p-ISSN 0854-9028 e-ISSN 2549-9343
AGRIC
JURNAL ILMU PERTANIAN
Fakultas Pertanian dan Bisnis Universitas Kristen Satya Wacana
Jl. Diponegoro 52-60 SALATIGA 50711 - Telp. 0298-321212 ext 354
email: jurnal.agric@adm.uksw.edu, website: ejournal.uksw.edu/agric
Terakreditasi Kementrian Riset, Teknologi dan Pendidikan Tinggi berdasarkan SK No 200/M/KPT/2020

LYCOPENE CONTENT IN SEVERAL AGES OF TOMATO CALLUS (*Lycopersiconesculentum Mill.* cv. Rampai)

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Received: 11 February 2022 | Accepted: 25 March 2022

ABSTRACT

Tomato (Lycopersiconesculentum Mill. cv. Rampai) is rich in lycopene, a carotenoid group compound with many biological activities, one of which is as an antioxidant. Conventional tomato cultivation is not optimal due to the susceptibility of tomato plants to pests and diseases, even though the need for lycopene continues to increase. Plant tissue culture method can be one solution for lycopene production. Callus tomato cv. Rampai was initiated from cotyledons on MS medium (Murashige& Skoog) with the addition of 1 ppm BA (benzyladenine) and 1.5 ppm IAA (indole-3-acetic acid). Callus aged one week to four weeks were harvested and lycopene was extracted by maceration method using hexane:acetone (9:1) as solvent. The presence of lycopene in callus was confirmed by TLC (thin-layer chromatography) (retention factor value/ Rf = 0.56) and UV-Vis spectrophotometry (wavelength that gives maximum absorbance = 472 nm). Lycopene content in tomato callus increased with increasing callus age and was directly proportional to the callus growth index. The highest lycopene level was found in callus aged 4 weeks, which was 0.3094 mg/100 g dry weight callus.

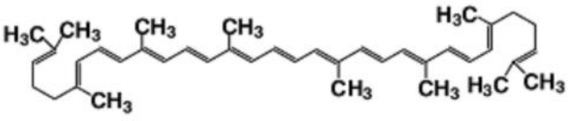
Keywords: ***carotenoid, plant tissue culture, thin-layer chromatography (TLC), UV-Vis spectrophotometry***

INTRODUCTION

Tomato (*Lycopersiconesculentum*Mill.) belongs to the Solanaceae family and is thought to have originated from South America (Gatahi, 2020). Tomatoes are widely developed in Indonesia because of their high economic value and contribute to the household economy if cultivated intensively using the right technology (Mariyono, 2019). There are many varieties of local tomato cultivars developed in Indonesia, one of which is the Rampai cultivar. Rampaicultivar's fruits are smaller than common tomatoes and have a high acid content so they are preferred (Refiliya*et al*., 2020). Tomato production in East Java in 2017 and 2018 reached 15.6 tons per ha and 16.5 tons per ha, respectively. However, this figure is quite low when compared to its potential production which reaches 33-35 tons per ha (Latifah*et al*., 2021). This low productivity is due to many abiotic and biotic stresses such as disease, high temperature, drought, salinity, and susceptibility to insects and other pests. Organisms such as fungi, bacteria, viruses, and nematodes can also cause disease in tomatoes (Arulananthu*et al*., 2019).

Tomatoes are widely used as fresh fruits or processed to produce various food products, such as pasta, juice, and others. In terms of nutrition, tomatoes contain carbohydrates (3%), protein (1.2%), total lipids (1%), minerals (calcium, magnesium, phosphorus, potassium, sodium, zinc, manganese, etc.), and vitamins (vitamins A and C, thiamine, riboflavin, niacin, pantothenic acid, and pyridoxine) (Salehi*et al*., 2019). In addition, tomatoes are also a source of phenolic components, carotenoids, and glycoalkaloids (Rao *et al*., 2018). One of the carotenoid components in tomatoes that have been widely explored is lycopene because of its antioxidant power. The amount of lycopene in tomatoes is reported to vary depending on the maturity level of the fruit and the variety (Thompson *et al*., 2000). From the results of a study conducted by Toma*et al*. (2008), it is known that cherry tomatoes have the highest lycopene content. Ripe tomatoes have lycopene levels of 1.9 - 6.5 mg/100 gramsfresh weight (Dominguez *et al*., 2020).

