Journal of Ecology of Health & Environment
An International Journal

http://dx.doi.org/10.12785/jehe/010101

Double-Pulse Laser Light Treatment Stimulate Germination and Changes the Oxidative Stress and Antioxidant Activities of Wheat (*Triticum aestivum*)

Abdelghafar M. Abu-Elsaoud *.

Department of Botany, Faculty of Science, Suez Canal University, Ismailia-41522, Egypt.

Received: 7 Jun. 2012, Revised: 21 Oct. 2012, Accepted: 23 Oct. 2012.
Published online: 1 May 2013.

Abstract: Photostimulation using photobiological methods can be considered as a valuable tool for enhancing the agricultural technologies especially in resolving global food problems. Current study aims to investigate the impact of double-pulse He-Ne laser on germination, growth, oxidative stress and antioxidant enzyme of wheat (*Triticum aestivum*). Wheat grains were irradiated with different doses ranging from 2 to 3600 seconds of He-Ne laser as a double-pulse; once before seed sowing and equal doses after week of seed germination and irradiated to the seedling. The activities of superoxide dismutase (SOD) and hydrogen peroxide (H_2O_2), and the content of malondialdehyde (MDA) were determined. Results showed remarkably increased germination percentage, germination rate, shoot and root lengths. The results showed that double-pulse He-Ne laser treatment of Egyptian common wheat cultivar could enhance the activities of Cu/Zn-superoxide dismutase (SOD) at low irradiation doses, and lead to a significant decline in response to higher irradiation doses. A significant decline in both hydrogen peroxide and lipid peroxidation (MDA-content) levels were also concluded. It can be concluded that the germination and growth wheat grains were enhanced after seed pretreatment with double shots and oxidative stress were decrease and antioxidant activities enhanced at some irradiation doses.

Keywords: double-pulse laser, He-Ne, wheat, germination, oxidative stress, antioxidant.

1 Introduction

The cumulative needs of agronomic crops from different areas worldwide for food production as well as different food industry requires the use of efficient tools improve the agricultural productivity and congregate the requirements of increasing human population (Campbell and Norman 1998). The Use of chemicals fertilizers and other chemical methods for improving plant crop production is thought to change the characteristics of soil, water and atmosphere leading to unfavorable conditions for growth and development of plants. The photostimulatory methods have been observed in different agronomical characters of major crop plants and are being used in single or combination of physical factors (Muthusamy et al. 2012). The effect of low-intensity laser irradiation on the chemical composition and structure of lipids in wheat tissue culture were extensively studied by Salyaev et al. (2007).

The effect of pulsed nitrogen laser radiation with wavelength of 337.1 nm on morphological and biochemical parameters in green-gram (*Vigna radiata* L.) seedlings. The seeds of green-gram were germinated and grown in petri dishes for seven days (Govil *et al.*, 1985). The shoot and root lengths, and fresh and dry weights of the seedlings were the maximum after radiation dose of 30 minutes, while protein exhibited highest content after 20 minutes, RNA and DNA contents with 5 min exposure time (Govil *et al.*, 1985).

The effect of laser radiations on accumulation of lipid peroxidation secondary products was studied in tissue culture of wheat (*Triticum aestivum* L.)(Salyaev *et al.*, 2003). Callus irradiation with helium-neon laser light of wavelength λ= 632.8 nm and intensity of 10 mW.cm^{-2} revealed an increase in the accumulation of thiobarbituric acid (TBA)-reactive products. The effect was less prominent within two days following the laser treatment, but even in this case the content of TBA-reactive products was greater than in the control. The data obtained confirm that the laser irradiation can induce lipid peroxidation processes in plant tissues (Salaev *et al.*, 2003).

*Corresponding author e-mail: abuelsaoud@science.suez.edu.eg; abdoacacia@gmail.com

Double-pulse laser irradiation treatment was not fully studied especially on oxidative stress and antioxidant defense mechanism. The aim of current study is to assess the effect of different doses of double-pulse He-Ne laser on the germination, growth, oxidative stress and antioxidant response of wheat (*Triticum aestivum* L.) cultivar sakha-161 from Egypt.

2 Materials and Methods

2.1 Plant Materials and double-pulse laser irradiation experiments

Table 1: Detailed experimental conditions used for seed pre-treatment of different wheat cultivars.

Experimental Condition	**Details**
Laser type	Helium neon laser (He-Ne)
Wavelength	632.8 nm
Power intensity	5.23 mW.cm^{-2}
Laser Exposition time (s)	Double-Pulses from 1-1800 s
Wave emission	Continuous (CW)
Irradiation times	Double-pulse
Beam size	1.5 mm
Distance from sample	20 mm
Laser polarization	Linear
Cultivars treated	*T. aestivum* L. cv.sakha-161

Seeds of *Triticum aestivum* L. cultivar Sakha-161 were obtained from the National Agricultural Research Centre, Giza, Egypt. Irradiation at wavelength 632.8 nm was obtained from the He-Ne laser (Model LG-79-1, Russian Federation) with a total maximum output power of 5.23 mW.cm^{-2} and beam diameter of 1.5 mm. Irradiation was performed at room temperature without thermostatic control. Detailed experimental conditions were presented in Table (1). Seeds of wheat cultivar sakha-161 were rinsed in tap water and then dipped into a sodium hypochlorite solution (1 % v/v) for sterilization. The arrangement of seeds followed the following requirements: seeds were placed in single monolayer. Each seed was irradiated once before sowing, and a second pulse after one week of seed sowing to maintain a double-pulse laser irradiation. It should be emphasized, that the exposure times and irradiation powers used in our experiments were insufficient to cause any thermal effects in seeds (McKenzie 1990).

The irradiation process was carried out with double-pulse helium neon laser as irradiation source. Each seed was irradiated once before sowing in hydrogel with the proposed irradiation doses: 1, 3, 10, 30, 60, 180, 600, 1200, and 1800 seconds (s), the second irradiation pulse were applied after 7-days of seed germination. The overall laser irradiation dose will be; 2, 6, 20, 60, 120, 360, 1200, 2400 and 3600s; respectively. Wheat seedlings were harvested fifteen days after sowing and various measurements of germination, morphological, and physiological parameters were performed. Seeds were sown on specially hydrogels, which are three-dimensional swollen networked structures. When placed in an aqueous medium, hydrogels swell and retain the volume of the adsorbed aqueous medium in a three-dimensional swollen network of hydrophilic homo-polymers or copolymers covalently or ionically cross-linked (Carbonell *et al.*, 2007; De Souza *et al.*, 2006; Flores *et al.*, 2007). Such aqueous gel networks are also known as aqua-gels. The original polymeric hydrogel network was developed by Wichterle and Lim in Czechoslovakia in 1954 (Gandhi, 1987). In our experiments, the capacity of hydrogels to absorb the aqueous media was exploited to supply wheat cultivars with continuous nourishment of water and salts required by the plant.

