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Enhancement synthesis of nucleic acid, protein and chlorophyll in callus of mustard *Brassica juncea* L treatment with Laser

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Abstract
This study deal with the effect of diode laser on embryonic and hypocotyls callus of the brown mustard *Brassica juncea* L. Embryo callus produced on the agar solidified medium (Murashige and Skoog,1962) MS + 0.3 mg /l^{-1} α-Naphthylacetic acid (NAA)+ 1.5 mg /l^{-1}6-Benzylaminopurine (BAP). Where hypocotyls callus was produced on (Murashige and Skoog, 1962) MS + 0.2 mg /l^{-1} α-Naphthylacetic acid (NAA) + 0.5 mg /l^{-1} 6-Benzylaminopurine (BAP). Laser diode at a wavelength of 650 nm and a power of 50 milliwatts / cm2 for durations of 10, 20, 30, 40 seconds was used to expose at a distance of seven cm from laser source.
Data indicates that laser treatment enhancement the nucleic acid DNA and RNA concentrations. Moreover protein and chlorophyll content was increased in both types of callus.

Keywords: *Brassica juncea* L**,** callus cultures, diode laser, DNA and RNA extraction

1. Introduction
The vegetable type in the current study is (Indian mustard) mustard brown Brassica juncea L, belongs to the Brassicaceae (mustard) family. It represents one of the most widespread groups in the plant kingdom and enjoys outstanding importance due to its medicinal properties [1]. Exposing plants to laser light has been shown to significantly enhance growth and increase nutritional values [2]. Mustard species showed the increase in biomass, followed by cauliflower and turnip species, compared to untreated He−Ne laser sprouts [3]. When plants are exposed to physical stress factors such as laser irradiation, plants tend to accumulate low molecular weight metabolites, such as amino acids, as a kind of adaptive response to changes [4]. The observed induction in plant growth after treatment with physical factors such as laser light may be partly due to the increased chlorophyll content and thus the increase in net photosynthetic activity after laser light application [5]. The current study proved that the laser contributes to raising the levels of nucleic acids, proteins, chlorophyll and, in general, fresh weights.
The aim of this article is to detect the role of diode laser on some parameters in callus of mustard pre-exposed to this factor.

2. Materials and Methods
2.1. Surface Sterilization of Seeds
Approximately 100 mustard seeds were dipped in 200 ml of water,then washed with distilled water 3-5 min (two to three times) to remove impurities and contaminants. Then it was immersed in 50 ml of 70 % ethyl alcohol with stirring for three min and washed thoroughly finally with sterile water they soaked in of sodium hypochlorite NaOcl solution that diluted by 1: 1 (v:v) for 10-15 min. With stirring and finally, seed was washed three times with Sterilized water surface sterilized seeds were placed on a sterile filter paper to get rid of water stuck in [6].
2-2 Production of Sterile seedings surface sterilized seeds were grown on the surface of 30 ml of agar - solidified MS medium in 50 ml glass vessels- sample were kept in culture room complete dark. After germination they transferred to conditions of 22±2 c^{o} 16/ 8 h dark regime [7].

2.3. Separation of embryos and hypocotyl explants
A sample of seeds were soaked in water for 18-24 hours. And then as mentioned priviosly sterilized by the seeds were placed on a sterile filter paper in a Petri dish. Seed coats were removed by sterile scalpel. Cotyledons were removed from around the embryos. Then embryos were excised with the removal of the upper position embryo, which represents the plumule and the lower position embryo, which represents the radicle to prevent its growth to intact plants [8]. Hypocotyl explants were cut from sterilized seedlings at age of 15 days [9].

2.4. Stimulation of callus from embryos
Embryos lacking the plumule and the radicle were plased of 15-55 days, reaching a weight from one gram to 3 grams or more. After the on the agar solidified medium MS + 0.3 mg /l-1 NAA+ 1.5 mg /l-1 BAP. In a 9.0 cm dia. plastic peteri-dish.Each dish contained 5.0 explants. Dishes were covered with lids and sealed by nescofilm strips speciemenl were incubated in culture room in the same conditios mentioned a bove [10].