Lycopene is an acyclic carotene with 11 conjugated double bonds (Figure 1). Apart from being an antioxidant, lycopene also has biological activities such as anti-inflammatory, hypoglycemic, photoprotective, anti-angiogenesis, anti-parasitic, anti-viral, and others. Consuming lycopene in daily food reduces the risk of suffering from chronic diseases, such as heart attacks, reduces oxidative stress levels in HIV patients undergoing antiretroviral therapy, and maintains healthy skin and bones (Madia*et al*., 2021). Until now, the demand for lycopene



Lycopene

Figure 1 Structure of lycopene compounds (Salehi*et al*., 2019)

for functional foods, supplements, and cosmetics continues to increase. At the same time, conventional tomato cultivation takes at least 60-100 days from seed until the fruit is ready to be harvested with productivity that tends to be low as described (Hamidi, 2017).

Plant tissue culture is one of the fields of biotechnology that can increase tomato productivity in a shorter time (Arulananthu*et al.*, 2019). Through plant tissue culture, the formation of tomato callus cells can be induced. Since the 1960s, the biosynthesis of secondary metabolites through callus cells has been extensively studied (Efferth, 2018). This study studied the lycopene content at several ages of tomato callus in order to obtain data on the optimum time of lycopene content in tomato callus cultures, specifically in Rampai cultivar. Thus, this research becomes the basis for future studies, such as increasing lycopene production in callus culture with the addition of certain elicitors and developing lycopene production capacity using bioreactors for commercial purposes.

MATERIALS AND METHODS

Materials

This research was carried out at the Plant Biotechnology Laboratory and the Biopurification and Molecular Biology Laboratory, Faculty of Biotechnology, Universitas Surabaya, Indonesia. The tools used in this study were autoclave, dissection apparatus, *laminar air flow* (LAF), culture bottles, glassware, analytical balance, stove, Buchner funnel, UV-Vis spectrophotometer, and *chamber* TLC. The materials needed in this study were tomato seeds (cv. Rampai), MS media (Murashige & Skoog), several growth regulators such as BA (6-benzyladenine), IAA (indole-3-acetic acid), IBA (indole-3 -butyric acid), NAA (1-naphthalene acetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), 96% ethanol, sodium hypochlorite, acetone, methanol, petroleum ether, n-hexane, ethyl acetate, plate silica gel, as well as standard lycopene compounds.

Sterilization and germination of seeds into cotyledons

Seed sterilization is carried out in stages in the LAF. Tomato seeds were soaked in 33% sodium hypochlorite for 5 minutes. Then the seeds were rinsed with sterile distilled water and continued with the second stage of sterilization. The seeds were soaked again in 25% sodium hypochlorite for 15 minutes, followed by rinse with sterile distilled water several times and are ready to use. The sterilized seeds were planted in MS + BA 0.5 ppm media with 10 seeds per bottle. The culture bottles were incubated in a culture room at a temperature of 25^0C and 24 hours light period. Observations were made every 2 days by giving attention to contamination, cells' growth, and cotyledons' formation.

Optimization of callus initiation from cotyledons

Cotyledons that have grown from germination results are cut aseptically. Cotyledon explants were placed in the various media to optimize callus initiation, as shown in Table 1. The placement was 5 explants per culture bottle, each replicated 3 times. Observations were made every 2 days by observing the growth of cotyledons and the formation of callus.

Callus proliferation and callus growth index curve

Callus that had been induced from the most

optimum medium was subcultured aseptically to the same optimum medium to proliferate callus. After obtaining a sufficient number of calluses, a callus growth index curve was made. A total of 2 grams of each callus were subcultured into optimum media; the day of subculture was week 0. The callus growth index curve was made until the fourth week, namely on the 1st. 2nd, 3rd, and 4th week. At the end of every week, callus was harvested for lycopene extraction. The callus growth index was calculated from the difference of growth callus mass (T_x- T_0) divided by the initial callus mass (T_0).

Extraction of lycopene from callus

The harvested callus was placed on a tray, air-dried until the mass was constant. Then the dried callus was ground into powder and stored in a tight and dry container until ready for extraction. A total of 10 grams of callus powder was macerated with 25 ml of a mixture of hexane:acetone 9:1 solvent for 24 hours. After that, the mixture was filtered using a Buchner funnel and the filtrate was taken. The filtrate obtained was then analyzed for the presence of lycopene by TLC and its concentration by UV-Vis spectrophotometry.