2.2 Germination and growth parameters

Standard germination data were recorded four times after 3, 5, 7, and 9 days following seed- sowing and various indices were calculated following Ciupak *et al.* (2007). Based on the results obtained, the percentage of germinated seeds (N_k) was calculated by the following formula:

$$N_k = \frac{n_k}{n_c} x100$$

Where: n_k = number of germinated seeds, n_c= total number of seeds sown; number of germinated seeds (n_k) was expressed as the absolute number of germinated seeds and is presented as such in all Figures and Tables.

Various growth parameters including shoot length, root length, and leaf area were measured, monitored and assessed using java based image processing software ImageJ version 1.47 (Maloof et al. 2013).

2.3 Hydrogen Peroxide (H_2O_2) level

To determine Hydrogen Peroxide (H_2O_2) concentration in leaves following irradiation with electromagnetic radiations, the FOX method was employed (Jiang *et al.*, 1990; Wolf, 1994). Based on the peroxide-mediated oxidation of Fe^{2+}, followed by the reaction of Fe^{3+} with xylenol orange (*o*-cresol-sulfonephthalein 3', 3''-bis [methylimino] diacetic acid, sodium salt; Farmitalia Carlo Erba, Milan, Italy), the FOX method accurately measures H_2O_2.

2.4 Lipid peroxidation (LP) level

Estimation of lipid peroxidation (LP) was carried out using spectrophotometric using the thiobarbituric acid-malondialdehyde (TBA-MDA) assay. The most prominent, and currently used, assay as an index for lipid peroxidation products is the thiobarbituric acid assay (TBA test). Based on the reactivity of a lipid peroxidation end product, malondialdehyde (MDA) acts as a biological marker when it interacts with TBA to produce a quantifiable red adduct (Williamson *et al.*, 2003). Lipid peroxides were extracted with 5 ml of 5% (w/v) metaphosphoric acid and 100 µL of 2% (w/v in ethanol) butyle hydroxytoluene. An aliquot of the supernatant was allowed to react with TBA at 95°C for 45 min. and then cooled to room temperature. The resulting TBA-MDA was extracted with 1-butanol and detected spectrophotometrically using a Shimadzu UV-160 scanning spectrophotometer.

2.5 Antioxidant enzymes activity

Superoxide dismutase (SOD) activity was determined by the photochemical method described by Winter *et al.* (1975). Cu/Zn-SOD was measured by the photochemical method as described by Giannopolitis and Ries (1977). Assays were carried out under illumination. One unit SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of p-Nitro Blue Tetrazolium Chloride (NBT) reaction at 560 nm.

2.6 Statistical analyses

Various statistical analyses were carried out including two-Way-Analysis-of-Variance (ANOVA) to assess variations among tested wheat cultivars and various He-Ne laser exposition doses. Significance was tested at p-value$\leq$0.05 level and is given asterisk (*) denoting the significance. Statistical analyses were performed using SPSS statistical software package (SPSS Inc., Version 15.00) and Microsoft Excel professional 2010 (a part of Microsoft Office Professional 2010 Package).

3 Results

The photostimulation and priming effect of double-pulse helium neon laser (He-Ne) on germination and growth of common wheat cultivar Sakha-161 was clearly revealed by the germination percent, number of germinated seeds (n_k) and shoot length, root length and number of roots. Both germination and growth constraints were measured and calculated after 72, 120 and 168 hours following seed sowing (Figures, 1-4). Shoot length (cm) was increasing with time from seed sowing in all studied laser radiation doses. Maximum shoot length was recorded at 120 and 360 seconds of laser irradiation dose (Figure, 1).

Root length (cm) clearly showed that laser light significantly enhanced the root length and hence the water and nutrient absorption especially laser irradiation doses of 2, 6, 60, 120, 360 s and higher doses. Biomass allocation in term of shoot/root ratio (S/R ratio, g.g^{-1}) was found to be changed significantly in response to laser irradiation.

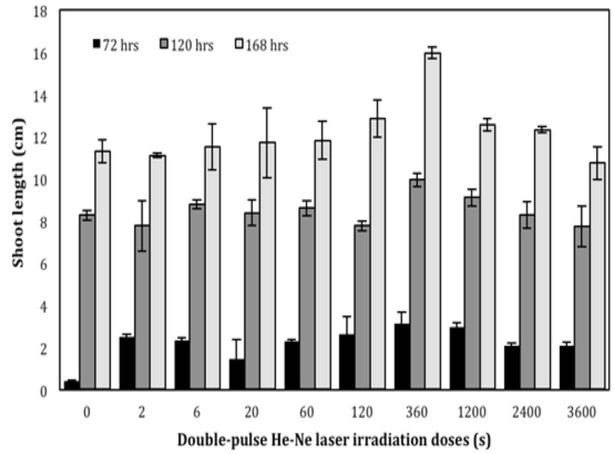

Figure 1. Shoot length (cm) in common wheat (*Triticum aestivum* L.) following irradiation with different does of laser light after 72, 120 and 168 hours of seed germination.

Biomass allocation based on shoot/root ratio revealed that nutrients were allocated toward the root system rather to the shoot causing enhanced root growth and morphology (Figures 1, 2).

Germinated were clearly enhanced after the first irradiation of different laser irradiation doses. Both germination percent and number of germinated seeds were increased with laser pre-sowing irradiation which were particularly visible on doses of 2, 6, 120 and 360 seconds of laser irradiation doses and after 120 and 168 h from seed sowing (Figures 3, 4).

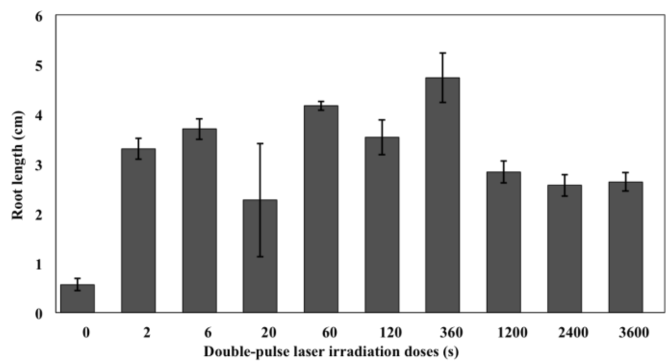

Figure 2. Root length (cm) in common wheat *Triticum aestivum* cv. Sakha-161 after irradiation twice with different doses of laser light.

