

# Ascorbic Acid and Carotenoid Contents in Tomato Callus: Effects of Explant Source and Subculture

Imam Purwanto, Yulita Nurchayati\*, Erma Prihastanti

Biology Department, Faculty of Mathematic and Science, Diponegoro University  
Jl. Prof. H. Jacub Rais, Tembalang, Semarang Tel. +6289508325317, Indonesia.

Corresponding author\*

yulita.yoko@gmail.com

Manuscript received: 10 December 2025. Revision accepted: 22 April 2026, Published: 18 May 2026.

## Abstract

The production of natural compounds in tomato can be achieved through callus culture by selecting the most suitable explant. The objectives of this study were to examine the effects of explant source and culture maintenance on the growth, ascorbic acid content, and carotenoid content of tomato callus. Callus induction was carried out on MS medium supplemented with 2,4-D. The resulting calli were divided into two groups: one subcultured into fresh medium and the other maintained without subculture. The study design used a completely randomized design of two factors, they were explants source (cotyledons, hypocotyls and root sprouts) and subculture treatment. The observed parameters were growth of callus, carotenoid and ascorbic acid content analyzed by spectrophotometer. The results showed that the highest callus weight (0.29 g) was obtained from hypocotyl explants. Subculture treatment significantly affected ascorbic acid content but did not influence carotenoid content. Callus from cotyledon explants produced the highest ascorbic acid content (15.2 g/100 g). Subculture treatment increased callus fresh weight, carotenoid content, and ascorbic acid content compared to the non-subculture treatment. In conclusion, hypocotyls were the best explant source for increasing biomass, while cotyledons were the most effective for enhancing ascorbic acid production.

**Keywords:** Callus culture; Explants; Secondary Metabolites; Subculture.

## INTRODUCTION

Tomatoes are an important dietary source of essential nutrients, including vitamins C, E, and B, along with minerals and antioxidants. Among these components, carotenoids are especially notable for their antioxidant activity and their role in lowering the risk of chronic diseases (Felfoldi et al., 2022). In Indonesia, various tomato cultivars such as Intan, Berlian, Ratna, Mutiara, and Permata have been developed through hybridization and other genetic improvement methods. The Permata variety, derived from a cross between TO 5186 (male) and TO 4142 (female), is one of the superior cultivars due to its resistance to major pests and diseases, and its adaptability to both lowland and highland environments across seasons (Handayani & Kurnia, 2018).

Tomatoes contain several important antioxidant compounds, particularly carotenoids and ascorbic acid (vitamin C). These metabolites are generally extracted directly from fresh plant tissues, mainly the fruit, which serves as their primary source. However, conventional extraction is often inefficient because it requires long cultivation periods until fruiting and large amounts of plant material to obtain sufficient metabolite yields. Moreover, Wang et al. (2022) note that traditional land-

based agriculture faces increasing limitations due to soil degradation, overuse of fertilizers, pesticide accumulation, and heavy metal contamination. These issues can hinder plant growth and reduce crop productivity, quality, and safety. Given these constraints, plant tissue culture offers a promising alternative, providing a controlled and aseptic environment that overcomes many of the challenges associated with conventional cultivation and supports more sustainable production of plant-derived compounds.

Tissue culture provides a sterile and controlled system for plant propagation, allowing growth independent of environmental conditions. This method enables rapid multiplication of uniform plant material and can enhance the synthesis of secondary metabolites (Cardoso et al., 2019). One of the commonly used approaches is callus culture. Callus consists of undifferentiated cells formed from explants through dedifferentiation when cultured *in vitro* on media containing specific plant growth regulators. Because callus cells are totipotent, they can regenerate into various tissues or organs, making callus induction a fundamental step in mass propagation and cell proliferation. Callus also provides a reliable source of material for plant regeneration and can be initiated from

many types of plant tissues (Rasud & Bustaman, 2020). Callus culture has been widely applied for secondary metabolite production in various species, including vincristine in *Catharanthus roseus* (Iskandar & Iriawati, 2016), alkaloids in mangosteen (Jamil et al., 2018), and andrographolide in *Andrographis* (Habibah, 2016). These studies highlight the potential of callus systems as effective platforms for producing valuable bioactive compound.

The choice of explant source is a key factor determining the success of tissue culture, as the type, physiological condition, and developmental stage of the explant strongly influence its response to in vitro conditions. Previous studies have shown that different explants can produce markedly different outcomes; for example, Oratmangun et al. (2017) reported that shoot explants of mangosteen performed better than node and internode tissues. Callus growth can also be optimized through appropriate culture maintenance, particularly via subculturing. Subculture refers to the periodic transfer of cultures to fresh media to ensure continued nutrient availability and support optimal tissue development (Rodinah et al., 2018). Nakasha et al. (2016) further demonstrated that repeated subculturing in *Chlorophytum borivilianum* significantly enhanced callus proliferation, with a notable increase observed after the fourth subculture cycle. Based on these findings, this study aims to compare the levels of ascorbic acid and carotenoids in tomato callus cultures maintained with and without subculturing, and to assess how different explant sources influence the accumulation of these bioactive compounds.