2.5. Stimulation of callus from hypocotyls
Seedlings obtained from sterilized seeds of 18 days old were used. The hypocotyl were separated by sterile scalpels, and cut into 1-1.5 cm pieces. Each five explants were placed in a 100ml glass bottle containing 50ml of agar- solidified medium MS + 0.2 mg /l-1 NAA+ 0.5 mg /l-1 BAP. Speciemeus were incubated in the culture conditions [9].

2.6. Exposure of callus to diode - laser
Many samples of embryo genic and hypocotyls callus (20 days old) were of one gram each were exposed separately to Diode laser(UK- SCIENTIFIC.LATD) of a wavelength 650 nm and a power of 50 mw/cm2 for each of 10, 20 30 and 40 seconds, at a distance 7 cm from the laser source, After exposing all samples, those exposed and unexposed samples (control)were cultived on the surface of agar- solidified medium MS provided the suitable concentration of NAA and BAP as mentioned in materials and methods.All specimens were incubated in cultue room conditions (22±2 Cº, 16 hrs. Light/ 8hrs. Dark, 2000 lux illumination) [11].

2.7. Determination of the fresh weights callus
Fresh weights of the induced callus embryos and hypocotyls explants of all treatments were estimated based on the weights of the differences in the of callus when treated and its weight after growth period of 15 and 30 days [12].

2.8. Determination of DNA and RNA extracted from callus
Extraction of DNA from each of embryo and hypocotyls callus. The ready-made Genomic DNA mini kit (supplied by Geneaid, Taiwan Company) was used for measurements DNA concentrations and purity. Also the prepared kit from (GEORGIA OMEGA - BIO TEK, INC Company) was used to measure RNA concentrations and purity. According to the method of work attached with the kit. After the extraction of DNA and RNA, their concentrations and purity were estimated by a Nano drop device at a wavelength of 260 nm. [13].

2.9. Estimation of proteins content in callus
The method [14]. Was adopted to estimate the amount of protein extracted from callus samples for all treatments at the age of 15 and 30 days of treatment. The amount of Protein arnountwas determined by adopting the standard curve prepared from gradual concentrations of 0.01- 0.05 mg/ml of bovine serum albumin (BSA).

2.10. Determination of Total Chlorophyll
Chlorophyll was extracted from treated and no treated calli and its quantity was estimated [15]. in all samples of embryos and hypocotyls calli and for all experiments as well as control samples at the age of 15 and 30 days of growth, The following equation was used to calculate the concentrations of total chlorophyll:

[mg total chlorophyll/ g. = 20.2(D 645) + 8.02(D663) x V/ L 1000 x W]

as

D: the optical density of the extract

V: final of acetone diluted at a concentration of 80%

W: used the fresh weight of the plant [16].

3. Results and Discussion
3.1. Production of embryogenic and hypocotyls callus.
The results indicated that both types of callus was produced readily on their induction media. Callus was friable in texture and yallow in color. Subculture was carried out every 30 days.

3.2. Influence of laser on growth of calli
The results indicated laser exposure to that callus showed a shrinkage in the fresh weights of culls in all treatments compared to the control sample, exposure for 20 seconds yield the maximum fresh for both types of callus. Average fresh weight of embryogenic callus at the age of 30 days was 5.38 grams and the average of the callus of the hypocotyls 4.86 grams, Table 1. Data showed that fresh weight of the two types of exposed callus enhanced the generation of vegetative shoot. Figure. 1, 2 results showed that there are some phenotypic differences between the masses of the control samples of Callus that were not exposed to laser radiation and other exposed samples.

Table 1: Increasing of embryos genic and hypocotyls callus of fresh weights of *Brassica juncea* L exposed to diode laser. Callus weights (gm).

