Physiologo-morphological features of common wheat under the influence of helium-neon laser

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Abstract. The research work is devoted to researching the comparative influence of electromagnetic radiations with wavelengths on various physiological and morphological parameters of four common wheat Triticum aestivum L. cultivars from Kazakhstan and Egypt. The comprehensive studies concerning various Functional mechanisms of living organisms under the action of electromagnetic radiations is a problem of special priority in biophysics and photobiology, revealing the general and specific plant resistance features to a given radiation source. These are of special importance when electromagnetic irradiations are carried out using various wavelengths, expositions and power intensities. The reactivity responses of living organisms to the action of various physical radiations usually take a part both metabolically and anatomo-morphologically, that is directed mainly to maintaining homeostasis. The purpose was based to reveal the morphological and physiological indicators of seeds and plants of four common wheat Triticum aestivum L. in a comparative aspect before and after the influence of electromagnetic radiations with wavelengths of 400.0 - 800.0 nm. The specific seed reactions feature of Triticum aestivum L have been established after the influence of electromagnetic radiations applying expositions and wavelengths.

1 Introduction

The work is devoted to researching the comparative influence of electromagnetic radiations with wavelengths from 400.0 to 800.0 nm, irradiation expositions from 1 to 1800 seconds and power intensities from 0.5 to 10 m $W.cm^{-2}$ on various physiological-morphological parameters of four common wheat Triticum aestivum L. cultivars from Kazakhstan and Egypt.

The influences of physical factors on living organisms cause the development of general reactions which would be reflected onto the physiological, morphological and biometrical indicators. This effect is very important in determining the degree of reactivity and stability to various physical factors [1]. The reactivity responses of living organisms to the action of

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various physical radiations usually take a part both metabolically and anatomo-morphologically, that is directed mainly to maintaining homeostasis [2]. However, still very little studies concerning the morphological, anatomical, physiological, biochemical, biophysical and biometrical features of living organisms especially plants in response to the action of electromagnetic radiations taking in consideration plants cultivar, radiation wavelengths, radiation expositions and radiation power intensity.

Also, of great interest is the action of laser radiations on morphological and physiological features of living organisms including low –intensity laser radiation in the visible area of spectrum. The thermal and shock effects of laser during the irradiation of biological objects is to be neglected. In the majority of research works helium – neon laser radiations with wavelength in red area of electromagnetic spectrum was used [3-6].

Effect of laser radiations on plants' germination and seedling growth. In an experiments of Sudha [7] they have investigated the effect of pulsed nitrogen laser radiation (337.1 nm) on morphological characteristics and biochemical contents in seedlings from treated greengram (*Vigna radiate* L.) seeds which were germinated and grown in Petri dishes for a week. The shoot and root lengths and fresh and dry weights of the seedlings were maximum with the 30 min exposure, while protein was maximum with 20 min, RNA and DNA contents with 5 min exposure time. Chlorophyll content was not affected by the irradiation.

In Laboratory experiments on the germination of tetraploid red clover seeds (var.Bona) carried out completely and randomly in four replications, the number of seeds germinating normally and abnormally, as well as the number of hard seeds and seeds infected with fungal disease was also determined in the experiment [8] . Laser treatment significantly decreased the share of hard seeds and did not influence the percentage of seeds germinating normally. Seed dressings significantly decreased seed infection with disease when compared to the control and objects with laser treatment. Clover seeds were most abundantly infected by fungi of the *Alternaria* type (*Alternaria alternate*). Strains of the *Phoma* and *Penicillium* type were eliminated by laser beam with power of 3mW cm^{-2}x 1 and 3 mW cm^{-2}x 3, and *Penicillium* by a dose of 6 mW cm^{-2} x 1; 6 mW cm^{-2} x 3. Laser treatment should not be applied in the case of massive seed infection with fungi of the *Alternaria* type since a significant increase was noted after laser irradiation with power of 3 mW cm^{-2} x 3; 3 mW cm^{-2} x 5 and 6 mW cm^{-2} x 5 [8] .

