How to Cite:
Yahya, R. T., & Sultan, S. J. (2022). Effect of exposing the seeds of tomato plant (Solanum lycopersicum L.) to diode laser rays on the germination, growth of hypocotyl stems callus of their seedlings and its content of lycopene. *International Journal of Health Sciences*, *6*(S1), 987-996. https://doi.org/10.53730/ijhs.v6nS1.4844

Effect of Exposing the Seeds of Tomato Plant (Solanum Lycopersicum L.) to Diode Laser Rays on the Germination, Growth of Hypocotyl Stems Callus of their Seedlings and its Content of Lycopene

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***Abstract*---**The current study succeeded in demonstrating the role of red laser beams at a wavelength of 650 nm and a power of 50 milliwatts / cm^2 when exposed to the seeds of the tomato plant *Solanum lycopersicum* L. at times of 0,5,10,15 minutes on their germination rate and seedlings growth, and then estimating the growth of its callus and its content of lycopene. Also the results showed that the time period of 10 minutes exceeded others in encouraging seed germination with a full percentage (100%) after 5 days of planting them on solid Murashige and Skoog (MS) medium with distinct growth of its seedlings, whose roots and vegetative total lengths reached 3.9, 7.1 cm after 20 days compared to seeds not exposed to radiation, the percentage reached 80% after 7 days, with seedlings growing at a rate of 2.3 and 3.4 cm, respectively. The study was able to initiate callus by cutting stems of its sub cotyledon seedlings on MS medium supplemented with 2.0 mg /l TDZ and 0.5 mg/l NAA. The results showed an increase in divisions of callus cells derived from seedlings exposed to 10 minutes of irradiation, declaring their preference for a fresh weight of 3.3 g after 50 days, accompanied by an increase in their lycopene content, recording 805.21 µg /g and superior to the rest of the treatments in fresh weight and lycopene content.

***Keywords*---**tomato plant (*Solanum lycopersicum* L.), diode laser rays, callus, seedlings, lycopene.

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
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Manuscript submitted: 27 Nov 2021, Manuscript revised: 18 Feb 2022, Accepted for publication: 09 March 2022

Introduction

The technology of plant tissue culture has a great interest by specialists and researchers for use in many scientific and research paths, whether agricultural, medical or industrial, especially the production of medical compounds from their plant sources and the ability to exploit many of them on a commercial level, in this field, one of the distinctive applications is the extraction of compounds are secondary and active metabolites of many plants such as phenolic compounds, steroids and pigments as by-products of various metabolic activities[1].For the success of this technology, it is necessary to provide the appropriate environmental conditions for the plant part grown outside the living body, similar to those conditions needed in the natural external environment. Light is one of the very important factors affecting many morphological and anatomical characteristics, and a large number of physiological processes in plants such as photosynthesis, flower formation, seed germination, chlorophyll biosynthesis and others which affected by the presence and intensity of light[2].

Laser is the amplification of light by stimulating radiation and is determined by the emitted wave length and intensity. Lasers have been used in various fields, including medical, industrial, agricultural techniques[3].The effect of laser beams varies according to their dose, time, type as well as the type of plant part used, as it was found that the red light is the most used in the process of activating the enzymes for the construction of gibberellic acid GA3[4].While the blue light stimulates the enzymes that synthesize the hormone cytokinin[5]. A study of the ability of He-Ne laser beams when exposed to Scorzonera plant seeds at different times for 1.0, 5, 10, 30 minutes showed its ability to increase the seed germination rate by increasing the exposure time and that the best was at 30 minutes[6].

Also in another study, exposing *Helianthus annuus* seeds to a red laser beam at a wavelength of 650 nm and with a capacity of 50 milliwatts / cm^2 for different times 5.0, 10, 15 and 20 minutes led to stimulating seed germination by 100% at the time of exposure 20 minutes, and also to increasing the rates of soft weights of callus resulting from its seedlings with an increase in the exposure times of callus[7].

