



## In Vitro Culture of Dragon Fruit (*Hylocereus polyrhizus*): Callus and Anthocyanin Production

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### Abstract

Tissue culture biotechnology has been widely used to produce secondary metabolites. Anthocyanin as the secondary metabolic content in Dragon Fruits was thought to be able to liberate free radicals and have pharmacological activities as antioxidants and anti-aging. Anthocyanin can be produced in vitro through callus production by tissue culture. The study aims to produce callus cultures that have the potential to produce secondary metabolites. This research was conducted in the tissue culture laboratory. The study used a completely randomized design method with a factorial pattern. Factor 1 : Type of explants ( M1): Explants from young shoots; (M2) Explants from dragon fruit callus. Factor II: The number of explant each culture tube. There are J1,J2, 3 and J4 with (1;2;3;4 explants in each culture tube. Murashige and Skoog + 15 % sucrose were used as media culture. The results of study showed that: callus began to form at 7 weeks after planting. (1) MS Media with the addition of 15 % sucrose had a significant effect on increasing the anthocyanin content in the callus of Dragon fruit formed. (2) Callus formed from Shoot Dragon fruit contain Anthocyanin 0,74 % ; (3) The Callus formed from Shoot and callus Dragon Fruits contain secondary metabolites of Anthocyanins ranging from 0,68%–0,76%. The highest Anthocyanin content (0,76%) was produced in treatment J3 ( 3 Explant each culture tube)

**Keywords:** anthocyanin, callus, dragon fruit, in vitro

### A. Introduction

Dragon fruit is a tropical plant with many benefits. The content of anthocyanins in fruit has pharmacological activity as an antioxidant and anti-aging (Inderwati, I. Sri Arijanti Jajuk H., Ristani W. Primawan P Nugrahadi, 2020). Red dragon fruit is a plant that contains substances that can increase endurance and improve metabolism (Dragon fruit has been reported for its high antiradical activities with the presence of phenolic compounds. Some significant characteristics of plant pigment are betacyanin, anthocyanin, and other flavonoids. (Prabowo I., Utomo, E. P., Nurvayzy, A., Widodo, A., Widjanto, E., & Rahadju, P., 2019). Red dragon fruit peel extract

(*Hylocereus polyrhizus*) containing phytochemical compounds is an effective antioxidant from natural plant sources, with anti-cardiogenic and anti-inflammation properties, and may help with other degenerative disorders.

With the development of biotechnology; secondary metabolite can be produced in Vitro with Tissue culture technology. (Rahmawati, 2006) reported that tissue culture techniques have been widely used in pharmaceutical field to produce secondary metabolites in larger quantities in short time for medical purposes. Mahmudah (2021) added that tissue culture is an alternative technology that can be used to produce secondary metabolites. The tissue culture method can be used to increase the content of metabolic compounds in callus by manipulating the level of component in media or adding precursor compound, and other alternatives; by increasing the productivity of cells maintained on a variety of aseptic artificial media (Rahmawati, 2006)

Mahmudah (2021) reported that the addition of combination of Plant Growth Regulator (PGR) to the culture of the young leaves of *Plectranthus scutellarioides* (Her plant) called callus contained the flavonoid quercetin with a concentration of 33.7 mg/gram callus. Indarwati, I., D, R, Suryaningsih, Sri Arijanti and A. W. Qurotin (2021), reported that in vitro tissue culture technology could be used to produce papain from papaya leaf callus; callus formed from sliced papaya varieties (California, Bangkok and Gantung) contain papain in range (11.06 % - 19.5%)

Indarwati *et al.* (2020) in her research reported that MS Media manipulation with addition of a sucrose elicitor in callus culture succeeded in increasing the Anthocyanin content in Dragon fruit callus formed. The addition sucrose Elicitor on MS media, the callus formed from the development of young shoots contained the highest Anthocyanins (0.14%) compared to other treatment while the addition of 5% carbohydrates elicitor callus that is formed only contains anthocyanins ranging (0.06% - 0.09%).

