Int. Agrophysics, 2009, 23, 327-332

INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Laser irradiation effects on field performance of maize seed genotypes**

C. Hernandez-Aguilar[1]*, A. Dominguez- Pacheco[1], A. Cruz-Orea[2], R. Ivanov[3], A. Carballo-Carballo[4], R. Zepeda-Bautista[1], and L. Galindo Soria[1]

[1]National Polytechnic Institute, Sepi-Esime, Zacatenco, Professional Unit 'Adolfo López Mateos' Col. Lindavista. México D.F., C.P. 07738, Mexico
[2]Department of Physics, CINVESTAV – IPN, A. P. 14-740, Mexico D.F., C.P. 07360, Mexico
[3]Academic Unit of Physics, Autonomy University of Zacatecas, A.P. 580, Zacatecas, Mexico
[4]College of Postgraduates IREGEP, Seed Program, Montecillo, Edo. de Mexico, C.P. 56180, Mexico

Received March 18, 2009; accepted June 20, 2009

A b s t r a c t. The paper presents a study on the effects of low intensity laser irradiation on field performance of maize hybrid seeds (*Zea mays* L.). Pre-sowing laser irradiation treatment on the seeds was done, during 20 s, by using a diode laser with output power of 27.4 mW at 650 nm wavelength. To evaluate the pre-sowing laser irradiation effects on the seed field performance, a randomized complete block experimental design with 5 replications was used for statistical analysis. The maize seed genotypes used in the present study were VC-AZ, VS-AM and MT-1415. Also, by using photoacoustic spectroscopy, the optical absorption coefficient (β) of each maize genotype was obtained. In order to evaluate the effects of laser irradiation on the seed field performance some vigour tests were done and the results are showed. In the case of MT-1415 a negative biostimulation for seedling emergence percentage, seedling emergence rate and chlorophyll *a* content was observed, compared to the control experiment *ie* without irradiation. For VC-AZ and VS-AM genotypes no significant effects were obtained for the same vigour tests, as compared to the control experiment. These results show that it is necessary to find the optimal irradiation parameters to induce positive biostimulation in maize seeds, which also depends on the seed genotype.

K e y w o r d s: *Zea mays* L., seed vigour, biostimulation, photoacoustic spectroscopy

INTRODUCTION

Ultraviolet and visible radiations are able to excite electronic states in biological molecules, leading to photochemical transformations at molecular level. Such processes can occur under the effect of light emitted from incoherent sources, but the use of laser light at specific wavelengths increases the ratio at which the molecules are excited, making it possible to study photobiochemical reactions over a broad range of time. For example, in picosecond pulses, which excite higher electronics states in target molecules, multi-photon processes can be studied (Letokhov, 1985). Illumination of biological tissue by coherent laser light unavoidably leads to strong intensity gradients of the radiation in the tissue due to speckle formation which causes inter- and intracellular gradient forces whose action may significantly influence the paths and speeds of biological processes (Ruvinov, 2003).

Low intensity red laser light is distinguished by characteristic parameters which produce biostimulation when used as pre-sowing treatment of seed (Benavides *et al.*, 2003; Hernandez *et al.*, 2005; 2006; 2008a; 2008b; Koper and Dziwulska, 2003; Muszyński and Gładyszewska, 2008; Podleśny *et al.*, 2001). The basis of the stimulation mechanism is the synergism between the polarised, monochromatic red laser beam and the phytochrome receptors (Koper *et al.*, 1996). The phytochromes are signal-transducing photoreceptors that switch between inactive and active forms in response to different wavelengths of light. This conversion is used to adapt plant development to the exigencies of the environmental light. Light signals provide information of crucial value at many stages of plant vegetation. Seed germination, seedling establishment, the development of the photosynthetic machinery itself, the architecture of vegetative plant, the timing of flowering, tuberisation and bud dormancy, also

*Corresponding author's e-mail: clauhaj@yahoo.com
**The paper was supported by CONACYT and Instituto Politécnico Nacional through COTEPABE, COFAA and EDI scholarships.

