Fine Growth on Explants of Manggis Leaf (Garcinia mangostana L.) Planted by In Vitro

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Abstract. This study was conducted to determine the effect of growth regulators (ZPT) on callus induction of mangosteen (*Garcinia mangostana* l.) from leaf sources grown in vitro. The study was carried out from February 2022 to June 2022. This study used a completely randomized design (CRD) with 2 treatment factors, namely: (a) the first factor concentration of 2,4D consisted of 4 levels, namely: 0 ppm, 1, 5 ppm, 3 ppm and 4.5 ppm. (b) the second factor of vitamin concentration consists of 3 levels, namely: 0 ppm, 1 ppm and 2 ppm. Callus appears first begins with the warping of the explant. Explants that grow are characterized by the appearance of white spots on one or several parts of the explants. The data from the observation of the fastest callus formation in the MediaV2+D3 treatment was the 2nd week. Meanwhile, the time for callus to appear was at the latest in the V2+D1,5 media treatment, which was the 12th week. The height of the callus pile observed at week 12 after planting was carried out by calculating the callus height that grew from all explants. The results of data analysis showed that the V1+D0 media treatment resulted in the highest number of Callus Pile Height 0.60 at 12 WAP observations. The lowest callus heap height yield was obtained from treatment with an average number of callus 0.33 roots. The average growth of the Callus Pile Height responded to an increase in the number of roots. The results of data analysis showed that the V2+D0 media treatment resulted in the highest number of callus widths of 1.36 at 12 WAP observations. The results of the lowest number of callus widths were obtained from the treatment of Media V0+D1,5 and V2+D1,5 with an average number of callus widths of 0.06. The average growth of callus width gave a response to an increase in the number of leaves. Callus color is influenced by growth regulators. The addition of hormones with increasing concentrations tends to show a light green color on longer-lasting callus. Explants that do not form callus will change color from green to brown and then die.

Keywords: In vitro, ZPT, Vitamin, 2.4 D

1. Introduction

Mangosteen is a fruit plant native to Indonesia which has a very high economic value. The mangosteen fruit has been dubbed because of the features and delicacy it has (Balai Research on Fruit Crops, 2006). In addition, it contains xanthones (alpha mangostin and gamma mangostin), anthocyanins and other phenolic compounds in the mangosteen rind which play an important role in the pharmaceutical and health fields (Permana et al., 2012). The

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development of mangosteen production in Indonesia in the period 2012 to 2017 continues to increase. The average growth is 2.49%. In 2016 the number of mangosteen production was 162,862 tons, then in 2017 it increased by 210,000 tons which was the highest production during the 2012-2017 period (Pusdatin, 2017).

As one of the leading horticultural commodities, the mangosteen fruit also has an important role in the agricultural sector, both in terms of the contribution to the national economy, farmers' income, employment and various aspects of people's lives. Mangosteen fruit is also much favored by consumers, both from within and outside the country and is able to penetrate the international market. The mangosteen fruit in recent years has become the mainstay of Indonesia's mainstay export in increasing the country's foreign exchange earnings and also has high economic value (Purwanto, 2008).

The vegetative propagation of mangosteen can be done both conventionally and in vitro. However, conventional propagation has a very low success rate. In vitro propagation is expected to provide mangosteen seeds en masse, uniformly, quickly, not to damage the parent tree and can be propagated throughout the year (Juanda and Cahyono, 2000). The tissue culture method requires growth regulators that function to control organogenesis and morphogenesis in the formation of shoots, roots and callus so as to accelerate the growth of plant explants. One type of growth regulator is cytokinin which plays a role in cell division and for shoot growth. The most active type of cytokinin is BAP, because it is not easily degraded and is inexpensive (Wattimena, 1992).