This study aims to produce callus culture that have the potential to produce better secondary metabolites (Anthocyanins) by modifying the variety of explant materials and the number of explants planted in the media (MS + 15 % sucrose).

## B. Literature Review

### 1. Overview of Anthocyanin on Dragon Fruit

Dragon fruit is a tropical fruit that many Indonesian people like. The exotic aesthetic characteristics of dragon fruit with attractive pulp in purple-red color make it also very popular in the European and United States markets. Dragon fruit, including tropical fruit that has high economic value. This is due that Dragon fruit not only used as an ornamental plant but also used fruit. Dragon fruit is unique its stems are triangular and have very short spines which differ from the general shape of stems that are round or rectangular. Judging from the flower of this plant has a funnel-shaped crown and stars blooming at dusk and bloom entirely at midnight with a fragrant smell.

Dragon fruit also has health benefits, because of the bioactive components contained in the fruit. Red dragon fruit is a plant that contains substances that can increase endurance and improve metabolism. Some research results on dragon fruit skins have been done. Dragon Fruit Skin is known to have antioxidant content of vitamin C, flavonoids, tannins, alkaloids, steroids, and saponin. Antioxidant compounds play a very important role in helping to overcome carcinogenic. There are natural antioxidants found from bio resources, plants, medicinal plants, and metabolites produced from various microbes that are currently used as natural active pharmaceutical ingredients (NAPIs) and nutraceuticals to improve human health (Kitts, D.D.; Wijewijkreme, A.N.; Hu, C. 2000).

Dragon fruit is good for health has been proven through an analysis conducted by "Taiwan Food Industry Development and Research Authorities". The benefits of consuming dragon fruits are : (1) Albumin which can release toxins; (2) Anthocyanin can free radicals and slow the ageing process/ anti-ageing; (3) Vitamin C which can beautify and make skin brighter; (4) Rich in fibre/ soluble fibre, so its useful for dieting; (5) Reducing diabetes; (6) Preventing colon cancer and launching bowel movement. Indarwati (2020) reported that Dragon fruit contain metabolic secondary (Anthocyanin) in vegetable and fruit. Dragon Fruits extract contain anthocyanin about 26,46 ppm. Anthocyanin are dyes that give red to blue colors. Anthocyanin belong to pigments called Flavonoids.

Dragon Fruit is also known as natural dyes because it contains anthocyanin, play a role in giving red colour so that are often used for various food Industries. Anthocyanins are found in all plant organs of Dragon fruits. The strong red colour has been used as natural dye, Dragon Fruit skins have been applied to the food and tested in white rats, the test results showed coloring dragon fruit can be used as a natural dye food (Handayani, E., Samudin, S. & Basri, Z., 2013). Zhang, Y., S.K.Vareed., M.G. Nair. (2005) reported that anthocyanins are one of the flavonoid groups that

have successfully known their benefits as bioactives that inhibit the growth of cancer cells in humans. The results of Yamuangmoru and Prom (2021) reported that Anthocyanin has been shown to lower the risk of several illnesses including cancer and obesity, as well as to have anti-viral, anti-inflammatory, and anti-aging properties.

Some of the secondary metabolites contained in the skin and flesh of Dragon Fruit can also be used as anti-microbial substances. Alkaloid compounds work by interfering with the components that make up peptidoglycan in bacterial cells, so that the bacterial cell wall layer becomes unstable (Suhartati, 2018). The destruction of peptidoglycan can be through the destruction of hydrogen bonds between the peptides that make it up which results in the cell wall layer being incompletely formed and bacteria can die (Ainurrochmah, 2013). Flavonoid compounds can inhibit bacterial growth naturally, these compounds can cause bacterial cell wall to be damaged and inhibit the movement of bacteria (Zubaidah, N., Juniarti, D. E., & Basalamah, F., 2018).

