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RESEARCH ARTICLE

EFFECT OF PLANT GROWTH REGULATORS ON IN VITRO CALLUS INDUCTION OF SHOOT EXPLANT MANGOSTEEN (*Garcinia mangostana* L.)

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Abstract

Introduction: The productions of generatively mangosteen still has problem because the seeds of mangosteen have recalcitrant characteristic, which means the seeds must be planted immediately, since the seeds does not have dormancy period.
Aim: 1) to determine Effect of Plant Growth Regulators on *in vitro* Callus Induction of mangosteen (*Garcinia mangostana* L.), 2) to know the best combination and composition of the media to *in vitro* callus induction of mangosteen (*Garcinia mangostana* L.).
Method: Researcher used complete randomized design (CRD) factorial which is consisting of 12 treatments with 3 times repetitions. The research procedures were: tool sterilization, explant sterilization, media making, explants planting.
Result: The fastest response of callus appearance is on sucrose (15; 30 gr / L) and NAA (1,5; 3 ppm) treatment which is producing callus with crumb texture and color is identical to green and white. The callus has 0,53a cm in height, 1,56a cm^2 in width, and 0,40a gr in weight. The percentage of callus formation is 100% and the explant response is growing all over the surface (sucrose 15 gr / L + NAA 1,5; 3 ppm).
Conclusion: The plant growth regulators have effect on *in vitro* callus induction of shoot explant mangosteen (*Garcinia mangostana* L.). The best media combination and composition on *in vitro* callus induction of shoot explant mangosteen (*Garcinia mangostana* L.) were sucrose (15; 30 gr / L) and NAA (1,5; 3 ppm).

Introduction:-

Mangosteen (*Garcinia mangostana* L.) is one type of plants that grows in the tropics and has high benefits. Mangosteen plants can be generatively propagated with seeds but less profitable because the seeds of the mangosteen are recalcitrant. Recalcitrant seeds do not have a dormancy period therefore it should be planted immediately [1]. Therefore, the generative production of mangosteen plants still has constraints that must be solved by using tissue culture methods to produce identical, fast and large quantities of seeds.

Tissue culture has same term with callus culture. Callus culture has purpose to obtain callus from isolated explants and grown it in a controlled environment. Callus culture is important to be used to study cell's metabolism and

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differentiation, cell morphogenesis, somaclonal variation, genetic transformation and secondary metabolite production [2]. The division of cells in callus is triggered by endogenous hormones and exogenous auxin and cytokinins which are added to the culture medium.

Plant growth regulators group auxin, namely NAA can stimulate the highest callus formation which is 83,36% [3]. The using of TDZ (cytokinin group) is also needed in order to produce callus with a friable structure. If the auxin concentration is higher than the cytokines then the callus would grow, and if the concentration of cytokines is higher than auxin, the shoot would grow [1]. Therefore, in this mangosteen callus induction, variation of NAA (auxin group) concentration was used and it was made higher than cytokines concentration. Addition of good BAP with NAA, 2,4-D, and IBA can produce 100% callus formation. The response of callus induction and production also depends on the types of explant and the media that is used [4].

Basides auxins and cytokines, organic compounds such as coconut water can also encourage callus formation. MS with 10% coconut water and 2,4-D was able to induce callus in green grape plants and the best medium was MS 0 + 2,4-D 1,5 ppm + 10% coconut water (D3) by responding to the appearance of callus at 11 days after planting, the percentage of callus was 76,67%, the cell was actively dividing, compact textured, and the color of callus was brownish green [5].

The purpose of this study is 1) to determine effect of plant growth regulators on *in vitro* callus induction of shoot explant mangosteen (*Garcinia mangostana* L.), 2) to know the best combination and composition of the media to *in vitro* callus induction of shoot explant mangosteen (*Garcinia mangostana* L.).

Material and Method:-

This research was conducted in the YAHDI tissue culture laboratory in Perum Pelabuhan Jl. Lambung No. 18 Tanah 600 Medan Marelan, started from May to July 2018. The tools used in the study were culture bottles, petri dishes, analytical scales, volume pipettes, measuring cups, glass funnels, cup glasses, autoclaves, refrigerators, pH meters, aluminum foil, spatulas, scalpels, knives, tweezers, scissors, labels, tissue, sprayer bottle, Bunsen burner, lamp, stirring rod, millimeter paper, culture rack, AC and *laminar air flow cabinet* (LAFC).