2. Research Methods

This research was conducted from February to June 2022, at the Yahdi tissue culture laboratory, Jl. Ambung, Tanah Six Hundred, Kec. Medan Marelan, Medan City, North Sumatra 20221. The tools used in this study were culture bottles, autoclave, rubber, plastic, beaker glass, aluminum foil spatula, Bunsen lamp, petri dish, tweezers, volume pipette, Laminar Air Flow Cabinet (LAFC). , PH meter, handsprayer, heater (stove), measuring cup, measuring kettle, scalpel, analytical balance, refrigerator, funnel, heating pan, stir bar, label paper, tissue, pen, ruler and culture rack. The materials used in this study were mangosteen seed ekaplan from the field, shoot explants obtained from mangosteen seeds grown in vitro, 98% alcohol, 70% alcohol, 5% chlorox, 10% chlorox, 15% aquadest, sterile distilled water. , detergent, fungicide (benlate/dithanae 45), bactericide (agrept), antiseptic/antibiotic (anoxylin 500 grams/tablet), 0.1N HCL, 0.1N KOH, MS medium, 2.4 D (0; 1.5; 3 ; 4.5 ppm) Vitamins (0; 1; 2 ppm). . The research implementation includes the preparation and sterilization of tools, media making, preparation of explants, planting, and maintenance. Observation parameters consisted of shoot formation time (DAT), shoot percentage (%), number of shoots, number of leaves, callus formation time (DAT) and callus percentage (%).

3. Results and Discussion

3.1 Mangosteen Callus Propagation

Callus Formation Time. Callus appears first begins with the warping of the explant. Explants that grow are characterized by the appearance of white spots on one or several parts of the explants. The data from the observation of the fastest callus formation in the MediaV2+D3 treatment was the 2nd week. Meanwhile, the time for callus to appear was at the latest in the V2+D1,5 media treatment, which was the 12th week. The time of callus emergence can be seen in Table 1. below this:

Table 1. Time of callus emergence of mangosteen (Garcinia mangostana L) leaves

No	**handling**	Callus Appearing Time/ week
1	Media $V_{0+}D_0$	Week-2
2	Media V0+D1$_{,5}$	Week-5
3	Media $V_{0+}D_3$	Week-4
4	Media $V_{0+}D_{4,5}$	Week-2
5	Media $V_{1+}D_0$	Week-6
6	Media $V_{1\_}D_{1,5}$	Week-4
7	Media $V_{1+}D_3$	Week-4
8	Media $V_{1+}D_{4,5}$	Week-3
9	Media $V_{2+}D_0$	Week-3
10	Media $V_{2+}D_{1,5}$	Week-5
11	Media $V_{2+}D_3$	Minggu ke-5
12	Media $V_{2+}D_{4,5}$	Minggu ke-5

Callus Height. The height of the callus pile observed at week 12 after planting was carried out by calculating the callus height that grew from all explants.

Table 2. Average Number and Total Height of Callus Piles of Mangosteen Plants (Garcinia mangostana L)

Vitamin (ppm) / **2,4D(ppm)**	**0**	**1,5**	**3**	**4,5**	Average
0	0,13	0,1	0,13	0,23	0,14
1	0,1	0,46	0,46	0,16	0,29
2	0,16	0,23	0,2	0,1	0,17
Average	0,13	0,26	0,26	0,16	0,2

The results of analysis of variance in the number of callus callus of mangosteen (Garcinia mangostana L) can be seen in Table 2 and the average yield of callus heap height of mangosteen (Garcinia mangostana L) can be seen in Table 3.

Table 3. Average Height of Mangosteen Leaf Callus (Garcinia mangostana L) at 12 Weeks After Planting Observation (MST)

No	**Handling**	**Average**
1	V0D0	0,13a
2	V0D1,5	0,1a
3	V0D3	0,13a
4	V0D4,5	0,23a
5	V1D0	0,1a
6	V1D1,5	0,46a
7	V1D3	0,46a
8	V1D4,5	0,16a
9	V2D0	0,16a
10	V2D1,5	0,23a
11	V2D3	0,2b
12	V2D4,5	0,1b

The results of data analysis showed that the V1+D0 media treatment resulted in the highest number of Callus Pile Height 0.60 at 12 WAP observations. The lowest callus heap height yield was obtained from treatment with an average number of callus 0.33 roots. The average growth of the Callus Pile Height responded to an increase in the number of roots (Figure 1)

