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EFFECTS OF 660 NM LASER IRRADIATION OF SOYBEAN SEEDS ON GERMINATION, EMERGENCE AND SEEDLING GROWTH

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A b s t r a c t. The aim of the experiments was to study the influence of irradiating soybean seeds (*Glycine max,* cultivar BRS 537) with a 660 nm laser diode array. The seeds were treated with laser light provided by a device assembled for the experiments with a power output of I = 3.5±0.2 mW cm^{-2}. The effects of biostimulation were analysed by determining the germination percentage and dry mass of normal seedlings in a germination experiment. Also, the seedlings emergence percentage, emergence rate and mean emergence time were determined in an indoor greenhouse experiment. The irradiation of the seeds with laser light showed the effects of positive biostimulation for two of the three treatments with light when compared to the control (without irradiation). The light dose of 1.6 J cm^{-2} (t = 457.14 s) significantly increased the germination percentage (5.5%), dry mass (58%) and emergence rate (29%) of normal seedlings and reduced the mean emergence time by 10%, while a dose of 3.2 J cm^{-2} (t = 914.28 s) significantly increased the dry mass (84%) and emergence rate (13%) at p-value ≤ 0.05.

K e y w o r d s: seed biostimulation, laser diode, soybean seeds, agriculture, indoor greenhouse

INTRODUCTION

In recent years, the chemical techniques applied in agriculture have been causing negative effects (Green 2019, Kumar *et al*. 2019). Among the problems caused by the current production model, the extensive and inappropriate use of pesticides stands out as one of the most harmful practices for the environment, as it is responsible for the contamination of rivers and groundwater (Evans *et al*. 2019). On the other hand, the development of technologies whose operating principles are based on physical methods (Govindaraj *et al.* 2017) rather than chemical methods should be considered over the next few years, with the aim of producing a more sustainable agriculture. In this context, physical methods such as light irradiation for seed biostimulation have emerged as a sustainable path with the potential to start changing the seed treatment field, this is due to the fact that it is based on the activation of photoreceptors by visible light and does not contribute to chemical waste generation (Hernández *et al.* 2010).

Through photoreceptors, plants are able to convert light energy from the sun into chemical energy that can be used during their stages of development. They can also direct plant growth towards light through a process known as photomorphogenesis (Jiao *et al.* 2007). Phytochromes are a family of photoreceptors that absorbs light at a wavelength that corresponds to the colour red (660 nm) when in their inactive form, and are reversibly interconverted to the active form that absorbs light at the far-red wavelength (730 nm) (Li *et al.* 2011, Jaedicke *et al.* 2012, Legris *et al.* 2019). Due the activation process of phytochromes, plants are able to distinguish different periods of the day based on the intensity and wavelength of the light they receive and direct their biological processes according to their needs, such as flowering and germination stages (Jiao *et al.* 2007, Legris *et al.* 2019).

According to Hernández *et al*. (2010), the biostimulation mechanism is the result of synergism between the monochromatic laser beam and the photoreceptors, which increases the bioenergetic potential of the plant. Some positive effects of this biostimulation process is the enhancement in some of the characteristics of the plant, like plant shoot and root length, dry mass, and also in chlorophyll a, chlorophyll b and carotenoid content (Alsahi *et al.* 2018). Moreover, the treatment of seeds with red light also has the potential to increase plant height (Osman *et al.* 2009), the emergence rate (Hernández *et al.* 2006) and the concentration of antioxidant enzymes such as superoxide dismutase (Asghar *et al.* 2016), which is an essential enzyme for cell antioxidant defence.

Dhakal *et al.* (2015) demonstrated that seedlings which germinated under the irradiation of red LED light and were inoculated with *Pseudomonas putida* on the fifth day of growth accumulated high levels of salicylic acid (SA) and presented upregulation of the pathogenesis-related gene *PR-1*, suggesting that red light induces resistance against bacterial rotting disease in seedlings, which is regulated by the SA-dependent pathway. The irradiation of seeds with red light also has the potential to protect the seedlings generated from these seeds against diseases caused by microorganisms, which may contribute to a reduction in the volume of pesticides used in the seed market in the future.