The detailed measurements of oxidative damage showed that both hydrogen peroxide and lipid peroxidation were increased slightly in response to different doses of laser light (Table 2-3; Figure 5, 6A,B). While, superoxide dismutase decreased significantly with the increasing irradiation doses of He-Ne laser. The average hydrogen peroxide level in untreated control was 1.5 µmole.g^{-1}.FW, which dropped to 0.026 after 2 shots of 1 s laser irradiation dose.

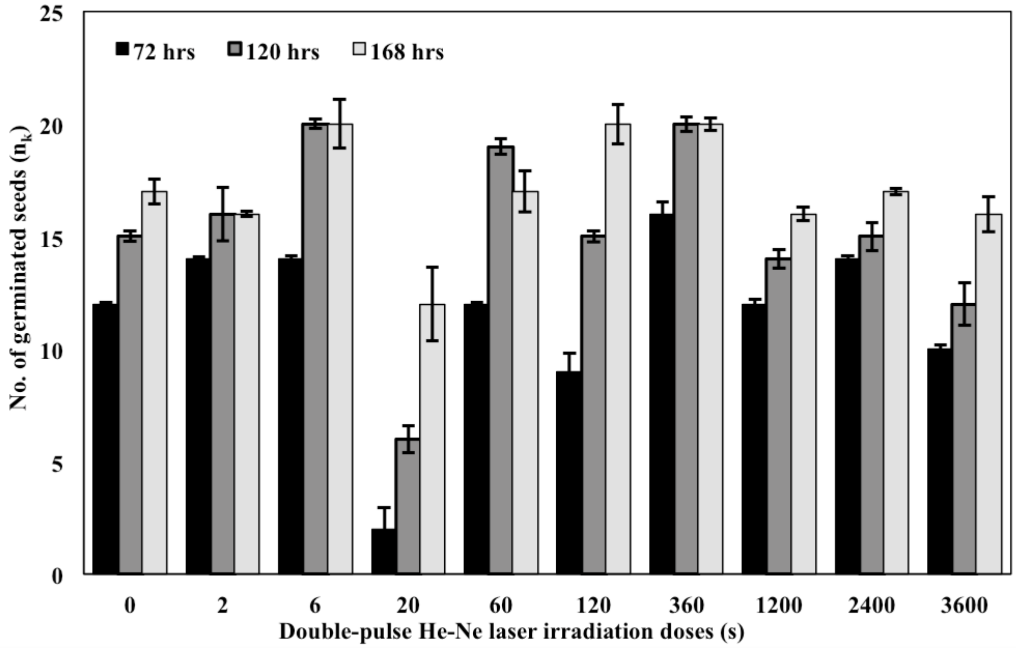

Figure 3. Total Number of germinated seeds in common wheat *T. aestivum* cv. Sakha-161 after irradiation twice with different doses of laser light.

Table 2: Analysis of variance and post-hoc Dunnett-t test statistic showing the effect of various exposure doses of double-pulse He-Ne laser (i.e. irradiated twice) on the Cu/Zn superoxide dismutase activities (Unit/g-tissue), hydrogen peroxide level (µmole.g^{-1}-FW), and lipid peroxidation level (nmole.MDA.g^{-1}.FW) of *T. aestivum* cv. Sakha-161.

He-Ne laser exposure doses (s)	SOD activities (Unit/g-tissue)			H_2O_2 (µmole.g-1 FW)			Lipid peroxidation (nmole.MDA.g^{-1}.FW)		
	MD	SE	Dunnett	MD	SE	Dunnett	MD	SE	Dunnett
2	-163.3	0.023	0.000***	-1.26	0.00	0.000***	-0.113	0.005	0.000***
6	-143.4	2.058	0.001***	0.21	0.00	0.002**	-0.287	0.005	0.000***
20	-168.1	2.740	0.002**	0.09	0.00	0.000***	-0.180	0.005	0.000***
60	-0.5	0.033	0.002**	0.21	0.00	0.002**	0.060	0.005	0.000***
120	10.99*	0.023	0.001***	0.09	0.00	0.008**	0.020	0.005	0.002**
240	5.75*	0.025	0.001***	0.11	0.00	0.006**	-0.163	0.005	0.000***
1200	2.69*	0.029	0.000***	0.01	0.00	0.32	0.360	0.005	0.000***
2400	1.12*	0.031	0.000***	0.11	0.00	0.000***	0.130	0.005	0.000***
3600	12.83*	0.023	0.000***	0.04	0.00	0.036*	0.033	0.005	0.000***
One-Way Analysis of variance (ANOVA)									
F-ratio	5325.4			28391			3037.5		
p-value	0.000***			0.000***			0.000***		

MD: mean different between treatment and untreated control.

Table 3: Analysis of variance and post-hoc dunnett-t test statistic showing the effect of various exposure doses of He-Ne laser (irradiated twice) on the Cu/Zn superoxide dismutase activities (Unit/g-tissue), hydrogen peroxide level (μmole.g-1 FW), and lipid peroxidation level (nmole.MDA.g-1.FW).

Laser irradiation doses (seconds)		Superoxide dismutase, SOD	Lipid peroxidation	H_2O_2
		(Unit/g-tissue)	nmole.MDA.g^{-1} FW	μmole.g^{-1} FW
0	Untreated control	14.97	0.811	1.500
2	2 x1s	178.30	0.696	0.026
6	2 x 3s	158.35	0.522	0.150
20	2 x 10s	183.04	0.631	0.033
60	2 x 30s	15.46	0.869	0.145
120	2 x 60s	3.98	0.830	0.134
360	2 x 180s	9.22	0.647	0.227
1200	2 x 600s	12.28	1.170	0.130
2400	2 x 1200s	13.85	0.939	0.196
3600	2 x 1800s	2.14	0.843	0.165
One-Way Analysis of variance (ANOVA)				
F-ratio		5325.4	3037.5	28391
p-value		0.000***	0.000***	0.000***

The application of double-pulse low intensity He-Ne laser light with power intensity 5.23 mW.cm^{-2} significantly decreased the cellular accumulation of H_2O_2 (Tables 2,3, Figure 4A, 5A). The hydrogen peroxide level in wheat leaves of plants pre-treated with He-Ne laser decreased with the influence of He-Ne laser. However, statistical assessment for the studied common wheat cultivars (*T. aestivum* L. cv. Sakha-161) by multivariate analysis based on analysis of variance test statistic (ANOVA) revealed that there were a significant decrease in the level of H_2O_2 after seed pretreatment with He-Ne laser (F=28391, *p*=0.000***). Dunnett’s test as a multiple-comparison-procedure was performed to compare each of the laser radiation treatments to the control (Table, 2). Many-to-one dunnett’s comparison revealed that all doses were significantly different from the control (not irradiated with He-Ne laser). Laser irradiation dose of 600 s showed no difference in hydrogen peroxide level with the control (Table 2).

