants of mangostana on the treatment 2 ppm 2,4-D and 15% coconut water with a relatively period of callus formation at the earliest on week 4.33; 0.4 g of fresh weight, yellow callus and compact textured. Moreover, callus can use for secondary metabolite production according to culture purpose.

References
[1] Wong. LP, Klemmer. PJ, Severe lactic acidosis associated with juice of mangosteen fruit, *Garcinia mangostana*, American Journal of Kidney Diseases, 51 (5): 829-833, 2008.
[2] Dorly. S, Tjitoosemito. R, Poerwanto. R, Juliari, Secretory duct structure and phytochemistry compounds of yellow latex in mangosteen fruit. *HAYATI J Biosci*, 15: 99-104, 2008.
[3] Harahap. F, Roedhy. P, Suharsono, Cicik. S, Suci. R, In Vitro Growth and Rooting of Mangosteen (*Garcinia mangostana* L.) on Medium with Different Concentrations of Plant Growth Regulator, *HAYATI Journal of Biosciences*, 21 (4): 151-158, 2014.
[4] Samson. JA, *The minor tropical fruit. in Tropical Fruit*. London and New York: Longman, pp. 223-224, 1980.
[5] Chen LG, Yang LL, Wang CC, Anti-Inflammatory activity of mangosteen from *Garcinia mangostana*. Food Chem Toxicol 46: 688-693, 2008.
[6] Chomnawang MT, Surassmo S, Wongsariya K, Bunyapraphatsara N, Antibacterial activity of Thai medicinal plants against methicillin-resistant *Staphylococcus aureus*. *Fitoterapia*, 80 (2): 102-104, 2009.
[7] Osman, M, Milan. AR, Mangosteen-Garcinia mangostana. Southampton Centre for Underutilised Crops. University of Southampton. Southampton, UK, 2006.

[8] Pedraza-Chaverri, J, Cardenas-Rodriguez, N, Orozco-Ibarra, M, Perez-Rojas, JM, Medicinal properties of mangosteen (Garcinia mangostana), Food Chem. Toxic, 46: 3227-3239, 2008.

[9] Wani, M, Snehal. PNM, Callus induction studies in Tridax procumbens L., International Journal of Biotechnology Applications, 2 (1): 11-14, 2010.

[10] Isda. M.N, Sulianyah. I, Induksi Kalus Centella asiatica melalui Aplikasi Akuksin dan Sitokin. Jerami, 2 (3): 162-165, 2009.

[11] Ariati. SN, Waeniati, Muslimin, Suwastika, IN, Induksi Kalus Tanaman Kakao (Theobroma cacao L.) Pada Media MS dengan Penambahan 2,4-D, BAP dan Air Kelapa. Natural Science: Journal of Science and Technology, 1(1): 74-84, 2012.

[12] Sitorus. EN, Hastuti. ED, Setiari. N, Induksi Kalus Binahong (Basella rubra L.) secara In Vitro pada Media Murashige & Skoog dengan Konsentrasi Sukrosa yang Berbeda. Bioma, 13 (1): 1-7, 2011.

[13] Hoesen. DSH, Witjaksono, Sukamto. LA, Induksi Kalus dan Organogenesis Kultur In Vitro Dendrobium lineale Rolfe. Berita Biologi, 9 (3): 333-341, 2008.

[14] Jahan. MT, Islam. MR, Khan. R, Mamun. ANK, Ahmed. G, Hakim. L, In Vitro Clonal Propagation of Anthurium (Anthurium andraeanum L.) using Callus Culture. Plant Tissue Culture and Biotechnology, 19 (1): 61-69, 2009.

[15] Shirin. F, Hossain. M, Kabir. MF, Roy. M, Sarker. SR, Callus Induction and PlantRegeneration from Internodal and Leaf Explant of Four Potato (Solanum tuberosum L.) Cultivars. World Journal of Agricultural Sciences, 3 (1): 1-6, 2007.

[16] Ibrahim. MSD, Rostiana. O, Khumaidah. N, Pengaruh Umur Eksplan terhadap Keberhasilan Pembentukan Kalus Embriogenik pada Kultur Meristem Jahe (Zingiber officinale Rosc), Jurnal Litri, 16 (1): 37-42, 2010.

[17] Reddy. JM, Bopaiah. AK, Abhilash. M, In Vitro Micropropagation of Anthurium digitatum using Leaf as Explant. Asian Journal of Pharmaceutical and Health Sciences, 2 (1):70-74, 2011.

[18] Sen. MK, Nasrin. S, Rahman. S, Jamal. AH, In vitro callus induction and plantlet regeneration of Achyranthes aspera L., a high value medicinal plant. Asian Pacific Journal of Tropical Biomedicine, 4 (1): 40-46, 2014.

[19] Jiménez. VM, Regulation of in vitro somatic embryogenesis, Rev. Bras. Fisiol. Veg, 13: 196-223, 2001.