Lycopene is a carotenoid hydrocarbon compound and one of the important carotenoids that has attracted researchers' attention in recent years. It is responsible for the red pigment found in the fruits of tomatoes, the color of red watermelons, and some types of fruits and vegetables such as red carrots and red peppers[8]. The ripe red tomatoes provide about 85% of lycopene. The researchers touched on the extraction of lycopene from the fruits of ripe tomatoes after their cultivation in the natural environment and its natural appreciation, and then using this dye in various biological tests[9].

From this point of view, the current study aimed to investigate the role of laser diode rays on the germination of tomato plant seeds and the behavior of its seedlings outside the living body in vitro and then the initiation of callus from those seedlings and measurement of its growth. The purpose of the study was to measure the lycopene content in callus cells.

Materials and Methods

Exposing tomato seeds to laser beams

The seeds of the tomato plant *Solaunm lycopersicum* L. were exposed to laser beams using a (UK-SCIENTIFIC) diode laser device with a wavelength of 650 nm and a power of 50 milliwatts /cm^2 for periods of time 0,5,10 and 15 minutes for each group of seeds separately and at a distance of 10 cm[7].

The culture of seeds and seedling production

The seeds (pre-exposed to the laser diode) were sterilized superficially individually by immersing them in 3% sodium hypochlorite solution NaOCl with stirring for 15 minutes, then washing them with sterile distilled water three times for one minute / time[10]. The seeds were transferred to flasks containing 30 ml of MSO medium[11] with two seeds /flask. The flasks were kept in the growth incubator, in dark conditions, at a temperature of 22+2 °C, and after the emergence of the radical and coleoptile, the flasks were transferred to light succession conditions for 16 hours of light / 8 hours. dark and intense 1500 lux.

Estimation of phenotypic characteristics of developing seedlings

The percentage of germination of seeds, their speed of germination, as well as the lengths of the vegetative and root groups were determined after 10, 20 days of germination for each type of seedling whose seeds were treated with laser beams separately.

Production of callus from the hypocotyl stems of seedlings

Hypocotyl stems about1.0 cm long were excised from laser-treated growing seedlings after 20 days separately and placed on a surface of 30 ml of solid MS medium supplemented with 2.0 mg/L TDZ and 0.5 mg/L NAA[12]. After the initiation of the callus, its mass was picked up and transferred to the same media to avoid the depletion of the components of the medium, and the process was repeated every 25 days.

Callus fresh weight

Determine the fresh weight of the callus by taking the difference between the weight of the flasks, which contain the nutritional medium alone only, and their weight after placing the callus on them after 25, 50 days.

Determination of lycopene content

The lycopene content in the tissues of the growing callus was estimated 25 and 50 days after the above by taking 250 mg of callus samples, each of them separately, and placing them in tubes containing 3.0 ml of a mixture of hexan:ethanol:aceton solution in a ratio of (2:1:1)(volume:volume:volume) ml. The tubes are placed in an ice beaker and shaken for 15 minutes using a shaking incubator (Shaking incubator, New Brunswick, USA). Then 3.0 ml of distilled water is added to each tube and shaken them for another five minutes. The samples are removed from the device and placed at room temperature for 5-10 minutes for the purpose of separating the layers of the solution into two layers. 1.0 ml of the top layer is taken and diluted with 10 ml of hexane solution. Then it was measured by a spectrophotometer (Ta2, UV Spectrophotometer PG instrument, German) at a wavelength of 503 nm using hexane as the control solution of the device. Then the amounts of lycopene were determined using the following formula:[13]

Lycopene (µg / g) = $(A_{503}x31.2x\ Dilution)/g\ of\ sample$

Results

Tomato plant seeds germination and seedling growth

The results showed a variation in the percentage and duration of germination of tomato plant seeds, depending on the times of exposing them to laser rays after 20 days (Table 1).

