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In vitro study: the addition of elicitor carbohydrates against the accumulation of anthocyanin on a callus dragon fruit

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ABSTRACT

Plants are the main source of chemical compounds that are used particularly for the pharmaceutical industry, food additives, perfumes. The production of secondary metabolite and elicitation can be used as one of the important tools in order to improve the synthesis of these compounds by *in vitro* technique. Dragon fruit contains Anthocyanins which are compounds that function as antioxidants; liberate of free radicals and slow the process of ageing/anti-ageing. In dragon fruit plant cell cultures, elicitors have increased metabolic production seconder. Although elicitation has been carried out in a large number of medicinal plants, we extensively research was conducted to improve the content of anthocyanins in callus dragon fruit. The study was conducted using a completely randomized factorial design with two factors, Factor I. Carbohydrate sources; G1 = Glucose, G2 = Fructose, G3 = Sucrose. Factor II. Concentration addition of carbohydrate K1 = 5%, = 10% K2, K3 = 15%, = 20% K4, K5 = 25%; Each treatment was repeated 3 times. Observations showed that the quality of callus formed inclined toward compact. The quantity of callus formed from the treatment source of carbohydrates and carbohydrate concentrations increase with the addition of 15% sucrose (G3K3) tend to produce callus quantity more / bigger. Results of analysis of the content of anthocyanins in callus dragon fruit age 56 dap and 84 dap shows as modifying carbohydrates Media MS with addition sucrose and sucrose concentration of 15% (G3K3) produced anthocyanins content tends to be higher (0.11% and 0.14%) compared to other treatments.

Key words : *Anthocyanin, Dragon fruit, Elicitor, Carbohydrates*

Introduction

Elicitation is a technique to improve the secondary these on tissue culture with additional special treatment (Sharma *et al*., 2011) elicitation is a process of induction to increase the formation of these secondary in plant tissue using an elicitor. Aijaz *et al.,* 2011. Hussain *et al.* (2012) Elicitor can be distinguished, namely: elicitor abiotic and elicitor Biotic. Elicitor abiotic inorganic, could be from a chemical compound of them are heavy metal; or physically the ultraviolet: use while elicitor biotic derived from organic compounds for example carbohydrates, protein or other compounds (Siddiqui *et al.,* 2013; Hakeem and Talat Parween (2013).

Plants are the primary source of chemical compounds used especially for, pharmaceutical industry food additive, natural or and perfume. Most of those compounds derived from the secondary metabolism extracted from a species of plants for phar-

maceutical industry (Toso, 2010) Biosintesa these secondary using culture techniques plant tissue has long been the attention of the, and has the goal that is valuable enough (Siddiqui *at al.,* 2013). Many studies have been reported for the anthocyanin content in vegetables and fruits. Dragon fruit, including tropical fruit that has high economic value. This is due to 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 starts blooming at dusk and blooms entirely at midnight with a fragrant smell. The content of secondary metabolites from dragon fruit can be used as medicine. The content, among others, contains vitamin C and levels of water-soluble fibre that is beneficial for the diet (Anonimous, 2004). Dragon fruit is used as a medicinal plant because the content of dragon fruit can balance blood sugar, gout, cholesterol-lowering and heat-lowering. In Thailand, dragon fruit is used as an addition to the production of ice cream, juice, grapes and fruit salads. Open flower buds can be cooked as a delicious vegetable, high in nutrients and low in fat. 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 fruit are: (1). Albumen which can release toxins; (2). Anthocyanin can free radicals and slow the ageing process / anti-ageing; (Wang, 1997) (3) Vitamin C which can beautify and make skin brighter; (4) Rich in fibre / soluble fibre, so it is useful for dieting.; (5) Reducing diabetes; (6) Preventing colon cancer and launching bowel movements (Anonimus, 2004). Anthocyanin is the primary source of chemical compounds used mainly for the pharmaceutical industry, food additives, fragrances (Chaiyasut *et al.,* 2016). Anthocyanins, which are derived from the phenylpropanoid pathway, belong to the flavonoids class of secondary metabolites. They are responsible for the purple, red and blue colouration of leaves, stems, flowers and fruits of many plants (Cominelli *et al.,* 2008; Das *et al.,* 2012; Espley *et al.,* 2007; Hatlestad, 2012. Anthocyanins Most of these compounds is derived from secondary metabolic products extracted from plant species. In producing the secondary metabolic compounds (anthocyanin content) in dragon fruit, it is attempted to propagate through tissue culture (Turfan *et.al.*, 2011)