The plant material that was used as explants was the shoot of the mangosteen plant from seeds cultured, Murashige and Skoog (MS) Media + 20% coconut water + TDZ 0,2 ppm + sucrose (0, 15, 30, 45 gr / l) and NAA (0, 1,5, 3 ppm), 98% alcohol, 70% alcohol, 15% and 20% chlorox, sterile distilled water, detergent, fungicide (Benlate), bactericide (Streptomycin), antibiotics (amoxilin 500 grams / tablet), gelatin and sugar.

Procedure

This study used basic media MS + TDZ 0,2 ppm + 20% Coconut water with the addition of a combination of sucrose (0; 15; 30; 45 g / L) and NAA (0; 1,5; 3 ppm). At the time of conducting research, sterilization of equipment explant sterilization and media making were conducted. The explant was sterilized gradually using detergents, fungicides and clorox. The research parameters observed were callus formation time, callus texture and color, callus height, callus width, callus weight, percentage of callus formation and explant response.

Research Design

This study was arranged based on complete randomized design (CRD) factorial, with 12 treatments with 3 times repetitions, and each one unit of experiment was using 1 explant to obtain 36 explants in 12 experimental units. This study used basic media in the form of MS + TDZ 0,2 ppm + 20% Coconut Water with the addition of a combination of Sucrose (0; 15; 30; 45 g / L) and NAA (0; 1,5; 3 ppm). The combination of media can be seen in table 1 below:

Table 1:-Media combination for *in vitro* callus induction of shoot explant mangosteen (*Garcinia mangostana* L.)

NAA Sucrose	0 ppm	1,5 ppm	3 ppm
0 gr/L	Sucrose 0 gr/L, NAA 0 ppm	Sucrose 0 gr/L, NAA 1,5 ppm	Sucrose 0 gr/L, NAA 3 ppm
15 gr/L	Sucrose 15 gr/L, NAA 0 ppm	Sucrose 15 gr/L, NAA 1,5 ppm	Sucrose 15 gr/L, NAA 3 ppm
30 gr/L	Sucrose 30 gr/L, NAA 0 ppm	Sucrose 30 gr/L, NAA 1,5 ppm	Sucrose 30 gr/L, NAA 3 ppm
45 gr/L	Sucrose 45gr/L, NAA 0 ppm	Sucrose 45gr/L, NAA 1,5 ppm	Sucrose 45gr/L, NAA 3 ppm

The data which was obtained from observation was analyzed by ANOVA statistics test, if the result shows significant difference effect, then *Duncan's Multiple Range Test* (DMRT) would be then performed as follow up test.

Result:-

The results showed the positive effect of plant growth regulators addition to callus induction from the source of mangosteen shoot explants, which can be seen from the time of callus formation, callus texture and callus color, callus height, callus widht, callus weight, percentage of callus formation and explant response.

Time of Callus Formation

Based on the results of the research, the callus formation of mangosteen shoot explants can be seen in the table below:

Table 2:-Callus formation time of mangosteen (*Garcinia mangostana* L.)

No	Treatments	Weeks After Induction (WAI)
1	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + NAA_0 ppm	5
2	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + $NAA_{1,5}$ ppm	5
3	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + NAA_3 ppm	5
4	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + NAA_0 ppm	4
5	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + $NAA_{1,5}$ ppm	3
6	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + NAA_3 ppm	3
7	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + NAA_0 ppm	4
8	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + $NAA_{1,5}$ ppm	3
9	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + NAA_3 ppm	3
10	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + NAA_0 ppm	4
11	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + $NAA_{1,5}$ ppm	4
12	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + NAA_3 ppm	4

Texture and Callus Color

Based on the results of the study the texture and color of callus explants of mangosteen shoots can be seen in the table below:

Table 3:-Texture and color of callus explant of mangosteen shoot (*Garcinia mangostana* L.)