the responses to neighbour competition, and the allocation of resources to root, stem, leaf, reproductive or storage structures, are all potentially controlled by the perception of environmental light signals by the phytochromes (Smith, 2000). A single short pulse of light maximizes conversion of the inactive Pr form to the active Pfr form (Shimizu-Sato *et al*., 2002). Therefore, the objective of the present work was the study of the effects of low intensity laser irradiation on three maize seed genotypes to evaluate the field performance with a single laser irradiation of 20 s, using a laser diode to irradiate the seeds prior to sowing. The optical absorption spectra of their seedling leaves were obtained by using photoacoustic spectroscopy in order to investigate qualitative differences in the optical absorption bands of chlorophylls *a* (at 680 nm) and *b* (at 650 nm). The use of laser diode would be very useful also in view of the fact that the price of semiconductor lasers has significantly decreased.

MATERIALS AND METHODS

Maize seeds from VC-AZ (Genotype A), VS-AM (Genotype B) and MT-1415 (Genotype C), produced at the Experimental Station 'Colegio de Postgraduados', Montecillo, Mexico, during the spring-summer season of 2007, were used. The seed samples were obtained from the maize ear for each genotype (maize ear central part) and the values of average weight of 50 seeds were 21.53, 18.66 and 11.09 g for A, B and C genotypes, respectively. The seed genotypes had different pigmentation.

Seeds of each genotype were selected at random to obtain their optical absorption coefficients (β) in the wavelength range from 500 and 750 nm. The photoacoustic (PA) experimental set-up consisted of 1 KW Xe lamp (Oriel), a mechanical chopper operating at 17 Hz, a monochromator (Oriel), and a homemade brass PA cell provided with an electret microphone. The PA signal from the microphone was fed into the input channel of a SR-850 (Standford Res.) lock-in amplifier interfaced to a personal computer that displayed simultaneously both the amplitude and the phase of the wavelength-dependent PA signal (Fig. 1 – instrumentation cell 1). The optical absorption coefficient β was determined from the PA signal amplitude by using the Rosencwaig and Gersho model (Fesquet *et al*., 1984). The total optical absorption coefficient for thermally thick ($a_s l_s >> 1$) samples was obtained from the normalized PA signal by using Eq. (1):

$$\beta = (a_s)\left\{q^2 + q\left(2 - q^2\right)^{1/2}\right\} / \left(1 - q^2\right), \qquad (1)$$

where: q is the normalized PA intensity, $a_s = (\pi f/\alpha)^{1/2}$ is the thermal diffusion coefficient at the modulation frequency f (Ramon *et al*., 2001), $\alpha = 4.44\ 10^{-3}$ cm^2 s^{-1} is thermal diffusivity of starch (Fernández *et al*., 2001) and l_s refers to the sample thickness.

Maize seeds were irradiated by using a 650 nm diode laser with 27.4 mW of output power. The maize seeds were selected randomly for the irradiation, inserted into sterile test tubes and fixed to a rotor. The seeds were exposed to irradiation while the rotor was in movement (Fig. 2). Laser power meter model 45-545 from Metrologic Instruments, Inc. was used to measure the laser output power. The seed irradiation time was 20 s with 6.08 mW cm^{-2} laser intensity. The seeds were soaked in water for 1 minute before irradiation.

The seed vigour, evaluated through the seed emergence test, was established at the Colegio de Postgraduados Experimental Station, Montecillo, Mexico. A loamy clay soil was used as substrate. Each plot consisted of 32 cm rows, 10 seeds per row and spaced at 3 cm. The seeds were