	Embryos genic callus			Hypocotyls callus		
Days after exposure	**1 Day**	**15 Day**	**30 Day**	**1 Day**	**15 Day**	**30 Day**
control	1	1.62 b	2.94 bc	1	1.62 b	2.94 bc
10 sec	1	1.66 b	3.48 abc	1	1.66 b	3.48 abc
20 sec	1	3.02 a	4.68 a	1	3.02 a	4.68 a
30 sec	1	1.98 b	3.98 ab	1	1.98 b	3.98 ab
40 sec	1	1.36 b	2.52c	1	1.30 b	2.52 c

Control: un exposed, Each value represents average of five replicates

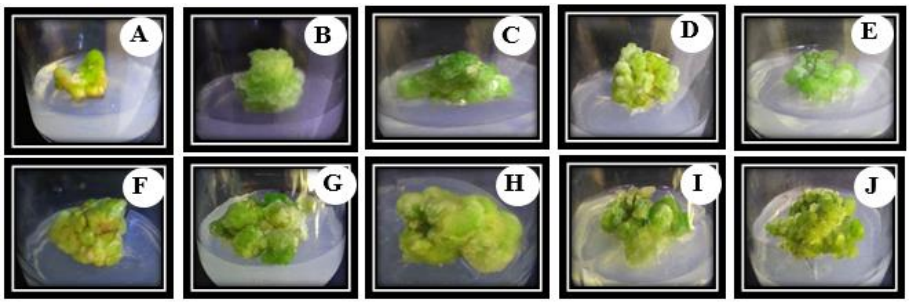

Fig 1: Influence exposur of embryogenic callus of *brassica juncea* L. to diode laser grown on MS + 0.3 mg /l^{-1} NAA+ 1.5 mg /l^{-1} BAP on biomass of callus after 30 days.

A: Non xeposed, after 15 days
B: Exposed for 10 sec, after 15 days
C: Exposed for 20 sec, after 15 days
D: Exposed for 30 sec,after 15 days
E: Exposed for 40 sec, after 15 days
F: Non xeposed, after 30 days
G: Exposed for 10 sec, after 30 days
H: Exposed for 20 sec, after 30 days
I: Exposed for 30 sec,after 30 days
J: Exposed for 40 sec, after 30 days

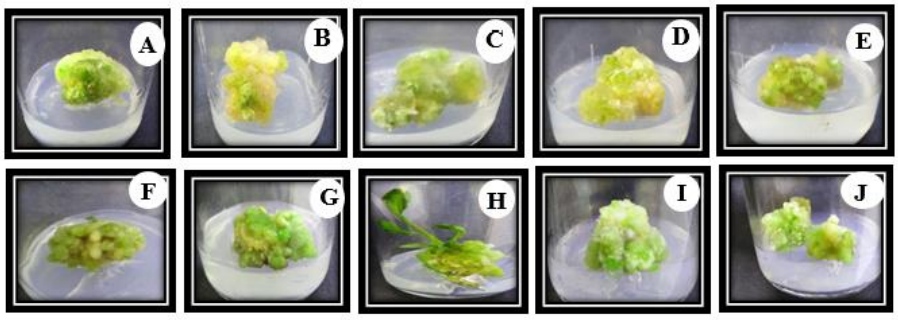

Fig 2: Influence exposur of hypocotyls callus of *brassica juncea* L. to diode laser grown on MS + 0.3 mg /l^{-1} NAA+ 1.5 mg /l^{-1} BAP on biomass of callus after 30 days.

A: Non xeposed, after 15 days
B: Exposed for 10 sec, after 15 days
C: Exposed for 20 sec, after 15 days
D: Exposed for 30 sec, after 15 days
E: Exposed for 40 sec, after 15 days
F: Non xeposed, after 30 days
G: Exposed for 10 sec, after 30 days
H: Exposed for 20 sec, after 30 days
I: Exposed for 30 sec,after 30 days
J: Exposed for 40 sec, after 30 days

3.4. Increasing nucleic acids DNA and RNA content in the Callus

The results Table 2, 3 exhibiting the positive effect of laser treatments in increasing the callus content of DNA and RNA, compared to the control. For both types of callus embryos and Hypocotyls, it was clear that 20 seconds achieved the highest increase in the amount of DNA, RNA after 30-day of exposure, where as embryos genec callus reported highn a concentration of DNA and RNA in Hypocotyls callus, concentration of DNA and RNA proved the accompanying increase in fresh weight of callus.