No	Treatments	Callus textures	Callus colors
1.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + NAA_0 ppm	Compact	Brownish white
2.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + $NAA_{1,5}$ ppm	Compact	Brownish yellow
3.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + NAA_3 ppm	Compact	Green
4.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + NAA_0 ppm	Loose	Yellow
5.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + $NAA_{1,5}$ ppm	Loose	White
6.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + NAA_3 ppm	Loose	Green white
7.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + NAA_0 ppm	Compact	Green
8.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + $NAA_{1,5}$ ppm	Loose	Green yellow
9.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + NAA_3 ppm	Loose	Green
10.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + NAA_0 ppm	Compact	Yellow
11.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + $NAA_{1,5}$ ppm	Compact	White
12.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + NAA_3 ppm	Compact	White

Height, Width, and Weight of Callus

The average height, width and weight of callus from mangosteen shoot explants can be seen in the table below:

Table 4:-The average height, width, and weight of mangosteen (*Garcinia mangostana* L.) callus formation in 12th weeks after induction

No	Treatments	Average height (cm)	Average width (cm^2)	Average weight (gram)
1	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + NAA_0 ppm	0,17c	0,56cd	0,17c
2	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + $NAA_{1,5}$ ppm	0,20bc	0,31d	0,18c
3	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + NAA_3 ppm	0,30abc	0,54cd	0,18c
4	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + NAA_0 ppm	0,23bc	0,60cd	0,16c
5	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + $NAA_{1,5}$ ppm	0,27bc	1,52ab	0,37ab
6	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + NAA_3 ppm	0,53a	1,56a	0,40a
7	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + NAA_0 ppm	0,27bc	0,63cd	0,19c
8	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + $NAA_{1,5}$ ppm	0,40abc	0,82bcd	0,27bc
9	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + NAA_3 ppm	0,43ab	1,20abc	0,26bc
10	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + NAA_0 ppm	0,23bc	0,49cd	0,23c
11	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + $NAA_{1,5}$ ppm	0,23bc	0,46cd	0,25bc
12	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + NAA_3 ppm	0,23bc	0,81bcd	0,24bc
	Total	3,49	9,5	2,9
	Average	0,29	0,79	0,24

Note : Numbers followed by the same letter in the same column indicate no significantly different (P> 0.05) in the DMRT test at 5% level.

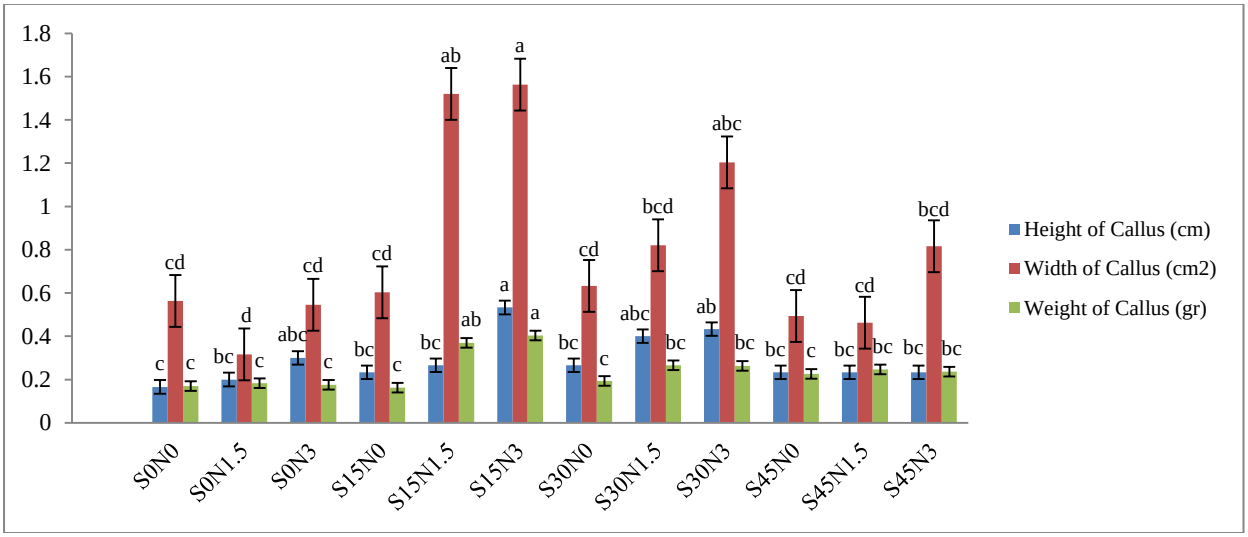

Figure 1:-Average Height, Width, and Weight of Callus of Mangosteen Shoot Explants (*Garcinia mangostana* L.)