Analysis of the presence of lycopene with TLC

The stationary phase used in thin-layer chromatography (TLC) is silica gel F254 plate, while the mobile phase or eluent is a mixture of ethyl acetate:methanol 4:6. A total of 25 ul of the filtrate (5 ppm) was

Table 1 Types of Growth Regulators in the Media for Optimization of Callus Initiation

Media	Plant growth regulators (ppm)				
	BA	IAA	NAA	IBA	2,4-D
1	-	1	-	-	-
2	-	2	-	-	-
3	-	3	-	-	-
4	0.1	0.5	-	-	-
5	0.1	1	-	-	-
6	0.1	1.5	-	-	-
7	0.5	0.5	-	-	-
8	0.5	1	-	-	-
9	0.5	1.5	-	-	-
10	1	0.5	-	-	-
11	1	1	-	-	-
12	1	1.5	-	-	-
13	1.5	0.5	-	-	-
14	1.5	1	-	-	-
15	1.5	1.5	-	-	-
16	1	-	0.5	-	-
17	1	-	1	-	-
18	1	-	1.5	-	-
19	-	-	-	1	-
20	-	-	-	2	-
21	0.1	-	-	-	0.2
22	0.1	-	-	-	0.5

spotted on a silica gel plate together with a standard lycopene compound. After that the silica gel plate is placed in the chamberTLC previously saturated by the eluent. The movement of the sample was observed under a UV lamp at a wavelength of 254 nm and the Rf (retention factor) value obtained between the sample and the standard compound was analyzed.

Analysis of lycopene levels with UV-Vis spectrophotometry

At first, scanning the absorbance of the filtrate and standard lycopene compounds was done to find the wavelength for maximum absorbance using a UV-Vis spectrophotometer. Absorbance scanning was carried out from a wavelength of 300-600 nm. Standard lycopene compound with a concentration of 0.5; 1; 1.5; 2; 2.5 ppm was then used to make a standard curve, then the lycopene content in each sample was determined based on the standard curve.

Statistical analysis

The research was designed by using completely randomized design to obtain which callus age contains the maximum amount of lycopene. Results of lycopene contentthat shown are the average value with the standard deviation. The statistical analysis carried out was Kruskal-Wallis (number of samples less than 30), run with the IBM SPSS Statistics 26.0 program, with a significance level of 5%. The *post hoc test* was conducted using pairwise comparison.

RESULTS AND DISCUSSION

Germination of seeds into cotyledons

Seed germination is carried out in the early stages of plant tissue culture to obtain sterile explants which are easier than taking explants from *in vivo* mature plants (Durosmo*et al*., 2014). Surface sterilization of seed explants requires chemicals toxic to microorganisms, but not toxic to seeds, so these chemicals are often required at low concentrations (Bharti *et al*., 2018). The sterilization method is also one of the things that affect the success rate of seed germination (Saeed *et al*., 2019). Thus, the sterilization method needs to be optimized because it varies quite a lot depending on the variety of seeds and the conditions of the explants. The surface sterilization of the seeds carried out in this study resulted in a bacterial contamination level of 2.5%, which was lower than that of a similar study (cv. SolanLalima) using 0.2% carbendazim for 10 minutes + 10% sodium hypochlorite for 20 minutes with a survivability rate of 61.88% (Bharti *et al*., 2018). However, another study showed that 2.5% sodium hypochlorite treatment for 10 minutes on tomato seeds of Jamila and Tomaland varieties resulted in a 0% contamination rate (Alatar*et al*., 2017).

The seed germination process can indeed take place on MS media without plant growth regulators. However, the addition of cytokinins can increase germination percentage and stimulate seed development (Diengdoh*et al*., 2017; Gegi*et al*., 2018). Tomato seeds appeared to have germinated on MS + BA 0.5 ppm medium at 2 weeks of age (Figure 2). These results parallel other studies that showed germination of differentiated seeds to form multiple shoots and leaves without root formation on MS + BA 2.5 ppm (Oceania *et al*., 2015).



Figure 2 Germination of tomato seeds on MS + BA 0.5 ppm medium at 2 weeks of age.