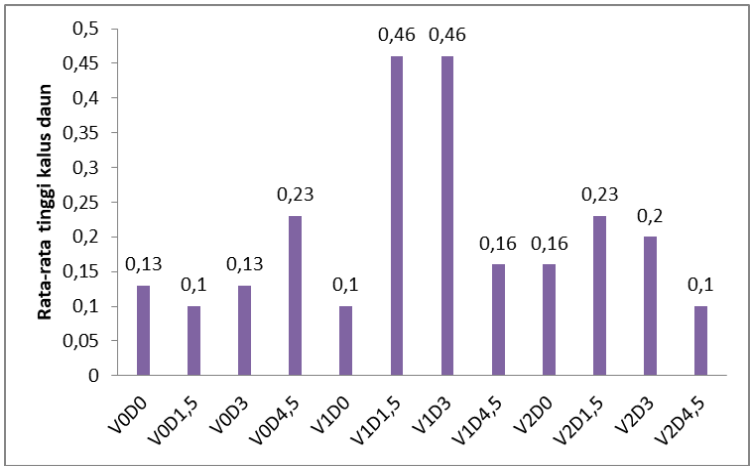
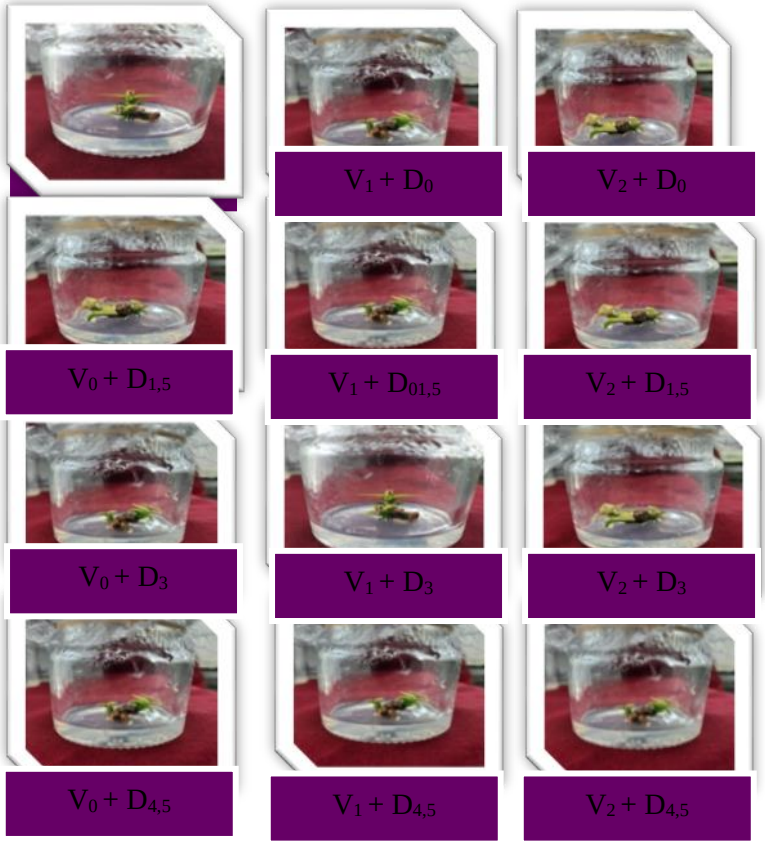


Figure 1 Histogram of Average Callus Height formed on callus leaves of the mangosteen plant (Garcinia mangostana L)



Source : Personally Documented at YAHDI Tissue Culture Laboratory, 2019

Figure 2. Callus observations of mangosteen (Garcinia mangostana L)

Callus Width. Callus width observed at week 12 after planting was done by calculating the callus width that grew from all explants.

Table 4. Average Number and Total Callus Width of Mangosteen Plants (Garcinia mangostana, L)

Vitamin (ppm) / 2,4D(ppm)	0	1,5	3	4,5	Average
0	1,1	1,6	3,06	3,16	2,23
1	3,36	2,1	3,1	1,06	2,40
2	1,63	4,76	3,33	3,33	3,26
Average	2,03	2,82	3,16	2,5	2,63

The results of the analysis of variance in callus width for mangosteen (Garcinia mangostana L) can be seen in Table 4 and the average callus width for mangosteen (Garcinia mangostana L) can be seen in Table 5

Table 5. Average Number of Callus Width (Garcinia mangostana L) at 12 Weeks After Planting Observation (MST)