## **2. Tissue Culture: Biotechnology to Produce Secondary Metabolites**

Tissue culture is the cultivation of plant cells into a whole plant. Tissue culture is often called in vitro culture or cell/tissue culture in glass tubes. In its development, tissue culture is widely used to modify plants and improve plants (Harahap, 2011). Further statement by Arijanti, Ribkahwati dan Retno D. (2009), Tissue culture is a method for isolating parts of plants such as protoplasm, cells, tissues, or organs and growing them in aseptic conditions so that these parts can regenerate into whole plants again. Through tissue culture, it is hoped that the seeds can produce the same plant seeds as their parents, as well as the seeds obtained, are free from pests and disease, and produce uniform seeds.

In tissue culture techniques, several media are often used in implementation but Murashige and Skoog (MS) and Vacin and Went media are relatively good media because nutrients, both macro and micro, and vitamins for plant growth and development can be fulfilled. In the method of propagation through in vitro culture, the growth and development of the explants are strongly influenced by the type of basic media and growth regulators. MS medium is the basic medium that is generally used for the propagation of a large number of plant species. The basic media is rich in minerals that stimulate organogenesis.

The use of in vitro culture technology, which was previously used for plant breeding and propagation, has now begun to be directed towards producing large amounts of secondary metabolites in a short time. The use of this technology can at the same time answer the problem of limited land, and maintain the balance of biodiversity by avoiding overexploitation of germplasm as a source of natural medicine.

Some of the advantages of using plant tissue culture techniques for the production of secondary metabolites include: (1) not depending on environmental factors such as climate, pests and diseases, geographical and seasonal barriers. (2) the production system can be arranged, when needed and in the desired quantity, so that it is close to the actual market conditions. (3) produce more consistent quality and yields and (4) reduce land use. Arijanti and Dwi Retno (2018) added that tissue culture can become a business opportunity. Furthermore, Indarwati et al. (2020) added that tissue culture technology has also been widely used to produce secondary metabolic compounds. Several tissue culture methods used to produce secondary metabolites include hairy root culture, cell suspension and callus culture. According to Wonganu (2007), callus is a tissue culture method that has high potential in providing secondary metabolites. Several research reports have proven that tissue culture biotechnology has been widely used to produce secondary metabolites. The addition of 5% sucrose can increase the production of Anthocyanins in callus *Populus hybrida*. The addition of 12.5% fructose in culture media can increase the content of polyphenols and citronellol in *Rose hybrida*. Furthermore Suryaningsih D. R., Prakoeswa S. A., & Eryanto A. (2021), reported the result of his research that the addition of the elicitor *Saccharomyces cerevisiae* to MS and VW Media increased the papain content in the callus of papaya leaves. Callus formed from sliced papaya leaves produces papain with a range (0,126 - 0,148 %).

The chemical industry or pharmaceutical industry is an industry that is supported by natural compounds from plants. Under certain circumstances, natural compounds from this plant cannot be replaced because of their healing activity. The potential for chemical synthesis in the plant world is enormous. More than 400 thousand plant species have identified the chemical and 10 thousand of them contain secondary metabolites which have potential as raw material for vegetable pesticides. (Saenong, M. Sudjak, 2016). Purbaningrum (2013) said, to obtain bioactive compounds in large quantities naturally, it is often difficult to deal with the supply of plants.

In an effort to produce secondary metabolic compounds (anthocyanin content) which is higher in dragon fruit callus, it is tried to propagate through tissue culture and biosynthesis of Anthocyanin compounds by adding 15 % sucrose, with two kinds of explant sources and the number of explants in culture tubes.