Lipid peroxidation (LP) refers to the cellular oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism, whereby increases in levels of LP become an indirect indicator of membrane and cellular damage. Irradiation of common wheat twice to different doses of He-Ne laser caused significant decrease in the lipid peroxidation level (MDA level: nmole.MDA.g^{-1}.FW). Laser irradiation dose of 60, 120, 360 s showed no difference in hydrogen peroxide level with the control (Table 2, Figure 4B, 5B). Statistical evaluation for wheat cultivars (*T. aestivum* L. cv. Sakha-161) by multivariate analysis based on analysis of variance test statistic (ANOVA) revealed that there was a significant variation in the level of lipid peroxidation after seed pretreatment with different doses of laser light (F=3037.5, *p*=0.000***). The mean difference between mean lipid peroxidation of an irradiated wheat and control were presented in table (2). Superoxide dismutase (SOD) enzyme catalyses the dismutation of superoxide into oxygen and hydrogen peroxide.

As such, it is an important antioxidant defense in nearly all cells exposed to oxygen. The role of superoxide dismutase is to act as a free radical scavenger preventing oxidative damage (Klebanov *et al.*, 1998; Zong-Bol *et al.*, 2008). In our study, the superoxide dismutase activity detected were clearly and significantly decreased after pre-sowing irradiation with laser light (Figure 5C, Tables 2,3,4). There were a significant different in Cu/Zn superoxide dismutase activity after seed pre-sowing irradiation with laser light (F=5325.4, *p*=0.000***). Dunnett’s multiple-comparison-test was applied to evaluate the difference in Cu/Zn-SOD between each laser radiation dose and the control (Table, 2). Dunnett’s test presented in table (2) revealed that all doses were significantly different from the control (not irradiated with He-Ne laser). Laser irradiation doses of 60, 360, 1200 and 2400 s showed no difference in hydrogen peroxide level with the control (Tables 2,3; Figures 5C, 6C). The superoxide dismutase level presented in table (3) showed

Table 4: Multivariate analysis based on ANOVA test showing the effect of irradiation doses of He-Ne laser on oxidative stress (lipid peroxidation and hydrogen peroxide levels) and antioxidant enzyme (superoxide dismutase)

Multivariate Test Statistic						
Effect		Value	Error df	Hypothesis df	F-ratio	Significance
Intercept	Pillai's Trace	1.0	10	11	1009564[b]	0.000***
Laser irradiation	Pillai's Trace	9.0	162	99	427.70	0.000***
Tests of Between-Subjects Effects						
Source of variation		SS	df	MS	F-ratio	Significance
Corrected Model	SOD	168850.4[d]	9	18761.2	5325.4	0.000***
	H_2O_2	5.110[h]	9	0.6	28391.2	0.000***
	LP (MDA)	0.911[l]	9	0.1	3037.5	0.000***
Intercept	SOD	104991.3	1	104991.3	29801.7	0.000***
	H_2O_2	2.3	1	2.3	116204.2	0.000***
	LP (MDA)	19.0	1	19.0	570254.4	0.000***
Laser irradiation doses (s)	SOD	168850.5	9	18761.2	5325.4	0.000***
	H_2O_2	5.1	9	0.6	28391.2	0.000***
	LP (MDA)	0.9	9	0.1	3037.5	0.000***
Error	SOD	70.5	20	3.5		
	H_2O_2	0.0	20	0.0		
	LP (MDA)	0.0	20	0.0		
Total	SOD	273912.2	30			
	H_2O_2	7.4	30			
	LP (MDA)	19.9	30			
Corrected Total	SOD	168920.9	29			
	H_2O_2	5.1	29			
	LP (MDA)	0.9	29			

significant high increase in the doses 2, 6, 20 s of laser irradiation. However, reached a level that is not different from the control after 60 s of laser irradiation, and then decreased to a significant level of 2.14 unit/g-FW of Cu/Zn superoxide dismutase (Table 2,3).

The results showed that double-pulse He-Ne laser treatment of Egyptian common wheat cultivar could enhance the activities of Cu/Zn-superoxide dismutase (SOD) at low irradiation doses, and lead to a significant decline in response to higher irradiation doses. A significant decline in both hydrogen peroxide and lipid peroxidation (MDA-content) levels were also concluded. It can be concluded that the germination and growth wheat grains were enhanced after seed pretreatment with double shots and oxidative stress were decrease and antioxidant activities enhanced at some irradiation doses.

4 Discussion

Results of the present study indicated that He-Ne laser generates increases in various germination and growth parameters of the studied wheat cultivars from Egypt, in addition to several biochemical and physiological parameters. Results were consistent with those published by Kutomkina *et al.* (1991), Govil *et al.* (1991), St.Dinoev *et al.* (2004), Chen *et al.* (2004), Abu-Elsaoud (2009), Muthusamy et al. (2012), Abu-Elsaoud and Tuleukhanov, 2013) and many others. The impact of laser light is considered as a photobiological phenomenon (Kutomkina *et al.*, 1991). The results from our experiments demonstrated clearly that not only the parameters of germination seeds were increased greatly, but also the growth and metabolism and development of wheat seedlings were significantly accelerated in response to laser irradiation. The role of laser irradiation is classified as a long-term effect.

The present understanding and available literature in the field of plant laser irradiation allowed postulating on potential mechanisms underlying this effect (Chen *et al.*, 2005). Helium neon laser radiation has a wavelength of 632.8 nm that is close to phytochrome red (P_r) absorbing wavelength, facilitating the activation of phytochrome most likely. In turn, enzyme activities modulated by phytochromes may be consequently augmented and result in accelerating phytochrome-mediated responses accelerating (e.g. the decomposition rate of lower entropy macromolecules). Accordingly, the entropy and internal energy of seeds were enhanced during germination process (Chen *et al.*, 2005).