Table 1
Effect of diode laser radiation on the germination of seeds of tomato plant *Solanum lycopersicum* L. on solid MS medium and averages of root and vegetative lengths of its seedlings after 20 days

Laser exposure time (min)	Germination percentage (%)	Germination period (day)	Average total lengths root (cm)		Average total lengths vegetative (cm)	
			after 10 days	after 20 days	after 10 days	after 20 days
(control) 0	80	7	1.0	2.3	2.1	3.4
5	90	6	1.8	3.4	3.1	6.7
10	100	5	2.3	3.9	3.5	7.1
15	80	7	1.3	2.8	2.6	3.9

The exposure time of 10 minutes stimulated seed germination by 100% after 5 days of planting them on MS medium, superior to the rest of the treatments, followed by the exposure time of 5 minutes with a percentage of 90% after 6 days compared to the control seeds (unexposed to radiation), as the percentage of germination reached 80% after 7 days, which corresponds to the percentage when the seeds were irradiated for 15 minutes. Similarly, the results showed the effect of laser rays on the growth behavior of seedlings resulting from those seeds (Table 1).

The highest average lengths of the root and vegetative groups were 3.9 and 7.1 cm, respectively, for those grown on MS medium and whose seeds were previously exposed for 10 minutes after 20 days. The seedlings were characterized by the elongation of their stems and the density of their root system (Fig.1-C). Followed by the rest of the treatments 15, 5 minutes at rates of varying lengths. Compared to the control seedlings, whose root and vegetative groups averaged lengths of 2.3 and 3.4 cm, respectively, and showed medium growth (Fig. 1-A).

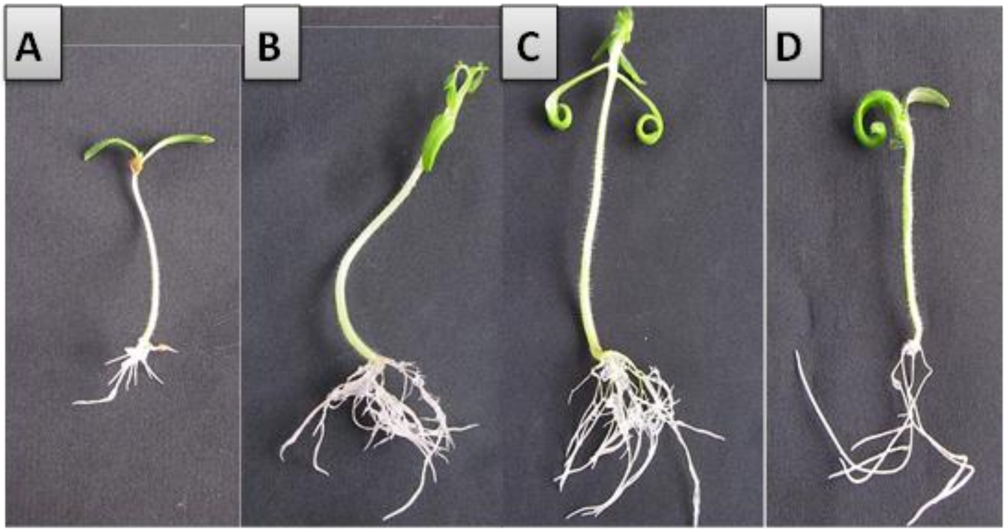

Figure 1. Growth of seedlings of tomato plant *Solanum lycopersicum* L. after 20 days on MS solid medium and its seeds exposed to diode laser radiation for different times:

A- 0 minutes (control) B-5 minutes C-10 minutes D-15 minutes.

Callus growth of tomato plant stems

Figure (2) indicates the variation in the growth of the hypocotyl stems callus of tomato seedlings after 25-50 days, according to the variation in the times of exposure of the seedlings produced to those seedlings to diode laser beams and for different times 0,5,10,15 minutes (Fig. 2).