Table 2: Extracted from embryogenic calli of *brassica juncea* L. prior-exposed to diode laser

Treatment	DNA		RNA	
	Conc. ng\50 mg	purity	Conc. ng\ 50 mg	purity
control	100	1.1	300	2.1
10 sec	210	1.4	2250	1.9
20 sec	720	1.7	9000	2.1
30 sec	300	1.2	3100	1.8
40 sec	390	1.3	3875	1.9

Each value represents the mean of three replicates

Table 3: Extracted from Hypocotyls calli of brassica juncea L. prior-exposed to diode laser

Treatment	DNA		RNA	
	Conc. ng\50 mg	purity	Conc. ng\ 50 mg	purity
control	420	1.8	750	1.4
10 sec	750	1.4	800	1.7
20 sec	1650	1.4	4625	2.3
30 sec	780	1.5	2500	2.1
40 sec	990	1.6	3150	2.0

Each value represents the mean of three replicates

3.5. Protein Estimation

Data Figure 3, shows that the total amount of protein extracted from the embryos and Hypocotyls callus was increased in close proportions when exposed to laser irradiation. The patterns of increase in the callus content of the plant parts of the protein were similar to the patterns of increase in the average fresh weights of the callus samples. The highest average protein content was 4.68 mg/g fresh weight of embryo genic callus and 5.38 mg/g fresh weight of Hypocotyls callus exposed of time 20 seconds to laser compared to other samples and standard parameters. Figure. 3-C.