## MATERIALS AND METHODS

### Study Area and Plant Material

The experiment was carried out January-May 2021 at the Tissue Culture Laboratory, Departement Biology, Diponegoro University. Tomato seeds were surface-sterilized using detergent and sodium hypochlorite, rinsed thoroughly with sterile distilled water, and germinated in vitro on moist tissue medium (2–3 seeds per culture bottle). The cultures were incubated under light conditions for 5–10 days, after which cotyledons, hypocotyls, and radicles were excised and utilized as explant sources.

### Callus Induction and Treatments

Each explants from sterilled sprouts were cut into the small size and were cultured on the Murashige and Skoog (MS) as the basal medium with the addition of 3% (w/v) sucrose and it was solidified with 2g/L gelzan. This basal medium also was added to 1,0 mg/L 2,4 D as initiation medium. The pH was adjusted to 5.8, and then it was sterilized with the autoclave (121°C, at 1,5 atm) for 15 minutes. Callus cultures were incubated in a

growth chamber at 25 °C temperature and 16 hours photoperiod of tube lamp (500 lux).

The cultures were divided into two treatments. In the first treatment, callus cultures were incubated for 35 days without subculturing. In the second treatment, 20 days old calli were subcultured into fresh MS medium with the same composition as the initiation medium and incubated for an additional 15 days. Calli from both treatments were harvested for observation of texture and color, as well as for analysis of ascorbic acid and carotenoid contents.

### Analysis of Ascorbic Acid and Carotenoids

#### Analysis of Ascorbic Acid Content

The content of ascorbic acid can be determined by the spectrophotometric method. The initial step is to determine the maximum wavelength by measuring the absorption solution value of 100 mg/L ascorbic acid in the 200-300 nm long wave range, and then make a standard curve for the absorption value of ascorbic acid solution, to get the standard equation curve  $y = 0.0057x + 0.0003$ . The following is absorption data and graphs of 100 mg/L ascorbic acid standard curves.

The next stage is determining the absorbance value of callus samples by spectrophotometry method based on the wavelengths that have been obtained. Callus samples were weighed as much as 0.1 g, then ground using mortar and pastel, after which they were mixed with a solvent such as 10 mL of CO<sub>2</sub>-free distilled water and filtered with filter paper then the sample solution was analyzed using a UV-vis spectrophotometer. After that, the determination of ascorbic acid levels was done by entering the absorbance value in the equation  $y = 0.0057x + 0.0003$ .

#### Analysis of Carotenoid Content

Carotenoid content was measured using the photometric method. Callus weighed 0.1 g, then crushed with mortar. The crushed callus was extracted with 10 ml acetone 80%. The extract was then filtered using filter paper. The filtrate was then inserted into the cuvette to measure the carotenoid content with a UV-Vis spectrophotometer at wavelengths of 480, 645, and 663 nm. After the absorption value was obtained, the carotenoid content can be calculated by using the following formula (Figure 1.)

$$\text{Karotenoid} = \frac{[(A_{480} + (0,114 \times A_{663}) - (0,638 \times A_{645})] \times V \times 10^3}{112,5 \times W}$$

Figure 1. Formula for determining carotenoid content (Hendry and Grime, 1993).

### Data Analysis

The experiments were carried out in five replication. The mean values with standard deviation (SD) were shown in the table and figures in the next section. The results were

derived from statistical analysis using analysis of variance (ANOVA). Levels of significance were indicated by Duncan multiple range test at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Result

#### Time of Callus Initiation

Based on the data obtained, callus initiation was observed to occur between the 8-10 (average of 9 days), after the explants were placed on the culture medium. This indicates that the explant tissues began responding to the in vitro conditions and hormonal stimuli within the first week of culture, leading to visible callus formation shortly thereafter (Table 1.)

**Table 1.** Time of initiation of tomato callus with different explant sources.

Explants Sources	Time of Callus Initiation (DAP)
Cotyledon	8
Hypocotyl	10
Root	10
Average	9,3

Description: Days After Planting (DAP)

Explants swelling at the age of 4-7 days after planting (DAP). Sliced scars in explants will form a group of cells that divides, grow and develop continuously, undifferentiated and irregular called callus (Figure 2).



**Figure 2.** Callus root explants of tomato sprouts aged 15 Days After Planting (DAP).

#### Fresh Weight of Callus

Fresh weight of callus is an important parameter for assessing growth, as it reflects an irreversible increase in tissue mass. Measuring the fresh weight of callus provides valuable information about the overall biomass accumulation, while the increase in tissue mass serves as an indicator of active metabolic processes occurring within the callus during the culture period. Based on the results of variance data (ANOVA) growth treatments showed significantly different data ( $P < 0.05$ ). Further analysis of the fresh callus weight data under different

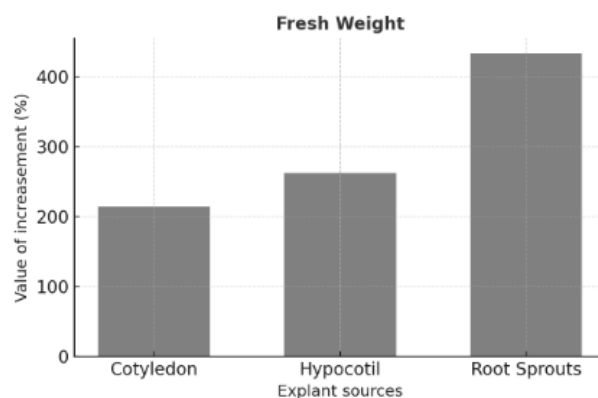
growth treatments was performed using Duncan's multiple range test to determine the presence of significant differences among treatments. Duncan test results showed that the subcultures of hypocotyl explant callus had the highest fresh weight value of 0.29 g, and the lowest fresh weight was callus from root explants without subculture of 0.03g (Table 2.).