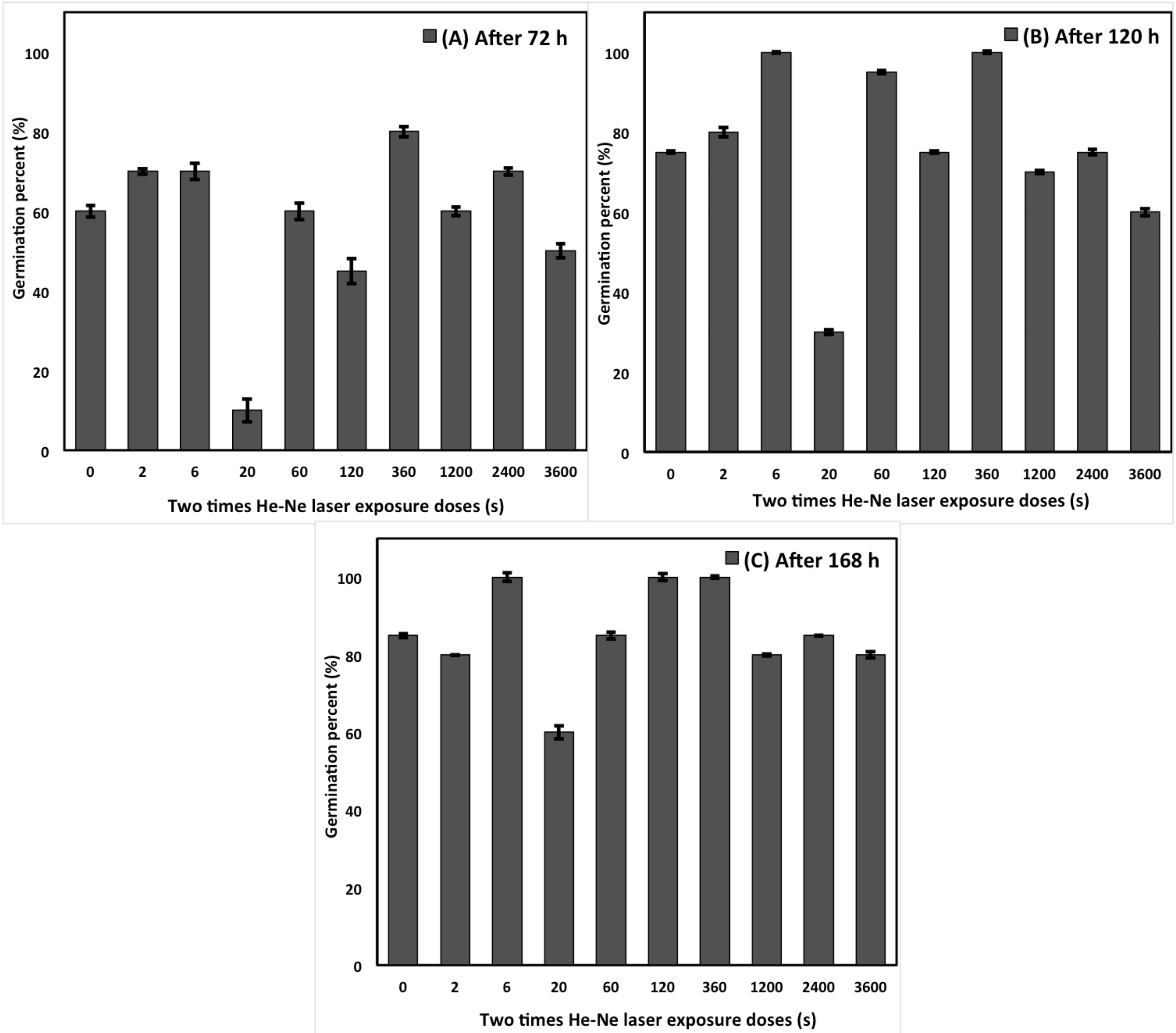

Figure 4: (A-C). Germination percent (%) in common wheat (*Triticum aestivum* L.) following irradiation with different does of laser light after (A) 72, (B) 120 and (C) 168 hours of seed germination.

The seed's intrinsic high order kinetic equilibrium was fragmented by laser light irradiation generating a disordered status. The seeds irradiated with laser light have to absorb further energy from the surroundings to acquire equilibrium and to retain the laser-driven disturbance of kinetic equilibrium in germinating seeds and enhanced internal energy of seeds (Chen *et al.*, 2005). The living organism must interchange energy with contiguous system so as to keep the high order energy status to compensate for broken order being broken (Yan and Zhan, 1997). Consequently, the biochemical and physiological metabolic processes of plants pre-treated with laser were accelerated, resulting in enhanced growth and notably augmented leaf area and biomass (Chen *et al.*, 2005). The laser, as a specific light, can be absorbed effectively by macromolecules to cause a variety of photochemical effects (Xiang, 1994); laser irradiation can boost the activities of superoxide dismutase, Ascorbic peroxidase and Catalase (Qi *et al.*, 2000; Li *et al.*, 1996; Cai *et al.*, 1994).

Two different parameters to monitor oxidative stress were assessed; lipid peroxidation (LP) and hydrogen peroxide (H_2O_2) levels. Irradiation with He-Ne laser caused a significant decrease in H_2O_2 content in the studied common wheat cultivar from Egypt. Lipid peroxidation levels were also decreased at low laser irradiation dose and increased at high doses. He-Ne laser irradiation induced a significant decrease in malondialdehyde (MDA) content, hydrogen peroxide, and increased the superoxide radical ($O_2^{-\cdot}$) production rate (Zong-Bol *et al.*, 2008). While, Mileva *et al.* (2000) found that He-Ne laser treatment led to a slightly decreased level of LP products, they concluded that the effect of low-intensity laser-irradiation may depend on the dose applied, individual tissues targeted and other factors.

The primary response of plant tissue to radiation is an increase in the content of lipid peroxidation products of peroxide oxidation.

Data demonstrated that laser light stimulates morphogenetic processes in plant tissues at later stages, as well, and believing that this stimulation may be conditioned metabolic changes caused by the change of content of a number of compounds formed as a result of the primary photoreactions (Dudareva *et al.*, 2007; Salyaev *et al.*, 2007). Such compounds might also include products of peroxide oxidation, with an increase in their amounts as response to the impact of laser radiation. This increase, in turn, affects membrane properties and changes its functional state, a measurable result of which would be an increase in the Ca^{2+} concentration inside the cells (Dudareva *et al.*, 2007).

