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The effect of low-intensive coherent seed irradiation on germinant growth of Scots pine and sugar beet

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Abstract: The success of forest and agricultural plant establishment program mainly depends on the quality of reproductive material. The study intends to offer engineers and farm owners a solution for small-size seed improvement before sowing. The effect of low-intensity coherent light on the seeds of various crops is theoretically and empirically hypothesized. The seedlots of Scots pine (*Pinus sylvestris* L.) and sugar beet (*Beta vulgaris* L.) of Russian diploid hybrid RMS-127 were germinated in a controlled environment. The germinants were produced from six seed fractions, previously irradiated with $1.274 \text{ W}\cdot\text{m}^{-2}$ at the 632.8 nm wavelength with 1, 2, 3, 5, 10, 15 min exposure to a standard laser system, plus untreated control. Pine germinants were measured on day 15, beetroot on day 10 after germination. An increase in exposure time reduced Scots pine germination energy and capacity, while for sugar beet the results were not conclusive. On the contrary, increasing the exposure time had a positive effect on both the height and biomass growth of both Scots pine and sugar beet germinants. The 10-min exposure time resulted in maximum values for sugar beet height and biomass and Scots pine height, while the 15-min exposure time produced maximum Scots pine biomass.

Keywords: seed light treatment; *Beta vulgaris*; *Pinus sylvestris*; seed germination; small-size seeds

Pre-sowing treatments affect the plant ontogenesis by changing the seed quality. Optical irradiation can change the germination of seeds due to activation or inhibition of physiological processes by photon energy (Salmia 1980), causing stress or improved growth. Optical radiation is an important element in evaluating (Novikov 2019; Novikov et al. 2019a, 2020) and biophysical activation of seeds. Seed activation can have a particular effect on the success of performance of seedlings in nurseries (Ivetić, Novikov 2019), planting (Novikov, Ivetić 2019; Novikov et al. 2019c), direct seeding (Grossnickle, Ivetić 2017), and aerial seed-

ing (Novikov, Ersson 2019). The light provides not only energy, but also important regulatory functions, driving various processes, including the gene expression. The basis of light regulation is the resonant absorption of photons by specific chromoproteins of higher plants, such as phytochrome and cryptochrome. The fundamental works of Fernbach and Mohr (1990), Konev and Volotovskiy (1979), and Voskresenskaya (1988) set the ways of transforming the light signal into a chemical signal, affecting the cell metabolism. In general, photoregulatory processes are well studied, but it is still unclear how ultra-weak photon streams

of biochemiluminescence participate in intercellular communication, and what causes the high biological efficiency of coherent radiation. Budagovsky studied the reaction of biological objects to laser irradiation in the absorption spectral ranges of 350–500 nm (cryptochrome) and 600–690 nm (phytochrome) and found that “the maximum stimulation effect varies in the range from fractions of a second to tens of minutes” (Budagovsky 2008), indicating the potential use in a rapid diagnostics of seeds. According to prof. Muluaem Tigabu (Swedish University of Agricultural Sciences, Southern Swedish Forest Research Centre, Alnarp, Sweden), “epigenetic responses could be detected in seeds in the range between 789 and 960 nm”.

Up to date, the influence of coherent laser radiation on the seeds and cuttings of about 100 species of tree crops has been investigated. Among the studied cultures, there was not a single breed that did not respond to laser irradiation to some extent. For example, the field germination was increased for Crimean pine and sycamore by 10%, *Robinia*, *Catalpa*, and *Cercis* by 20%, and black walnut by 36% (Maksimenko et al. 2020). Laser treatment of seeds increased the germination of rhododendron seeds 2–4 times (Skvarko 1997), as well as the weight and length of shoots of *Caragana*, *Gleditsia*, and *Robinia* (Aladjadjiyan 2003). Laser irradiation of ordinary lilac seeds had a positive effect on the growth and development of plants and improved their decorative properties (Sagitova, Dzevitskaya 1984).

The main objective of the study is a comparison of the effects of low-intensity coherent laser light on the seeding quality of seeds and the biomass of seedlings of agricultural and forest plants.

This situation is especially relevant for small-seeded crops, such as Scots pine and sugar beet, since the small content of spare substances in them can cause significant fluctuations in the sowing characteristics in the positive or negative direction, even with a slight exposure to radiation doses.

MATERIAL AND METHODS

Samples. Scots pine (*Pinus sylvestris* L.) seeds were obtained from cones collected in autumn 2019 from a natural forest stand of the Voronezh region, Russia. After extraction from the cones, the seeds were dewinged (Figure 1) and processed on a gravity separator (Novikov, Saushkin 2018; Novikov, Ivetic 2019; Novikov et al. 2019b, c). The samples were stored in glass jars with tightly closed lids at a temperature from 5 to 8 °C and humidity of 5–8% before the research. Before the experiment, the seeds were kept 24 h in the laboratory at a temperature of 20 °C. The moisture content of seeds before the experiment was 10–12% due to their hygroscopic properties. For the experiment seeds from the seedlot were separated into three fractions according to their spectrometric properties. The optical criterion for seed separation in the wave range of 650–715 nm (Novikov et al. 2021) is presented in Table 1.