**Table 2.** Fresh callus weight from different explant sources and subculture treatment.

Treatment	Explant sources (g)		
	Cotyledon	Hypocotyl	Root
Without subculture	0,07 <sup>d</sup>	0,08 <sup>d</sup>	0,03 <sup>e</sup>
Subculture	0,22 <sup>b</sup>	0,29 <sup>a</sup>	0,16 <sup>c</sup>

Description: The numbers followed by different superscripts in one column stated significantly different based on the Duncan Test at a 95% confidence level.

Based on the graphs of tomato callus increase in weight (Figure 3.), root sprouts explants had the highest percentage increase of 433.3% followed by hypocotyl explants and the cotyledons respectively 262.5% and 214.2%.



**Figure 3.** Percentage changes in initial fresh weight and final fresh weight of callus after 15 days subculture treatment.

#### Carotenoid Content

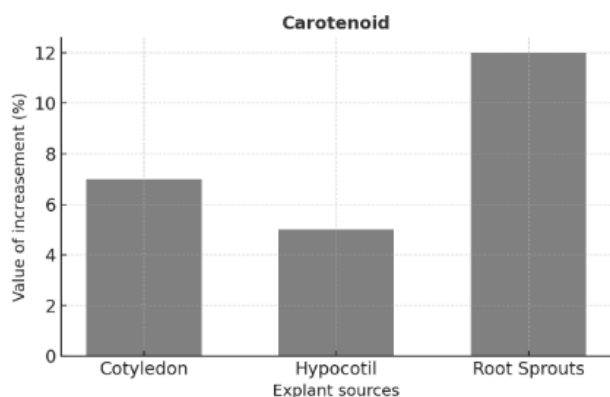
Carotenoids are classified as secondary metabolites that can be synthesized naturally by plants. These pigments are produced through specific biosynthetic pathways and are subsequently accumulated within plastids. ANOVA showed no significant data of subculture treatment to the callus carotenoid content ( $P > 0.05$ ). Based on the results (Table 3), the average carotenoid content in both subcultured and non-subcultured callus was relatively similar, indicating no significant difference between the treatments. The value of carotenoid content in cotyledon callus without subculture was 171.9 mg / L and after subculture it became 180.1 mg / L, in the callus of hypocotyl without subculture the value of carotenoid content was 97.7 mg / L and after subculture it became 100.9 mg / L, whereas in callus from the germination root without subculture the carotenoid content value was 116.6 mg / L after subculture the value was 128.3 mg / L.

**Table 3.** Carotenoid content of callus tomatoes with different explant sources and subculture treatments.

Treatment	Explant sources (mg/L)		
	Cotyledon	Hypocotyl	Root
Without subculture	171,9 <sup>a</sup>	97,7 <sup>b</sup>	116,6 <sup>b</sup>
Subculture	180,1 <sup>a</sup>	100,9 <sup>b</sup>	128,3 <sup>b</sup>

Description: The numbers followed by different superscripts in one column stated significantly different based on the Duncan Test at a 95% confidence level.

The graph of changes in carotenoid content (Figure 4), shows the callus from root sprouts explants has the highest percentage increase in carotenoid content.

**Figure 4.** Percentage increase in callus carotenoid content from different explant sources.

### The Content of Ascorbic Acid

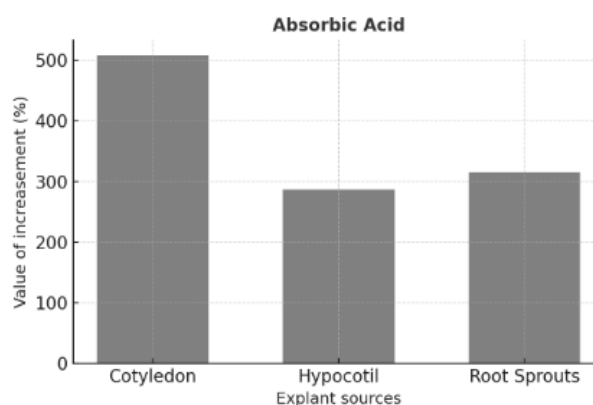
Based on statistically test, growth treatments and different of explant source showed significantly different ( $P < 0.05$ ), meaning that the subculture treatment affects the content of ascorbic acid from all explant sources. Further analysis of the content of callus ascorbic acid showed that the callus from cotyledon explant followed by subculture had the highest ascorbic acid content of 15.2 g / 100g, and the low ascorbic acid content was the callus from cotyledon explant without subculture which was 2.5 g / 100g (Table 4).