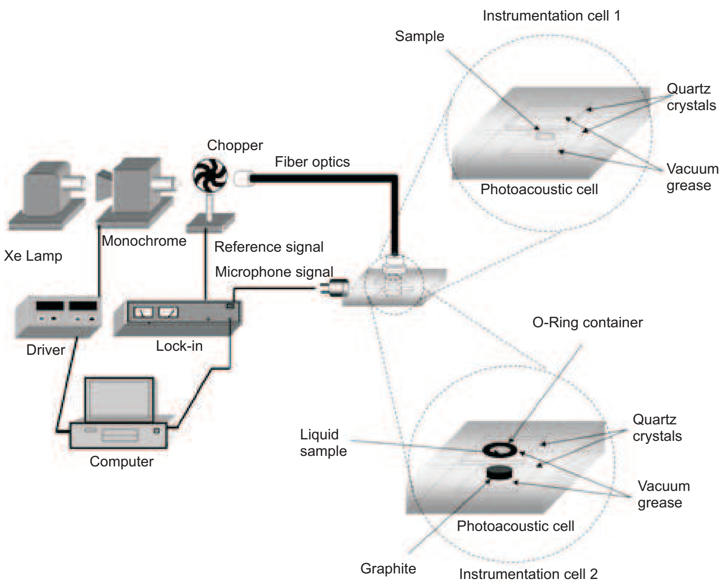

Fig. 1. Experimental setup of the photoacoustic spectrometer used to obtain the optical absorption spectrum of a) the studied maize seeds (instrumentation cell 1) and b) chlorophyll liquid samples (instrumentation cell 2).

sown immediately after the laser pre-sowing treatment (including the control of each genotype) by placing the pedicel in the soil, leaving the silk scar exposed. The seeds were covered with 5 cm soil. The seedbed, with dimensions of 1.3 x 0.40 x 0.15 m, was watered every day at the same hour. A micro-tunnel, made of metal and semitransparent plastic, covered the seedbed and placed to inclemency outdoors under environment conditions and natural light cycle. The average temperature was 15°C. The seedling emergence began on the 7th day after sowing. From that day, daily countings of emerged seedlings were made each 12 h during the next 5 days. Finally, the total number of emerged seedlings, the seedling emergence rate according to Maguire (1962), and the seedling dry weight on the 21th day, after drying at 40°C during 72 h, were used in the statistical analysis.

The vigour test was carried out in a randomized complete block design with 5 replications, with the plot including 10 seeds. Variance analyses were based on the GLM procedure in SAS (2008). Differences among treatments were tested for significance by Tukey test at 5% probability level (Steel and Torrie, 1980). On the third day after the beginning of emergence, significant statistical differences were found in the seedling emergence rate and emergence establishment percentage. The seedling dry weight was evaluated on the 21st day after initial emergence. Only normal seedlings according to the ISTA rules (1993) were evaluated. The accuracy in these measurements was an essential component of the methodology used in the present research (Tekrony, 2003).

On 21st day after the initiation of emergence, one seedling was taken at random from each treatment and chlorophyll was extracted from one leaf of this seedling. This chlorophyll was diluted with 3 ml of alcohol, using 0.3 ml for the measurement process. Liquid chlorophyll for each seedling was placed on the photoacoustic cell within the o-ring container that allowed the measurement in transmission mode in order to obtain their complementary optical absorption spectra (Fig. 1- Instrumentation cell 2). These spectra were obtained in the range of 600 to 700 nm by using the photoacoustic spectrometer. This procedure was repeated 15 times for each treatment in order to complete 5 repetitions with 3 seedlings for each repetition, and the whole procedure was done for each treatment and genotype. Data were subjected to variance analysis (ANOVA) using the SAS GLM procedures (SAS, 2008). The Tukey test at 5% probability level was conducted for comparing treatments. Each optical absorption spectrum was normalized to its initial PA signal amplitude value, at 600 nm, to avoid some little differences in the light intensity from the Xenon lamp. The data of the PA signal amplitude corresponding to chlorophyll *a* (at 680 nm) and *b* (at 650 nm) were introduced in the SAS program, for all repetitions and treatments, including the control sample (seeds without irradiation).

RESULTS

Figure 3 shows the optical absorption spectra of the three maize seed genotypes used in the present study, each point corresponding to the average of three measured seeds. The values of β at 650 nm are 5.26, 1.54 and 0.88 cm^{-1} for genotypes A, B and C, respectively. This wavelength is important because the laser used for the seed irradiations emits at this wavelength. The optical absorption spectrum of genotype C seeds shows less optical absorption than the others. The short-pulse laser irradiation had significant effects on seedling emergence rate, seedling emergence (%) and optical absorption of chlorophyll *a* (at 680 nm) for Genotype C. In the case of Genotypes A and B there was no significant effect on the parameters evaluated (Table 1).