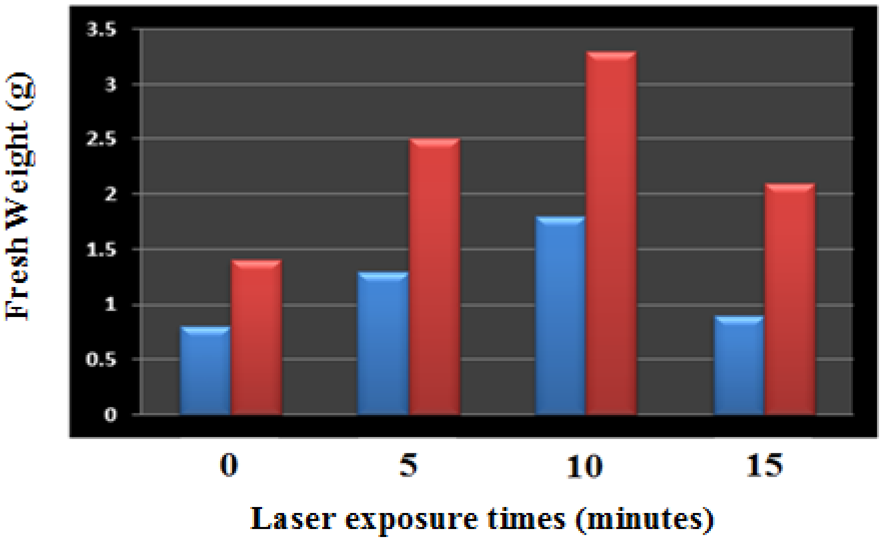

Figure 2. Average fresh weight of seedling callus of tomato plant *Solanum lycopersicum* L. exposed to different laser times and grown on MS solid media supplemented by adding 2.0 mg/l TDZ + 0.5 mg/l NAA after 25 , 50 days.

*Each value represents an average of four replicates
The results showed the efficiency of laser beams with exposure time of 10 minutes in stimulating the growth of seedlings from the previous figure, which later led to an increase in callus growth after 50 days and reached 3.3 g , the callus was distinguished by its hard texture and the dark green color predominated on it (Fig.3- C). Then the exposure times 5 and 15 minutes, as the average fresh weight of the callus reached 2.5 and 2.1 g, and the callus showed a brittle texture and was yellowish-green and dark green, respectively (Fig.3- B, D). While the average fresh weight of the callus growing on the standard MS medium alone was 1.4 g, and the callus was brittle in texture and dark green in color (Fig.3- A).

