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Assessment of Diode Laser Pretreatments on Germination and Yield of Wheat (*Triticum aestivum* L.) under Salinity Stress

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Abstract The results of the effect of laser radiation on germination and yield of wheat under NaCl concentrations (i.e., 0, 100, 200 and 300 mM NaCl) are reported in irrigated water Final Germination Percentage, Mean Time for Germination, Mean Daily Germination, Daily Germination Speed and Yield were determined for Two diode\lasers, emitting 660nm semi-coherent red beam and 980nm infrared beam, and one second harmonics of Neodymium-Yttrium-Aluminum Garnet (Nd:YAG) laser emitting 532nm were chosen as illumination sources. The seeds of wheat were irradiated for 12 minutes. The standard germination test and yield improved significantly by increasing the intensity dose of laser irradiation. All different types of evaluated laser radiation (Red, Infra-Red and Nd:YAG lasers) improved standard germination test and yield considerably compared to control samples. Results showed that the best efficiency was related to Nd:YAG laser with two times illumination. Consequently, application of semi-coherent laser radiation, as a pretreatment, can significantly enhance the resistance of wheat to salinity stress comparing to the controlled samples.

Keywords: *wheat, laser, salinity, seed, germination*

1. Introduction

Salinity stress remains one of the most serious environmental problems, which substantially hampers crop productivity in arid and semi-arid areas of the world [24]. In addition, soil salinity is one of the problems of agriculture, and is receiving much attention from plant breeders [22]. Salinity inhibition of plant growth is the result of osmotic and ionic effects [11], and different plants have developed different mechanisms to cope with such effects. There are also many investigations showing that salinity induces the water deficits in many crop species [10]. Biochemical, physiological and morphological characteristics of crop species, directly affected by salinity conditions, are well established in plants such as bean, eggplant, wheat, onion, pepper, corn, sugarcane, potato and cabbage [15]. Seed quality is affected by many parameters, such as environmental factors, genetics, fertility of soil and etc., which limits their growth and productivity. These factors include drought and salinity. Seed germination is an important stage in the life of plants [8]. Germination, seed and seedling vigor are affected by different factors, including stress [24]. For example, the salt effect on germination is often thought of as a decline in germination of seeds successfully in a given time. It might be more illuminating to consider the depression in percentage of germination as an extreme phenomenon of inhibition of seedling growth [3]. Light plays a critical role in the plant growth process and it is widely accepted that germination process is sensitive to irradiation with various wavelengths of visible and infra-red light. Furthermore, investigations indicated that germination of seeds may be triggered by millisecond-exposures to sunlight and a 5seconds-exposure to the weak while moonlight can saturate the germination of photosensitized seeds [9]. Laser biostimulation is a physical phenomenon based on the absorption of light energy by grains. The energy supply increases the energy potential of seeds, which in turn impacts the physiological processes in germinating seeds. Thus, accelerating maturity, increases resistance to stress as well as raising the biological and processing quality of the yield [26,27]. Recently, results of laser irradiation of plants have indicated that laser can be used to pre-treat plant seeds. This process enhances the rate at which seeds turn into seedlings, deactivate seed dormancy, increase the field rate of seedling emergence, improve the germination rate and vigor index, and boost young seedling growth. This also makes plants precocious, and strengthens plant adaptation to the environment, etc., even under poor surroundings [6,25]. The beneficial effect of the pre-sowing laser biostimulation of seeds on germination, initial growth and development and yield has been proven by numerous studies on some cereals (i.e., wheat, rye, white lupine, maize), root crops (sugar beet) and vegetables (tomatoes, cucumbers) [12]. Moreover, Pre-sowing stimulation of seeds with laser light caused a significant increase in the content of specific protein, phosphorus and molybdenum in the dry matter of the plants, and a decrease in the content of crude fiber [7]. Thus, the present research was aimed to evaluate the effect of diode laser on wheat germination under salinity stress in order to be used in agriculture in enhancement of wheat germination percentage.