Optimization of tomato callus initiation

Several types of explants can induce callus formation. Several studies have shown that callus can be induced from leaf discs (Sharma & Srivastava, 2013), roots and stems (Kumar *et al*., 2017), as well as hypocotyls and cotyledons (Muthuvel*et al*., 2005; Chaudhry *et al*., 2007; Sakthivel &Manigandan, 2011). In this study, the cotyledons formed were separated from the stem and planted on 22 types of media variation (Table 1). Cotyledonsare expected to provide a better response because the tissue is in juvenile stage (Sakthivel& Manigandan, 2011). Therefore, in this study cotyledons are used as an explant for callus initiation.

The absence of callus in the control medium (C) indicates that callus initiation requires the role of growth regulators, such as auxins and cytokinins (Table 2). This result was linear with other study (Ikeuchi*et al*., 2013). This study used auxins (NAA, IAA, IBA, 2,4-D) and cytokinins (BA) at various concentrations to induce callus formation. In media 1-3, the addition of auxinwithout cytokinins induced the emergence of roots from callus that had appeared in the first week (Figure 3A). The higher the concentration of IAA, the more roots were induced to grow from the callus (Table 2).

In media 4-6, the addition of low concentrations of cytokinins (BA 0.1 ppm) with auxin (IAA) at 0.5 – 1.5 ppm showed results that were not different from those without the addition of cytokinins. The same thing also happened to media 8-9, an increase in cytokinin (BA) concentration to 0.5 ppm still indicated the induction of root formation rather than a callus. When the concentration of auxin (IAA) with cytokinin (BA) was the same (media 7), it was also found that roots were still obtained even though the rate of root formation was slower than in media with a concentration of auxin (IAA) greater than cytokinin (BA).

In media 10-11, it shows the formation of shoots from the callus that had formed initially. This indicated that a higher cytokinin concentration than auxin triggered shoot formation (Figure 3B). Media 12 with a concentration of cytokinin (BA) 1 ppm and a slightly higher concentration of auxin (IAA) (1.5 ppm), was the optimum medium for callus formation because at the end of the fourth week, 100% callus was obtained (Figure 3C). This result parallels the results obtained in another study (Jatoi*et al*., 2001) which reported 100% tomato callus induction on media containing BA and IAA. However, there are differences in results with the research of Durrani*et al*. (2017) who reported that the relatively higher concentration of cytokinins than auxin was responsible for increasing the percentage of callus formation.

Table 2 Callus, shoots, and roots formation on various media variations

	Number of callus, shoots, and roots formed from explants																	
We	MS^0 (control)			Media 1			Media 2			Media 3			Media 4			Media 5		
ek	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R
1	-	-	-	1	-	-	13	-	-	1	-	-	1	-	-	1	-	-
				0						3			1			5		
2	-	-	3	1	-	1	13	-	1	1	-	13	1	-	1	1	-	1
				0		0			3	3			4		0	5		5
3	-	2	5	1	-	1	13	-	1	1	-	13	1	-	1	1	-	1
				0		0			3	3			4		4	5		5
4	-	2	6	1	-	1	13	-	1	1	-	13	1	-	1	1	-	1
				0		0			3	3			4		4	5		5
We	Media 6			Media 7			Media 8			Media 9			Media 10			Media 11		
ek	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R
1	1	-	-	9	-	-	6	-	-	7	-	-	1	-	-	1	-	-
	5												4			0		
2	1	-	1	12	-	0	1	-	6	1	-	9	1	9	-	1	9	-
	5		5				5			5			4			3		
3	1	-	1	12	-	1	1	-	15	1	-	1	1	1	-	1	1	-
	5		5			2	5			5		5	4	2		3	3	
4	1	-	1	12	-	1	1	-	15	1	-	1	1	1	-	1	1	-
	5		5			2	5			5		5	4	4		3	3	
We	Media 12			Media 13			Media 14			Media 15			Media 16			Media 17		
ek	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R
1	14	-	-	7	-	-	9	-	-	1	-	-	1	-	-	15	-	-
										4			5					
2	14	-	-	1	9	-	1	8	-	1	-	1	1	-	-	15	-	-
				0			3			5		5	5					
3	14	-	-	1	1	-	1	1	-	1	-	1	1	-	1	15	-	1
				2	2		3	1		5		5	5		5			5
4	14	-	-	1	1	-	1	1	-	1	-	1	1	-	1	15	-	1
				2	2		3	3		5		5	5		5			5
We	Media 18			Media 19			Media 20			Media 21			Media 22					
ek	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R			
1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-			
	5			1														
2	1	-	15	1	6	-	-	-	-	1	-	-	14	-	-			
	5			4						3								
3	1	-	15	1	6	-	1	-	-	1	-	-	14	-	-			
	5			4			3			4								
4	1	-	15	1	13	-	1	-	1	-	-	-	-	-	-			
	5			4			3		3									