**Table 4.** Content of ascorbic acid callus tomatoes with different explant sources and subculture treatments.

Tretments	Explant Sources (g/100g)		
	Cotyledon	Hypocotyl	Root
Without Subculture	2,5 <sup>f</sup>	3,7 <sup>d</sup>	3,3 <sup>e</sup>
Subculture	15,2 <sup>a</sup>	14,3 <sup>b</sup>	10,4 <sup>c</sup>

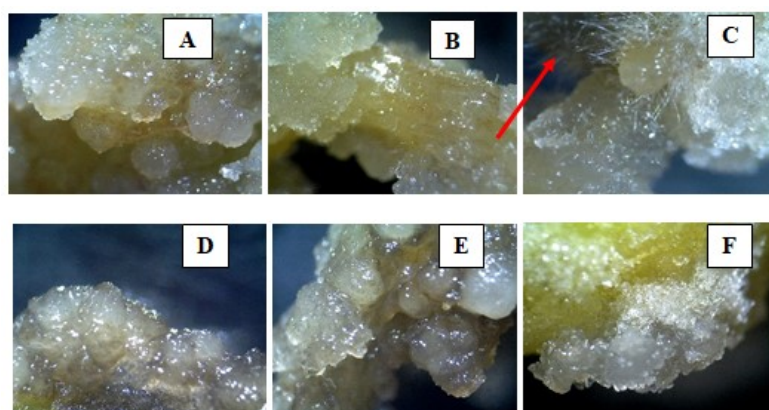
Description: The numbers followed by different superscripts in one column stated significantly different based on the Duncan Test at a 95% confidence level.

The percentage increasement in ascorbic acid content (Figure 5) shows that the cotyledon explant callus had the highest increasement compared to callus root and hypocotil explants respectively, namely 508%, 315.1%, and 286.4%.

**Figure 5.** Percentage increase in callus ascorbic acid content from different explant sources.

### Callus Texture

Based on observations that have been made (Figure 6), the overall callus texture of all treatment explants without subculture or subculture is crumb, where the callus texture is very easily separated and some aqueous callus crusts, this is because the callus absorbs the nutrient and water content in the medium to support growth and division of callus cells.

**Figure 6.** Texture of tomato callus aged 35 DAP without subculture. (A: cotyledonary explant without subculture). (B: hypocotyl explants without subculture). (C: root germ explants without subculture). Texture of tomato callus aged 35 DAP with 15 days of subculture. (D: cotyledonary explants with 15 days of subculture). (E: hypocotyl explants with 15 days of subculture). (F: explant root sprouts with 15 days of subculture). (Arrows indicate absorption of hair on the callus).

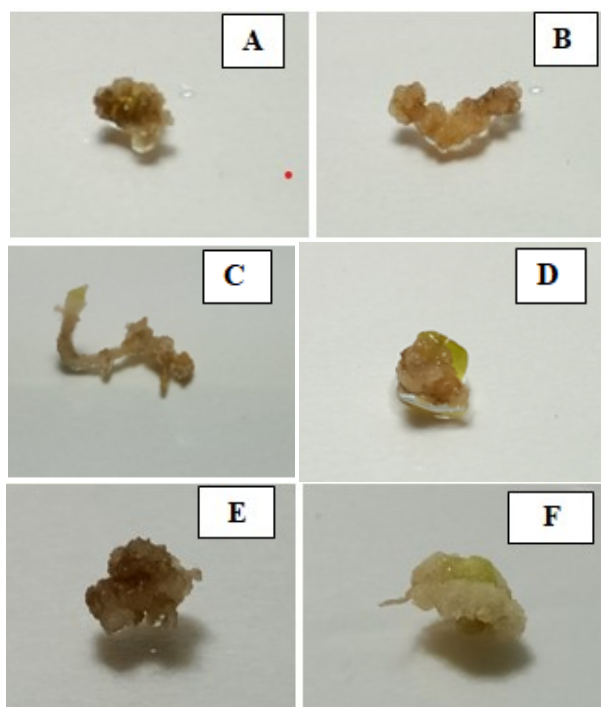
Besides, the callus from root explants formed hair absorption such as those in a circle and pointed at the arrow (Figure 7), which functions to absorb water and nutrients for the callus.



**Figure 7.** Absorbance of hair in callus root explants 35 DAP without subculture treatment.

### Callus Color

The color of the callus was examined at the end of the observation period both visually and using an Optilab microscope to obtain a clearer view of its pigmentation. As the callus aged, its color gradually changed, turning brown and eventually black upon visual inspection (Figure 8), reflecting physiological and biochemical changes associated with prolonged culture.



**Figure 8.** Color of tomato callus aged 35 DAP without subculture. (A: cotyledon explant without subculture). (B: hypocotyl explants without subculture). (C: root germ explants without subculture). Color of tomato callus aged 35 DAP with 15 days of subculture. (D: cotyledon explants with 15 days of subculture). (E: hypocotyl explants with 15 days of subculture). (F: explant root sprouts with 15 days of subculture). (Arrows indicate absorption of hair on the callus).