The laser irradiation effects on the kinetics of soil-emerged seedlings for seed of Genotype C are presented in Fig. 4. A significant increase can be seen in the seedling emergence percentage of control seeds when compared with the irradiated sample, then for this case the seedling emergence percentage shown, for this irradiation, indicates a negative effect of biostimulation, decreasing 10.20% with respect to the control samples.

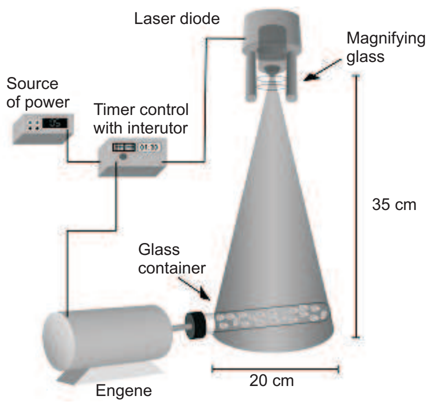

Fig. 2. Experimental setup used for pre-sowing seed treatment.

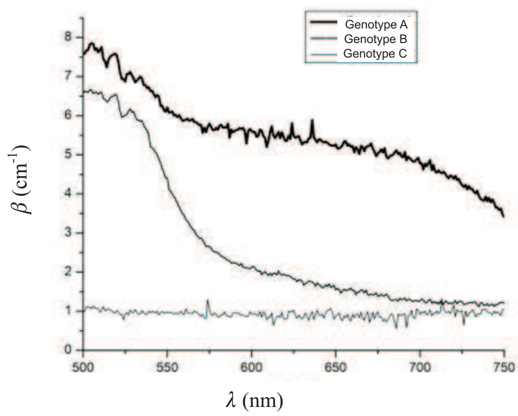

Fig. 3. Optical absorption spectra, obtained from PA signal of maize seed A, B, and C genotypes, as a function of wavelength.

T a b l e 1. Mean values of the obtained variables from the vigour test under micro-tunnel conditions

Treatment	I (mW cm^{-2})	*t* (s)	SER	EP	SDW	Ch*a*	Ch*b*
VC-AZ (Genotype A)							
1 (control)	0	0	1.30a	96a	30.86a	1.10a	1.12a
2	6.08	20	1.33a	98a	28.53a	1.27a	1.23a
VS-AM (Genotype B)							
1 (control)	0	0	1.37a	98a	27.34a	1.09a	1.07a
2	6.08	20	1.34a	100a	29.37a	1.12a	1.09a
MT-1415 (Genotype C)*							
1 (control)	0	0	1.20a	98a	16.00a	1.48a	1.28a
2	6.08	20	1.10b	88b	15.95a	1.26b	1.14a

Mean values with the same letters are statistically equal (Tukey, $\alpha = 0.05$). *Significant differences at 5% probability error level, I – intensity, *t* – time, SER – seedling emergence rate, EP – emergence percentage (%), SDW – seedling dry weight (g), Ch*a* – PA signal amplitude of chlorophyll *a* (at 680 nm), Ch*b* – PA signal amplitude of chlorophyll *b* (at 650 nm).

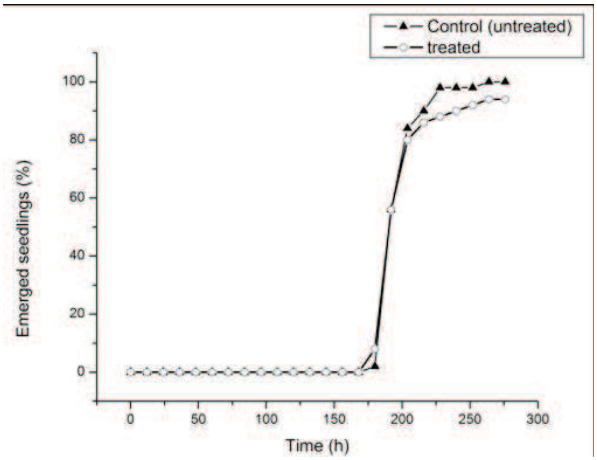

Fig. 4. Kinetics of soil-emerged seedlings from C genotype seed.