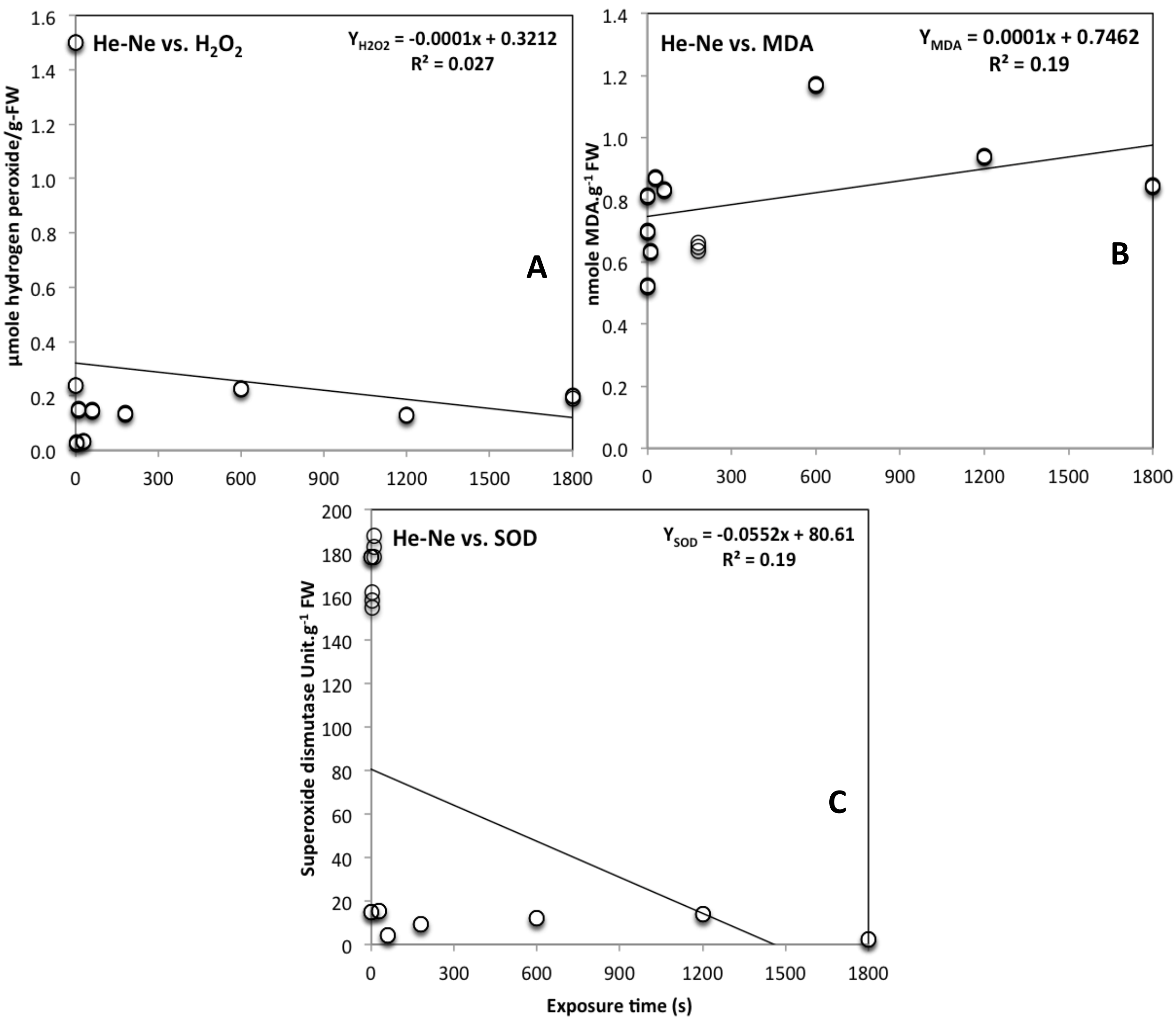

Figure 5: (A-C). Simple linear regression trendline showing the interrelationship between irradiation doses of double-pulse He-Ne laser and (A) Cellular hydrogen peroxide, (B) Lipid peroxidation and (C) superoxide dismutase; respectively.

The sequence of events following laser radiation exposure may occur as follows: 1) photon adsorption by endogenous photosensitizers and further peroxidation of lipids (photoperoxidation) followed by (2) calcium ion introduction into the cell. Activation of intracellular processes and/or secondary product accumulation may act as a signal not only for the start-up of relevant protection mechanisms, but, probably, for some secondary responses, perhaps even at the transcription level. This probability is indirectly confirmed by the stimulating effect of laser light on morphogenetic processes in wheat and wild crops tissue cultivar (Dudareva *et al.*, 2007).

Based on data from the stimulation of morphogenetic processes in wheat tissue culture by low-intensity laser radiation (Salyaev *et al.*, 2007), it was suggested that the changes observed should be accompanied by molecular shifts and structural reconstruction in those tissues subjected to radiation. Chirkova (2002) indicated that such reconstructions should take place primarily in cell and organellar membranes. These reconstructions produce a profound impact on all forms of functional activity in the membrane, particularly with lipids to a considerable extent. In lipids, fatty acids are the primary subject influenced by both genetic and environmental conditions. Stress may cause shifts in the proportion between various groups of fatty acids, and the degree of their non-saturation may change. The length of fatty acids chains, positional situation of double bonds, or the number of polar groups may also change (Dudareva *et al.*, 2007). Our result agreed with that of Zong-Bol *et al.* (2008) who stated that He-Ne laser irradiation significantly decreased malondialdehyde (MDA) content and superoxide radical ($O_2^{\cdot-}$) production rate in wheat seedlings exposed to moderate drought stress.