### C. Methodology

#### 1. Place and Time

In vitro experiment was done to produce callus as an extraction material, to determine the Anthocyanin content in callus. The study was conducted at the Tissue Culture Laboratory; Faculty of Agriculture, University of Wijaya Kusuma Surabaya

#### 2. Material and Method

Materials : : explants from callus (in culture tubes) and shoots of dragon fruits plants, MS Media (tabel 1); Growth Regulators, NAA, BAP Coconut Water, Glucose, Fructose, Sucrose, 70%, and 90% Alcohol.

Equipment required during this research are: Sartorius Scales, Autoclave, Oven, LAF, pH meter, Tweezers, Scalpel, Erlenmeyer, Measuring cup, Measuring pipette; Petri dish, Dropper Pipette, tweezers, spatula, Culture tube, Magnetic stirrer, and Gas Chromatography.

#### 3. Research Design

The study was conducted using a completely randomized design with two factors. The Factor I: Types of Planting Material / plantlet there are 2 levels; M1 = shoot explant; M2 = callus of eksplant dragon fruit. Factor II. Number of explant dragon fruit; there 4 levels; J1 ;J2 ;J3 and J4 each with 1;2;3 and 4 explant each culture tube Each treatment was repeated 4 times, with 10 replications each.

#### 4. Culture Condition

Sterile culture tubes with Autoclave 17 psi 30 minutes essential media utilized by MS media. + 15 % sukrosa ( Indarwati, *et al.* 2020) Sterile youthful shoot explants from Dragon fruit plants cut into  $\pm 1$  cm pieces and absorbed betadine, planted in culture tubes that as of now contain media as indicated by treatment. The steril explant was planted in the MS medium under Laminar Air Flow Cabinet. In the wake of planting it is set on a brooding rack which comprises hatching stages.

**Table 1 .Composition of Murashige and Skoog media ( Arijanti and Dwi Retno S., 2018)**

Ingredients	Material requirement (mg/l)
1. Macro: Nutrien	
KNO <sub>3</sub>	1900
NH <sub>4</sub> NO <sub>3</sub>	1650
CaCl <sub>2</sub> 2H <sub>2</sub> O	440
MgSO <sub>4</sub> 7H <sub>2</sub> O	370
KH <sub>2</sub> PO <sub>4</sub>	170
2. Micro Nutrien	
MnSO <sub>4</sub> 7H <sub>2</sub> O	22.3
ZnSO <sub>4</sub> / 7H <sub>2</sub> O	8.6
H <sub>3</sub> BO <sub>3</sub>	6.2
KI	0.83
CuSO <sub>4</sub> . 5H <sub>2</sub> O	0.025
NaMoO <sub>4</sub> . 2H <sub>2</sub> O	0.25
CaCl <sub>2</sub> . 6H <sub>2</sub> O	0.025
FeSO <sub>4</sub> . 7H <sub>2</sub> O	27.8
NaEDTA . 2H <sub>2</sub> O	37.3
3. Vitamin	
Mio-inositol	100
Thamin HCl	0.1
Nikotinik acid	0.5
Piridoksin HCl	0.5
Glisin	2
4. Carbohydrate	30.000 + 15 % Sucrose

## 5. Variable

- a) **Callus quality.** Seen at timespans weeks outwardly utilizing scoring: 1 = no callus; 2 = reduced callus; 3 = friable callus
- b) **Callus Quantity:** Observed at timespans weeks outwardly by scoring scor 1 = no callus; 2 = growing of explants; 3 = little callus (<1 times the explant size); 4 = medium callus (1-2 times the explant size); 5 = many callus (> multiple times the explant size)
- c) **The content of secondary metabolites in the callus ( Anthocyanin ) :** Observed ruinously through Anthocyanin content examination at about two months subsequent to planting (56 days) Secondary Metabolite Analysis of the material extricated utilizing total liquor at that point broke down by gas chromatography

## 6. Technique of Data Analysis

The data obtained were processed using Variance Analysis (Test F) using a completely randomized design patterns at the level of 5%. If there were any real differences between treatments. If there is an influence that is a significant difference between treatments then the test is continued with a comparison test between treatments using the Least Significant Difference Test (LSD) at the 5% level.