Optimization of growth medium was conducted using medium Murashige-Skoog (MS). Anthocyanins are obtained from callus Hylocereus polyrhizus with variations in carbohydrate sources of glucose, fructose and sucrose. The results of this study are expected to obtain information on the use of carbohydrates on callus growth and development and accumulation of secondary metabolites. The content of secondary metabolites that you want to increase is anthocyanin levels. Anthocyanin is an antioxidant, so anthocyanin content that is produced as a moisture base material will be developed.The purpose of this study was to Know accumulation of anthocyanin content in callus *Hylocereus polyrhizus (*Dragon Fruit) grown in Murashige and Skoog media by modifying carbohydrate sources using sucrose, glucose and fructose, with various concentrations.

Research Methods

Place and time

The study was conducted at the Culture Laboratory of the Faculty of Agriculture, University of Wijaya Kusuma, Surabaya. April - November 2018

Materials and Tools

The materials used during this research are: explants originating from shoots/shoots of young dragon fruit plants, as well as the basic media of Murashige and Skoog (MS). NAA and BAP Growth Regulators, Coconut Water, Glucose, Fructose, Sucrose, 70% and 90% Alcohol. Tools: Equipment needed during this research are: Sartorius Scales, Autoclave, Oven, LAF, pH meter, Tweezers, Scalpel, Erlenmeyer, Measuring cup, Measuring pipette, Drop pipet, Petridist, Spatula, Culture tube and magnetic stirrer Hot plate, Gas Chromatography

Methods

The study was conducted using a completely randomized design with two factors, Factor I: Carbohydrate Sources there are three levels: G1 = Glucose; G2 = Fructose; G3 = Sucrose. Factor II. Concentration of adding carbohydrates there are 5 levels; K1 = 5%; K2 = 10%; K3 = 15%; K4 = 20%; K5 = 25%. Each treatment was repeated three times, with five replications each. Composition of MS media that are the macromolecule of the soil consist of KNO_3 (1900

mg/L), NH—4NO_3 (1650 mg/L), $CaCl_2$ $2H_2O$ (440mg/L), $MgSO_4$ $7H_2O$ (380 mg/L), KH_2PO_4 (170mg/L); The micro molecule of the soil consist of MnSO-4 -7H-2-O (22.3 mg/L), $ZnSO_4$ $7H_2O$ (8.6 mg/l), $H_3BO_3^-$ (6.2 mg/L), Kl (0.83mg/L), $CuSO_4$ $5H_2O$ (0.025 mg/L), $NaMoO_4$ $2H_2O$ (0.25 mg/L), $CaCl_2$ $6H_2O$ (0.025 mg/L), FeSO-4 $7H_2O$ (27.8 mg/L), NaEDTA $2H_2O$ (37.3 mg/L); Vitamin of the soil consist of Mio-inositol (100 mg/L), Thiamin HCL (0.1 mg/L), Nicotinic acid (0.5 mg/L), pyridoxine HCL (0.5 mg/L), Glycin (2 mg/L) and Carbohydrate (30.000 mg/L + according to treatment). Chew YL 2009

Implementation

The equipment used was wrapped in brown paper and sterilized at 121 ° C for 30 minutes. Sterile culture tubes with Autoclave 17 psi 30 minutes. Primary media used by MS media with an elemental composition modified with the addition of various carbohydrates with several concentrations according to the treatment.

Cultivation

Sterile young shoot/shoot explants, then cut into ± 1 cm pieces and soaked in betadine, planted in culture tubes that already contain media according to treatment. After planting it is placed on an incubation rack which consists of incubation stages.

Incubation

After planting the culture tube is placed in an incubation rack, and the formation of callus is observed.Variable: The observed variables are as follows

a. Callus quality. Observed at intervals of 2 weeks visually using scoring: 1 = no callus; 2 = compact callus; 3 = callable friable ; b. Callus Quantity: Observed at intervals of 2 weeks visually by scoring: Scor 1 = no callus; 2 = swelling of explants; 3 = little callus (<1 times the explant size); 4 = medium callus (1-2 times the explant size); 5 = many callus (> 2 times the explant size). c. The content of secondary metabolites in the callus. Observed destructively through anthocyanin content analysis at eight weeks after planting (56 days) Secondary Metabolite Analysis of the material extracted using absolute alcohol then analyzed by gas chromatography.