At present, soybean (*Glycine max* (L) Merrill) is one of the most important leguminous crops worldwide and it's highly recommended for human and animal consumption (Bischoff *et al.* 2016) due to its relatively high protein, carbohydrate, amino acid and oil content (Sharma *et al.* 2011). In Brazil, soybean is the most important crop, it generates is the highest income in its agricultural sector, at present, the country is the world's largest soybean exporter (Abraham *et al.* 2020).

The main objectives of this study were to analyse the effect of pre-sowing laser irradiation of soybean seeds on the germination percentage and dry mass of normal seedlings in a germination experiment, the emergence percentage, emergence rate and mean emergence time of normal seedlings were also measured in an indoor greenhouse experiment.

MATERIALS AND METHODS

The effects of laser biostimulation were analysed using the Brazilian soybean cultivar BRS 537 (with a productive cycle of 120 days). Before using the seeds, they were stored in a closed and previously sterilized box to prevent microorganism contamination. Furthermore, no pretreatment was performed on the seeds. A device containing an array of five 660 nm laser diodes was assembled for the experiments and the resulting power output was measured with a power meter (Coherent, USA) where I = (3.5±0.2) mW cm^{-2} (Fig. 1).

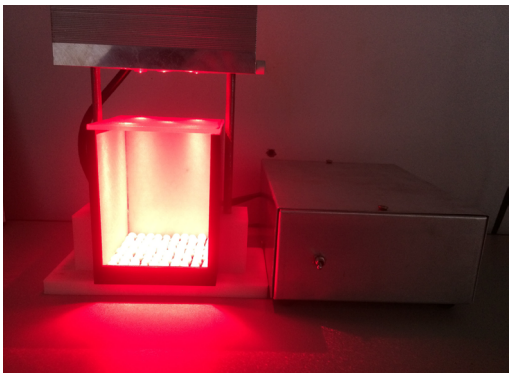

Fig. 1. Laser instrumentation used in the experiments. The equipment consists of five 660 nm laser diodes placed in an aluminium sink, an acrylic part to diffuse the light, a compartment capable of holding a hundred soybean seeds and a power source.

The seeds were positioned at a distance d = (130.02 ± 0.02) mm from the light source and irradiated on their upper surface in three different light doses: T1 (0.8 J cm^{-2}), T2 (1.6 J cm^{-2}) and T3 (3.2 J cm^{-2}). The control consisted of non-irradiated seeds. The irradiation parameters are shown in Table 1. To increase the precision of the irradiation process, the laser device was connected to a Raspberry Pi 3, which was responsible for calculating the irradiation time (t) based on the intensity (I) and the desired light dose (LD), and also for activating the equipment only at the appropriate irradiation time.

Table 1. Irradiation parameters of the treatments applied in the experiments. The precision of the irradiation time is due to the fact that the equipment was connected into a Raspberry Pi, which was only responsible for activating the equipment for the duration of the irradiation time, which was calculated using the intensity and light dose parameters

Treatments	Light Dose (J cm^{-2})	Intensity (mW cm^{-2})	Irradiation time (s)
Control	0	0	0
T1	0.8	3.5	228.57
T2	1.6	3.5	457.14
T3	3.2	3.5	914.29

As the light treatment finished, the seeds were sown in two different experiments. In both cases, the control consisted of non-irradiated seeds. The first experiment was performed in a Biological Oxygen Demand incubator (BOD incubator) to examine the effects of laser biostimulation on the germination percentage and dry mass of normal seedlings with the treatments T1, T2 and T3. The second experiment (Fig. 2) was performed in trays with commercial soil in an indoor greenhouse to only examine the emergence parameters for treatments T2 and T3, since treatment T1 did not affect the germination percentage of normal seedlings in the germination experiment, and therefore it was excluded from the second part of the study. The indoor greenhouse lighting consisted of 40 plant growth LED lamps and two white ceiling LED lights. The resulting light spectrum was measured with a spectrometer (Ocean Optics, USA) (Fig. 3). The greenhouse illumination intensity was measured with a lux meter (Minipa, Brazil) at different points of the cultivation bench and since it was not homogeneous (I = 14303±903 lux), the trays were positioned randomly along the bench.

Fig. 2. Indoor greenhouse laboratory with artificial lighting used in the tray experiment.