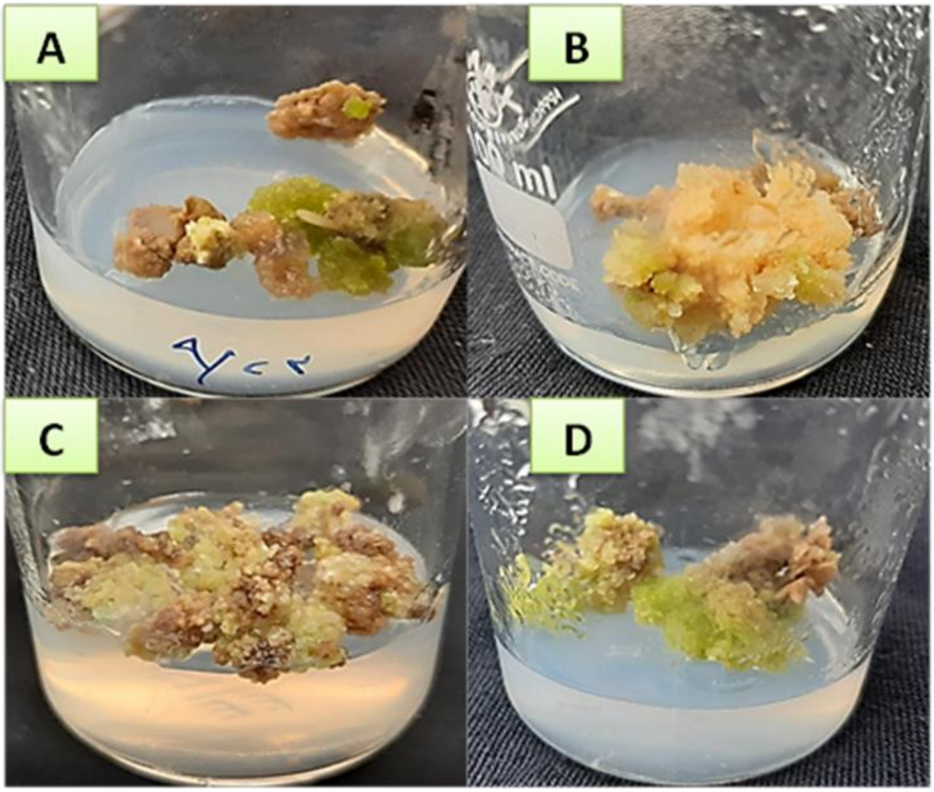

Figure 3. hypocotyl stems callus growth of seedlings of tomato plant *Solanum lycopersicum* L. exposed to different diode laser times:
A- 0 min (control) , B – 5 min , C – 10 min , D – 15 min after 50 days on MS solid medium supplemented with 2.0 mg/l TDZ and 0.5 mg/l NAA

Total lycopene content in tomato callus

The results of estimation of lycopene content in the hypocotyl stems callus tissues of tomato seedlings, whose seeds were previously exposed to diode laser beams, showed the reflection of the percentages of their contents from this dye according to the different exposure times after 25 and 50 days (Fig. 4). The callus initiate mainly from seeds exposed to radiation for 10 minutes recorded a superiority in its content of lycopene by 805.21 µg/g over the rest of the other treatments 5 and

15 minutes after 50 days (Fig. 4). Compared to the callus developed from seedlings whose seeds were not exposed to radiation, which left a callus with a lower content of 373.48 µg/g.

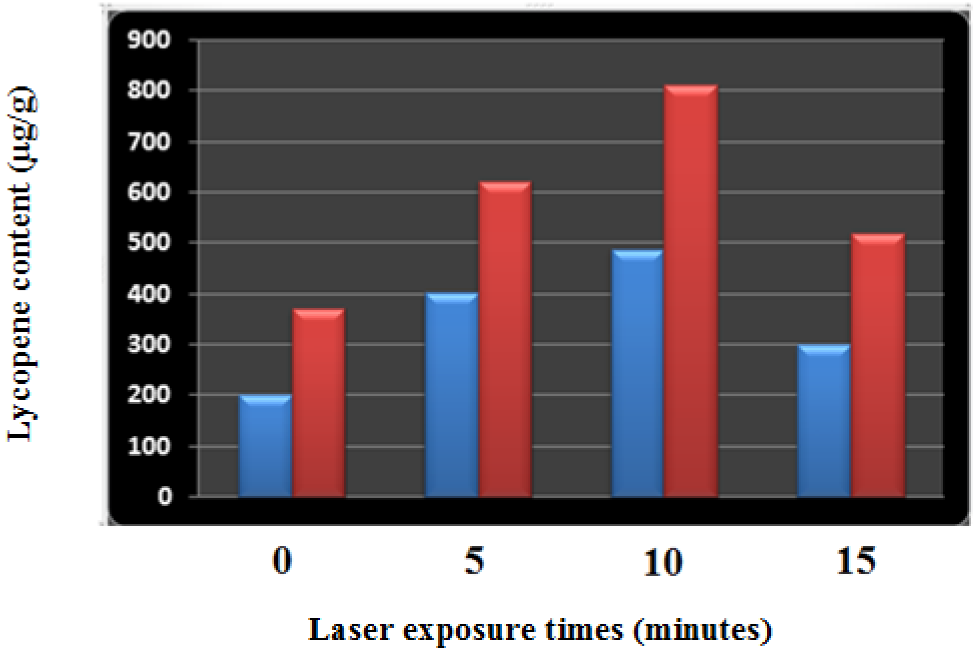

Figure 4. Lycopene amount in the callus of seedlings of tomato plant *Solanum lycopersicum* exposed to different laser times on MS solid medium and supplemented by addition of 2.0 mg/l TDZ and 0.5 mg/l NAA after 25 , 50 days.
*Each value represents the average of three replicates

Discussion

Radiation is one of the most important factors affecting the vital components of living cells such as proteins, nucleic acids, lipids, carbohydrates, and others[14]. Exposing tomato seeds to a red diode laser showed different results in their germination rates and speed. And that stimulating most of the exposure times for their germination compared to the comparison treatment, may be due to their ability to quickly break latency to the seeds and thus speed up their germination, as well as its role in stimulating the enzymes necessary for seed germination, and that the red light is the most used in stimulating vegetable seeds to germinate and activate the necessary phytochrome for that[15].