2. Materials and Methods

Spring wheat (*Triticum aestivum* L. cv. Kavir) seeds were obtained from Seed and Plant Improvement Institute (Karaj, Iran). Seeds with the same size selected and after sterilization for 10min in 0.1% $HgCl_2$ washed in flowing water for 50min. Two diode\lasers, emitting 660nm semi-coherent red beam (power intensity: 110mW.mm^{-2}; beam diameter: 10mm) and 980nm infrared beam (power intensity: 250mW.mm^{-2}; beam diameter: 10mm), and one second harmonics of Neodymium-Yttrium-Alminum Garnet (Nd:YAG) laser emitting 532nm (power intensity: 75mW.mm^{-2}; beam diameter: 10mm) were chosen as illumination sources and directly irradiated the seeds of a spring wheat, respectively. The seeds were irradiated for 12 minutes, and this protocol was performed for one and two times (having distanced 24 hours). The seeds were exposed to laser one by one. The non-irradiated seeds were used as the control or reference seed.

The experiment was conducted during 2009 at center laboratory of Aboureihan campus (University of Tehran, Iran). After irradiation of seeds, they were germinated on moist filter paper for seven days in an incubator at 25℃ and irrigated with four salinity solutions (0, 100, 200 and 300mM NaCl). All the experiments were conducted in 9cm Petri plate on filter paper beds in the incubator. 20 seeds were sown in 9cm diameter Petri plate on filter paper beds, irrigated with 5ml solution of respective treatment. Each treatment was replicated thrice. The filter paper beds were irrigated daily with 5ml solution of the respective treatment. Qualitative description of the results of laser irradiation was based on the analysis of several germination indices.

The following indices were calculated for each treatment replication: Final Germination Percentage (FGP); Mean Time for Germination (MTG); Mean Daily Germination (MDG); Daily Germination Speed (DGS).-Final Germination Percentage (FGP) is the maximum percentage which occurred for seed lots.-Mean Time for Germination (MTG) was calculated in hours as follows:

$$\text{MTG}=\frac{\sum(nidi)}{\sum ni} \quad (1)$$

n_i– number of germinated seeds during a given time interval;
t_i– number of hours counted from the beginning of the germination test;
n – total number of seeds germinated at the end of the germination test.
-Mean daily germination (MDG) is contrast to day germination speed (time to need for germination a seed), MDG is ‘mean germination days’ and it is speed germination day index.

$$\text{MDG}=\frac{\text{Final germination percentage}}{\text{Germination term}} \quad (2)$$

-Daily germination speed (DGS) is time that seeds needed for germination. a seed when ever reduce it, increase germination speed. The speed of germination is based on the rate of radical protrusion per unit time.

$$\text{DGS} = 1/\text{MDG} \quad (3)$$

Then, they were sown separately in plastic pots (25cm × 30cm) and watered with the solution of different salinity concentrations. All the pots were uniformly filled with the same weight of soil and for uniform soil moisture, the same water amount was added to each pot. Every replicate experiment comprised six basins, each containing four seedlings. The pots with seedlings were grown in an artificial greenhouse.

Statistical analysis

Factorial analysis was used based on Randomized Complete Block Design layout with three replications. All data were subjected to analysis of variance (ANOVA), using the general linear model of SAS (version 9.1). Mean of treatments tested by Duncan’s multiple range test (DMRT). Significance levels of sources ($p < 0.05$ or $p < 0.01$) have been determined.

3. Results

3.1. Effect of Different Laser Pretreatment on Standard Germination Test and Yield under Salt Stress

Table 1. Mean squares from analysis of variance forstandard germination test and yieldof wheat (*Triticum aestivum* L.) irradiated with threetypes of lasers (Red, Infra-Red and Nd:YAG) at two exposures, one (12 minutes) and twice (12 minutes per each with 24 hours distance)at four NaCl concentrations (0, 100, 200 and 300 mM)

Sources of Variation	DF	MS				
		FGP	MTG	MDG	DGS	Yield
Laser	3	905.90**	0.92**	35.23**	0.10**	2755.89**
Exposure	1	376.04**	0.24**	15.04**	0.00^{ns}	6.01^{ns}
Salinity	3	23664.93**	8.57**	946.59**	0.29**	85722.68**
Laser × Exposure	3	67013.5^{ns}	0.04^{ns}	2.68^{ns}	0.0002^{ns}	140.01^{ns}
Laser × Salinity	9	234.18**	0.15**	9.36**	0.05**	192.35*
Exposure × Salinity	3	69.57**	0.03^{ns}	2.78**	0.01*	72.24^{ns}
Laser × Exposure ×Salinity	9	72.37**	0.03^{ns}	2.89**	0.03**	444.39**
Error	64	25.03	0.04	1.001	0.005	78.83

ns: not significant,*****: p <0.05,******: p <0.01.
(FGP): Final Germination Percentage,(MTG): Mean Time for Germination,(MDG): Mean Daily Germination,and (DGS): Daily Germination Speed.