No	Handling	Average
1	Media V0+D0	1,13a
2	Media V0+D1,5	0,56a
3	Media V0+D3	0,73ab
4	Media V0+D4,5	0,73ab
5	Media V1+D0	0,66bc
6	Media V1+D1,5	1,3bc
7	Media V1+D3	0,6cd
8	Media V1+D4,5	0,83cd
9	Media V2+D0	1,36cd
10	Media V2+D1,5	0,5d
11	Media V2+D3	1,26d
12	Media V2+D4,5	1,23cd

The results of data analysis showed that the V2+D0 media treatment resulted in the highest number of callus widths of 1.36 at 12 WAP observations. The results of the lowest number of callus widths were obtained from the treatment of Media V0+D1,5 and V2+D1,5 with an average number of callus widths of 0.06. The average growth of callus width gave a response to an increase in the number of leaves (Figure 3)

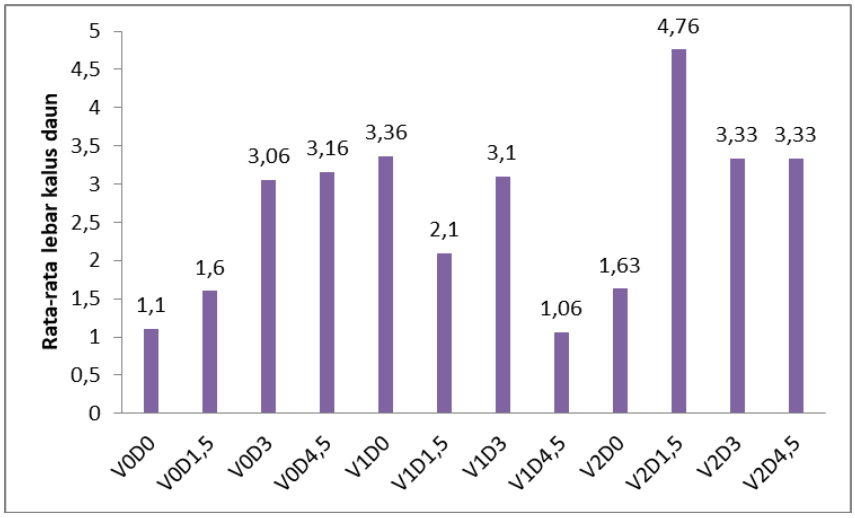


Figure 3. Histogram Average callus width formed on callus of mangosteen (Garcinia mangostana L)

Callus Biomass. The callus biomass observed at week 12 after planting was carried out by calculating the callus biomass that grew from all leaf explants

Table 6. Average and Total Callus Biomass of Mangosteen (Garcinia mangostana L) Plants

Vitamin (ppm) / 2,4D(ppm)	**0**	**1,5**	**3**	**4,5**	**Averaage**
0	0,18	0,12	0,07	0,04	0,10
1	0,19	0,18	0,10	0,10	0,14
2	0,26	0,1	0,09	0,16	0,15
Average	0,21	0,13	0,08	0,1	0,13

The results of analysis of variance of callus biomass of mangosteen (Garcinia mangostana L) can be seen in Table 6 and the average yield of callus biomass on mangosteen (Garcinia mangostana L) can be seen in Table 7.

Table 7. Average Amount of Callus Biomass (Garcinia mangostana L) at 12 Weeks After Planting Observation (MST)

No	Handling	Average
1	Media V0+D0	0,18a
2	Media V0+D1,5	0,12ab
3	Media V0+D3	0,07bc
4	Media V0+D4,5	0,04bc
5	Media V1+D0	0,19bc
6	Media V1+D1,5	0,18bc
7	Media V1+D3	0,10bc
8	Media V1+D4,5	0,10cd
9	Media V2+D0	0,26cd
10	Media V2+D1,5	0,1cd
11	Media V2+D3	0,09cd
12	Media V2+D4,5	0,16d

The results of data analysis showed that the V2+D0 media treatment resulted in an increase in callus biomass of mangosteen plants (Garcinia mangostana L was highest with an average of 0.26 at 12 WAP observations. The lowest callus biomass addition of mangosteen plants (Garcinia mangostana L) was obtained from the V0 medium treatment. +D4,5 with an average callus biomass of 0.040. The average growth of callus biomass for mangosteen (Garcinia mangostana L) is presented in (Figure 4)