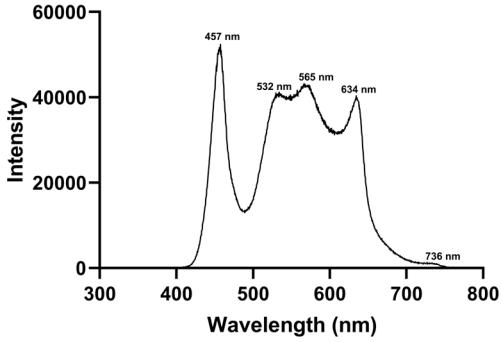

Fig. 3. Artificial lighting spectrum of the indoor greenhouse laboratory. The spectrum has peaks at 457, 532, 565 and 634 nm, and a very small peak at 736 nm (infrared LED of the plant growth LED lamps).

The experiments were carried out using the Completely Randomized Design (CRD) method. The data for all of the parameters were statistically analysed with an analysis of variance (ANOVA) and the treatments that presented significant differences were distinguished using Tukey's test with 5% significance (p-value ≤ 0.05).

Germination percentage and dry mass

After light irradiation, 100 seeds were placed on two germination papers, and then the seeds were covered by a third sheet of paper and moistened with distilled water in the proportion of 2.5 times the dry weight of the paper, following the Brazilian Rules for the Analysis of Seeds (Brasil, 2009). The papers were rolled up in order to make a germination roll, placed in a plastic bag to avoid humidity loss and then stored in a BOD incubator (Eletrolab EL212/4, Brazil) at 25°C, 60% humidity with a photoperiod of 12 h light/12 h darkness for 7 days. Four replicates were used for each treatment. The germination percentage (%) was obtained by counting the number of normal seedlings in a germination roll after 7 days of incubation and the error was calculated using the standard deviation of the four replicates. For each of the four replicates, 25 normal seedlings were randomly selected and stored in paper bags inside a drying oven at 60°C for three days. After this period, the dry mass (mg) of the 25 seedlings was weighed using an analytical balance and the value obtained was divided by 25, thereby revealing the average dry mass of one seedling. The standard deviation also represented the error in this case.

Emergence rate, emergence percentage and mean emergence time

After light irradiation, 35 seeds were sown in trays with the commercial soil BioPlant and irrigated with 100 mL of water every two days for a 7-day period. Moreover, the indoor greenhouse temperature was set at 25°C and the photoperiod was artificially illuminated. Four replicates were used for each treatment and the standard deviation represented the error. The number of seedlings which emerged from the soil surface was counted every day at the same time for the entire duration of the experiment. The emergence percentage (%) for each day is obtained by multiplying by 100 the number of seedlings which emerged from the soil surface from the beginning of the experiment until the day on which the seedlings were counted and then dividing the result by 35 (total number of seeds per replicate). According to Ranal *et al.* (2006), the emergence rate (adimensional) is calculated using Eq. (1):

$$EmergenceRate = \sum_{i}^{t} \frac{n_i}{t_i} \tag{1}$$

In Eq. (1), n_i is the number of seedlings that emerged from the soil until day i and t_i represents the number of days which passed since the beginning of the experiment. The mean emergence time (days) were calculated using Eq. (2) according to Carvalho *et al*. (2009):

$$MeanEmergenceTime = \frac{\sum_i^t n_i t_i}{\sum_i^t n_i} \quad (2)$$

In Eq. (2), n_i is the number of seedlings that emerged from the soil on day i and t_i represents the number of days after the beginning of the experiment.

RESULTS

The germination percentage of the normal seedlings was 5.5% higher for the treatment with a light dose of 1.6 J cm^{-2} (T2) when compared to the control (Fig. 4).

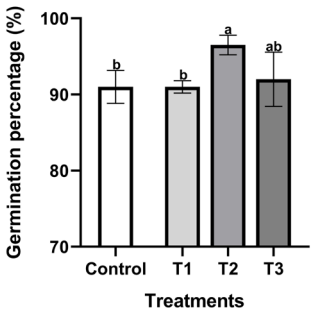

Fig. 4. Germination percentage (%) of normal seedlings in the germination experiment for three different light doses: T1 (0.8 J cm^{-2}), T2 (1.6 J cm^{-2}) and T3 (3.2 J cm^{-2}). The control consisted of non-irradiated seeds. Mean values with the same letter above the bars do not differ significantly in terms of p-value ≤ 0.05.