## D. Result

### 1. Observation of Callus Quality.

The result of observation on callus quality showed that there was no significant different between the types of explants and the number of explants. Callus began to form at the age of 7 weeks after planting with callus quality toward compact as presented in Table 2. The observation of the growth of the quality starting from the age of 1 to 12 weeks after planting can be seen in table 2.

Table 2 it can be seen that there until the age of 6 weeks the treatment types and the number of explants) had the same effect on quality of callus with score of 1. The value of callus quality began develop at week 7 tends to be slow, because for 7 weeks the explant only experienced swelling. While the number of explants one to four had no effect on the formation of callus quality. There has not been a competition for the use of nutrients in culture media nutrients due to the slow growth. It is suspected that factors from outside and from within the tissue culture environment have not provided maximum support.

**Table 2. Average scoring Quality callus Dragon Fruit at Various Age Obsevation (WAP)**

Treatment	1	2	3	4	5	6	7	8	9	10	11	12
M1J1	1,00	1,00	1,00	1,00	1,00	1,00	1,10	1,1	1,30	1,44	1,62	1,80
M1J2	1,00	1,00	1,00	1,00	1,00	1,00	1,04	1,04	1,12	1,12	1,14	1,30
M1J3	1,00	1,00	1,00	1,00	1,00	1,00	1,06	1,12	1,30	1,36	1,42	1,50
M1J4	1,00	1,00	1,00	1,00	1,00	1,00	1,08	1,16	1,42	1,56	1,66	1,70
M2J1	1,00	1,00	1,00	1,00	1,00	1,00	1,02	1,04	1,16	1,16	1,20	1,30
M2J2	1,00	1,00	1,00	1,00	1,00	1,00	1,02	1,04	1,16	1,16	1,20	1,30
M2J3	1,00	1,00	1,00	1,00	1,00	1,00	1,10	1,12	1,32	1,44	1,48	1,54
M3J4	1,00	1,00	1,00	1,00	1,00	1,00	1,10	1,12	1,28	1,32	1,34	1,40
LSD: 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Note : NS : Non Significant ; WAP : Week after Planting

### 2. Observation of Callus Quality.

Analysis of callus quantity showed that there were no interactions between the single factor types of explant and the number of eksplant. The result of observations of callus quantity showed that there was No. significant difference in the treatment of explant sources and the number of explants on quantity of Dragon Fruit Callus. The real difference only occurred in the singgle factor explant sources at weeks 2 to 5 on the quantity of callus formed as presented in table 3 below.

In table 3. Shows the quantity of callus which tends to be better in explants derived from stem than those from callus. The highest number of callus was form in the number of explants 4 although it was not significantly different from the other treatments. It is suspected that the number of explants 4 with slow callus growth has not seen any nutritional competition for the

growth and development of explants. Callus is mass of cell formed on surface of the explant or in the incision / wound. The appearance of callus on the cut surface is a protective response for plants to repair damaged tissue (Arijanti, *et al.*, 2018).

**Table 3. Average scoring quantity callus due at Various Age Obsevation (WAP)**

Treatment	1	2	3	4	5	6	7	8	9	10	11	12
M1	1,00	1,00b	1,12b	1,35b	1,76b	2,00	2,00	2,11	2,27	2,33	2,44	2,49
M2	1,00	1,29a	1,48a	1,88a	2,00a	2,00	2,00	2,08	2,20	2,27	2,28	2,32
LSD 5 %	NS	S	S	S	S	NS	NS	NS	NS	NS	NS	NS
J1	1,00	1,14	1,28	1,56	1,84	2,00	2,00	2,08	2,20	2,30	2,30	2,40
J2	1,00	1,16	1,30	1,62	1,88	2,00	2,00	2,06	2,14	2,18	2,19	2,20
J3	1,00	1,14	1,30	1,62	1,88	2,00	2,00	2,10	2,30	2,40	2,44	2,50
J4	1,00	1,14	1,32	1,66	1,92	2,00	2,00	2,14	2,34	2,44	2,48	2,52
LSD: 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Note: S : Significant ; NS = Not Significant WAP : Week After Planting