Table 1 shows the results obtained from the analysis of standard germination test and yield in the seeds of wheat at different kinds of lasers and numbers of exposure to irradiation under salinity conditions.

The results showed that different kinds of lasers and salinity levels had significant effect (p<0.01) on the final germination percentage, mean time for germination, mean daily germination, daily germination speed and yield. However, radiation dosage effect is significant only on final germination percentage, mean time for germination and mean daily germination.

In addition, there was significant interaction effect among evaluated factors with the exception of laser×exposure in all sources and Exposure×Salinity and Laser×Exposure×Salinity in MTG.

3.2. Effect of Different Salinity Concentrations on Standard Germination Test and Yield Accumulation

Considering the data presented in Table 2, clearly higher salt treatments greatly increased mean time for germination, daily germination speed and yield, but decreased final germination percentage and mean daily germination in wheat.

Table 2. Mean comparisons of effect of different salinity concentrations (0, 100 200 and 300mMNaCl) on standard germination test and yieldof wheat (*Triticum aestivum* L.)

Salinity	FGP(%)	MTG	MDG	DGS	Yield(g/plant)
0 mM	95.208±0.63a	1.517±0.29d	19.041±0.12a	0.052±0.0003b	127.60a
100 mM	95.625±0.81a	1.803±0.21c	19.125±0.16a	0.052±0.0004b	125.82a
200 mM	65.208±1.51b	2.181±0.06b	13.040±0.30b	0.077±0.0018b	24.83b
300 mM	29.375±3.28c	2.898±0.09a	5.875±0.65c	0.282±0.053a	8.80c

Statistical analysis determined by ANOVA. Mean values followed by the same letters in the same column are not significantly different at P<0.05 (Duncan’s Multiple Range Test).
±Standard Error
(FGP): Final Germination Percentage, (MTG): Mean Time for Germination, (MDG): Mean Daily Germination, and (DGS): Daily Germination Speed

3.3.1. Effect of One Time Laser Radiation on Standard Germination Test and Yield under Salt Stress

Based on the data presented in Table 3, it was observed that germination of wheat pretreated by three types of lasers for 12 minutes in one time of radiation increased significantly. Nd:YAG laser had the highest increasing level of final germination percentage and mean daily germination and had the lowest decreasing level of daily germination speed mean while Red laser showed the lowest decreasing level of mean time for germination (Figure 1).However, the Infra-red laser had the higher effect on yield (Figure 4).

3.2.2. Effect of Two Times Laser Radiation on Standard Germination Test and Yield under Salt Stress

From Table 4, clearly laser treatment greatly improved germination percentage for two times radiation with different types of laser compared to control plants. Furthermore Nd:YAG laser had the highest effect on standard germination test and yield, but there is not significantly difference between Nd:YAG and Red laser and Infrared lasers in FGP and MDG (Figure 2, Figure 4).

3.3.3. Effect of Different Exposure Radiation on Standard Germination Test and Yield under Salt Stress

Table 5 presents the effects of two types of exposure radiation on the standard germination test during salinity circumstances. Results showed more efficiency of two period irradiation exposure rather than one period radiation in FGP, MTG and MDG; but there was no significant difference in DGS and yield.

Figure 3 shows the *final germination percentage* of *wheat* seeds under 0, 100, 200 and 300mM NaCl after laser pretreatment, separately. The results clearly demonstrated that prior laser irradiation on seeds, regardless of laser type, had a significant effect (p<0.01) on germination percentage under the same salinity concentration. Results showed that maximum germination percentage (100%) recorded mainly at Nd:YAG and Red laser of two times exposure under 100mM NaCl.

Table 3. Mean comparisons of standard germination testand yield in wheat (*Triticum aestivum* L.) exposed once(for 12 minutes) to laser treatment

Laser	FGP(%)	MTG	MDG	DGS	Yield(g/plan)
Nd:YAG	73.333±7.34a	2.014±0.13c	14.666±1.46a	0.079±0.01b	76.46ab
Red laser	72.500±8.80a	2.016±0.16c	14.500±1.16b	0.085±0.01b	68.47b
Infrared laser	68.750±8.49b	2.195±0.14b	13.750±1.68b	0.100±0.02b	79.97a
No-laser	62.916±10.43b	2.376±0.23a	12.583±2.08b	0.215±0.08a	50.62c