Table 2. Germination percentage (GP: %), seedling dry mass (DM: mg), emergence rate (ER: adimensional), mean emergence time (MET: days) and emergence percentage (EP: %) of normal seedlings from day 4 (when emergence began) to day 7. Mean values with the same letter within the same column do not differ significantly at p-value ≤ 0.05

Treatments	GP (%)	DM (mg)	ER (adimensional)	MET (days)	EP (4 days) (%)	EP (5 days) (%)	EP (6 days) (%)	EP (7 days) (%)
Control	91.0 b	17.1 c	14.0 b	5.24 a	17.1 a	61.4 ab	68.8 b	83.6 a
T1	91.0 b	13.0 d	–	–	–	–	–	–
T2	96.5 a	27.1 b	18.0 a	4.71 c	44.3 a	72.9 a	80.7 a	86.4 a
T3	92.0 ab	31.4 a	15.8 ab	4.98 b	35.0 a	58.6 b	75.7 ab	83.6 a
F	5.61	127.78	4.50	20.05	3.97	4.73	10.35	0.2428
p-value	0.0122	0.0001	0.0441	0.0005	0.0582	0.0395	0.0046	0.7894
$CV_{(\%)}$	2.39	6.85	12.06	2.36	43.11	10.79	5.05	7.97

Despite the small increase in this parameter due to the light dose, a biostimulation effect on the germination of the seeds was observed (Table 2). For T1 (0.8 J cm^{-2}), the irradiation of soybean seeds with laser light did not significantly affect the germination percentage of normal seedlings in the germination experiment. The germination percentage of treatment T3 was statistically similar to that of treatment T2 after the application of Tukey's test because the standard deviation of the four replicates was too high in this case.

Considering the three different treatments with laser light, two of them were responsible for causing seedling growth after 7 days in the germination experiment. The T3 treatment achieved the largest increases in dry mass values for normal seedlings and was capable of increasing this parameter by 84% when compared to the control seedlings, followed by the T2 treatment which caused increases of 58% (Fig. 5).

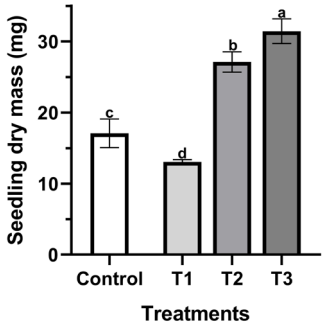

Fig. 5. Dry mass (mg) of normal seedlings in the germination experiment for three different light doses: T1 (0.8 J cm^{-2}), T2 (1.6 J cm^{-2}) and T3 (3.2 J cm^{-2}). The control consisted of non-irradiated seeds. Mean values with different letters above the bars differ significantly in terms of p-value ≤ 0.05.

These increases may be observed in soybean seedlings generated from the seeds of treatments T2 and T3, which presented longer hypocotyls and primary roots, and the greater development of secondary roots (Fig. 6).

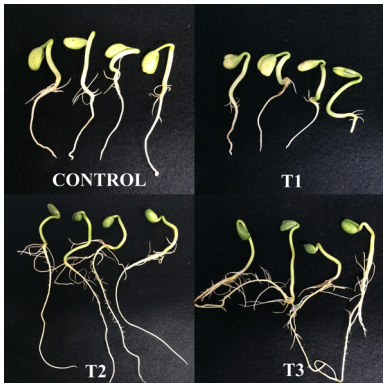

Fig. 6. Representative soybean seedlings of each treatment obtained in the germination experiment.

Irradiating soybean seeds with a light dose of 0.8 J cm^{-2} (T1) decreased the average dry mass of the normal seedlings in this experiment by 24%, suggesting that the biostimulation process may result in negative effects as already reported by (Hernández *et al.* 2005, 2010). Positive and null effects were also reported by the authors (Hernández *et al.* 2005, 2009, 2010).

The emergence percentage in the indoor greenhouse trays experiment was calculated from the fourth day after sowing (when emergence began) to the seventh (Fig. 7). Treatment T2 (1.6 J cm^{-2}) led to the highest values of emergence percentage since the fourth day, when its value was about 2.5 times larger than the control but without significant differences (high standard deviation), up to the seventh day. However, there were no significant differences between all the treatments after the first 7 days of the experiment, once the emergence percentage of the control seedlings reached a value close to the ones of the treatments with light (Table 2).