C = number of callus formed; S = number of shoots formed; R = number of roots formed

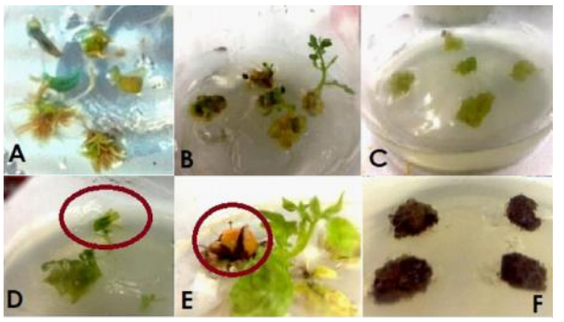


Figure 3 Explants 4 weeks old forming roots (**A**); shoots (**B**); callus (**C**); in vitro flowering (**D**); fruit in vitro subculture results at week eight (**E**); and browning phenomenon in callus (**F**).

Meanwhile, the relatively higher auxin concentration than cytokinin increased callus formation and shoot regeneration in tomatoes. This difference is due to the different varieties of tomatoes used. Differences in varieties indicate differences in genotypes that impact differences in cell responses to plant growth regulators (Lu *et al*., 1997).

The concentration of cytokinin (BA) which was much higher than the concentration of auxin (IAA) as shown in media 13-14 triggered the formation of shoots, even from the second week. High auxin concentration (IAA, NAA) (1 - 1.5 ppm) in media 15 and media 17-18 indicated that root formation occurred more quickly. Meanwhile on media 14 (NAA 0.5 ppm + BA 1 ppm) still showed root formation, although slower. IBA-type auxin on 19 media showed that it was unsuitable for forming a callus. IBA-type auxin at a concentration of 1 ppm was not sufficient to induce roots. Shoots formation in this media was suspected of originating from endogenous hormones. The emerging shoots then develop and form in vitro flowering in the fourth week (Figure 3D). Other subcultures of the formed flowers produced tomato fruit at the eighth week of culture (Figure 3E).

On media 20, a higher concentration of IBA-type auxin (2 ppm) indicated the formation of roots. In media 21 and 22 it was shown that auxin with type 2,4-D was unsuitable to form a callus, indicated by the presence of browning (Figure 3F). This is follows previous studies that reported that 2,4-D was not effective for tomato callus induction (Durrani*et al*., 2017).

Callus growth index

Callus growth resembles bacterial growth. The process of callus growth exhibits typical phases where the specific growth rate is zero initially, resulting in a lag phase. Then this growth rate will increase over time over a certain period until it reaches the maximum value. The growth curve will also show the final phase,where an asymptote occurs when the growth rate approaches zero. Over time, callus growth will reach a stage where the cells begin to die and enter the death phase. Overall, this growth rate profile appears as a sigmoidal curve (Hussein *et al*., 2016; Pan *et al*., 2020).

The growth index curve of tomato callus on media 12 (MS + BA 1 ppm + IAA 1.5 ppm) in this study was also sigmoidal (Figure 4). The callus growth phase in this study was not determined with certainty. Indeed, there is an increase in the callus growth index every week, but accurately determine the period of each phase (starting from the lag phase, exponential, to stationary) requires more intensive data collection, for example every 2-3 days. At the end of the fourth week, the callus appeared to be still growing even though the growth rate was slowing. This means that until the end of the fourthweek, the nutrients in the media are still sufficient to support cell division and increase callus mass. Callus obtained at the end of the first week showed a green color with the addition of yellow in some spots. The green color is chlorophyll, a cytokinin activity in the induction of chlorophyll formation (Sari, 2019). By the end of the fourthweek, the number of yellow spots on the callus increased, indicating the increasing lycopene content in the tomato callus since the first week (Figure 5).