Statistical analysis determined by ANOVA. Mean values followed by the same letters in the same column are not significantly different at P<0.05 (Duncan’s Multiple Range Test).
±Standard Error
(FGP): Final Germination Percentage, (MTG): Mean Time for Germination, (MDG): Mean Daily Germination, and (DGS): Daily Germination Speed

Table 4. Mean comparisons of standard germination test and yield in wheat (*Triticumaestivum*L.) exposed twice (12 minutes per each with 24 hours distance) to laser treatment

Laser	FGP(%)	MTG	MDG	DGS	Yield(g/plant)
Nd:YAG	81.250±5.64a	1.951±0.14b	16.250±1.12a	0.065±0.004b	82.24a
Red laser	75.416±7.77b	.861±0.14b	15.083±1.55b	0.078±0.01b	67.30b
Infrared laser	73.750±8.40b	2.010±0.12b	14.750±1.68b	0.089±0.01b	76.77a
No-laser	6^2.916±10.43c	2.376±0.23a	12.583±2.08c	0.215±0.08a	50.62c

Statistical analysis determined by ANOVA. Mean values followed by the same letters in the same column are not significantly different at P<0.05 (Duncan’s Multiple Range Test); ±Standard Error
(FGP): Final Germination Percentage, (MTG): Mean Time for Germination, (MDG): Mean Daily Germination, and (DGS): Daily Germination Speed

Table 5. Mean comparisons of different exposure radiation, one (12 minutes) and twice (12 minutes per each with 24 hours distance) on standard germination test and yield in wheat (*Triticum aestivum* L.)

Dosage of laser	FGP(%)	MTG	MDG	DGS	Yield(g/plant)
1 period	73.333a	2.050b	14.666a	0.112a	75.77a
2 period	69.375b	2.150a	13.875b	0.120a	74.63a

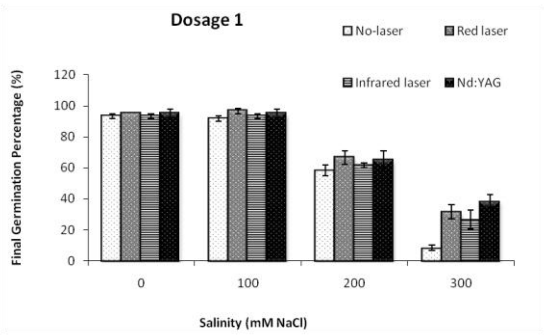

Figure 1. Effects of different kind of lasers with one exposure(12 minutes)on final germination percentage in wheat at NaCl concentrations (0, 100, 200, 300 mM)

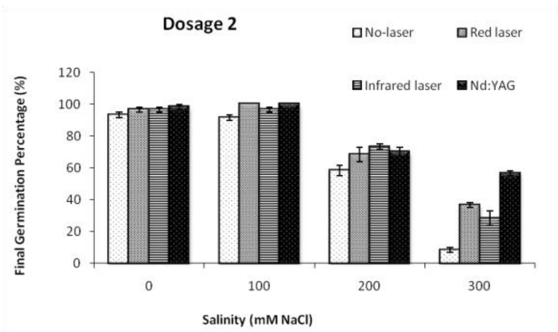

Figure 2. Effects of different kind of lasers with twice exposure (12 minutes per each with24 hours distance) on final germination percentage in wheat at NaCl concentrations (0, 100, 200, 300 Mm)

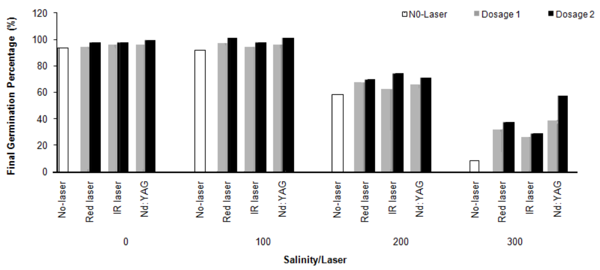

Figure 3. Final germination percentage(FGP)of wheat seeds under 0, 100, 200 and 300mM NaCl after laser pretreatment, separately