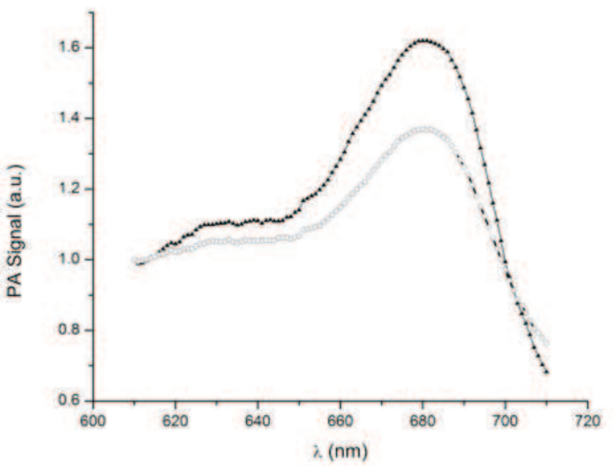

Fig. 5. PA signal amplitude in the range of the optical absorption bands of *a* and *b* chlorophylls. Explanations as on Fig. 4.

In relation to the PA signal amplitude, which is directly proportional to the optical absorption, for chlorophylls *a* (at 680 nm) and chlorophyll *b* (at 650 nm), it was observed in chlorophyll *a* that the optical absorption decreased by 14.86%, when compared with the optical absorption of control sample, as shown in Fig. 5. This result indicates that chlorophyll concentration in the leaves of seedlings decreased in the case of seedlings coming from irradiated seeds. The experimental results indicated that there was negative biostimulation by the interaction of the laser light on the maize seeds and the short irradiation time (20 s).

DISCUSSION

The results of this study show that the effects produced by laser light applied prior to sowing depend on the maize seed genotype and optical absorption coefficient. Negative biostimulation was found for genotype C when laser irradiation at 650 nm was applied during 20 s with intensity of 6.08 mW cm^{-2}. Also for this genotype the variance analysis, at 5% level of significance, showed a decrease in seedling emergence rate, seedling emergence during early stages of growth (third day), and also on 21 day. On the other hand, the optical absorption of the seedlings at 680 nm (chlorophyll *a*), which is directly proportional to the PA signal amplitude, decreased when compared to the optical absorption, at the same wavelength, of the control sample. The decrease in seed vigour parameters, due to laser irra- diation, resulted in unfavourable bio-stimulation, which was associated with the decrement in seed vigour by the interaction of the laser light on the maize seeds at single irradiation of 20 s on only one genotype investigated. This indicated that short times of irradiation of seeds with laser light have an influence on the course of metabolic processes in cells, as well as on their photosynthetic activity, which depends also on the seed genotype. This indicates that the cell is able to absorb, transform and use the energy of laser light photons (Szymer and Klimont, 1999).

The optical absorption coefficient β for seed genotype C, obtained by PAS, indicates that the light intensity penetrates deeper than those shown for the genotypes A and B due to their β values; consequently, for the genotype C, the optical penetration length is higher. Analysis of the optical absorption spectra for genotypes A and B indicates the presence of natural pigments of flavonoid type in the pericarp, in the aleurone layer, or in both structures of the seed (Fernández *et al*., 2001). Flavonoids are secondary metabolites that are present in high levels in seeds and grains (Winkel, 1998). In the present study the genotype A had bluish coloration and genotype B had yellowish coloration.

Ouf and Abdel-Hady (1999) indicates that the effects of laser irradiation are increased when the seed is artificially coloured. From the results obtained in this study, the seed with natural blue and yellow pigments did not have positive or negative biostimulation effect with short time of pre-sowing irradiation in any of the evaluated variables. This suggests that for processes of biostimulation the optimum dose varies with the seed natural pigment and maize genotypes.