In laboratory studies on the germination of Alfalfa seed (American variety 'Legend') completely and randomly in four replications in 2002 carried out by [9]. The factors studied were as follows: 1) irradiation with divergent He-Ne laser light with a surface power density in the irradiation plane of 0,3 and 6 mW. cm^{-2} applied 1, 3 and 5-times; 2) seed dressings: Funaben T, Sarfun T 65 DS in a controlled environment. The number of seeds germinating normally and abnormally as well as hard seeds and seeds infected with fungal disease was determined. Seed treatment with He-Ne laser light did not significantly influence the share of those seeds germinating normally. However, it did decrease the percentage of those seeds germinating abnormally, the hard seeds and also those seeds infected with disease but only when double the power was used. Laser light with surface power density and multiplication of R 6x3 and R 6x5 destroyed fungi from the *Penicillium* kind completely, whereas in a dose of R 3x3, R 3x5 and R 6x1 it significantly stimulated the growth of fungi from the *Alternaria* type. All seed dressings destroyed fungi from the *Penicillium* and also *Alternaria* types completely (except for Super-Homai 70DS dressing) [9].

The influence of He-Ne laser irradiation on seeds thermodynamic parameters and seedlings growth of *Isatis indogotica* were investigated by [10]. In his study, the seed embryos of *Isatis indogotica* were exposed to He-Ne laser irradiation (5.23 mW mm^{-2} , radiated for 5 min) to determine whether or not He-Ne laser caused changes to thermodynamic parameters of seeds and had a long-term physiochemical effects. A microwave (180 mW mm^{-2}) was also employed in place of laser to pretreated seeds for 8s as a parallel experiment with the laser pretreatment in order to identify the temperature effects of laser. The thermodynamic parameters were calculated according to thermograms of seed germination tested with a calorimeter at 25˚C for 40 h. The changes in physiological characters (net photosynthetic rate, stomatal conductance, water utilization efficiency and concentration of chlorophyll), biochemical characters (soluble saccharides, soluble protein, pyruvic acid, and the activities of three kinds of enzymes) and the growth parameters of seedlings (biomass and leaf area) were measured when the seedlings, which seeds were pre-treated with HE-Ne laser, developed to the stage of 24-25 days. It was found that the thermodynamic parameters in ΔH, $(\Delta S)c$, $(\Delta S)e/\Delta t$ of seeds pre-treated with He-Ne laser and microwave were similar during 40 h germination at 25˚C, while they changed significantly compared with that of the control.

Similary, the physiological characters of the seedlings, e.g. stomatal conductance, water utilization efficiency, net photosynthetic rate and chlorophyll concentration, and the biochemical characters of the seedlings, eg. the concentration of soluble saccharides,soluble protein and the activities of α-amylase,GTP AND GOT were increased significantly by the laser pre-treated [10]. Meanwhile, the biomass and leaf area of the seedlings were significantly improved. These changes suggested that the pretreatment with He-Ne laser had not only a short-term biological effect, which enhanced inner energy of seeds, but also a long-term effect, which contributed to the acceleration of the grouth and development of seedlings. The present study has benefits in the elucidation of mechanisms underlying the effect of laser irradiation on organisms, as well as in the application of laser in agriculture and horticulture [10].

2 Materials and Methods

*Seed presowing irradiation experiments.*Seeds from the four selected cultivars from Kazakhstan and Egypt were rinsed in tap water and then dipped into a sodium hypochlorite solution (1 %v/v) to sterilize. Seed arrangement was carried out according to the following requirements: First, seeds were laid out in a monolayer. Each seed was irradiated once prior to sowing. It should be emphasized, here, that the exposure times and irradiation powers used in our experiments were insufficient to cause any thermal effects in seeds [11-12]. The control samples, labeled as 01, 02, 03 and 04 (for cultivars Akcay, Kazakhstanskaya-10, Eretrospermum-350 and Sakha-168, respectively) were each treated in exactly the same manner, except for the variation in irradiation procedure.