While the percentage was reduced when the seeds were treated for 15 minutes, which is attributed to the negative effect of rays by increasing the exposure time, which may cause inhibition of the enzymes necessary for germination, and this is consistent with the findings of researchers in inhibiting the germination of cucumber seeds[16].

The advantage of seedling growth of the proper tomato plant and its seeds exposed to laser radiation over other untreated ones may be due to the ability of the phytochrome dye to absorb red light, stimulating vegetative growth and helping the phytochrome proteins to show and amplify the effects of light and thus play the role of an active enzyme [17]. Also, the red light has a role in stimulating the building of the complex ring of gibberellin hormone GA3, which is directly responsible for the elongation and growth of seedlings[18]. The development of callus from stems explants of tomato seedlings, as a result of the balance between TDZ and NAA, had a clear effect in encouraging those plant cuttings to produce callus[19].

The increase in the fresh weight of the callus (initiate from stems of seedlings whose seeds were mainly exposed to rays) when compared to the control treatment is due to the positive role of those rays in stimulating successive divisions of cells and the preference for growth according to the ability of plant tissues to survive different exposure times, and this is what was found in the increase in the fresh weight of sunflower plant callus exposed its plant parts to red laser rays[20]. *Pisum sativum* L. plant[21]. The increase in the fresh weights of the callus was accompanied by an increase in the content of its cells of lycopene, with the same variation and superior to the callus initiate mainly from seedlings whose seeds were not exposed to radiation. This may be explained by radiation stimulation to build nucleic acids, DNA, RNA and proteins[22], which contribute to increasing the gene expression of genes responsible for building and accumulating lycopene[23]. Also, the ability of red laser light to stimulate photoreceptors (phytochrome) that modulates the activation of HY5 and PIF5 enzymes in order to increase the expression of lycopene synthesis genes[24], [25].

Conclusion

This study proved the positive effect of red laser beams at a wavelength of 650 nm and a power of 50 milliwatts / cm^2 on the seeds of the tomato plant *Solanum lycopersicum* L. at times of 0,5,10,15 minutes on all their germination and growth parameters.

Acknowledgment

The authors are very grateful to the University of Mosul/ College of Science and AlNoor University College for their provided facilities, which helped to improve the quality of this work.

Authors' declaration:

Conflicts of Interest: None.
We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.