[20] Evans. DA, Sharp WR, Flick CE, Growth and behavior of cell cultures: embryogenesis and organogenesis. In: Thorpe TA (ed.) Plant Cell Culture: Methods and Applications in Agriculture, New York: Academic Press, pp. 45-113, 1981.

[21] Vasil. IK, Thorpe. TA. Plant cell and tissue culture. Dordrecht: Kluwer Academic Publishers, 1994.

[22] Murashige. T, and Skoog, F, “A revised medium for rapid growth and bioassays with tobacco tissue cultures”, Physiology of plant, 15: 473-497, 1962.

[23] Mampuara, YSW, Kapiita Selekta Kultur Jaringan Tumbuhan, Surabaya: Airlangga University Press, 2014.

[24] Arianto. D, Basri. Z, Bustamil. MU, Induksi Kalus Dua Klon Kakao (Theobroma cacao L.) Unggul Sulawesi pada Berbagai Konsentrasi 2,4-Dichlorophenoxy Acetic Acid secara In Vitro, Agrotek, 3(3): 211-220, 2013.

[25] George. E, F, Sherrington. PD, Plant Propagation by Tissue Culture: Handbook and Directory of Commercial Laboratories. London: Cambridge University Press, 1992.

[26] Sen. MK, Nasrin. S, Rahman. S, Jamal. AH, In vitro callus induction and plantlet regeneration of Achyranthes aspera L., a high value medicinal plant. Asian Pacific Journal of Tropical Biomedicine, 4 (1): 40-46, 2014.
[27] Rashmi. R, Trivedi. MP, Effect of Various Growth Hormone Concentration and Combination on Callus Induction, Nature of Callus and Callogenic Response of *Nerium odoratum*. *Applied Biochemistry and Biotechnology*, 172 (5): 2562-2570, 2014.

[28] Surachman. D, Teknik Pemanfaatan air kelapa untuk perbanyakan nilam secara in vitro, *Buletin Teknik Pertanian*, 16 (1): 31-33, 2011.

[29] Hendaryono. DPS, A. Wijayani, *Teknik Kultur Jaringan Tumbuhan*, Yogyakarta: Kanisius, 1994.

[30] Dwi. NM, Waeniati. Muslimin. INS, Pengaruh Penambahan Hormon 2,4-D Pada Medium Ms Dalam Menginduksi Kalus Tanaman Anggur Hijau (*Vitis vinifera* L.), *Jurnal Natural Science*, 1(1): 53-62, 2012.

[31] George. EF, and P.D. Sherrington, *Plant propagation by tissue culture*. England: Exegetic Ltd, Edington, Westbury, Wilts, 709 pp, 1984.

[32] Indah. PN, Erma vitalini. D, Induksi Kalus Daun Nyamplung (*Calophyllum inophyllum* Linn.) pada Beberapa Kombinasi Konsentrasi 6-Benzylaminopurine (BAP) dan 2,4-Dichlorophenoxyacetic Acid (2,4-D), *Jurnal Sains Dan Seni Pomiits*, 2 (1): 237-3520, 2013.

[33] Hayati. SK, Nurchayati. Y, Setiari. N, Induksi Kalus dari Hipokotil Alfaalfa (*Medicago sativa*) secara In Vitro dengan Penambahan Benzyl Amino Purine (BAP) dan *α*-Naphtalene Acetic Acid (NAA), *Bioma*, 12 (1): 6-12, 2010.

[34] Amid. A, Johan. NN, Jamal. P, Zain, WNW, Observation of Antioxidant Activity of Leaves, Callus and Suspension Culture of *Justicia gendarusa*, *African Journal of Biotechnology*, 10 (81): 18653-18656, 2011.

[35] Ajijah. N, Tasma. IM, Hadipoentyanti. E, Induksi Kalus Vanili (*Vanili planifolia*) dari Eksplan Daun dan Buku. *Buletin RISTRI*, 1 (5) : 277-234, 2010.

[36] Zulkarnain, Kultur Jaringan Tanaman: Solusi Perbanyakan Tanaman Budi Daya, Jakarta: Bumi Aksara, 2009.

[37] Sari. N, Rahayu. ES, Sumadi, Optimization of Type and Concentration of PGR in Embryogenic Callus Induction and Regeneration into Plantlets on *Carica pubescens* (Lenne & K.Koch). *BioSantifika: Journal of Biology & Biology Education*, 6 (1): 51-59, 2014.

[38] Nurchayati. Y, Santosha, Nugroho. LH, Indrianto. A, Growth Pattern and Copper Accumulation in Callus of *Datura metel*. *BioSantifika: Journal of Biology & Biology Education*, 8 (2): 135-140, 2016.

[39] Gao. J, Li. J, Luo. C, Yin. L, Li. S, Yang. G., &He, G, Callus Induction and Plant Regeneration in *Alternanthera philoxeroides*, *Molecular Biology Reports*, 38 (2): 1413-1417, 2011.