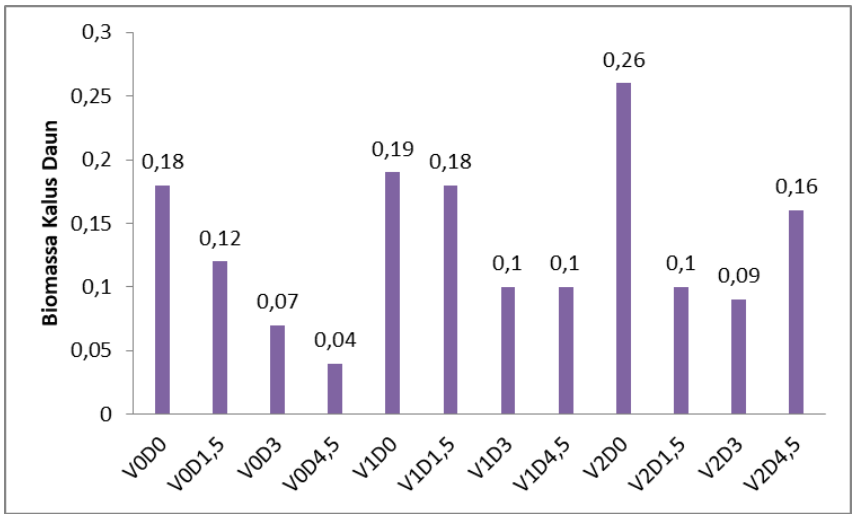


Figure 4. Histogram Average amount of callus biomass formed on explants of mangosteen leaves (Garcinia mangostana L)

Callus Color and Texture. Based on the results of research on callus color, various callus colors were obtained. The callus that is formed also produces a callus that is compact in texture and crumbs.

Table 8 Callus Color and Texture

No	Handling	Callus Color	Callus texture
1	Media $V_{0+}D_0$	Dark green	Unified
2	Media $V_0+D_{1,5}$	Green orange	Crumb
3	Media $V_{0+}D_3$	Light green	Crumb
4	Media $V_{0+}D_{4,5}$	Light green	Crumb
5	Media $V_{1+}D_0$	Brown	Crumb
6	Media $V_{1\_}D_{1,5}$	Light green	Crumb
7	Media $V_{1+}D_3$	Dark green	Unified
8	Media $V_{1+}D_{4,5}$	Dark green	Unified
9	Media $V_{2+}D_0$	Light brown	Crumb
10	Media $V_{2+}D_{1,5}$	Light green	Crumb
11	Media $V_{2+}D_3$	Brown	Unified
12	Media $V_{2+}D_{4,5}$	Dark green	Unified

Callus color is influenced by growth regulators. The addition of hormones with increasing concentrations tends to show a light green color on the callus that lasts longer. Explants that do not form callus will change color from green to brown and then die. Based on observations of callus in the form of compact texture and crumbs. The treatment with media 2,3,4,5,6, and 10 resulted in crumb callus. This shows that optimal hormone administration produces crumb callus.

4. Conclusion

The results showed that the data from the observation of callus formation was the fastest in the MediaV2+D3 treatment, which was the 2nd week. Meanwhile, the time for callus to appear was at the latest in the V2+D1,5 media treatment, which was the 12th week. The results of data analysis showed that the V1+D0 media treatment resulted in the highest number of Callus Pile Height 0.60 at 12 WAP observations. The lowest callus heap height yield was obtained from treatment with an average number of callus 0.33 roots. The results of data analysis showed that the V2+D0 media treatment resulted in the highest number of callus widths of 1.36 at 12 WAP observations. The results of the lowest number of callus widths were obtained from the treatment of Media V0+D1,5 and V2+D1,5 with an average number of callus widths of 0.06. The results of data analysis showed that the V2+D0 media treatment resulted in an increase in callus biomass of mangosteen plants (Garcinia mangostana L was highest with an average of 0.26 at 12 WAP observations. The lowest callus biomass addition of mangosteen plants (Garcinia mangostana L) was obtained from the V0 medium treatment. +D4,5 with an average number of Callus Biomass 0.040. Callus color was influenced by growth regulators.

The addition of hormones with increasing concentrations, callus in the form of compact and crumbly textures indicated that optimal hormone administration resulted in crumb callus.

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