## Discussion

### Time of Callus Initiation

Time of emergence (initiation) callus of a third source of explants is relatively similar. Initiation of callus is characterized by the presence of swelling in parts of explants that contact directly with the media culture, besides it is also marked by the emergence of new tissue in the form of meristematic smaller nodules on the injured explants in pale yellow/white. Some root sprouts explants also appear new roots and root hairs. It is showing no response from the explants were grown on media culture and absorb nutrients from the media. Hormones are one of which the triggers the induction. According to Huh et al, (2017), callus induction occurs as a physiological response of plant tissues to mechanical injury or incision made on the explants. This response is regulated by the action of plant hormones, which may originate from endogenous sources within the tissue or be supplied exogenously through the culture medium, ultimately triggering cell dedifferentiation and unorganized cell proliferation.

The explant part that is formed to form a callus, caused cells that come in contact with the media are pushed to become meristematic and then actively carry out division such as wound closure tissue. The source of the explants used affects callus velocity (Ulva et al, 2019). The balance of growth regulators incorporated into the culture medium plays a crucial role in regulating the induction of callus, as well as the formation of shoots and roots during in vitro culture. In this study, the three types of explants used were derived from different parts of the sprout, all of which possess meristematic characteristics. This meristematic nature contributes to the high capacity of the explants to undergo active cell division, which is subsequently followed by cellular dedifferentiation, thereby facilitating callus formation and further morphogenetic processes.

### Fresh Weight of Callus

Physiologically, callus tissue is composed primarily of water and a mass of actively dividing, undifferentiated cells. The increase in callus weight is largely attributed to its high water content, which contributes significantly to the overall biomass accumulation during the culture period (Jamil et al., 2018). According Al-Oqab et al. (2023) reported that frequent subculture of *Digitalis purpurea* callus in media supplemented with nutrients and growth regulators promotes cell growth and differentiation, leading to higher production of secondary metabolites. Pillai & Siril (2019), subcultures can influence callus growth and the production of secondary metabolites by altering the physiological and nutritional conditions of the culture environment.

This is presumably because the sprout roots are still meristematic and the presence of endogenous auxins in the roots that can affect the process of cell division at the root. Besides, the presence of 2,4-D (1 mg/L) in the media is thought to be the optimal auxin concentration in

supporting the callus growth process at the root explant source. According Nafisah et al. (2019), auxin plays a crucial role in regulating water uptake within plant tissues, which in turn promotes cell elongation and enlargement. This physiological process contributes significantly to the increase in fresh weight of the callus, as the enhanced absorption of water by the cells leads to greater tissue expansion and biomass accumulation during the culture period.

### Carotenoid Content

The results showed that there were no significant differences in carotenoid content between culture and subculture treatments for each callus type. This indicates that the subculture treatments applied did not influence carotenoid accumulation, suggesting that the levels of this compound remained consistent across different culture conditions. This condition is presumed to occur because the callus was still in the exponential growth phase, during which intensive cell division and enlargement take place. At this stage, metabolic activity is mainly directed toward growth processes rather than secondary metabolite biosynthesis, resulting in carotenoid production that has not yet reached its optimal level. Besides, environmental factors in the form of suboptimal light (500 lux lamps) are thought to affect carotenoid production. Hussein et al. (2016) stated that the growth curve of *Jatropha curcas* L. callus exhibits a sigmoidal pattern, consisting of lag, exponential, and stationary phases. The modified Gompertz model was used to describe callus growth, with a coefficient of determination ( $R^2$ ) of 0.99. Parameters obtained from model fitting indicated a maximum growth rate ( $\mu_m$ ) of  $0.193 \text{ d}^{-1}$ , a lag time ( $\lambda$ ) of 2.91 days, and a maximum callus production ( $Y_{max}$ ) of 0.38 g per 25 mL of culture.

The average carotenoid content of cotyledon callus explants showed the highest value of 176 mg / L followed by callus from root explants (122.4 g / mL) and hypocotyl explant callus (99.3 mg / L), this is because certain carotenoid (especially violaxanthin, which included xanthophyll) are found in the membranes of chloroplasts. The pigments in the chloroplast lamella are mostly two kinds of chlorophyll (a and b) and two kinds of yellow to orange pigments classified as carotenoids (carotene and xanthophyll). Therefore, in addition to its photosynthetic function, the leaf is also a place of carotenoid biosynthesis. Besides, carotenoid-forming genes are already present in the cotyledons. According Schaub et al. (2018), stated that the research used *Arabidopsis* cotyledons to produce non-green callus and examine carotenoid biosynthesis. The callus was able to synthesize carotenoids, though at lower levels than green tissues, showing that cotyledon explants can be used for carotenoid production in vitro. Oleszkiewicz et al. (2018) reported that callus derived from carrot root accumulated carotenoids up to nine times higher than pale-yellow callus, suggesting that root-derived callus has a strong

capacity for carotenoid production. The elevated carotenoid content can be attributed to the genetic, metabolic, and biochemical characteristics inherent to root tissues. This finding is consistent with the statement of Fitriana et al. (2017), who reported that variations in secondary metabolite content among plant tissues are influenced by genetic factors, metabolic activity, and the availability of biosynthetic precursors in each tissue. Genetically, carrot roots (*Daucus carota* L.) possess specific gene expressions that regulate and enhance carotenoid biosynthesis. Metabolically, root cells exhibit higher enzymatic activity in the carotenoid formation pathway compared to other plant parts. In addition, the availability of biosynthetic precursors in root tissues is greater, supporting a more efficient synthesis and accumulation of carotenoids.