On the other hand, other authors have found positive influence to 10 s irradiation with He-Ne laser on lettuce, causing a highly significant stimulation of germination (Paleg and Aspinall, 1970). In our research, negative biostimulation effects in genotype C were found. Also, it should be noted that natural or artificial seed colour is important to obtain effects of biostimulation. In this sense we found differences in biostimulation effects when the maize seeds were or were not photosensitized by soaking in red methyl before laser irradiation (Hernandez *et al*., 2006). Also, biostimulation effects were found by using natural seed (Hernandez *et al*., 2007) where, during the irradiation, the seeds were oriented in such a way that the light impinged on the seed embryo. In the present study the seeds were in movement, in order to reach a uniform illumination on the seed, and only the genotype C (MT-1415) had negative biostimulation till the third day of emergence in two of the evaluated vigour variables: seedling emergence rate and emergence percentage. Furthermore, in the evaluated chlorophylls, at 680 nm wavelength, there was also a negative biostimulation. On the other hand, in previous studies with coloured maize seeds, we observed qualitative differences in the optical absorption spectra, obtained by PAS, of chlorophylls *a* and *b* of seedling leaves, whose coloured seeds were irradiated with laser at different times; in those studies both negative and positive effects were found, depending on the combination of the irradiation parameters (Hernandez *et al*., 2005). In the present research were obtained negative effects, when compared with the control, only for genotype C. From our results we observed that is very important to estimate the specific combination of laser irradiation parameters for each genotype, in order to produce positive biostimulation effects. In this research we observed negative biostimulation effects produced by seed treatment using laser light, so it is necessary to find the optimum parameters for the pre-sowing treatment of seeds in order for this procedure to be useful in agriculture.

Laser irradiation is distinguished by some parameters which determine its usefulness in agricultural science: exposure time, intensity, dose, and irradiation wavelength (Hernandez *et al*.; 2006). The laser most frequently reported in seed irradiation for biostimulation is He-Ne laser. Recently, the price of semiconductor lasers has significantly decreased (Steele, 2002), which makes semiconductor lasers a valid option in seed enhancement technology. The diode lasers are relatively cheap sources of laser irradiation and also it is possible to fit them together to irradiate more seeds in the seed industry. In this research it can be seen that it is important to define specific irradiation doses for each maize genotype and seed lot. Therefore, future research will have to be carried out on a wider range of germplasm and irradiation parameters.

CONCLUSIONS

1. The effects caused by laser irradiation (at 650 nm) on maize seeds were sensed by PAS technique (measurement in transmission mode), which demonstrated to be a useful technique for the study of the effects in liquid chlorophyll of seedling leaves which came from irradiated maize seeds.

2. It was found that the effects of laser irradiation treatments, during 20 s, on maize seeds depend on the seed genotype. Then no significant (at 5%) biostimulation effects were found for A and B genotypes, and a negative biostimulation effect for genotype C.

3. Laser irradiation could be a valid low-cost seed enhancement technique that can modify the quality of the seed in short time (20 s). It will be important to define specific irradiation doses for each genotype and seed lot in order to improve maize seed lot field performance.

REFERENCES

Benavides M., Garnica S., Michtchenko A., Hernández A.C., Ramírez R., and Hernández D., 2003. Stress response and growth in seedling developed from seeds irradiated with low intensity laser. Agrofaz, 3(1), 269-272.

Fernández M.J.L., Zelaya O., Cruz-Orea A., and Sanchez F., 2001. Phase transitions in amylose and amylopectin under the influence of $Ca(OH)^2$ in aqueous solution. Analytical Sci., 17, 338-341.

Fesquet J., Girautl B., and Razafindrandriatsimaniry M.D., 1984. Determination of absorption coefficients of thick semiconductor samples using photoacoustic spectroscopy. Appl. Optics, 23(16), 2784-2787.

Hernandez A.C., Carballo C.A., Artola A., and Michtchenko A., 2006. Laser irradiation effects on maize seed field performance. Seed Sci. Technol., 34, 193-197.

