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Estimation of Lycopene and Beta-Carotene Contents in Different Tomato Plant Tissues

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ABSTRACT

Present study succeeded in induced the callus from hypocotyledon stem segments of tomato (*Solanum lycopersicum)* seedlings and produced its cultures then differentiated to vegetative shoot. The extraction and estimation of the lycopene and beta-carotene contents of these tissues were followed. The results indicated that callus was inducible from these segments on solid Murashige and Skoog (MS) medium supplemented with 2.0 mg L^{-1} 2,4- Dichlorophenoxyacetic acid (2,4-D) after 7 days at 100%, with aggregate formed on the segments and then it's become large in size after 12 days, its separated and transferred to a medium with the same components above producing callus cultures due to the rapid and continuous division of cells. What distinguished in study was the spontaneous differentiation of vegetative shoot from callus after the second subculture at a rate of 85%. The methods of preparing the extracts proved successful and the quantities of lycopene and beta-carotene were estimated. The best tissue in their produced was the callus, which reached 3.615 mg100 g^{-1} and 1.301 mg 100 g^{-1} at the third hour of extraction, respectively.

Keywords: *Solanum lycopersicum*, callus, differentiation, lycopene, beta-carotene.

INTRODUCTION

Lycopene is a natural organic compound, a product of secondary metabolism, and is related to the organic pigment carotene, giving a red color to many fruits and vegetables such as tomato, watermelon, pink grapefruit, sea buckthorn and other plants (Imran *et al*., 2020; Ena *et al*., 2023). Recent research and studies indicate that lycopene, in addition to its pigmentary function for a range of fruits and vegetables, also has independent importance as a bioactive substance or dietary supplement. It is an acyclic hydrocarbon compound containing 13 double bonds, 11 of which are conjugated (Arballo *et al*., 2021). It is insoluble in water and almost insoluble in methanol and ethanol, but freely soluble in chloroform and tetrahydrofuran (Doyle, 2020). Common names for this compound include all-trans-lycopene and (all-E)-lycopene, while its chemical formula is $C_{40}H_{56}$ (Przybylska, 2025; Shafe *et al.*, 2024). It is highly effective in treating many diseases such as prostate, lung, stomach, cataract, coronary heart disease, atherosclerosis and inhibiting the formation of free radicals in cells (Bin-Jumah *et al*., 2022; Rejali *et al.*, 2022; Abir *et al.*, 2023).

Beta-carotene pigment is widely found in fruits and vegetables and it a powerful coloring agent with beneficial effects on human health due to its ability to scavenge free radicals, reduce the risk of cancer and promote eye and bone health (Szabo *et al*., 2025). 80-90% of carotenoids are obtained through the consumption of fruits and vegetables, and humans can absorb beta-carotene, alpha-carotene, lycopene, lutein, and zeaxanthin in their diet (Maiani *et al*., 2009). Since the identification of beta-carotene's effectiveness and its function as vitamin A, research has been conducted on methods to improve the productivity and quality of beta-carotene extracted from plant sources using various techniques (Pataro *et al*., 2018). For increasing of these compounds, researchers used modern technologies to achieve this, including plant tissue culture. which mean using any small part of the plant and culture it on a sterile nutrient medium under sterile conditions and it's grown under controlled conditions of temperature, light, and humidity (Plaza *et al*., 2023). This technique provides to the produce that natural compound in laboratories within a small space without the more cost and not depend on a specific season, it also provided simple bioreactor systems leads to increased production of pharmaceutically important compounds, including pigment compounds (Kumar *et al.*, 2021).

One of the studies achieved by (Goenawan *et al*., 2022) able to estimate lycopene in tomato callus grown on MS medium supplemented with 1.0 ppm BA and 1.5 ppm IAA after 2-4 weeks of growth. The results indicated the superiority of four-week-old callus in its lycopene content. Similarly, Kareem and Karrar (2018) were able to estimate the compound in tomato plant callus tissues. Other studies have also been able to extract and estimate the quantities of beta-carotene in various tomato plant tissues (Toma *et al.*, 2008; Nurchayati *et al*., 2023).

This study aimed to induce callus from hypocotyledon stem segments seedlings of tomato and produce its cultures, as well as the possibility of differentiating vegetative shoots from it, for the purpose of extracting and estimating their content of lycopene and beta-carotene.