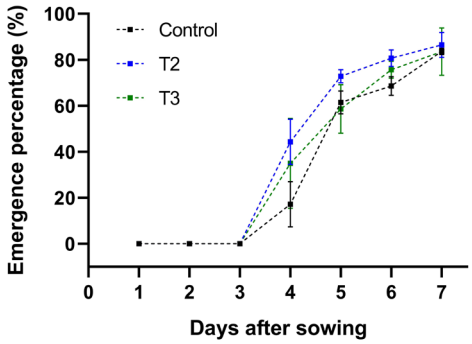

Fig. 7. Emergence percentage (%) of normal seedlings over 7 days in the tray experiment using indoor greenhouse for two different light doses: T2 (1.6 J cm^{-2}) and T3 (3.2 J cm^{-2}). The control consisted of non-irradiated seeds. The standard deviation (SD) of four replicates was used as an error bar.

The emergence rate was 29% higher for the seedlings of treatment T2 when compared to the control (Fig. 8). Treatment T3 also resulted in seedlings capable of emerging from the soil sooner than the control seedlings, with increases of 13% in the emergence rate. Once the light dose increased to 3.2 J cm^{-2} in treatment T3, the emergence rate declined by almost 14% when compared to the results obtained by using the light dose T2, thereby suggesting that perhaps the emergence rate requires lower light doses in order to obtain better results (Table 2).

Since treatment T2 presented the highest value for the emergence rate, seedlings from this treatment emerged faster from the soil when compared to the other treatments, thus reducing the mean emergence time (Fig. 8). In this case, the mean emergence time was reduced from 5.24 days (control seedlings) to 4.71 days, a 10% reduction that corresponds to half a day. As in the previous case, treatment T3 was also capable of significantly reducing the mean emergence time but in a less impactful way (5% reduction).

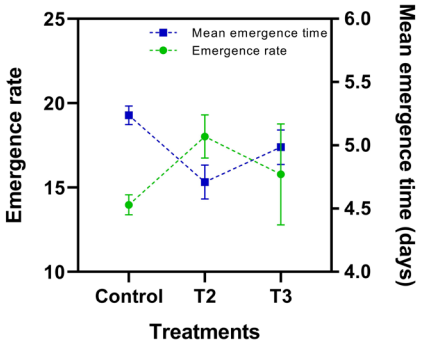

Fig. 8. Emergence rate (adimensional) and mean emergence time (days) of normal seedlings in the indoor greenhouse trays experiment at two different light doses: T2 (1.6 J cm^{-2}) and T3 (3.2 J cm^{-2}). The control consisted of non-irradiated seeds. The standard deviation (SD) of four replicates was used as an error bar.

DISCUSSION

The biostimulation effects caused by red light irradiation were first reported by Wilde *et al.* (1969) five decades ago when they managed to accelerate the germination and seedling growth processes through the irradiation of seeds with laser light as mentioned by Hernández *et al*. (2010). Since then, numerous studies in the seed biostimulation area have been carried out in order to better understand the effects caused by this physical method and also to determine the phytochrome mechanisms which are involved (Hernández *et al.* 2010, 2016, Asghar *et al.* 2017, Li *et al.* 2011, Jaedicke *et al.* 2012, Wang *et al.* 2003).

A He-Ne laser (632.8 nm) was used by Muszyñski *et al*. (2008) to irradiate radish seeds with a light dose of 1.27 J cm^{-2} and the authors observed small increases (9%) in the germination percentage as obtained in our experiments (5.5%). Ri *et al.* (2019) obtained an increase of 3.1% in the germination percentage of rice seeds (*Pyongyang-53*) irradiated with a laser diode of 650 nm for 0.28 seconds (5 mW). However, Aftab *et al*. (2020) demonstrated that the irradiation of wheat seeds with two 630 nm diode lasers at a light dose of 1.2 J cm^{-2} was capable of increasing the germination percentage by 25%, suggesting that different species of seeds require different light dose optimization processes, since the wavelength of the light and the dose were very similar in both cases. Sacala *et al*. (2012) investigated the biostimulation process through the irradiation of sugar beet seeds with 670 nm laser light and their results showed that this physical method was capable of increasing the dry mass of the roots of plants whose seeds were treated with a light dose of 1.75 J cm^{-2}, thereby supporting the results obtained for seedling growth in our experiments. Increases in dry matter content were also observed by Mozdzen *et al.* (2020) when the authors

treated triticale grains (x *Triticosecale* Wittm. ex A. Camus) with He-Ne laser light for 3 hours and by Podleśna *et al.* (2015) in pea plants (*Pisum sativum*) whose seeds were also treated with He-Ne laser light. Janayon *et al.* (2019) treated mung bean seeds (*Vigna radiata* L.) for 2 minutes (632.9 nm, 10 mW) and observed an increase of 29.2% in seedling mass, which corroborated our results indicating the effect of laser irradiation in the early stages of growth.