Hernandez A.C., Carballo C.A., Cruz-Orea A., Ivanov R., San Martin E., and Michtchenko A., 2005. Photoacoustic spectroscopy applied to the study of the influence of laser irradiation on corn seeds. J. Physique, 125, 853-855.

Hernandez A.C., Carballo C.A., Michtchenko A., and Lopez B.J., 2007. Pre-treatment laser light on maize seed vigor. Int. Ejournal Eng. Mathematics: Theory Appl., 1, 87-94.

Hernandez A.C., Carballo C.A., Cruz-Orea A., Ivanov R., and Dominguez-Pacheco A., 2008a. Optical absorption coefficient of laser irradiated wheat seeds determined by photoacoustic spectroscopy. Eur. Physical J., 153, 515-518.

Hernandez A.C., Mezzalama M., Lozano N., Cruz-Orea A., Martěnez E., Ivanov R., and Dominguez-Pacheco A., 2008b. Optical absorption coefficient of laser irradiated wheat seeds determined by photoacoustic spectroscopy. Eur. Physical J., 153, 519-522.

ISTA, **1993**. International rules for seed testing. Seed Sci. Technol., 21, 288.

Koper R. and Dziwulska A., 2003. Effects of laser stimulation of white lupine seeds (in Polish). Acta Agrophysica, 82, 99-106.

Koper R., Wojcik S., Kornas-Czuczwar B., and Bojarska U., 1996. Effects of the laser exposure of seeds on the yield and chemical composition of sugar beet roots. Int. Agrophysics, 10, 103-108.

Letokhov V.S., 1985. Laser biology and medicine. Nature, 316, 325-330.

Maguire D.J., 1962. Speed of germination, an aid in selection and evaluation for seedling emergence and vigour. Crop Sci., 2, 176-177.

Muszyński S. and Gładyszewska B., 2008. Representation of He-Ne laser irradiation effect on radish seeds with selected germination indices. Int. Agrophysics, 22, 151-157.

Ouf S.A. and Abdel-Hady N.F., 1999. Influence of He-Ne laser irradiation of soybean seeds on seed myclofora, growth, nodulation, and resistance to *Fusarium solani*. Folia Microbiol., 44, 388-396.

Paleg L.G. and Aspinall D., 1970. Field control of plant growth and development through the laser activation of phytochrome. Nature, 5275, 970-973.

Podleśny J., Misiak L., and Koper R., 2001. Concentration of free radicals in faba bean seeds after the pre-sowing treatment of the seeds with laser light. Int. Agrophysics, 15, 185-189.

Ramón-Gallegos E., Stolik S., Ponce-Parra C., López-Bueno G., Pérez-Zapata A.J., Calderón A., Munoz- Hernández R.A., Cruz-Orea A., Sánchez-Sinencio F., 2001. Photoacoustic spectroscopy applied to the study of photoporphyrin IX induced in mice. Analytical Sci., 17, 361-364.

Ruvinov A.N., 2003. Physical grounds for biological effect of laser irradiation. J. Physics, 36, 2317- 2330.

SAS, **2008**. Statistical Analysis System for Windows. Release 8.01. SAS Institute Press, Cary, NC, USA.

Shimizu-Sato, Enamul H., Tepperman J.M., and Peter Q., 2002. A light-switchable gene promoter system. Nature Biotechnol., 20, 1041-1044.

Smith H., 2000. Phytochromes and light signal perception by plants – an emerging synthesis. Nature, 407, 585-591.

Steele R., 2002. Review and forecast of the laser markets. Diode Laser. Laser Focus, 38, 61-80.

Steel R.D. G. and Torrie J.M., 1980. Principles and Procedures of Statistics. Mc Graw-Hill Press, New York, USA.

Szymer J. and Klimont K., 1999. The influence of laser biostimulation on the quality of French bean (*Phaseolus vulgaris* L.) seeds. Biul. IHAR, 210, 165-168.

Tekrony D.M., 2003. Precision is an essential component in seed vigour testing. Seed Sci. Technol., 31, 435-447.

Winkel B.S., 1998. Flavonoids in seeds and grains: physiological function, agronomic importance and the genetics of biosynthesis. Seed Sci. Res., 8, 415-422.