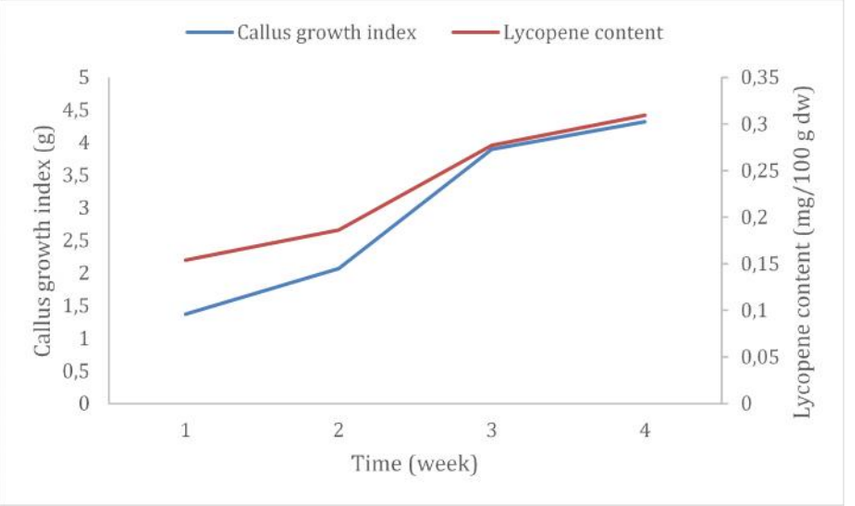


Figure 4 Growth index curve and lycopene content of several callus ages

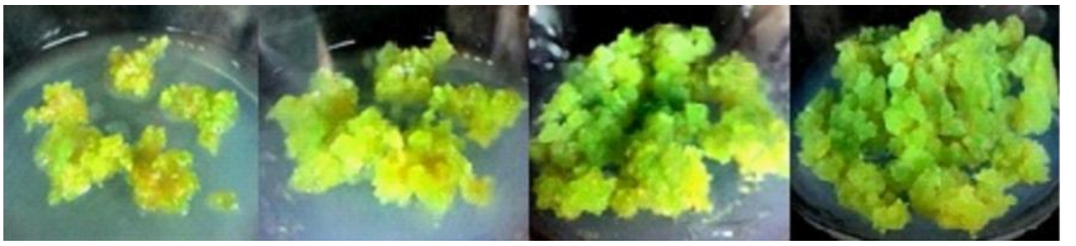


Figure 5 Callus growth at the end of the first week (far left) to the end of the 4th week (far right)

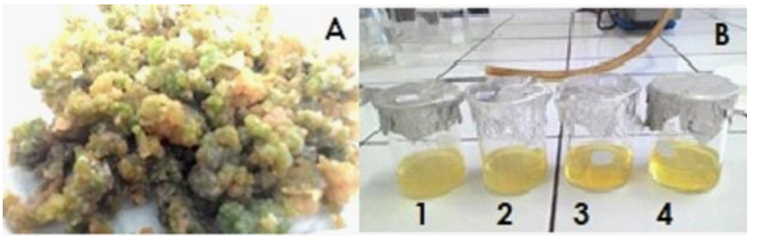


Figure 6 Extraction of lycopene from dried callus (A) produces a yellow-orange colored liquid extract (B). Figures 1, 2, 3, and 4 in the figure show the extracts from the first, second, third, and fourth-week callus, respectively.

Analysis of lycopene presence by TLC

Extraction of lycopene from a dry powder of tomato callus in the first week until the fourthweek using maceration with hexane: acetone solvent 9:1, an orange-yellow liquid extract was obtained (Figure 6). The extract was then analyzed for the presence of lycopene using TLC and obtained *Rf* value of 0.56, exactly the same as *Rf* value for standard lycopene compounds (Figure 7). The same *Rf* value for both the extracts from the first week to the fourth-week callus indicate that lycopene has been formed since the first week.