Callus quantity shows the number of callus formed as a result of cell division. In general, the growth and development of callus is influenced by the elements in the media. Each explant of different species has different media requirements in this case the nutrients needed for growth. Success in plant tissue culture techniques is highly dependent on the media used. It is known that tissue culture media contain macro, micro, vitamins and carbohydrates (glucose) as carbon substitutes (Rahmawati S., 2006).

### 3. Observation of Callus Weight.

The results of the observation of callus weight at the age of 12 weeks showed a significant difference in a single factor. The average weight of callus at 12 weeks of age is presented in Table 4. From Table 4. it can be seen that the heaviest callus was produced in the treatment with explant from callus. Compared with other treatments, it was suspected that in explant one there was no nutritional competition between explants so that the allocation of nutrients was able to increase callus weigh.

**Table 4. Callus Weight Effect of Treatment Types and Number of Explants**

Treatment	Callus Weight ( g)
M1	0.61 a
M2	0.60 b
LSD 5 %	S
J1	0,80
J2	0,55
J3	0,54
J4	0,51
LSD 5 %	NS

Note : S : Significant ; NS : Non Significant

### 4. Anthocyanin Content in Callus

The result of observasion made on the anthocyanin conten in callus aged 8 and 12 weeks showed no significant difference between the treatment of explant sources and the number of explants as shown in table 5.

**Table 5. Anthocyanin Content Effect of Treatment on Explant Material**

Treatment	Anthocyanin content on callus (%)	
	8 WAP	12 WAP
M1	0,68 a	0,74 a
M2	0,65 b	0,72 b
LSD 5%	S	S
J1	0,62	0,68 c
J2	0,67	0,73 bc
J3	0,70	0,76 a
J4	0,68	0,74 b
LSD 5%	NS	NS

**Note S : Significant ; NS : Non Significant ; WAP : Week after Planting**

From Table 5. It can be seen that at 12 WAP, the type of explant source treatment had a significant effect on the anthocyanin content formed. Explants grown from young shoots of callus that were formed contained anthocyanins (0,74%) higher than explants from callus dragon fruit (0,72%). While in single factor; number of explant treatment; It can be seen that the the results of the analysis of anthocyanin content of the planted callus with the number of explants 3 and 4 explants each culture tube. Produce Anthocyanins equally well by addition of explants . In the observation of 12 WAP, it can be seen that the treatment J3 could produce anthocyanin secondary metabolites. The results of the analysis showed that The callus formed contained the highest anthocyanin tends to be more (0,76 %); highest compared to other treatment.

## E. Discussion

Callus is a selamorphous collection that occurs from dividing cells and consists of parenchyma cells (Slater, A., N. Scott & M. Fowler., 2003). From research observations on the analysis of the quality and quantity of callus in Table 2 and Table 3; it can be seen that callus of young shoot an callus explant show slow growth. Callus quality scores began to increase at week 7; while a score of 2 on the quantity of callus (growing of explants) began to appear at week 6 sedangkan score 2 pada kuantitas calus (growing of explant) mulai terlihat pada minggu ke 6. Callus formation is one indicator of the occurrence of explosive growth in tissue culture. The results showed that callus began to form at week 6. Callus began to form on young shoot incisions that were in contact with MS media. This showed that the explant is starting to adapt and begins to respond to callus growth.). In line with the the statement of Hos ( 2008) in callus culture there are 3 stages of induction, proliferation, and differentiation. In the induction stage, cells begin to divide; the proliferative stage (cell division occurs very quickly); Stage of differentiation (the process of metabolism or organogenesis occurs). In the Induction stage, it begins with the absorption of water so that cell wall loosens and the cell size enlarges and actively divides.