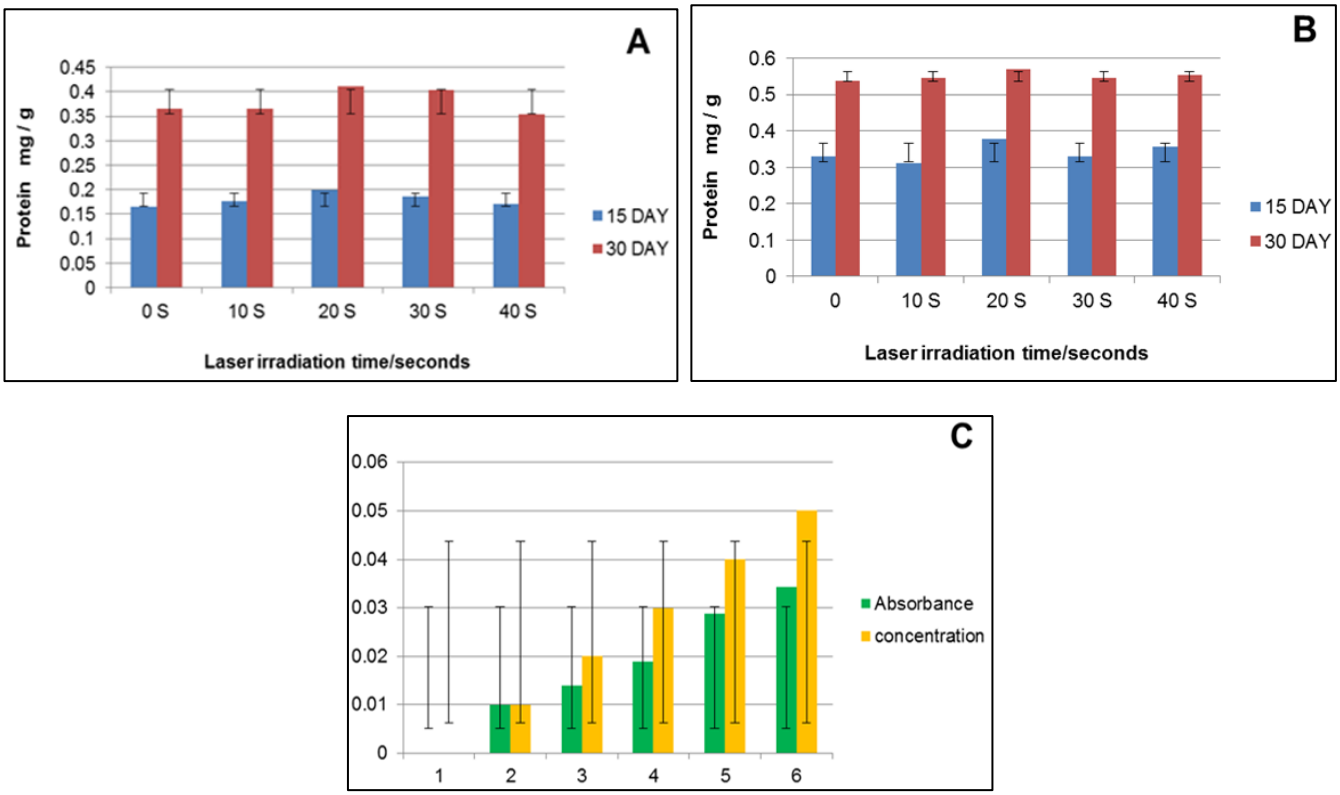

Fig 3: Protein Content in calli of Brassica juncea L prior exposed to laser. Embryos callus (A). Hypocotyls callus. (B). Concentration gradient for (BSA) Bovine Serum Albumin. (C). each value represents the mean of three replicates

3.6. Total amount of chlorophyll for callus

Data Figure 4, shows the superiority of all treatments except for the 40-second treatment in fetal calluses Figure.4-A. The increase in chlorophyll pigment was delayed at the age of 15 days, increased to the superiority after 30 days exposure for 20 seconds recorded the highest concentration of chlorophyll after 15 and 30 days of exposure the laser. As for the calluses of the hypocotyls, all treatments were also superior in them in close concentrations compared to the comparison sample. Figure.4-B.

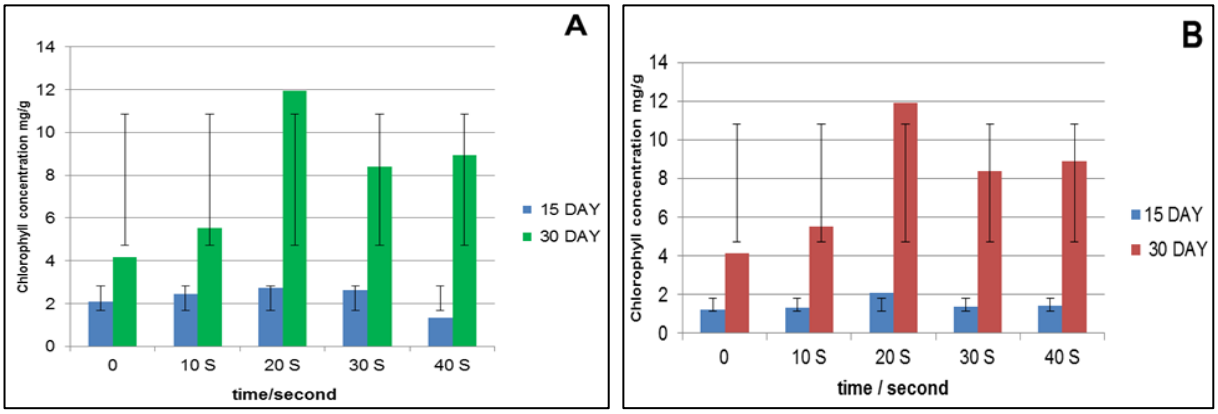

Fig 4: Contents of total chlorophyll Extracted from laser exposed callus of Brassica juncea L Embryos callus. (A) Hypocotyls callus. (B) Each value represents the mean of three replicates