Note :

S = Sucrose N = NAA (*Naphthalene Acetic Acid*)

Percentage of callus formation and explant response

Table 5:-Percentage of callus formation and explant response on mangosteen (*Garcinia mangostana* L.) callus induction

No	Perlakuan	Persentase Pertumbuhan Kalus	Respon Eksplan
1.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + NAA_0 ppm	100%	*
2.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + $NAA_{1,5}$ ppm	100%	*
3.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_0$ gr/l + NAA_3 ppm	100%	*
4.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + NAA_0 ppm	100%	*
5.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + $NAA_{1,5}$ ppm	100%	***
6.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{15}$ gr/l + NAA_3 ppm	100%	***

7.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + NAA_0 ppm	100%	*
8.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + $NAA_{1,5}$ ppm	100%	**
9.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{30}$ gr/l + NAA_3 ppm	100%	**
10.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + NAA_0 ppm	100%	*
11.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + $NAA_{1,5}$ ppm	100%	**
12.	MS + Coconut $Water_{20\%}$ + $TDZ_{0,2}$ ppm + $Sucrose_{45}$ gr/l + NAA_3 ppm	100%	**

Note:
(*) : Swollen callus orcallus growth only at one end of explant
(**) : Callus growth on parts of explant surface
(***) : Callus growth on entire explant surface

Discussion:-

Time of Callus Formation

The results of the research in table 2 above show that the fastest response in inducing mangosteen callus is found in sucrose treatment (15; 30 gr / L) and NAA (1,5; 3 ppm). Furthermore, it was followed by sucrose treatment (15; 30; 45 gr / L) and NAA (0; 1,5; 3 ppm) which induced callus treatment in the fourth week after planting. Then the slowest response in mangosteen callus induction was found in 0 gr / L and NAA sucrose treatment (0; 1,5; 3 ppm). The fastest response explains that the concentration of media when inducing callus is optimal. This indicates that the concentration of sucrose can affect the appearance of callus (not too high or too low). The increasing concentration of sucrose from 30 gr/lto 40 gr/l actually reduced the response of anther in forming callus [6]. Mean while 40 grams of sucrose was the best concentration in the culture of sunflower anther [7]. Auxin addition was very effective to induce callus formation. However, the role of cytokines was needed for proliferation of callus so the combination of auxin and cytokines was very good to stimulate callus growth [8]. The addition of auxin at high concentrations stimulates callus formation, on the contrary if the ratio of auxin and cytokine media was lower, it would stimulate the growth of explants regeneration to produce organs [9]. The progression rate of callus was influenced by the work of auxin endogenous and exogenous cytokines hormones which correlated with each other. Improper administration of growth regulators could inhibit callus growth on explants. The inhibition of callus formation was due to endogenous and exogenous hormones found in explants unable to stimulate callus growth quickly [10].

Texture and Callus Color

Based on the results of the study in table 3, mangosteen callus induction was seen like crumb textured on sucrose treatment (15; 30 gr / L) and NAA (0; 1,5; 3 ppm) with callus color in 15 gr / L + NAA sucrose treatment 0 ppm is yellow, sucrose 15 gr / L + NAA 1,5 ppm is white, sucrose 15 gr / L + NAA 3 ppm is greenish white, sucrose 30 gr / L + NAA 1,5 ppm is greenish yellow and sucrose 30 gr / 3 ppm L + NAA is green. Furthermore, callus which produces compact texture is found in sucrose treatment (0; 30; 45 gr / L) and NAA (0; 1,5; 3 ppm) with varying callus colors, is 0 gr / L + NAA 0 ppm sucrose in white brownish, sucrose 0 gr / L + NAA 1,5 ppm yellow brownish, sucrose (0; 30 gr / L) + NAA (0; 3 ppm) green, sucrose 45 gr / L + NAA 0 ppm yellow, sucrose 45 gr / L + NAA (1,5; 3 ppm) is white.