In line the opinion of Iwase A, M Ohme -Takagi, & K Sugimoto (2011) added that growth begins with swelling of the explant slices of stem shoots, then a wavy callus is formed (swelling) and small white granules appear to form a compact callus. Early callus formation from incision scars in contact with tissue culture media; cells undergo division followed by the proliferation process on the surface of the slice until a callus is formed. This is accordance with the opinion of Prakoeswo, S.A. Ribkahwati & D. R. Soeryaningsih, (2010) that the emergence of difference in callus quality depends on environmental condition of growth and which is influenced by explant sources. It is assumed that up to 7 weeks the planting material/plant-let that was tried was still in the stage of adapting to the media and the environment. This is in accordance with the opinion of Purwaningsih, Y. (2013) that the use of callus explants in tissue culture shows an easily observed morphology and can produce secondary metabolites in the form of anthocyanin pigments.

From table 4 it can be seen that the source of the explant that was tried had a significant effect on the weight of the explant formed. Explant grown from young shoots showed better callus growth. Fresh tissue have meristematic properties and are actively dividing. The success of engineering the tissue culture of the plant is highly dependent on the medium used. Further Indarwati *et al.* (2020), added that MS media has a real effect and is very good to use as a culture medium to produce metabolic sekundair (Anthocyanin) from the young shoot of dragon fruit stems.

The results of observation made on the anthocyanin content in callus (table 5.) showed that the type of explant and the number of explants planted on MS +15% sucrose media had a significant effect on the secondary metabolites produced. The analysis was carried out at the age of 8 weeks, the Anthocyanin content formed was in the range (0,65%-0,69%). Observations at week 12 Anthocyanin content increased in the range ( 0,68% - 0,76%). The accumulation of secondary metabolites is linear with the formation of the callus quantity. In a high metabolic process, it will be followed by formation of primary metabolites in greater quantities so that it can be used to synthesize the formation of secondary metabolites. In line with the opinion Pan Y, L Lin, S Xiao, Z Chen, S Sarsaiya, S Zhang, YS Guan, H Liu, & D Xu. (2020); this is due to the condition of medium containing optimal nutrients, in its body cells cleave to grow larger, elongate to form callus by using the energy contained in the culture medium to increase growth

and formation of secondary metabolites. In this study callus growth was seen towards compact, followed with the formation of high Anthocyanin secondary metabolites (0,76 %).

Once of factors that determine the success of plant regeneration is the availability of carbohydrate sources. For plants bred through tissue culture, carbohydrates serve as a source of carbon needed to produce energy. In addition the source of carbohydrates as a energi is very depend on the konsentration of carbohydrates. The addition of 15% sucrose on MS media aims to provide an additional source of energi for metabobolic processes so that it can increase the formation on secondary metabolotes. The result of analysis of Planting Material (Shoot and callus of Dragon Fruit) which were cultured on (MS Media + 15% Sucrosa) produced secondary metabolits antochyanin (0,74 % and 0,72%). Treatment of the number of expansion 1,2,3 and 4 explant in each culture tube at week 12 callus analyzed resulted in Anthocyanins (0,68 %-0,76%).

## F. Conclusion

There is no interaction between the source of explant materia and the number of explants each culture tube on the content of secondary metabolites in callus. MS Media with the addition of 15 % sucrose had a sinificant effect on increasing the anthocyanin content in the callus of Dragon fruit formed. Callus formed from Shoot Dragon fruit contain Anthocyanin 0,74 %. The Callus formed from Shoot and callus Dragon Fruits contain secondary martabolites of Anthocyanins ranging from 0,68% – 0,76 %. The highest Anthocyanin content (0,76%) was produced in treatment J3 (3 Explant each tube).

## G. Acknowledgements

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