3.7. Discussion

In this study the positive role of diode laser exposing embryos and hypocotyls calli of Brassica juncea L for different periods of time stimulated t. growth and proliferation in terms of increased fresh weight, nucleic acids and protein moreover increased amount of chlorophyll pigment. This is may due to the laser effect that activate the division process in the plant cell, which in turn leads to shortening the growth and development of t.s in a record time, and gives t.s better resistance to conditions that are not suitable for growth

compared to that are not exposed to laser [17]. The activity of some enzymes such as, Poly peroxidase has been observed. Phenol oxidase and Catalyase are oxidation enzymes with increasing radiation doses to a certain extent. The activity of such enzymes is associated with an increase in hydrogen peroxide [18, 19]. The increase in low doses was explained by the destruction of radiation the enzyme inhibitors in the cell and the enzyme gain energy that makes it more active and sustained the formation of free radicals is a material for the reaction such as hydrogen peroxidase. The use of radiation leads to the formation of free radicals from lipids containing peroxidase, which an active reaction material for such enzymes. In a study in which it was proven that the red diode laser rays caused an increase in the cell contents of nucleic acids, proteins and folate extracted from the of the sunflower callus [20]. It was noted that the protein content in the callus of Silybum marinum L induced from leaves and cotyledons exposed to the green laser ND-YAG achieved the highest levels. For this callus, the results of exposure to the red laser light He-Ne of the same plant were also shown to its role in reducing the time of callus development from all plant parts. [21]. Other study indicated that laser irradiation significantly enhanced the resistance of wheat plant to salt stress based on the comparison samples [22]. The growth stimulus obtained from exposure to laser beams may due to the presence of phytochrome pigment, which has proved its presence in all plants, but it is present in high concentrations in young and non-specialized plant t.s, including meristems and even root cells. For each case to be converted inversely to the other by absorbing light P660, P730 [23]. The laser rays may affect the phytochrome and transfer it from the hibernation phase to the control phase on various physiological activities, including the production of some substances and enzymes that help accelerate growth and make maximum use of the components of the nutrient medium, thus achieving better growth in a shorter time [24].

4. Conclusions and Recommendations

1. The readiness of the brown mustard plant Brassica juncea L to respond to the growing medium.
2. The laser increases the levels of nucleic acids, proteins and chlorophyll.
3. Benefiting from the effects of shock in encouraging the differentiation of the callus, which shows difficulties in its differentiation.
4. Propagation of brown mustard by laboratory rapid breeding method.
5. Expanding the study of the effects of physical factors to cause histological or physiological changes or both.