Hernández *et al.* (2006) treated *Zea mays* seeds with a GaAlAs laser (660 nm) with a light dose of 1.2 J cm^{-2} and observed increases of 43% in the emergence rate. This light dose is very close to that of the T2 treatment (1.6 J cm^{-2}) which showed the best results in the emergence experiment, but the increased value (29%) was smaller than that reported by the authors who used another seed species. Others authors also demonstrated through other research results that the irradiation of wheat seeds with 660 nm laser light increased the length of seedlings stems by 12% when compared to the control seedlings after a light dose optimization process that included changing equipment irradiance (Hernández *et al.* 2017). For leguminous plants, Podleśna *et al.* (2003) studied the effects of fava bean and white lupine seed irradiation on emergence and discovered that the laser irradiation process could accelerate the dynamics of emergence in both cases when compared to the control (non-irradiated seeds). Other authors observed that non-irradiated pea seeds presented the same dynamic of emergence as seeds irradiated with a He-Ne laser, but with a delay of 2 days from the beginning of the emergence process, which demonstrates an increase in the emergence rate of the irradiated seeds (Podleśna *et al.* 2015).

The early stages of plant development are very important for the healthy growth of the plant in the following stages. Seed vigour is a commonly used factor in the seed industry for measuring their quality and biological potential (Wang *et al.* 2020). Since this factor is calculated using parameters such as the germination percentage and the hypocotyl/primary root length, new studies must be conducted in order to better understand how the laser irradiation of seeds increases their vigour and will soon become a new technology in the seed treatment field. Moreover, the irradiation of seeds with 660 nm laser light seems to induce resistance against diseases caused by microorganisms in seedlings (Dhakal *et al.* 2015), which could be an interesting and sustainable area of research, since it is a very common practice in seed processing in Brazil to treat seeds with fungicides to prevent contamination with soil fungus like *Rhizoctonia solani*, *Fusarium spp*., *Colletotrichum truncatum* and many others (França Neto *et al.* 2010, Hernández *et al.* 2011). Furthermore, irradiating seeds with red laser light seems to improve the nutritional value of seedlings through the accumulation of vitamins, minerals, pigments and antioxidants, and is also capable of boosting both the antioxidant capacity and anti-inflammatory activities through the inhibition of cyclooxygenase-2 and lipoxygenase activities, which could be a promising approach in the food industry as reported by (Almuhayawi *et*

al. 2020). More recently, Dziwulska-Hunek *et al.* (2020) treated sweet corn seeds of three different cultivars with red light from two halogen floodlights and two of the three cultivars treated with the same light dose presented increases of 25% in production yield (ton ha^{-1}) and in the cob length, thereby demonstrating that laser irradiation of seeds can increase productivity in agriculture. In this way, new studies concerning the activation of the phytochrome must be performed with the aim of consolidating the laser biostimulation methods for agriculture, in particular, in the seed treatment field. Moreover, the particular effects of light coherence provided by laser light must be studied in order to understand how this particular factor can influence the biostimulation processes, since the development of large scale irradiation equipment is most likely to occur through the use of non-coherent light sources like LEDs, since they provide good quality light and they are not expensive like diode lasers. However, laser irradiation could serve a useful purpose both at the present time and in the near future (Hernández *et al.* 2016).

CONCLUSIONS

1. 660 nm laser irradiation is capable of increasing the germination potential of seeds when the light dose is adequate.

2. Some of the positive effects of laser biostimulation occurs at the initial stages of growth of seedlings, and increases the rate of development of seedling structures and consequently their dry mass.

3. The irradiation of seeds with 660 nm laser light can result in negative growth effects when the light dose is not adequate.

4. The results indicate that the pre-sowing laser irradiation of seeds can increase the emergence rate of seedlings, thus reducing the mean time they require to emerge from the soil.

Conflict of interest: The authors declare no conflict of interest.

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