The irradiation process was carried out for each of the four selected wheat cultivars (Akcay, Kazakhstanskaya-10, Eretrospermum-350 and Sakha-168) with selected irradiation sources (He-Ne, IR, MCRPL and IWL). Each seed was irradiated once before sowing in hydrogel with the proposed irradiations doses: 1,3,10, 30,60, 180, 600, 1200 and 1800 seconds (s). Wheat seedlings were harvested fifteen days after sowing and measurements of germination, morphological and physiological parameters were performed. Hydrogels are three-dimensional swollen networked structures [13-15]. 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 homopolymers or copolymers covalently or ionically crosslinked. Such aqueous gel networks are also known as aquagels [14]. The original polymeric hydrogel network was developed by Wichterle and Lim in Czechoslovakia in 1954 [15-17]. In our experiments, the capacity of hydrogels to adsorb the aqueous media was exploited to supply wheat cultivars with continuous nourishment of water and salts required by the plant.

Petri-dishes were numbered with Latin numerals and triple code: the first number of the triple code represents the irradiation dose (10 doses: 0 to 9): the second number represents the source of radiation (4 radiation sources: 1 to 4); and, the third number represents the cultivar (4 cultivars: 1 to 4). Then, seeds were arranged in Petri-dishes at the same time.

Germination parameters. Standard germination data were collected and involved the counting out of thirty individual seeds onto hydrogel. Germination parameters were measured four times after sowing: at post-sowing day 3,5,7 and 9. The following germination parameters were calculated: Germination percent (%).

Based on the results obtained, the percentage of germinated seeds (N_K) was calculated by the following formula [6]:

$$N_K = \frac{n_k}{n_c} \square 100\ \% \tag{1}$$

where : n_k= number of germinated seeds, n_c = total number of seeds sown

Number of geminated seeds (n_k)

Number of geminated seeds (n_k) was expressed as the absolute number of germinated seeds and is presented as such in all Figures and Tables.

Germination rate S_k *(seed.* h^{-1}*)*

The germination rate was calculated by the following equation:

$$S_{k=} \frac{n_{max}}{\Delta t} \tag{2}$$

where: $n_{max=}$ maximum number of germinated seeds recorded during one count, Δt =time interval between two successive counts [13].

3 Results and Discussion

Germination percent (%). Data of germination and germination percent after three days of cultivation on hydrogel (germination %) are outlined in Table 1.

Table 1. Effect of He-Ne laser seed irradiation on germination percent (%) of four different wheat cultivars from Kazakhstan.

He-Ne laser exposition time (s)	Cultivars				Variance
	Akcay	Kaz-10	Eret-350	Sakha-168	
Control	60.0	36.7	43.3	46.7	8.7
1	66.7	60.0	40.0	43.3	14.9
3	50.0	60.0	46.7	13.3	36.9
10	40.0	16.7	36.7	16.7	14.3
30	46.7	33.3	26.7	10.0	20.9
60	36.7	43.3	70.0	16.7	43.7
180	10.0	16.7	23.3	6.7	4.9
600	60.0	30.0	23.3	20.0	30.0
1200	33.3	30.0	6.7	16.7	13.7
1800	60.0	10.0	36.7	46.7	40.3
Variance	26.1	26.5	26.0	21.9	
Two Way ANOVA					
Among cultivars		F-ratio	3.0	p-value	0.013*
Among treatments		F-ratio	4.6	p-value	0.009**
*Singnificant at p<0.05, **highly significant at p<0.01, ***Very high significant at p<0.0001.					