### The Content of Ascorbic Acid

Ascorbic acid (vitamin C) is an important antioxidant and a secondary metabolite synthesized by various plant organs. Although the highest concentrations are typically found in fruit, which functions as the primary storage organ, ascorbic acid is also present in other plant tissues, including leaves and flowers. Determination of ascorbic acid content can use various methods, one of which is by using the spectrophotometry method. It is presumed that the callus had entered the final stage of the stationary phase, during which growth activity ceases and the availability of nutrients in the medium becomes limited. Under these conditions, the callus experiences physiological stress due to nutrient depletion and the cessation of active cell proliferation. According to Perangin et al. (2019), plants have evolved multiple strategies to cope with various stresses, including the production of toxic secondary metabolites. This is following the research of Pillai and Siril (2019), where the stable berberine production of *Tinospora cordifolia* was achieved by continuous subculture of the callus. Other studies also conducted by Indriani et al. (2019) demonstrated that the frequency of subculture significantly affects both callus growth and carotenoid accumulation in tomato (*Lycopersicon esculentum* Mill.).

Callus derived from leaf explants was found to produce higher levels of ascorbic acid compared to other explant sources. This may be attributed to the fact that leaves, as the primary photosynthetic organs, are capable of generating substantial amounts of carbohydrates, which function as essential precursor molecules in the biosynthetic pathways of ascorbic acid. The abundance of these precursors likely supports increased metabolite synthesis within the callus tissue. This is in line with the study by Wei et al. (2019), which showed that treating mung bean seeds with sucrose significantly increased vitamin C content, total phenolics, and antioxidant activity in the sprouts, indicating that sucrose can enhance ascorbic acid biosynthesis during germination.

This means that callus from explants cotyledons can be used as the best production of ascorbic acid. The cotyledon is considered a main site for ascorbic acid biosynthesis. During dedifferentiation into callus, its genetic traits related to ascorbic acid production likely remain active, allowing the callus to retain this ability. This agrees with Julianti et al. (2021), who found that cotyledon explants have high metabolic activity and respond well to carbon sources like sucrose. This finding is also supported by the statement of Nurchayati et al. (2023), who reported that callus induced from tomato cotyledon explants showed high antioxidant activity and notable ascorbic acid content, indicating that the cotyledon maintains its biosynthetic potential after dedifferentiation.

### Callus Texture

The texture of the induced callus can be categorized into two types, namely friable (crumb) callus and compact callus, based on its physical characteristics during the culture process. Crumb callus is characterized by an easy to separate callus texture. If the callus is taken with tweezers, the callus cells will easily stick to the tweezers. Conversely, compact callus shaped solid clumps that are difficult to separate. Callus texture is one indicator to assess the quality of a callus. Ariani et.al (2016), stated the texture of callus is considered an important indicator for assessing its quality, as it provides insight into the physiological state of the cells. By evaluating callus texture, it is possible to determine whether the cells within the tissue are actively undergoing division or have reached a stage where cell division has slowed or ceased, indicating a state of stagnation.

The process that occurs in explants after being planted on culture media that contains nutrients and hormones, namely the first explant will experience swelling in some parts, because it has absorbed water and nutrient content from the media, followed by discoloration to yellow or whitish color discharge in some parts of the explant injury, this is because there has been a process of cell division in explants, especially in the vicinity of the explant injury, it becomes one of the signs of initiation of callus formation in explants after which the cells will continue to divide by the size and weight of the callus will increase. Crumb callus texture is influenced by ZPT which is used for callus induction. The use of MS media with the addition of 2,4-D 1 mg/L is capable of producing crumb-textured callus tomatoes. This is following the opinion of Sundarasekar et al. (2012) that 2,4-D is more effective than other plant growth regulators for inducing the formation of friable (crumb) callus, as it promotes cell dedifferentiation and proliferation in a manner that favors the development of this desirable callus texture. Nurchayati et al. (2016), stated that high-quality callus is characterized by a friable (crumb) texture, which allows the tissue to be easily disaggregated into single cells, thereby facilitating its use in suspension culture systems.

### Callus Color

The color of the callus was examined at the end of the observation period both visually and using an Optilab microscope to obtain a clearer view of its pigmentation. According Rasud and Bustaman (2020), the color of callus reflects the visual appearance of its cells and provides an indication of their cell division activity. Changes in callus color signify transitions in the growth phase of the cells, from young and actively dividing cells (white) to more mature cells (pale yellowish). These color changes are primarily caused by the synthesis of phenolic compounds within the callus cells. Initially, the callus exhibited a yellowish or pale white coloration, indicating the development of plastids containing pigment compounds within the tissue. According to Ulva et al. (2019), the change in color of the callus to brown can be caused by the callus no longer has swelling. The callus that continues to divide remains white even though other parts are light brown in color, while the callus whose size remains at the end of the observation will be light brown to brown. Wu et al. (2024), reported that callus browning in *Camellia hainanica* occurs as a result of the accumulation and subsequent oxidation of flavonoid compounds. This process is further regulated by the expression of specific genes and proteins that play a role in controlling the oxidative mechanisms responsible for the observed tissue discoloration.