Our findings that lycopene was found in the tomato callus is in accordance with several studies. In general, secondary metabolites

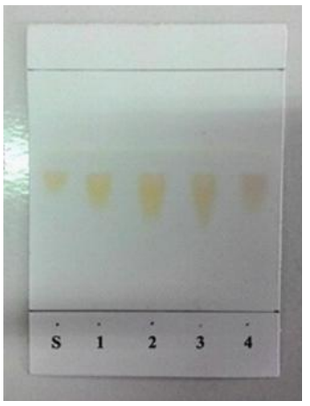


Figure 7 Results of TLC analysis of lycopene extract. S: standard lycopene compounds; 1-4 consecutively show the extract from the first to fourth-week callus.

localized to some *in vivo* plant tissues can be synthesized in undifferentiated callus cell cultures. On the other hand, when the secondary metabolites produced by *in vivo* plants are localized specifically to certain tissues or organs, the organ culture method is preferred to produce secondary metabolites through plant tissue culture (Thurimurugan*et al*., 2018). Lycopene is a secondary metabolite compound produced in almost all plant tissues *in vivo*, although the optimum production is found in the pericarp of tomato fruit and its calyx. Thus, undifferentiated tomato callus cells can also produce lycopene, as reported in similar studies (Robertson *et al*., 1995; Kareem & Karrar, 2018).

Analysis of lycopene levels by UV-Vis spectrophotometry

In addition to using TLC, the presence of lycopene in tomato callus was confirmed using UV-Vis spectrophotometry. This analysis was also carried out to estimate the level of lycopene in the callus samples every week, so it can be known the maximum level of lycopene from callus age. The maximum absorbance of the standard compound lycopene is at a wavelength of 472 nm (Figure 8). The results obtained in this study are linear with those obtained in other studies, which state that the absorption peaks of lycopene compounds are at wavelengths of 420 nm, 470 nm, and 500 nm (Bunghez*et al*., 2011). Lycopene extracts from both the first week to the fourth-week callus showed spectra that were quite similar to the spectra shown by standard lycopene compounds (Figure 8). This confirmed the presence of lycopene at all callus ages. There were non-significant differences in the UV-Vis spectra of callus in different weeks. This is because physical and biochemical changes may occur during callus growth, resulting in different profiles and content of metabolites (Farjaminezhad & Garoosi, 2019).

Lycopene has been detected from the one-week callus. The highest lycopene level was obtained in fourth-week callus, which was 0.3094 mg/100 g DW callus (Table 3). When aligning the growth index curve and callus lycopene content every week (Figure 4), it appears that callus production is in line with callus growth every week. The results of other studies indicate an indication of an increase in the production of carotenoids from subcultures, and even continues to increase along with the number of subcultures carried out (Indriani*et al*., 2020). From the weekly lycopene contentprofile and callus growth

Table 3 Lycopene Levels of Several Callus Ages

Time (week)	Lycopene content in callus (mg/100 g DW)
1	0.1538 ± 0.0009^{a}
2	0.1863 ± 0.0013^{b}
3	0.2771 ± 0.0019^{c}
4	0.3094 ± 0.0023^{d}

The letters next to the numbers indicate a 5% significance level difference.