The most interesting result obtained was from cultivar Eretrospermum-350 which showed the highest germination percent among all four cultivars after three days. Eret-350 appeared to reach a maximum of 70% after 60s He-Ne laser irradiation, and the lowest germination percent to reach a minimum of 6.7% after 1200s He-Ne laser irradiation. Generally, He-Ne laser seed irradiation with low doses (expressed as irradiation times) enhanced the germination percent of wheat cultivars, especially after 1, 3, 60 and 600s, while irradiation with 10, 180, 1200 and 1800s reduced the germination percent of all wheat cultivars examined.

The Egyptian cultivar Sakha-168 clearly exhibited a different behavior with various He-Ne laser doses (Figure 1).

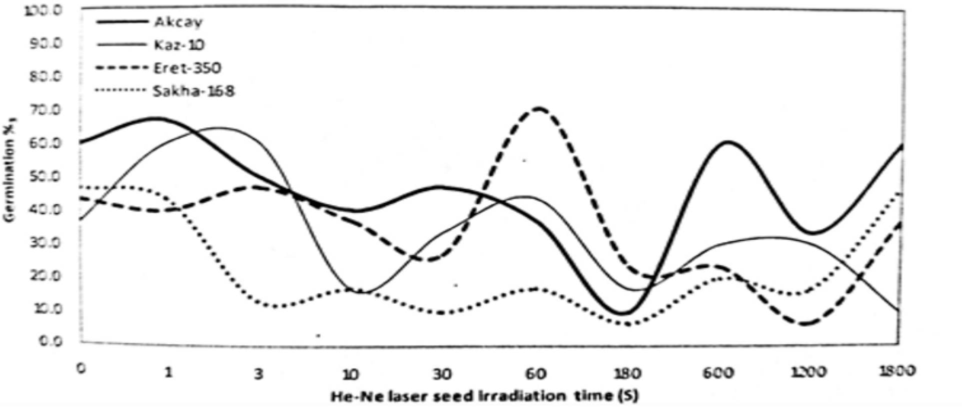

Fig. 1. Effect of He-Ne laser seed irradiation on germination percent (%) of four different wheat cultivars from Kazakhstan and Egypt.

Triticum aestivum L. cultivar Sakha-168 showed enhanced germination percent only at 1 and 1800s, while other doses reduced the germination percent. Statistical analyses confirmed this variation among cultivars and confirmed a highly significant difference among cultivars (F=3.0, p=0.013*). There were also highly significant differences among He-Ne laser radiation doses assessed using two-way ANOVA (F=4.6, p=0.009**; Table 2 and Figure 2).

Table 2. Effect of He-Ne laser seed irradiation on germination percent (%) of four different wheat cultivars from Kazakhstan and Egypt

He-Ne laser exposition time (s)	Cultivars				Variance
	Akcay	Kaz-10	Eret-350	Sakha-168	
Control	90.0	60.0	70.0	70.0	158.3
1	56.7	46.7	33.3	66.7	202.8
3	53.3	56.7	83.3	10.0	921.3
10	16.7	26.7	46.7	26.7	158.3
30	53.3	66.7	26.7	23.3	439.8
60	56.7	56.7	93.3	23.3	817.6
180	40.0	26.7	50.0	100.0	2321.3
600	63.3	50.0	60.0	43.3	84.3
1200	76.7	53.3	10.0	30.0	832.4
1800	83.3	23.3	70.0	63.3	666.7
Variance	456.9	242.0	681.6	1311.2	
Two Way ANOVA					
Among cultivars		F-ratio	0.8	p-value	0.581
Among treatments		F-ratio	0.4	p-value	0.729
*Singnificant at p<0.05, **highly significant at p<0.01, ***Very high significant at p<0.0001.					