Data Processing: The observations were analyzed with ANOVA factorial with SPSS, and if the results showed that there were real differences would be analyzed by the 5% LSD test.

Results and Discussion

Fine Quality

Analysis of callus quality showed that there were no significant interactions and differences in the single factor of carbohydrate source treatment at 14, 28.42.56, and 70 DAP. The interaction between the treatment of the use of various carbohydrate sources (glucose, fructose, and sucrose) with the treatment of the addition of carbohydrate concentrations were seen in the callus quality parameters at 42 ht observation. The results of observations of the growth of the quality of the Dragon fruit callus starting from the age of 14 to 70 days after planting can be seen in Table 1.

In Table 1. It can be seen that the quality of callus formed from the treatment of various types of carbohydrate utilization and the concentration of carbohydrate enhancement showed a score of 1.00 at callus age 14 and 28 DAP; all treatments have the same effect. Explain from young shoots planted has not shown the growth of callus formation. Callus formation begins at 42 DAP At the age of 42 observations, there was an interaction between the two factors that were tried. The combination of G3K3 (15% addition of sucrose) shows the highest score and is significantly different from the others. Elevated glucose increase 12-lipoxygenase activity and expression in porcine aortic smooth muscle cells. Diacylglycerol de novo synthesis is secondarily due to increased formation of precursors derived from glucose metabolism. Elevation of glucose concentration can increase Activation of Protein Kinase C (Krisantini and Wiendi (2018). the addition of sugar in the media will increase the growth faster (Rai, 2015) but increase the level of contamination (Martin, 2003).

Up to observations 84 DAP; the use of sucrose with a concentration of 15% (G3K3) tends to give a higher score than other treatments. A callus that formed from explant is callus toward Compact (Scor = 2.40). The use of sucrose carbohydrate source in MS media tends to produce callus quality towards compact. *In vitro* explorer shoot/shoot dragon fruit plants with the addition of various sources of carbohydrates showed explants to swell at the age of 42 DAP (Kristl, 2011).

Fig. 1. The results of observing the quality of cal-

Table 1. The value of score on the quality of a callus the fruit of the dragon python which formed from treatment sources of carbohydrate and concentration on the addition of carbohydrates

Treatment	The AgeDAP (Day After Planting)								
	28	35	42	49	56	63	70	77	**84**
G1K1	1.00	1.06	1.16 c	1.26	1.50	1.73	1.93	2.00	2.06
G1K2	1.00	1.06	1.26 a	1.33	1.53	1.73	1.93	2.00	2.06
G1K3	1.00	1.00	1.13 d	1.20	1.53	1.66	1.86	2.00	2.06
G1K4	1.00	1.06	1.20 b	1.26	1.46	1.66	1.86	2.00	2.06
G1K5	1.00	1.00	1.00 f	1.13	1.13	1.26	1.33	1.40	1.40
G2K1	1.00	1.06	1.16 c	1.16	1.23	1.46	1.43	1.60	1.66
G2K2	1.00	1.06	1.16 c	1.23	1.40	1.60	1.80	1.93	2.06
G2K3	1.00	1.06	1.20 b	1.20	1.23	1.46	1.66	1.66	1.73
G2K4	1.00	1.00	1.13 d	1.13	1.26	1.46	1.53	1.63	1.66
G2K5	1.00	1.00	1.13 d	1.06	1.13	1.26	1.33	1.20	1.40
G3K1	1.00	1.06	1.20 b	1.20	1.33	1.53	1.66	1.80	1.86
G3K2	1.00	1.06	1.13 d	1.13	1.33	1.26	1.53	1.63	1.66
G3K3	1.00	1.13	1.26 a	1.33	1.60	1.80	2.06	2.26	2.40
G3K4	1.00	1.00	1.06 e	1.13	1.26	1.26	1.53	1.63	1.66
G3K5	1.00	1.00	1.06 e	1.06	1.13	1.26	1.33	1.40	1.40
LSD 5 %	**NS**	**NS**	**0.021**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Information: figures who was accompanied by the same letters shows that markedly dissimilar
NS : Non Significant

lus on a single factor show that; the use of glucose, fructose and sucrose as a source of carbohydrates has the same effect on the quality of the callus of the formed Naga fruit. Carbohydrate Concentration Level seems to have a significant effect on the quality of Dragon Fruit Callus formed. The use of a Concentration of 15% (K3) has the same effect as a Concentration of 10% (K2); tend to give a higher Scor Value and significantly different from other concentrations. Up to the age of 84, DAP callus that tends to lean towards the compact callus (Scor 2.07).