References

1. Shengwei Z and Jingsam, S. Rapid plant regeneration from cotton(Gossypium hirsutum L.). Chin. Sci. Bulletin. 2000 ; 45(19):1772-1773.
2. Kazemzadeh - Beneh H, Mahna N, Safari E, Zaare-Nahandi F and Motallebi-Azar A. Effects of diode and He-Ne laser on in vitro production of anthocyanin in apple cell suspension culture. Internat. J. Horticul. Sci. Technol. 2015; 2(2): 205-212.
3. El-Sherif F, Yap YK and Ibrahim HI. Laser irradiation induces DNA polymorphism and alters phytochemicals compositions as well as growth and yield of Curcuma longa. J. Diseases and Medic. Plants. 2019; 5 (2) : 29-38.
4. Cope KR and Bughee B. Spectral effects of three types of white light emitting diodes on plant growth and development: Absolute versus relative amounts of blue light. Hort. Sci. 2013; 48(4):504-509.
5. Kazemzadeh -Beneh H, Mahna N, Safari E and Motallebi - Azar A. Blue diode and red He-Ne lasers affect the growth of anthocyanin producing suspension cells of apple. Internat. J. Horticult. Sci. Technol. 2018; 5(2):231-239.
6. Krawiec M , Dziwulska-Hunek A , Palonka S , Kapłan M and Baryła P. Effect of laser irradiation on seed germination and root yield of scorzonera (Scorzonera hispanica L.). Acta Agroph., 2016 ; 23(4): 621-631.
7. Ghanem S N. Effect of laser radiation in the initiation and growth of sunflower (Helianthus annus L.) calli , dihydrofolate reductase enzyme activity and their contents nucleic acids, proteins and folate. 2017; M.Sc Thesis, College of Science, University of Mosul, Iraq.
8. Shi J and Mageur LM. Lycopene in tomatoes; chemical and physical properties affected by food processing. Crit. Rev. Food Sci. Nutr. 2000 ; 40: 1-42.
9. Zuorro A. Enhanced lycopene extraction from tomato peels by optimized mixed-polarity solvent mixtures. Molecules, 2020; 25(9): p.2038.
10. Al- Taee N E J, Mohammed A A H and Ahmed J M Y. Production of transgenic tomato (Lycopersicon esculetum) plants by Agrobacterium tumefaciens C58C1 mediated transformation. Internat. J. Sci. Technol., 2015; 10(2):105-110.
11. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962; 15: 473-497.
12. Osman MG, Elhadi EA and Khalafalla MM .Callus formation and organogenesis of tomato (Lycopersicon esculentum Mill, C.V. Omdurman) induced by thidiazuron. African J. Biotech. 2010, 9(28):4407-4413.
13. Fish WW, Perkins-Veazie P and Collins JK.. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. J. Food Comp. Anal., 2002; 15, 309 -/317.
14. Metwally SA. Physiological and anatomical studies on the effect of gamma and laser irradiation and some bio regulators treatments on the growth, flowering and keeping quality of gerbera. 2010; Ph.D. Thesis, Faculty of Agriculture, Zagazig University.
15. Ri P and Jang Y.Study on laser pre-sowing treatment of rice seeds by free-falling method, IPA. 2019; 6 (4) : 515-521.
16. Klimek-Kopyra A , Dłużniewska J , Ślizowska A and Dobrowolski J W. Impact of coherent laser irradiation on germination and mycoflora of soybean

seeds—innovative and prospective seed quality management. Agriculture, 2020 ; 10(8): 314.

17. Samuilov FD and Garifullina RL. Effect of laser irradiation on micro-viscosity of aqueous medium in imbibing maize seeds as studied with a spin probe method. Russ. J. Plant Physiol. 2007 ; 54(1): 128-131.
18. Kamiya H and Ozawa S. Dual mechanism for presynaptic modulation by axonal metabotropic glutamate receptor at the mouse mossy fibre-CA3 synapse. J. Physiol., 1999 ; 15 (2): 497–506.
19. Shilpa AK and Kaur R . Establishment and regeneration of callus cultures in tomato (Solanum lycopersicum L.) from various explants. Ann. Res. Rev. Biol., 2017; 12(2): 1-6.
20. Ghanem S N and Abood SA. Diode laser radiation effects in initiation and growth of sun flower plant (Helianthus annuus L.) callus. Rafidain J. Sci., 2019; 28(1): 24-34.
21. Yaseen E T and Abood SA. Role of diode laser radiation pretreatments in growth of Pisum sativum L. plant seedlings and callus under salinity stress. Rafidain J.Sci. , 2020 ; 29(1):50-67.
22. Janayon RVB and Guerrero RA. Laser irradiation of mung bean (Vigna radiata L.) at two wavelengths for enhanced seedling development. Internat. J. Optics. 2019 ; 3:1-7.
23. Pizarro L and Stange C . Light-dependent regulation of carotenoid biosynthesis in plants. Cien. Inv. Agr., 2009 ; 36:143-162.
24. Bao-xing X, Jing-jing W, Yi-ting Z , Shi-wei S, Wei S, Guang-wen S, Yan-wei H and Hou-cheng L. Supplemental blue and red light promote lycopene synthesis in tomato fruits. J. Integrative Agricult.,2019; 18(3):590–598.
25. Leivar P and Monte EP. Systems integrators in plant development. Plant Cell., 2014 ; 26(1):56-78.