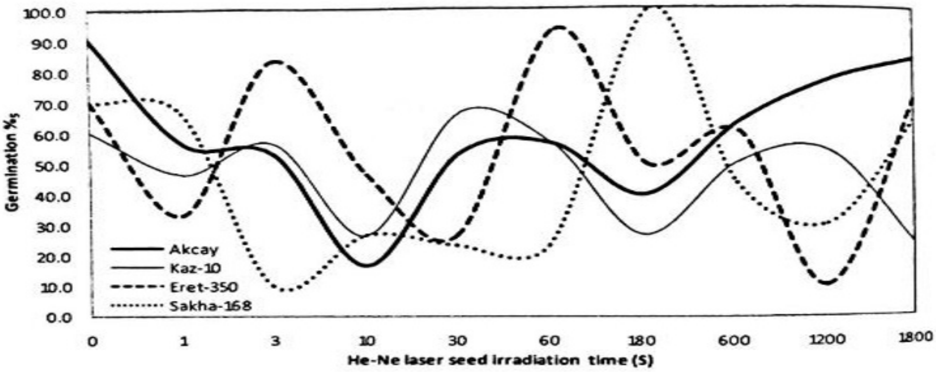

Fig.2. Effect of He-Ne laser seed irradiation on germination percent (%) of four different wheat cultivars from Kazakhstan and Egypt.

He-Ne laser one-shot irradiation with 632.8 nm enhanced the germination percent of Triticum aestivum cultivars after 5 days of cultivation and a maximum germination percent of 100% was recorded in the Egyptian cultivar Sakha-168 after irradiation for 180s. However, statistical analyses did not confirm significant differences among cultivars (F=0.8, p=0.581).

Data presented in Figure 3 illustrates the main differences in behaviour and response of various cultivars to 632.8 nm laser seed irradiation. The three Kazakhstan cultivars (Akcay, Kaz-10 and Eret-350) showed enhanced germination percent after irradiation for 3, 30, 60 and 600s. While, Egyptian cultivar Sakha-168 showed enhanced germination percent after seed laser irradiation for 1 and 180s.

Seven days post-seed sowing, wheat cultivar Kaz-10 showed maximal germination percent (100 %) after 1s He-Ne irradiation. While after 3s He-Ne irradiation, all three Kazakhstan cultivars showed maximum germination percent while the Egyptian cultivar Sakha-168 attained minimum germination (23.3%) (Table 3).

Table 3. Effect of He-Ne laser seed irradiation on germination percent (%) of four different wheat cultivars from Kazakhstan and Egypt.

He-Ne laser exposition time (s)	Cultivars				Variance
	Akcay	Kaz-10	Eret-350	Sakha-168	
Control	93.3	96.7	100.0	73.3	143.5
1	90.0	100.0	80.0	83.3	77.8
3	100.0	100.0	100.0	23.3	1469.4
10	93.3	73.3	86.7	56.7	262.0
30	66.7	100.0	56.7	56.7	422.2
60	83.3	100.0	100.0	50.0	555.6
180	76.7	43.3	86.7	70.0	343.5
600	93.3	86.7	76.7	70.0	107.4
1200	100.0	76.7	26.7	56.7	966.7
1800	80.0	46.7	93.3	76.7	388.0
Variance	116.2	486.5	540.2	294.4	
Two Way ANOVA					
Among cultivars	F-ratio		0.8	p-value	0.661
Among treatments	F-ratio		3.4	p-value	0.033*
*Singnificant at p<0.05, **highly significant at p<0.01, ***Very high significant at p<0.0001.					