MATERIALS AND METHODS

Surface sterilization of seeds

Tomato (*Solanum lycopersicum*) plant seeds provided from local markets in Mosul/ Iraq, were sterilized by immersed them in 3% sodium hypochlorite (NaOCl) solution for 15 minutes with stirring. They were rinsed three times with sterile distilled water 1 minute/ 3 times and dried by placing them on sterile filter paper (Ling *et al*., 2020). Sterilized seeds cultured on 100 ml flask placing 30 ml of solid MS medium at 6-8 seeds per flask. The samples were kept in a growth chamber under dark conditions at a temperature of 24 ± 2 °C. After seed germination (emergence of radicle and plumule), which took five days, they were transferred to photoperiod conditions of 16 hours of light at an intensity of 2000 lux / 8 hours of darkness.

Callus initiation from hypocotyledon stem segments of tomato seedlings

Healthy 15-day-old tomato seedlings were removed from MS medium and their hypocotyl stems were cut into segments approximately 1.5-2 cm long. Three or four segments were placed on the surface of a flask containing 30 ml of nutrient medium supplemented with 2.0 mg L^{-1} 2,4-D. The samples were incubated in the growth chamber under the previously mentioned conditions.

Production of callus cultures

The callus masses formed were excised and subcultured on the same induction medium to obtain callus cultures. The process was repeated every 20 days.

Differentiation of vegetative shoots

To determine the possibility of differentiating vegetative shoots, 1.0 gram of callus pieces were taken and transferred to solid MS medium containing 2.0 mg L^{-1} of 2,4-D. The samples were incubated in the growth chamber under the previously mentioned conditions.

Measurement of lycopene and bet-carotene contents in different tomato plant tissues

To obtain the lycopene alcoholic extract from the tomato plant tissue samples under study, one gram of each of the following is taken from vegetative shoots of seedlings grown on solid MS medium, 17 days old, callus grown on solid MS medium containing 2.0 mg L^{-1} 2,4-D, 20 days old and from vegetative shoots differentiated from callus in (2), 15 days old.

Sample is placed in a separate bottle and mixed with 10 ml of solvent solution (hexane: acetone: ethyl alcohol) in a ratio of (1:1:2) using a shaking incubator for 10 minutes. Then, 1.5 ml of water is added to separate the hexane layer from the acetone and ethyl alcohol layer. The mixture is then mixed again for another 5 minutes, and the previous step is repeated. The upper layer containing lycopene is removed and stored in a dark glass bottle with a lid. The process is repeated by adding another number of solvents until the residue becomes colorless. The layers containing the pigment are collected together and dried using a rotary evaporator at 40 °C. The dried pigments are dissolved in petroleum ether and the volume is made up to 50 ml. Spectrophotometric absorbance is measured at a wavelength of 472 nm for each sample individually and the following formula is applied to obtain the lycopene content (Thompson *et al*., 2000):

Lycopene content (mg 100 g^{-1}) = 3.1206 × sample absorbance value × sample volume × dilution factor inverse × 100.

Then for estimation of beta-carotene content, 25 grams of samples of above (lycopene paragraph) were taken, and each was placed individually in 4 °C conditions for three days (Dutta *et al*., 2005), then transferred to 250 ml bottles. 100 ml of 96% ethanol was added. The samples were placed in a water bath at 60 °C with shaking every 10 minutes. After each hour, 5 ml was taken (for 4 hours) and placed in new bottles, and 20 ml of petroleum ether solution was added. The volume was then brought up to 50 ml with distilled water. At this point, two layers were observed: An upper and a lower layer (Fikselova *et al*., 2008). The supernatant was taken and spectrally read using a spectrophotometer (CECIL, Scanning instructions, Aquarus, German) at a wavelength of 450 nanometers. The amount of beta-carotene was calculated according to the following equation:

(Fikselova *et al*., 2008) where:

A = absorbance.

d = dilution.

W = weight of sample (gm).

V = volume (ml).

Coefficient of absorbance (2592 for petroleum-ether).

RESULTS AND DISCUSSION

Production of sterile tomato seedlings

The surface sterilization method for *S. lycopersicum* tomato seeds demonstrated high efficiency, as evidenced by a 100% germination rate after 5 days, producing sterile seedlings with distinct vitality. The efficiency of seed sterilization is often measured by the absence of contamination after planting on the culture medium and the germination of seedlings with good vitality for subsequent use (Sen *et al*., 2013).

Callus induction and its culture production

The results of cultured hypocotyledon stem segments of tomato seedlings on solid MS medium supplemented with 2.0 mg L^{-1} 2,4-D showed a distinct response with 100% callus induction. The stem segments began to swell after 7 days Fig. (1-A), then completely transformed into a callus mass after 12 days Fig. (1-B). The callus masses continued to grow and divide rapidly after being separated and transferred to flask contained solid MS medium supplemented with the same medium above, typical callus cultures produced after 25 days Fig. (1-C). The callus was characterized by its friable texture and yellowish-green color, while the MSO medium failed to stimulate callus induction.