### CONCLUSIONS

Based on the results of research on the subculture treatment and the different sources of explants on growth, the content of carotenoids and tomato callus ascorbic acid, some conclusions can be drawn as follows the different sources of explants affect biomass, carotenoid content and tomato callus ascorbic acid. Subculture treatment for 15 days can increase biomass and ascorbic acid content, but does not affect the carotenoid content of tomato callus.

**Acknowledgements:** The authors thank Diponegoro University for providing research on the tomato callus culture. Gratitude is also conveyed to Dr. Rini Budihastuti, M.Si, for the comments on the manuscript, and Rizqi Fadlia Julianti, S.Si for technical support.

**Authors' Contributions:** Yulita Nurchayati and Erma Prihastanti designed and conceived the experiments. Imam Purwanto and Yulita Nurchayati experimented. Imam Purwanto, Yulita Nurchayati, and Erma Prihastanti contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed by Imam Purwanto. All authors provided critical feedback and contributed to developing the research, analysis, and manuscript.

**Competing Interests:** The authors declare that there are no competing interests.

**Funding:** Thank you to the head of the department and the head of the Biology Laboratory of Diponegoro University who facilitated this Final Project research. Thank you to LPPM UNDIP with Riset Pengembangan and Penerapan (RPI) 2025 for funding this research and publication