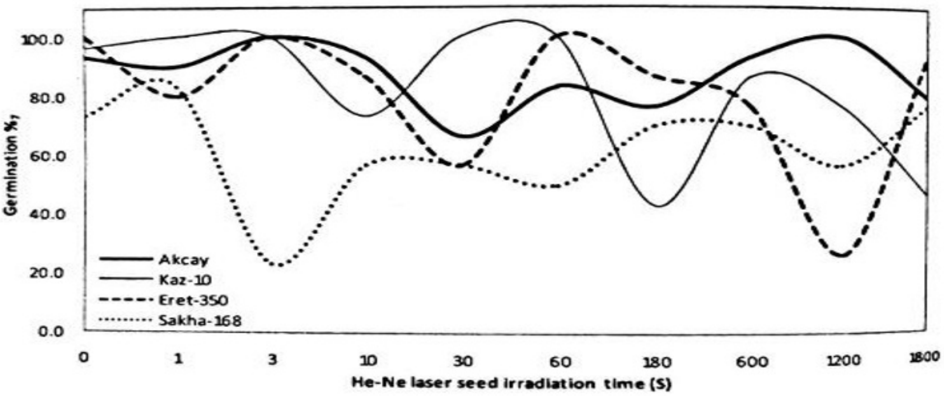

Fig. 3. Effect of He-Ne laser seed irradiation on germination percent (%) of four different wheat cultivars from Kazakhstan and Egypt.

These variations among Egyptian and Kazakhstan wheat cultivars appear to confirm that there is an internal energy difference among these cultivars. Chen et al. [10] stated that the pretreatment of seeds with He-Ne laser had not only a short-term biological effect, which enhanced inner energy of seeds, but also a long-term effect, which contributed to the acceleration of the growth and development of seedlings [10].

Also, the authors stated that changes of enthalpy (ΔH) are commonly believed to be an indicator of charge in internal molecular force. Our results suggest He-Ne laser pretreatment enhances the internal energy and enthalpy of seeds, and the differences between Kazakhstan habitats and environmental conditions may contribute to a significant difference among these cultivars in germination and further in seeding development (Table 2; F=3.0, p=0.013*) .

4 Conclusions

Thus, on the bases of the carried out researches it is possible to draw the following conclusions:

1. Studying the influence of seed irradiation by laser beam with wavelength of 632.8 nm, power intensity of 10 mW.cm and exposition durations from I to 1800 seconds. Wheat cultivars were cultured on hydrogel to allow establishment of iradiation dependence effect between radiation expositions and various wheat cultivars. The establishment of optimum laser radiation expositions allowed to increase seed germination to 100% (at 1, 3, 30, 60 and 1200 s), accelerating the growth rate (doses> Is), and. stimulating various physiological and biochemical indications (> Is).
2. The influence of wheat cultivars seed irradiation by infra-red beam with wavelength of 760.0-800.0 nm, power intensity of 1.0 mW.cm and exposition durations from 1 to 1800 s, were allowed to reveal dependence effect between wheat cultivars and irradiation expositions. It is shown that, Kazakhstan cultivars were more sensitive to IR-beams, while, Egyptian cultivar "Sakha-168" was less sensitive. Optimum IR-light expositions, increased seed germination to 100% (180 and 1200 s), stimulated wheat growth (1, 180, 600 and 1200 s) and also enhanced the level of various physiological and biochemical processes 1 s).
3. The definition of the influence of seed irradiation by monochromatic red polarized light (MCRPL) with wavelength of 632.8 nm, power intensity of 0.5 mW.cm and exposition durations from 1 to 1800 seconds, has allowed to assessing the dependence effect

influence between the four wheat cultivars and irradiatian germinations to 100% (60, 180 and 1200 s), stimulating wheat growth (180-1800 s). and increasing the level of physiological and biochemical activities (> l s).

4. Comparative analysis of physiological, biochemical, biophysical, morphological and anatomical features of the four examined wheat cultivars under the influence of electromagnetic radiations with wavelengths from 400.0 to 800.0 nm, power intensities from 0.5 mW.cm to 10.0 mW.cm and exposition durations from 1-1800 seconds, has allowed to establish that, these electromagnetic radiations have stimulatory effect and degree of activation depending mainly on iradiation exposition and wheat cultivar. It is revealed that, the Egyptian cultivar "Sakha-168", compared to Kazakhstan cultivars: "Akcay", "Eret-350", "Kaz-10", had no remarkable reaction with small irradiation expositions of laser and MCRPL.

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