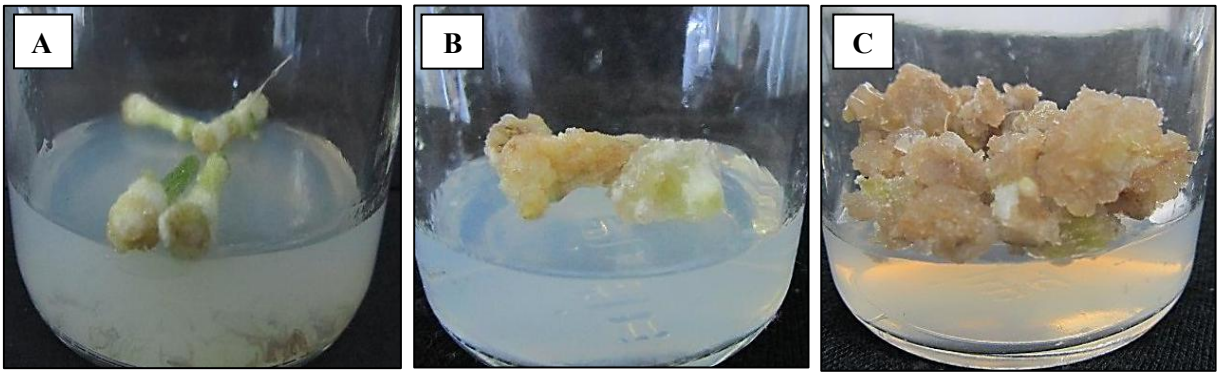

Fig. (1): Production of callus cultures from the hypocotyledon stems of *S. lycopersicum* seedlings grown on MSO medium containing 2.0 mg L^{-1} of 2,4, -D.

A- Callus initiated after 7 days of culture.

B- The development of callus from (A) and covered the explants after 12 days.

C- Cultures from continued growth of callus in (B) at the age of 25 days.

Growth regulators play a role in stimulating callus induction. The presence of auxins in the solid MS medium, either alone or combined with cytokinins, has a significant role in promoting callus induction (Azis *et al*., 2015). The presence of the growth regulator 2,4-D alone in the culture medium can have a positive effect on cell divisions in some plant species and callus initiation (Taiz and Zeiger, 2006). Some studies have indicated its role in callus induction from tomato plants (Gerszberg *et al*., 2016; Rumiyati *et al*., 2017; Setiaji *et al.,* 2020). The study conducted by (Mohammed and Yahya, 2018) successfully obtained complete white lupine (*Lupinus albus*) plants from the differentiation of sub-cotyledonary stem callus of its seedlings, the solid MS medium supplemented with 1.0 mg L^{-1} NAA was superior in inducing callus with a rate of 90% after 20 days of cultivation, compared to other selected media.

Spontaneous vegetative shoot formation from tomato callus

The results showed that after 6 days of the second subculture of callus on the same callus induction medium, green growths appeared spontaneously and increased in growth, later developing into vegetative shoot with a high average number of 5 shoot per callus piece and an average length of 3.2 cm, with a rate of 85% (based on 25 re-cultivated callus pieces) after 20 days Fig. (2). The resulting shoot were well-grown, bearing true leaves.

Fig. (2): Spontaneous differentiation of vegetative shoots from callus grown on solid MS medium supplemented with 2.0 mg L^{-1} 2,4-D after 46 days.

The spontaneous formation of vegetative shoot on the same callus induction medium is attributed to the genetic makeup of the plant species used, as well as the efficiency of its cells in forming vegetative shoot. This is in addition to the levels of plant hormones within the tissues, which stimulated differentiation (Rajoriya *et al*., 2018). It is also due to the inherent self-potential of the cells, which allows each cell to produce supplemented with plant (Parray *et al*., 2018).

Estimation of lycopene content in different tomato plant tissues

The results revealed a variation in the lycopene content extracted from different tomato plant tissues. The lycopene content extracted from the hypocotyledon stem callus grown on solid MS medium containing 2.0 mg L^{-1} 2,4-D was the highest, reaching 3.615 mg 100 g^{-1}. This was compared to the lycopene content extracted from the vegetative shoot differentiated from the callus and from the seedlings, which were 2.512 and 1.747 mg 100 g^{-1}, respectively, as shown in (Table 1).

Table 1: Lycopene content extracted from different tomato plant tissues.