## REFERENCES

- Al-Oqab, M. A., Zaid, S., Al-Ammouri, Y., and Alawdi, S. H. (2023). Effect of nutrient media enhanced with plant growth regulators on genetic stability in sub-cultures of *Digitalis purpurea* callus. *Journal of Applied Biology & Biotechnology*, 11(2), 1–9. DOI: <https://doi.org/10.7324/JABB.2023.110227>.
- Ariani R, Anggraito YU, Rahayu ES. (2016). Respons Pembentukan Kalus Koro Benguk (*Mucuna Pruriens* L.) pada Berbagai Konsentrasi 2,4-D dan BAP. *Indonesian Journal of Mathematics and Natural Science*, 39(1), 20–28. DOI: <https://doi.org/10.15294/ijmns.v39i1.7695>.
- Cardoso, J. C., Oliveira, M. E. B. D., dan Cardoso, F. D. C. (2019). Advances and Challenges on the in Vitro Production of Secondary Metabolites from Medicinal Plants. *Horticultura Brasileira*, 37(2), 124–132. DOI: <https://doi.org/10.1590/S0102-053620190201>.
- Felfoldi, Z., Ranga, F., Roman, I.A., Sestras, A.F., Vodnar, D.C., Prohens, J., and Sestras, R.E. (2022). Analysis of Physico-Chemical and Organoleptic Fruit Parameters Relevant for Tomato Quality. *Agronomy*, 12, 1232. DOI: [10.3390/agronomy12051232](https://doi.org/10.3390/agronomy12051232)
- Fitriana, N., Semiarti, E., & Rumiati. (2017). Respon Pertumbuhan dan Kandungan Karotenoid Kalus dari Kecambah Wortel (*Daucus carota* L.) dengan Jenis Eksplan yang Berbeda. Skripsi S1 Biologi, Universitas Gadjah Mada.
- Handayani, S. U. dan T. D. Kurnia. (2018). Seleksi 25 Genotif F1 Tomat (*Lycopersicon esculentum*) Berdasarkan Potensi Hasil dan Karakter Morfologi Buah. Fakultas Pertanian dan Bisnis. Universitas Kristen Satya Wacana.
- Huh, Y. S., Lee, J. K., and Nam, S. Y. (2017). Effect of plant growth regulators and antioxidants on in vitro plant regeneration and callus induction from leaf explants of purple passion fruit (*Passiflora edulis* Sims). *Journal of Plant Biotech.* 44(3), 335-342. DOI: <https://doi.org/10.5010/JPB.2017.44.3.335>.
- Hussein, S., Halmi, M. I. E. and Kiong, A. L. P. 2016. Modelling the growth kinetics of callus cultures from the seedling of *Jatropha curcas* L. according to the modified Gompertz model. *Journal of Biochemistry, Microbiology and Biotechnology*, 4, 20-23. DOI: <https://doi.org/10.54987/jobimb.v4i1.284>
- Indriani, R., Prihastanti, E., Budihastuti, R., & Nurchayati, Y. (2020). Effect of Subculture Frequency Toward Growth and Carotenoid Content from Tomato (*Lycopersicon esculentum* Mill.) Callus. *Jurnal Biodjati*, 5(2), 303-315. DOI: <https://doi.org/10.15575/biodjati.v5i2.5840>
- Iskandar, N.N. and Iriawati. (2016). Vinblastine and Vincristine Production on Madagascar Periwinkle (*Catharanthus roseus* (L.) G. Don) Callus Culture Treated with Polyethylene Glycol. *Makara Journal of Science*, 20/1, 7-16. DOI: <https://doi.org/10.7454/mss.v20i1.5656>
- Jamil, S.Z.M.R., E.R. Rohani1 · Syarul Nataqain Baharum, and N. M. Noor. (2018). Metabolite profiles of callus and cell suspension cultures of mangosteen. *Biotech*, 8, 322. DOI: <https://doi.org/10.1007/s13205-018-1336-6>
- Julianti, R.F., Nurchayati, Y., & Setiari, N. (2021). Pengaruh Konsentrasi Sukrosa dalam Medium MS terhadap Kandungan Flavonoid Kalus Tomat (*Solanum lycopersicum* syn. *Lycopersicon esculentum*). *Metamorfosa: Journal of Biological Sciences*, 8(1), 141–149. <https://doi.org/10.24843/metamorfosa.2020.v08.i01.p015>
- Nakasha, J. J., Sinniah, U. R., Kemat, N., & Mallappa, K. S. (2016). Induction, subculture cycle, and regeneration of callus in safed musli (*Chlorophytum borivilianum*) using different types of phytohormones. *Pharmacognosy Magazine*, 12 (Suppl 4), S460-S464. DOI: [10.4103/0973-1296.191457](https://doi.org/10.4103/0973-1296.191457).
- Nurchayati, Y., Prihastanti, E., & Budihastuti, R. (2023). In-vitro Callus Development and the Bioactive Compounds of Tomato (*Lycopersicon esculentum* Mill.). *Al-Kauniyah: Jurnal Biologi*, 16(1), 89–97. <https://doi.org/10.15408/kauniyah.v16i1.21565>
- Nurchayati, Y., Santosa, L. H. Nugroho, A. Indrianto. (2016). Growth Pattern and Copper Accumulation in Callus of Datura metel L. *Biosaintifika*, 8(2), 135-140. DOI: [10.15294/biosaintifika.v8i2.5177](https://doi.org/10.15294/biosaintifika.v8i2.5177).
- Oleszkiewicz, T., Klimek-Chodacka, M., Milewska-Hendel, A., Zubko, M., Stróz, D., Kurczyńska, E., Boba, A., & Baranski, R. (2018). Unique chromoplast organisation and carotenoid gene expression in carotenoid-rich carrot callus. *Planta*, 248(6), 1455–1471. <https://doi.org/10.1007/s00425-018-2988-5>
- Perangin Y. Yayuk, P. Yenni, A. Murni, S.R. Nurhayati. (2019). Pemanfaatan kandungan metabolit sekunder yang dihasilkan tanaman pada cekaman biotik. *Jurnal Agriland*. 7(1), 39-47. DOI: <https://doi.org/10.30743/agr.v7i1.3471>.
- Pillai, S. K., and E.A. Siril. (2019). Enhanced Production of Berberine Through Callus Culture of *Tinospora cordifolia* (Willd.) Miers ex Hook F. and Thoms. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 1-9. DOI: <https://doi.org/10.1007/s40011-019-01106-9>.
- Rasud, Y., and Bustaman. (2020). Induksi Kalus secara In Vitro dari Daun Cengkeh (*Syzigium aromaticum* L.) dalam Media dengan Berbagai Konsentrasi Auksin. *Jurnal Ilmu Pertanian Indonesia*, 25(1), 67-72. DOI: <https://doi.org/10.18343/jipi.25.1.67>.
- Rodinah R, Hardarani N, and Ariani, H.D. (2018). Modifikasi Media Dan Periode Subkultur pada Kultur Jaringan Pisang Talas (*Musa paradisiaca* VAR. SAPIENTUM L.). *Jurnal Hexagro*, 2(2), 1–6. DOI: <https://doi.org/10.36423/hexagro.v2i2.129>
- Schaub, P., Rodriguez-Franco, M., Cazzonelli, C.I., Álvarez, D., Wüst, F., and Welsch, R. (2018). Establishment of an Arabidopsis callus system to study the interrelations of biosynthesis, degradation and accumulation of carotenoids. *PLoS ONE*, 13. DOI: <https://doi.org/10.1371/journal.pone.0192158>.
- Ulva, M., Y. Nurchayati, N. Setiari, and E. Prihastanti. (2019). Pertumbuhan Kalus Tomat (*Lycopersicon esculentum* Mill.) Varietas Permata F1 dari Jenis Eksplan dan Konsentrasi Sukrosa yang Berbeda secara In Vitro. *Life Science*, 8 (2), 160-169. DOI: <https://doi.org/10.15294/lifesci.v8i2.37103>.

- Wang, G., Ren, Y., Bai, X., Su, Y., and Han, J. 2022. Contributions of Beneficial Microorganisms in Soil Remediation and Quality Improvement of Medicinal Plants. *Plants*, 11(23), 3200. DOI: <https://doi.org/10.3390/plants11233200>.
- Wei, Y., Wang, X., Shao, X., Xu, F., and Wang, H. (2019). Sucrose treatment of mung bean seeds results in increased vitamin C, total phenolics, and antioxidant activity in mung bean sprouts. *Food Science & Nutrition*, 7(12), 4037–4044. DOI: <https://doi.org/10.1002/fsn3.1269>
- Wu, K., Liu, Y., Xu, Y., Yu, Z., Cao, Q., Gong, H., Yang, Y., Ye, J., and Jia, X. (2024). Unveiling the molecular mechanisms of browning in *Camellia hainanica* callus through transcriptomic and metabolomic analysis. *International Journal of Molecular Sciences*, 25(20), 11021. DOI: <https://doi.org/10.3390/ijms252011021>.

**THIS PAGE INTENTIONALLY LEFT BLANK**