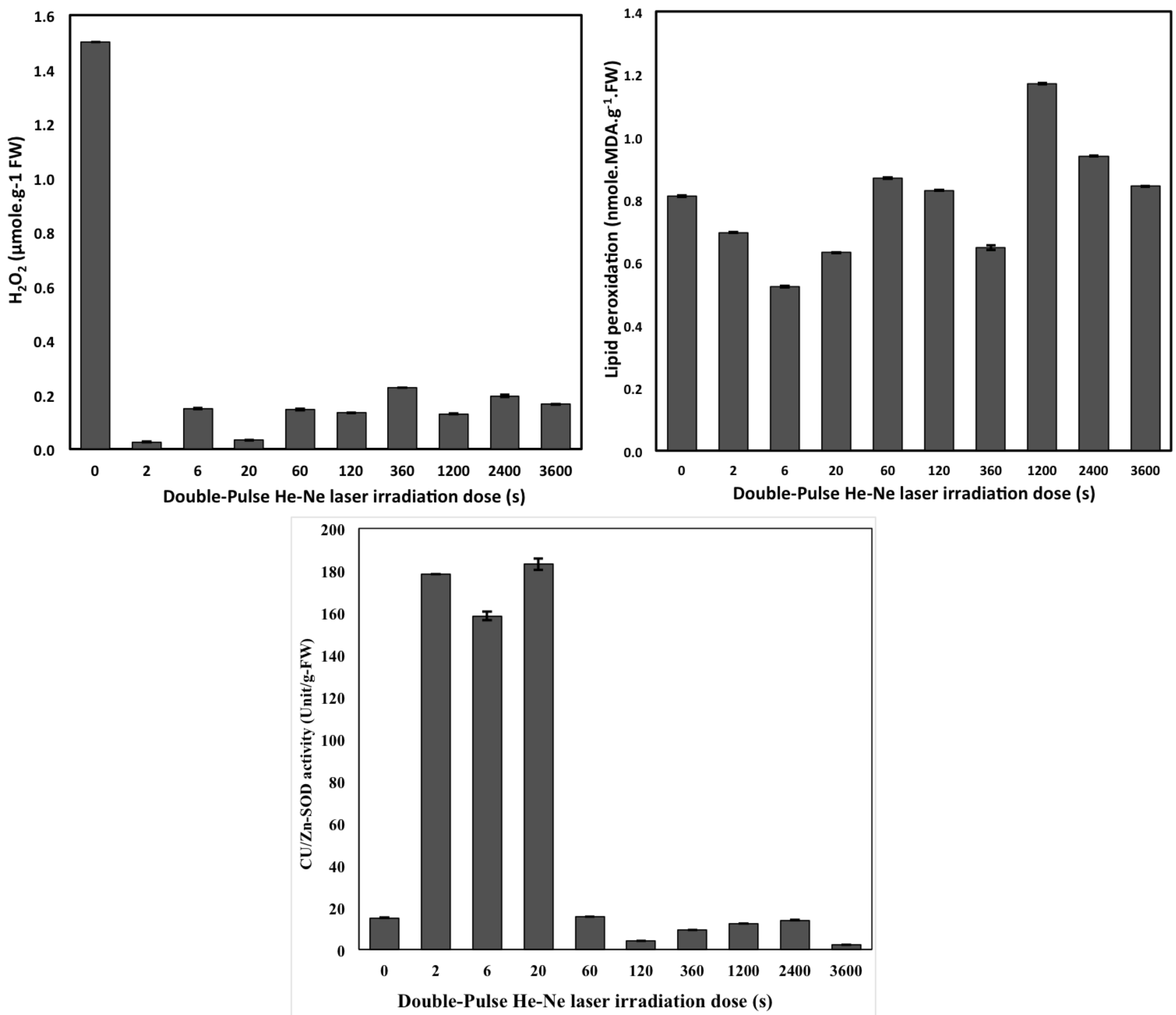

Figure 6: (A-C). Effect of different irradiation doses of double-Pulse helium-neon laser on (A) Cellular hydrogen peroxide level, (B) Membrane Lipid peroxidation and (C) Cu/Zn Superoxide dismutase activities (Unit/g-FW) in common wheat (*Triticum aestivum* L.). Bars on histograms represent the standard error for mean.

Conversely, our results disagreed with Mileva *et al.* (2004) who stated that the low-Intensity laser irradiation did not affect the oxidative stress in experimental cataract.

In conclusion the results showed that double-pulse He-Ne laser irradiation enhanced the germination and growth of wheat cultivar Sakha-161. Moreover, several doses of double-pulse laser stimulated the activities of Cu/Zn-superoxide dismutase (SOD) especially low laser doses. A significant decline in both hydrogen peroxide and lipid peroxidation (MDA-content) levels were also concluded.

References

[1] Abu-Elsaoud, A.M. 2009. Comparative physiological and morphological studies on common wheat under the action of electromagnetic radiations with wavelengths from 400.0-800.0 nm. Ph.D. thesis, Al-Faraby Kazakh National University189pp.

[2] Abu-Elsaoud, A.M., and S.T. Tuleukhanov. 2013. "Can He-Ne Laser Induce Changes in Oxidative Stress and Antioxidant Activities of Wheat Cultivars from Kazakhstan and Egypt?" Science International 1 (3): 39–50. doi:10.5567/sciintl.2013.39.50.

[3] Cai, S.W., Zhao, X.S., Lu, F.T., Ma, X.L. 1994. The influence of He–Ne laser irradiation on the active oxygen metabolism of corn seedlings, Chin.J.Laser. - Vol. 21. - P. 767 - 769.

[4] Campbell, Gaylon S., and John M. Norman. 1998. An Introduction to Environmental Biophysics. New York, NY: Springer New York. http://link.springer.com/10.1007/978-1-4612-1626-1.

[5] Carbonell, M.E., Martinez, R., Flores, M. 2007. Influencia de campos magneticos estacionarios de 125mT y 250 mT en la germinacion de semillas de girasol, EIDENAR-Revista Ingenieria de Recursos Naturales I Ambiente-UNIVALLE, 2005. In Aladjadjiyan, A. The use of physical methods for plant growing stimulation in Bulgaria / Journal of Central European Agriculture. - Vol. 8, N 3. – P. 369 - 380.

[6] Chen Yi-Ping, Yuea, M., Xun-Ling, W. 2004. Influence of He–Ne laser irradiation on seeds thermodynamic parame ters and seedlings growth of Isatis indogotica, Plant Sci. Vol. 168. - P. 601 - 606.

[7] Chen, Yi-Ping, Ming Yue, Xun-Ling Wang, 2005. Influence of He-Ne laser irradiation on seeds thermodynamic parameters and seedlings growth of Isatis indogotica, Plant Science. Vol. 168. - P. 601 - 606.

[8] Chirkova V. (2002). Physiological basis of plants resistance. - Saint-Petersburg: Pub House Saint-Petersburg Univ., Russian Federation, P. 240.

[9] Ciupak, A., Szczurowska, I., Gładyszewska, B., Pietruszewski, S. 2007. Impact of laser light and magnetic field stimulation on the process of buckwheat seed germination, Techn. Sc. Vol. 10. P.1-10.

[10] De Souza, A., Garcia, D., Sueiro, L., Gilart, F., Porras, E., Licea, L. 2006. Presowing magnetic treatments of tomato seeds increase the growth and yield of plants, Bioelectromagnetics. Vol. 27. - P. 247-257.