Samples	Lycopene Content (mg 100 g^{-1})
Vegetative shoots of seedlings grown on solid MS medium at 17 days old	1.747
Callus grown on solid MS medium containing 2.0 mg L^{-1} 2,4-D at 20 days old	3.615
Vegetative shoot differentiated from callus at 15 days old	2.512

Results have shown that utilizing a ternary system solution consisting of acetone, ethanol, and hexane successfully increased lycopene extraction. The presence of two highly polar compounds, acetone and ethanol, exhibits a better synergistic effect compared to a single highly polar compound, acetone (Tarrahi and Rezanejad, 2017). This allows for greater penetration of the solvent mixture into the cellulosic tissues of tomato waste. Additionally, lycopene pigment is more soluble in hexane than in petroleum ether and ethyl lactate (Rasheed and Al-Anbari, 2024).

The increased production of lycopene in callus tissue may be attributed to its undifferentiated cellular nature and its function of continuous division, which enhances the synthesis of secondary metabolites, including lycopene and others (Nurchayati *et al*., 2023).

Estimation of beta-carotene content in different tomato plant tissues

The results showed a direct increase in beta-carotene content with increasing extraction time up to the third hour in samples of vegetative shoot separated from seedlings, stem callus and vegetative

shoot resulting from spontaneous callus differentiation. Its percentage began to decrease at the fourth hour, as shown in (Table 2).

Table 1: Beta-carotene content extracted from different tomato plant tissues.

Samples	Extraction time (hours)			
	1	2	3	4
	Quantity (mg 100 g^{-1})			
Vegetative shoot separated from 15-day-old seedlings grown on MS medium	0.413	0.451	0.723	0.508
Callus grown on MS medium supplemented with 2,4-D growth regulator at 20 days old	1.213	1.221	1.301	1.210
Vegetative shoot resulting from spontaneous callus differentiation	0.719	0.821	0.845	0.689

The table indicated that the highest beta-carotene content was 1.301 mg 100 g^{-1}, extracted from stem callus grown on MS medium supplemented with 2.0 mg L^{-1} 2,4-D growth regulator at the third hour, compared to vegetative shoot of seedlings and vegetative shoot resulting from spontaneous callus differentiation, where the highest beta-carotene content was 0.723 and 0.845 mg 100 g^{-1}, respectively, both at the third hour. The lowest beta-carotene content in all samples was observed at the first hour of extraction.

Beta-carotene is a vital compound known as provitamin A, playing a role as an antioxidant with other benefits (Szabo *et al*., 2025). The variation in its presence depends on the extraction method. Therefore, the method used in this study proved successful (Mohammed and Al-Mallah, 2013). The actual increase depends on the enhanced effects of callus on bioavailability, while the decrease in quantity may be due to oxidation in cells (Mohammed and Masyab, 2020). Additionally, a study indicated the role of temperature in the compound's presence, as high temperatures can reduce it by degrading the compound, as previously mentioned, the rapid cell division of callus led to increased compound synthesis compared to vegetative shoot (Nurchayati *et al*., 2023).

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تقدير محتوى الليكوبين والبيتا–كاروتين في انسجة نبات الطماطم المختلفة

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الخلاصة

نجحت الدراسة الحالية في استحداث الكالس من قطع السيقان تحت الفلقية لبادرات نبات الطماطم (*Solanum lycopersicum*) وانتاج مزارعه ومن ثم تمايز الافرع الخضرية منه. يليها استخلاص وتقدير محتوى تلك الانسجة من مركبي الليكوبين والبيتا–كاروتين. واشارت النتائج الى استحداث الكالس من تلك القطع بعد 7 ايام من زراعتها على وسط MS الصلب المدعم باضافة 2.0 ملغم لتر$^{-1}$ 2,4- Dichlorophenoxyacetic acid (2,4-D) بنسبة بلغت 100% بتكوين انتفاخات على القطع ثم تحولها الى كتل كبيرة من الكالس بعد 12 يوم. هذه الكتل انتجت مزارع من الكالس بعد فصلها ونقلها الى وسط بذات المكونات اعلاه بسبب سرعة انقسامات خلايا الكالس واستمرارها. وما تميزت به الدراسة هو التمايز التلقائي للافرع الخضرية من الكالس بعد اعادة الزراعة الثانية بنسبة بلغت 85%. وابدت طرق تحضير المستخلصات نجاحها وقدرت كميات الليكوبين والبيتا–كاروتين وكانت أفضل الانسجة تفوقا في بنائها هي نسيج الكالس اذ بلغت 3.615 ملغم 100غرام$^{-1}$ و1.301 ملغم 100 غرام$^{-1}$ بعد ثلاث ساعات من الاستخلاص على التوالي.

الكلمات الدالة: *Solanum lycopersicum*، كالس، تمايز، الليكوبين، البيتا–كاروتين.