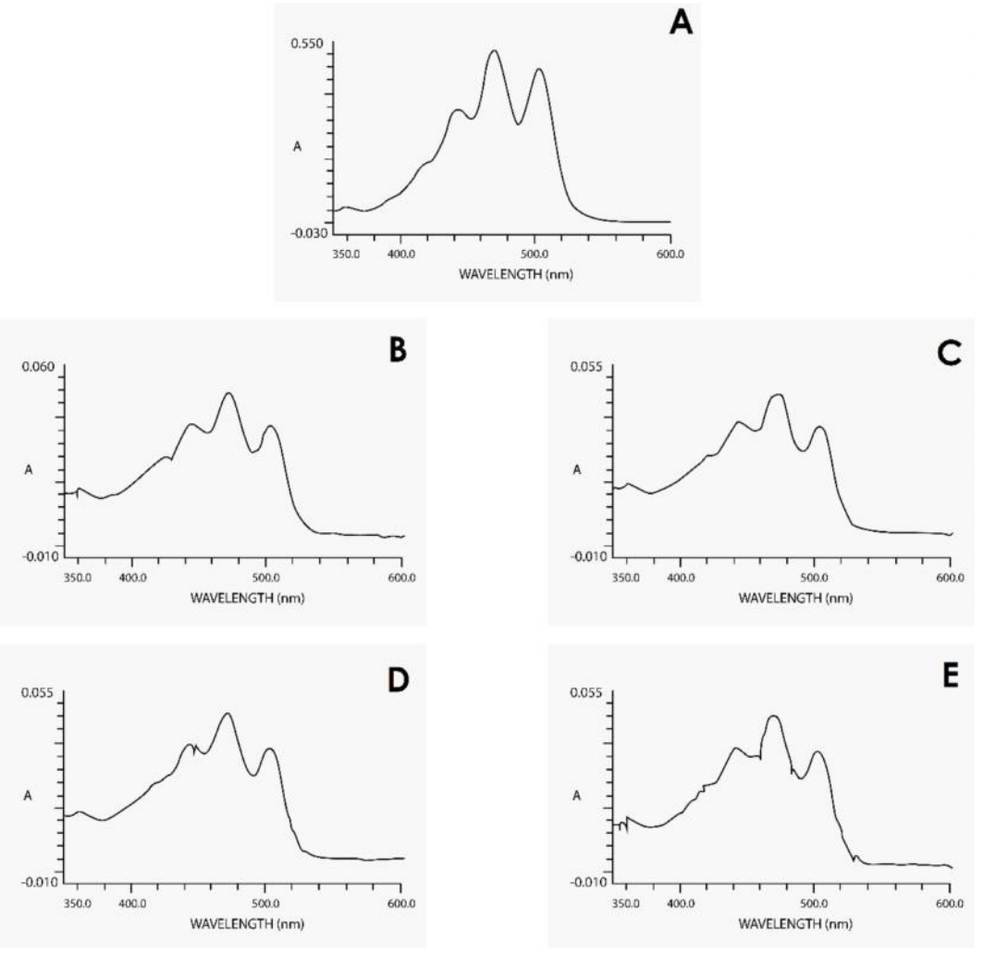


Figure 8 UV-Vis spectra of standard lycopene compounds (A), first week to fourth-week callus (B-D). The maximum wavelength appears at 472 nm.

index, it was suspected that tomato callus cells had entered the early exponential growth phase from the first week to the second week, as evidenced by the statistically significant increase in lycopene content and mass of callus between the first-week callus and the second-week callus (Figure 4). This increase even continued until the end of the fourth week.

Callus cells are composed of parenchyma cells with a high rate of division. The production of lycopene, which is a secondary metabolite compound, usually increases at the end of the exponential phase to the stationary phase due to an increase in vacuoles and plastids in the cell (Orban*et al*., 2008). Thus, it is possible that the stationary phase has not occurred yet so that the callus can still grow and produce more lycopene after the fourth week. However, it should be considered that the lycopene content in the callus will reach the optimum point at a certain callus age (Behbahani*et al*., 2011). Re-subculture can be done so that the callus can still produce lycopene.

As much as 4.32 grams of FW callus cells were obtained through plant tissue culture in the fourth-week.The lycopene content of

the fourth-week callus was 0.31 mg/100 g DW. Assuming the DW of callus was 10-15 of the FW of callus according to other studies (Papry*et al*., 2015; Jasim, 2016), then the total lycopene obtained from fourth-week callus was around 1.3 - 2.0 µg. Meanwhile, as previously written, conventional lycopene extraction performed on ripe tomatoes (aged 60-100 days) can yield 1.9 – 6.5 mg/100 grams of FW (Dominguez *et al*., 2020). If converted, then in 60-100 days of callus culture, about 0.062 – 0.103 mg/100 grams of FW lycopene can be obtained.

This result may appear to be smaller than the yield obtained by conventional extraction from tomato fruit. However, through plant tissue culture, there are several additional steps that can be taken to increase the lycopene content in callus culture, for example by adjusting the medium, for example by adding PEG (polyethylene glycol) and adjusting the salt concentration (Indriani*et al*., 2020); regulate culture conditions, such as light intensity; and perform genetic manipulation. Plant tissue culture can also overcome the problems that arise in conventional tomato cultivation, especially problems related to susceptibility to pests and diseases. Therefore, plant tissue culture is still profitable and has the potential to be explored for large-scale lycopene production in the future.

CONCLUSIONS

Tomato callus can be initiated from cotyledon explants on MS media with the BA 1 ppm and IAA 1.5 ppm. Tomato callus contains lycopene, confirmed by TLC (*Rf* value = 0.56) and UV-Vis spectrophotometry (maximum wavelength = 472 nm). Lycopene content in tomato callus increased with increasing callus age and was directly proportional to the callus growth index curve. The highest lycopene level was found in callus aged 4 weeks, which was 0.3094 mg/100 g DW callus. This study shows the production of lycopene by plant tissue culture is feasible to be explored in the future.

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