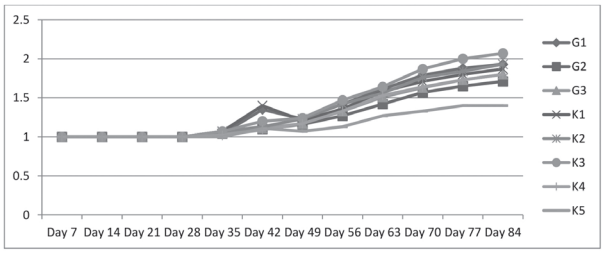

Fig. 1. The analysis of single factor of callus quality shoots stems dragon fruit formed from treatment sources of carbohydrate and concentrate the addition of carbohydrates

Alse Quantities

The results of observations on the growth of the Dragon fruit callus quantity starting from the age of 14 to 70 DAP. The results of the analysis showed no interaction between the treatment of the use of various carbohydrate sources (glucose, fructose, and sucrose) with the treatment of the addition of carbohydrate concentration seen in the callus quantity parameters. The average Scor Scores of the Callus Quantity of Dragon Fruit at various age observations are presented in Table 2.

From Table 2. It appears that our observations score quantity a callus formed from treatment sources of carbohydrate and concentrate the addition of carbohydrates; treatment that everyone tries effect at about the many a callus formed. But with the addition of sucrose 15% (G3K3) tends to produce more callus quantity.

The growth and development of a callus on the quantity of callus influenced by elements at the in media. Every explant of different species needs different mediums also; in this nutrient required for growth. In this research look that the use of sucrose influential is better on the growth of a callus of shoot explant fruit naga. This following statements from Rahmawati (2006) that the success of in engineering tissue culture of the plant is highly dependent on medium used.

The media of tissue culture contains elements macro, micro, vitamin and carbohydrates a substitute for carbon. The use of sucrose as a source of

Table 2. The quantity a callus scor dragon fruit formed from those sources of carbohydrate and concentrate with the addition of carbohydrates

Treatment	The age (days afterm planting)											
	35	14	21	28	35	42	49	56	63	70	77	84
G1K1	1.00	1.06	1.40	1.66	2.00	2.8	2.26	2.50	2.73	2.93	3.00	3.06
G1K2	1.00	1.13	1.53	1.73	2.00	2.26	2.33	2.53	2.73	2.93	3.00	3.06
G1K3	1.00	1.06	1.40	1.53	1.93	2.13	2.20	2.46	2.66	2.83	3.00	3.06
G1K4	1.00	1.20	1.66	1.80	2.00	2.20	2.26	2.46	2.66	2.83	3.00	3.06
G1K5	1.00	1.13	1.46	1.60	1.80	2.00	2.06	2.13	2.26	2.33	2.40	2.40
G2K1	1.00	1.06	1.46	1.66	1.93	2.10	2.16	2.23	2.46	2.53	2.60	2.66
G2K2	1.00	1.20	1.66	1.80	1.93	2.10	2.23	2.40	2.60	2.80	2.93	3.06
G2K3	1.00	1.06	1.46	1.66	1.86	2.13	2.26	2.33	2.46	2.63	2.66	2.73
G2K4	1.00	1.13	1.40	1.60	1.80	2.00	2.13	2.26	2.46	2.53	2.60	2.66
G2K5	1.00	1.06	1.33	1.46	1.73	1.93	2.06	2.16	2.26	2.33	2.40	2.40
G3K1	1.00	1.13	1.53	1.73	1.93	2.13	2.20	2.33	2,53	2.63	2.80	2.86
G3K2	1.00	1.20	1.60	1.73	1.86	2.00	2.13	2.33	2.46	2.53	2.60	2.73
G3K3	1.00	1.20	1.80	1.93	2.13	2.26	2.33	2.60	2.80	3.06	3.26	3.40
G3K4	1.00	1.20	1.46	1.60	1.80	2.00	2.13	2.26	2.46	2.53	2.60	2.73
G3K5	1.00	1.20	1.33	1.46	1.73	1.93	2.06	2.13	2.26	2.33	2.40	2.40
LSD 5 %	NS	NS	NS	NS	NS	NS	TN	NS	NS	NS	NS	NS

Information: figures who was accompanied by the same letters shows that markedly dissimilar
NS: Non Significant

carbon influential better to the growth of a callus fruit dragon. The result of the observation quantity a callus on the single treatment and concentration the addition of carbohydrates served in Fig. 2.