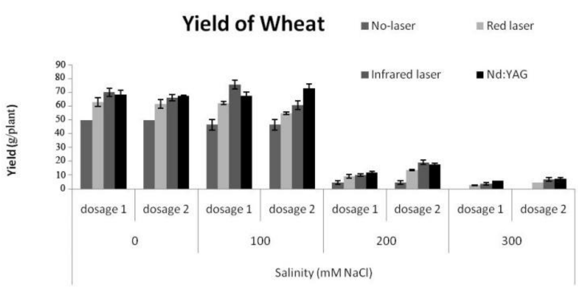

Figure 4. Effects of different kind of lasers anddifferent exposure,one (12 minutes) and twice (12 minutes per each with 24 hours distance)at four NaCl concentrations (0, 100, 200 and 300 mM)on yield of spring wheat

4. Discussion

Salinization is the annoyance of intensive agriculture and is one of the most important environmental stresses impeding plant growth [13]. The results of this investigation demonstrated that laser pretreatment of wheat seeds would provide protection for wheat from salinity stress damage. During this investigation, it was demonstrated that, with increasing of salinity level, the germination decreased in wheat (cv. Kavir). Many investigations have shown that high concentration of salt has detrimental effects on germination of seeds and plant growth [8,20]. Furthermore, the results showed that salinity stress caused the significant decrease in germination and seedling growth of wheat [20]. Poor germination and seedling establishment are the results of soil salinity, and it may be that NaCl reduced the rate of germination due to the reduced water potential and the resulting slower rate of imbibitions [2]. Furthermore, the results showed that laser successfully improved germination of wheat cultivar. Researchers [14,16] have shown previously that germination capacity is increased due to irradiation of spring wheat seeds by laser light. One of the reasons for the germination increase can be due to additional energy in plant at irradiation with laser beams. [6,19] also has illustrated that the enthalpy change during the germination process of seeds pretreated with laser was notably higher than the control. Moreover, change of enthalpy, often referred to as heat content, is related to internal energy. The laser, as a specific light, can be absorbed effectively by macromolecule and cause seeds pretreated with suitable laser irradiation have to absorb more energy from the surrounding than that of the control samples in during the individual development because the laser broke the kinetic equilibrium of germination seeds and increased the internal energy of seeds. Furthermore, throughout this study, it is demonstrated that not only the thermodynamic parameters of germination seeds were increased greatly but also the physiological metabolism and the growth and development of seedlings were accelerated because of stimulation seeds with laser radiation [5].

From this viewpoint, the laser offers a pure ecological source of energy that ensures high yield. For this reason, the laser started to beused as a bio-stimulator in plant production. In this study, with increasing germination, yield of spring wheat was increased as well. Positive influence of laser beams on the yield increase was reported for cereals 15-20%, vegetables to 40%, potatoes 30%, tobacco 20%, and poppy 20% [21].

There were also significant differences in times of irradiation. The best results of positive influence of the laser beam on the standard germination test and yield were obtained for two times. Therefore, with pretreatment for a long time with laser, plant had developed further resistant to salinity stress. Consequently, based on laser exposure time, the obtained results could be different. [1] stated that different times of radiation had the diversities' effect to germination of plants. For this reason, for useful effect of laser to plants choosing the best time of irradiation is important.

Furthermore, for the same salinity concentrations, different types of laser had significantly affection on the standard germination test and yield. As a result, these methods can be used for quick determination of the stress responses in the plants. [17] showed a relationship between drought tolerance and laser treatment. These investigators exposed wheat seeds to a CO_2 laser, then simulated drought conditions in the growing seedlings. The results showed that laser treatment increased the drought resistance of the plant, enhancing the activity of many of the same enzymes. Experiments on optimization of the exposure time to the CO_2 laser showed a period of 3min was optimal [27]. In addition, many studies have indicated that proper laser pretreatment could alleviate the inhibitory effect of enhanced UV-B radiation, delaying the senescence process [18].

5. Conclusions

1. The types of laser (Red, Infra-Red and Nd:YAG lasers) induce bio-stimulation effect into germination and yield in spring wheat (*Triticum aestivum* L.).

2. The best efficiency was related to Nd:YAG laser, one second harmonics of Neodymium-Yttrium-Aluminum Garnet laser emitting 532nm (power intensity: 75mW.mm^{-2}; beam diameter: 10mm).

3. The seeds were irradiated by Red, Infra-Red and Nd:YAG lasers, for 12 minutes, and this protocol was performed once and twice having distanced 24 hours. The results showed that irradiation for two times (12 minutes per each) had more efficiency rather than one time radiation.

4. Stimulating effect of laser light can be useful for improving wheat germination and yield in the field conditions, especially in the regions where the high salinity occurs.

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