The crumb or fragile callus texture was considered good because it simplifies the callus separation into single cells and it would increase oxygen aeration between cells [11]. Visually, the crumb texture callus that forms on the explant, has characteristics of tenuous bonding between cells, easily separated and if taken with nippers, the callus was easily broken and some were attached to the nippers. Crumb texture callus appear to have small, clustered cells and if the cells were taken, they are easily released [12]. The compact callus texture was an effect of cytokines and auxin which affecting water potential in cells. This the absorption of water from medium into cell is increasing so the cell becomes more rigid [13]. Callus with solid texture have characters of solid cells bonding that are difficult to separate and tend to clot solid.

The color of callus induces the presence of chlorophyll in the tissue, the more green callus color, the more chlorophyll content. Light or white color could also induce, and indicated callus conditions were still good enough [11]. Addition of cytokines concentration tends to show more durable green (bright) color in callus [12]. The white color callus indicates the callus does not contain chlorophyll. White callus was an embryonic tissue that does not contain chloroplasts, but has a high content of starch [13].

Height, Width and Weight of Callus

Based on table 4, it shows that the callus formation height response from mangosteen shoot explants correlates with callus width and weight. In the treatment of 15 gr / L + NAA sucrose (1,5; 3 ppm) produced callus height 0,53a cm, callus width 1,56a cm^2, and callus weight 0,40a gr. Meanwhile, the numbers followed by the same letter in the same column showed no significant difference in sucrose treatment (0; 30; 45 gr / L) and NAA (0; 1,5; 3 ppm). Auxins such as NAA were effective in stimulating callus formation because of its strong activity to suppress organogenesis and maintained callus growth, while sucrose was the main energy source for explants to grow [14]. Sucrose is important carbon source used as a constituent of cells. Thus, sufficient sucrose can promote cell division, cell enlargement and cell differentiation properly [8].

Lower callus weight occurs because explants only experience callus formation on some explant surfaces. This was related to relatively longer callus initiation time due to an imbalance in concentration of sucrose and NAA thus, from the beginning growth of explants takes place slowly in inducing activity of metabolic enzymes. Low acidity can also activate certain enzymes in cell walls which can degrade various proteins or polysaccharides that spread on soft and flexible cell walls, so cell enlargement can occur [15].

Percentage of Callus Formation and Explant Response

Based on table 5 it can be seen that all treatments produced 100% callus formation from mangosteen shoot explants, but with different explant responses. On 15 gr/l sucrose and NAA (1,5; 3 ppm), callus grows on the entire surface of the explant. On sucrose (30; 45 gr / l) and NAA (1,5; 3 ppm), callus growth on some explant surfaces. While in other treatments, callus only form at one end of explant. Callus culture requires sucrose and coconut water as an energy source in order to proliferate and form callus. Coconut water is a group of organic compounds containing amino acids, organic acids, sugars, vitamins and some growth regulating substances such as auxins, cytokines, zeatin, zeatin ribosides [16].

Growth regulating agents also play a role in faster callus formation and perfect callus growth. Callus formation can occur on surface of explants and wounds, and is characterized by swelling of the explants and appearance of white, green or yellow spots (Figure 2). The formation process of callus starts from a small bulge on the edge of the explant which will grow into white callus and after a certain time callus will fill the entire surface of the explants [17].

Figure 2. Responses of mangosteen shoot callus on treatment a) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_0 gr/l + N_0 ppm, b) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_0 gr/l + $N_{1,5}$ ppm, c) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_0 gr/l + N_3 ppm, d) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{15} gr/l + N_0 ppm, e) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{15} gr/l + $N_{1,5}$ ppm, f) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{15} gr/l + N_3 ppm, g) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{30} gr/l + N_0 ppm, h) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{30} gr/l + $N_{1,5}$ ppm, i) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{30} gr/l + N_3 ppm, j) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{45} gr/l + N_0 ppm, k) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{45} gr/l + $N_{1,5}$ ppm, l) MS + $CW_{20\%}$ + $T_{0,2}$ ppm + S_{45} gr/l + N_3 ppm.

Conclusion:-

According to research result, it was concluded that: 1) plant growth regulators have affect *in vitro* callus induction of shoot explant mangosteen (*Garcinia mangostana* L.) 2) the best media combination and composition for mangosteen *in vitro* callus induction was sucrose (15; 30 gr/L) and NAA (1,5; 3 ppm).

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