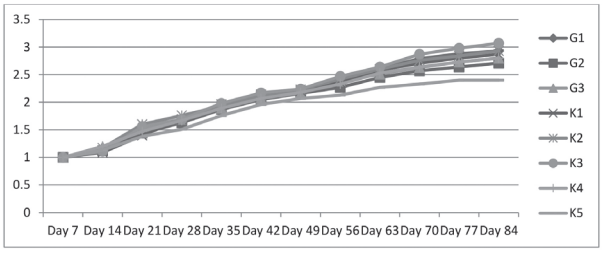

Fig. 2. The quantity a callus dragon fruit formed from treatment sources of carbohydrate and concentrate the addition of carbohydrates.

Fig. 2. Show the use of sorts of sources of carbohydrate effect at a callus against significant/ the quality of a callus. Variation concentrates carbohydrates real bearing on the quantity a callus on observation age 56 and 84 DAP. The addition of the concentration of carbohydrates 15% (K3) a callus plant bearing a dragon has score 3.07 callus formed still a little.

The analysis of anthocyanin content formed at the age of 56 days after planting shows there will be no interaction between the treatment of the concentration of carbohydrates and the sources of carbohydrates in terms of anthocyanin formed. The average percentage in a callus anthocyanin content on callus dragon fruit is shown in Table 3.

Table 3. The average moisture content of anthocyanin on callus formed from the kind of treatment and the additional sources of carbohydrates (%) at the age of 56 DAP and 84 DAP

Treatment	Anthocyanin content on calus (%) on the observations	
	56 DAP	84 DAP
G1K1	0.04	0.06
G1K2	0.06	0.08
G1K3	0.07	0.09
G1K4	0.06	0.08
G1K5	0.06	0.08
G2K1	0.06	0.08
G2K2	0.07	0.09
G2K3	0.08	0.11
G2K4	0.10	0.13
G2K5	0.09	0.12
G3K1	0.08	0.11
G3K2	0.08	0.12
G3K3	0.11	0.14
G3K4	0.09	0.12
G3K5	0.09	0.12
LSD 5 %	NS	NS

NS : Non Significant

Table 4. The results of the analysis of anthocyanin content single factor in a callus fruit dragon formed from treatment sources of carbohydrate and concentration the addition of carbohydrates(%)

Treatment	Anthocyanin content on callus (%) on the observations	
	56 DAP	84 DAP
G1	0.06 c	0.08 c
G2	0.08 b	0.11 b
G3	0.09 a	0.12 a
LSD 5 %	0.005	0.005
K1	0.06 d	0.08 c
K2	0.07 c	0.10 b
K3	0.09 a	0.11 a
K4	0.08 b	0.11 a
K5	0.08 b	0.11 a
LSD 5%	0.007	0.007

NS : No Significant

From Table 3. It appears that the use of combination treatment (G3K3); sources of carbohydrate sucrose with concentrated 15%, a callus dragon fruit capable of producing anthocyanin 0.11%, tends to be higher than the use of the other.

From Table 4. It can be seen that each single factor who attempted had have real impact on establishing the womb anthocyanin in a callus fruit dragon at the age of 56 and 84 hst DAP, The kind of treatment carbohydrate source it can be seen that the use of sucrose influential carbohydrates than the best treatment G2) (fructose and glucose ( ) G1. At age 84 and 56 DAP Sources of carbohydrate to the use of sucrose callus dragon fruit is capable of producing anthocyanin 0.09 % and increased to 0.12 % the highest and 0.11 % markedly dissimilar to the use of other sources of carbohydrate.

It can be seen that the kind of the concentration of the addition of the carbohydrates that the one we are trying had to have a real impact on the formation of anthocyanin on a callus the fruit of the dragon symbol of egypt.

Conclusion

Overall, the current study results conclude Glucose as sources of carbohydrate tends to produce quantity a callus that tends to be a lot of; Concentrate the addition of carbohydrates 15 % produce the quantity a callus. Most votes;.The addition of 15%, 20% and 30% sucrose produce anthocyanin content higher; The addition of sucrose 15 % yielding fat a callus and anthocyanin content higher (0.14 %) compared to other treatment

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