[11] Dudareva, L.V., Lankevich, S.V., Sumtsova, V.M., Rudikovcka, E.G., Salyaev, R.K. 2007. Possible paths of the impact of low-intensity laser radiation on membrane structures in plant cells, Annual wheat newsletters. Vol. 53. P. 70 - 72.

[12] Flores, M., Carbonell, M.V., Martinez, E. 2007. Exposure of maize seeds to stationary magnetic fields: Effects of germination and early growth, Environmental and Experimental Botany. Vol. 59, N 1. P. 68-75.

[13] Gandhi, O.P. 1987. Physics in Medicine and Biology, IEEE Engineering in Medicine and Biology 9th conference. - Boston, Vol. 6. - P. 1.

[14] Giannopolitis, N., Ries, S.K. 1977. Superoxide Dismutase. 1. Occurrence in higher plants, Plant Physiol. Vol. 59. - P. 309 - 314.

[15] Govil S.R.; Agrawal D.C.; Rai K.P.; Thakur S.N. 1985. Growth responses of Vigna radiata seeds to laser irradiation in the UV-A region. Physiologia Plantarum: Vol. 63 Issue 1 pp.-133.

[16] Govil, S.R., Agrawal, D.C., Rail, K.P., Thakur, S.N. 1991. Physiological Responses of Vigna radiata L. to nitrogen and argon laser irradiation, Indian J. Plant Physiol. Vol. 1, N 1. P. 72-76.

[17] Jiang, Z.Y., Woollard, A.C.S., Wolff, S.P. 1990. Hydrogen peroxide production during experimental protein glycation, FEBS Lett. Vol. 268. P. 69-71.

[18] Klebanov, G., Kapitanov, A.B., Tessikin, Y.A. 1998. The antioxidant properties of lycopene, Biol. Membr. (Mosc.). Vol. 2. P. 22–28.

[19] Kutomkina, E.V., Karu, T.L., Mushegian, M.S, Duda, V.I. 1991. He-Ne laser induced germination of Endospores of Anaerobactor polyendosporus, Laser in the life Sciences. Vol. 4, N 3. P. 147-151.

[20] Li, Y.W., Feng, W.X. (1996). The effects of He–Ne laser treatment on seeds germination and growth of atractylodesma crocephala, Chin. J. Appl. Laser. Vol. 16. P. 37-41.

[21] Maloof, Julin N., Kazunari Nozue, Maxwell R. Mumbach, and Christine M. Palmer. 2013. "LeafJ: An ImageJ Plugin for Semi-Automated Leaf Shape Measurement." Journal of Visualized Experiments, no. 71 (January). doi:10.3791/50028.

[22] McKenzie, A. L. 1990. "Physics of Thermal Processes in Laser-Tissue Interaction." Physics in Medicine and Biology 35 (9): 1175–1209.

[23] Mileva, M., Bakalova, R., Zlateva, G. (2004). The low-Intensity laser irradiation does not affect the oxidative stress in experimental cataract, Medical Laser Application. Vol. 9, N 3. P. 150 - 154.

[24] Mileva, M., Zlateva, G., Karabasheva, S., Hadjimitova, V., Antonov, I. 2000. Effect of He-Ne laser treatment on the level of lipid peroxidation products in experimental cataract of rabbit eyes, Methods and findings in experimental and clinical pharmacology. 22 (9) 679-681.

[25] Muthusamy A., Kudwa P.P., Prabhu V., Mahato K.K., Babu V.S., Rao M.R., Gopinath P.M., Satyamoorthy K. 2012. Influence of Helium-Neon laser irradiation on seed germination in vitro and physic-biochemical character in seedling of Brinjal (Solanum melongena L.) var. Mattu Gulla. Photochem Photobiol. 2012 Sep-Oct; 88(5):1227-35. doi: 10.1111/j.1751-1097.2012.01162.

[26] Qi, Z., Cai, S.W., Wang, X.L. 2000. The effect of He–Ne laser irradiation on soluble protein synthesis, Chin. J. Northwest Univ. Vol. 30. P. 45 - 48.

[27] Salyaev R.; Dudareva, L.; Lankevich S.; Makarenko S.; Sumtsova V.; Rudikovskaya E. 2007. Effect of low-intensity laser irradiation on the chemical composition and structure of lipids in wheat tissue culture. Doklady Biological Sciences, MAIK Nauka Interperiodica, 412 (1) 87-88.

[28] Salyaev, R. K., L. V. Dudareva, S. V. Lankevich, E. G. Ekimova, and V. M. Sumtsova. 2003. “Effect of Low-Intensity Laser Radiation on the Lipid Peroxidation in Wheat Callus Culture.” Russian Journal of Plant Physiology 50 (4): 498–500. doi:10.1023/A:1024720707041.

[29] Salyaev, R. K., L. V. Dudareva, S. V. Lankevich, S. P. Makarenko, V. M. Sumtsova, and E. G. Rudikovskaya. 2007. “Effect of Low-Intensity Laser Irradiation on the Chemical Composition and Structure of Lipids in Wheat Tissue Culture.” Doklady Biological Sciences 412 (1): 87–88. doi:10.1134/S0012496607010280.

[30] St. Dinoev, Antonov, M., Stoyanov, T., Georgieva, C. 2004. Spectral Impact of Low-Power Laser Radiation on Wheat and Maize Parameters, Bulgarian Academy of Sciences, Problems of Engineering Cybernetics and Robotics. - Sofia, 54: 74 - 85.

[31] Williamson, K.S., Hansley, K., Floyd, R.A. 2003. Fluorometric and colorimetric assessment of thiobarbituric acid reactive lipid aldehydes in biological matrices, In: methods in biological oxidative stress. http://www.springerlink.com/content/wu014l581n211785

[32] Winter, B., Christine, C., Rosemary, E., Brian, M., Carrell, R. 1975. The Estimation of red cell Superoxide Dismutase activity, J. Lab. Clin. Med., 75: 337-341.

[33] Wolff, S.P. 1994. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides, Methods Enzymol. 233: 182-189.

[34] Xiang, Y. 1994. Laser biological effects, Chin. Laser Biol. 1994. 3: 243–245.

[35] Yan, L.F., Zhan, Y.L. 1997. Microbiology / China Agriculture University Press. Beijing, China, P. 21 - 26.

[36] Zong-Bo1, Qiu, Liu Xiao, Li Fang-Min, Xiang-Jun, T., Ming, Y. 2008. Influence of He-Ne laser irradiation on protective enzyme activities and lipid peroxidation in wheat seedlings by drought stress damage, Journal of Plant Ecology. 32 (5) 1002-1006.