5. References

1. YH Chu, CL Chang, HF Hsu. Flavonoid content of several vegetables and their antioxidant activity, J Sci of Food and Agri. 2000; 80(5):561-566.
2. M Hasan, MM Hanafiah, TZ Aeyad, IHH AlHilfy, MNM Said. Laser irradiation effects at different wavelengths on phenology and yield components of pretreated maize seed, Appl. Sci. 2020; 10(3):1189.
3. YP Chen, M Yue, XLWang. Influence of He-Ne laser irradiation on seeds thermodynamic parameters and seedlings growth of Isatis Indogotica, Plant Sci. 2005; 168(3):601-606.
4. LV Dudareva, VN Shmakov, AM Sobenin, EG Rudikovskaya, RK Salyaev. Low-Intensity Laser Radiation Changes Amino Acid Composition of Wheat Callus Tissues, Dokl. Biochem. Biophys. 2012; 446(1):260.
5. R Perveen, Y Jamil, M Ashraf, Q Ali, M Iqbal, MR Ahmad. He-Ne laser-induced improvement in biochemical physiological, growth and yield characteristics in sunflower (Helianthus Annuus L.), Photochem. Photobiol. 2011; 87(6):1453-1463.
6. SA Al-Badrani, AR AL-Baker. Initiation callus cultures from the seedlings of Silybum Marianum and Estimate their protein content, Rafi J of Sci. 2021; 30(1):21-37.
7. ET Yaseen, SA Abood. ROLE of diode laser radiation pretreatment in growth of pisum sativum L, plant seedling and callus under salinity stress. Rafe. J Sci. 2020; 29(1):50-67.
8. ST Akinyosoye, JA Adetumbi, OD Amusa, MO Olowolafe, JO Olasoji. Effect of seed size on in vitro seed germination, seedling growth, embryogenic callus induction and plantlet regeneration from embryo of maize (Zea mays L.) seed, Nigerian J Genet. 2014; 28(2):1-7.
9. AK Thakur, BK Singh, V Verma, JS Chauhan. Direct organogenesis in Brassica juncea var. NRCDR-2 and analysis of Genetic Uniformity Using RAPD markers, national academy science letters. 2013; 36(4):403-409.
10. GQ Zhang, WJ Zhou, HH Gu, WJ Song, EJJ Momoh. Plant regeneration from the hybridization of Brassica juncea and B. napus through embryo culture, J Agron. and Crop Sci. 2003; 189(5):347-350.
11. ET Sultan. Assessment of diode laser radiation and folic acid pretreatments on germination, seedlings and callus growth of pisum sativum L. plant under salinity. Ph.D thesis,College of Science, University of Mosul. Iraq, 2020.
12. AA Mohammed, RT Yahya. Regeneration of White Lupin, Lupinus albus, Plants form Hypocotyl Stems. Rafi. J of Sci. 2018; 27(4):95-102.
13. SR Gallagher, PR Desjardins. Quantitation of DNA and RNA with absorption and fluorescence spectroscopy, Current protocols in molecular biology. 2006; 76(1):A-3D.
14. OH Lowry, NJ Rosebrough, AL Farr, RJ Randall. Protein measurement with the folin-regents. J Biol. Chem. 1951; 193:265-275.
15. HK Lichtenthaler. Chlorophylls and carotenoids: Pigments of photosynthetic bio-membranes, Method Enzymol. 1987; 148:350-382.
16. Al-Mallah MK, Al-Nuama QS. Thermal shock in increasing the content of protein and chlorophyll in callus carnation stalks Dianthus caryophyllus L, J of Educ. and Science. 2007; 20(1):563-569.
17. Ri P, Choe S, Jang Y. Study on laser pre-sowing treatment of rice seeds by free-falling transport method. Information Processing in Agriculture. 2019; 6(4):515-521.
18. Tazawa S. Effects of various radiant sources on plant growth" (Part 1). Japan Agricultural Research Quarterly. 1999; 33:163-176.
19. Dinoev S. Laser-a controlled assistant in agriculture, Bulgarian academy of sciences, Problems of Engraining Cybernetics and Robotics. 2006; 56:83-91.
20. SN Ghanem, SA Abood. Diode Laser Radiation Effects in Initiation and Growth of Sun Flower Plant (Helianthus annuus L.) Callus, Rafi J of sci. 2019; 28(1):24-34.

21. AR Meshaal, SHM Saleh. Support for green laser Nd-YAG and red laser He-Ne Composition and differentiation callus Wild plant Kalangum Silybum marianum L, J of Educ. and sci. 2019; 28(1):132-146.
22. L Ferdosizadeh, SA Sadat-Noori, A Zare, S Syghafi. Assessment of diode laser pretreatments on germination and yield of wheat (Triticum aestivum L.) under salinity stress, World J. Agric. Res. 2013; 1(1):5-9.
23. B Tomas, D Vince-Prue. Photoperiodism in plants 2nd.ed. San Diego Academic press, 1997.
24. Liu F, Chen H, Han R. The Effects of He-Ne Laser and enhanced Ultraviolet-B radiation on proliferating-cell nuclear antigen in wheat seedlings, Ameri J of Plant Sci. 2015; 6(8):1206-1214.