Sugar beet (*Beta vulgaris* L.) seeds (Figure 1) of the RMS-127 hybrid selected by the All-Russian Research Institute of Sugar Beet and Sugar named after A. L. Mazlumov were obtained in 2019 under irrigation conditions in the territory of the Republic of Crimea. Sampling and storage of samples before the research were carried out in canvas bags in the laboratory at room temperature, according to GOST 22617.0-77. The humidity of the received

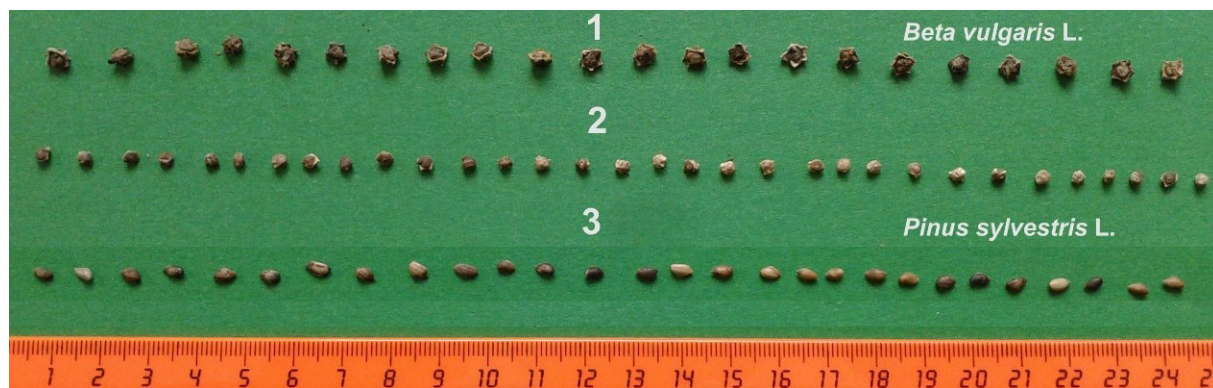


Figure 1. The samples of Scots pine and sugar beet seeds used in this study. The figure shows natural (1), sanded (2) sugar beet seeds, and dewinged (3) Scots pine seeds

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Table 1. Colour and mass characteristics of seed samples used in the study

Samples	Optical separation criteria in the 650–715 nm wavelength range (Novikov et al. 2021)	Colour classification of Mansell	Mass of 1 000 seeds (g)
<i>Pinus sylvestris</i> L.			
CTRL P	–	–	7.10
D	0.0706–0.1549	7.3YR 2.6/1.7	7.42
L	0.1871–0.3010	4.9 Y 7.5/4.2	8.15
LD	0.3468–0.5229	9.8YR 6.0/4.1	7.60
<i>Beta vulgaris</i> L.			
CTRL B	–	–	8.43

CTRL P – bulk; D – dark; L – light; LD – light-dark; CTRL B – control

seeds was determined immediately before the experiment. The seeds were brought to a completely dry state in a drying cabinet at a temperature of 130 °C for one hour, according to GOST 22617.3-77. Before conducting the experiment, the seed moisture content was between 10 and 12%. Sanding of seeds (Figure 1) was carried out on a laboratory installation to the degree of the pericarp removal 40–60%.

Seed processing. Before biophysical activation, an experiment was performed to determine the germination energy and laboratory germination of seeds not exposed to Low-intensity Coherent Radiation (LCR), as well as to determine the parameters of the biomass of seedlings obtained from them – the height of the germinant in centimetres and the mass of the germinant in grams. The experiment was performed with four variants of Scots pine seeds (seed fractions of three colours dark (D), light (L), light-dark (LD) and bulk control (CTRL P) and one variant of sugar beet seeds (CTRL B) in the following order. The mass of 1 000 seeds was determined of each sample group (see Table 1). Next, the germination rate and energy of germination were determined.

The mass of 1 000 seeds of pine and beet is comparable in values, the spectrometric group D of Scots pine seeds of a given year of harvest and origin has the lowest mass value, and the spectrometric group L – the largest.

Determination of germination of Scots pine seeds was carried out in accordance with Government Standard of the Russian Federation (GOST) 13056.6-97 “Seeds of Trees and Shrubs. Method for Determination of Germination”. Determination of germination energy and laboratory germination of sugar beet seeds was done in accordance with GOST 22617.2-94 “Sugar Beet

Seeds. Methods for Determination of Seed Germination, monogermity and Quality”. Standards developed at the country level are harmonized with the international ISTA standards in terms of the general test methodology.

The determination of biomass was performed simultaneously with the determination of germination by measuring the germinant length (in cm) using a ruler and the germinant mass (in 10^{-3} g) using an electronic scale.

Then the seeds from the same batch were activated by Low-intensity Coherent Radiation (LCR) with an exposure for 1, 2, 3, 5, 10 and 15 min. The source of low-intensity coherent radiation was the LOS-25A device (Budagovsky 2008), illustrated in Figure 2. The light output power from module 3 is 15 mW, from module 4–10 mW. Diameter of the visible spot is 0.1 m, a distance between the optical hinge 4 and the seed surface – 0.4 m (established experimentally). Power density was calculated as $1.274 \text{ W}\cdot\text{m}^{-2}$. Before the experiment, the device was warmed up for 0.5 h.

Data processing. The main indicators of descriptive statistics were calculated for the distribution of seed energy, seed germination, germinant heights and biomass (Tables 2–5). Mean values were separated using Tukey’s HSD test for unequal number of samples, with a significance level of alpha of 0.01.

RESULTS

The results of determining the seeding qualities of seeds and parameters of the germinant biomass before LCR-activation are shown in Table 2 and 3, respectively.

The germination energy of beet seeds not treated with laser light (Table 2) is comparable with

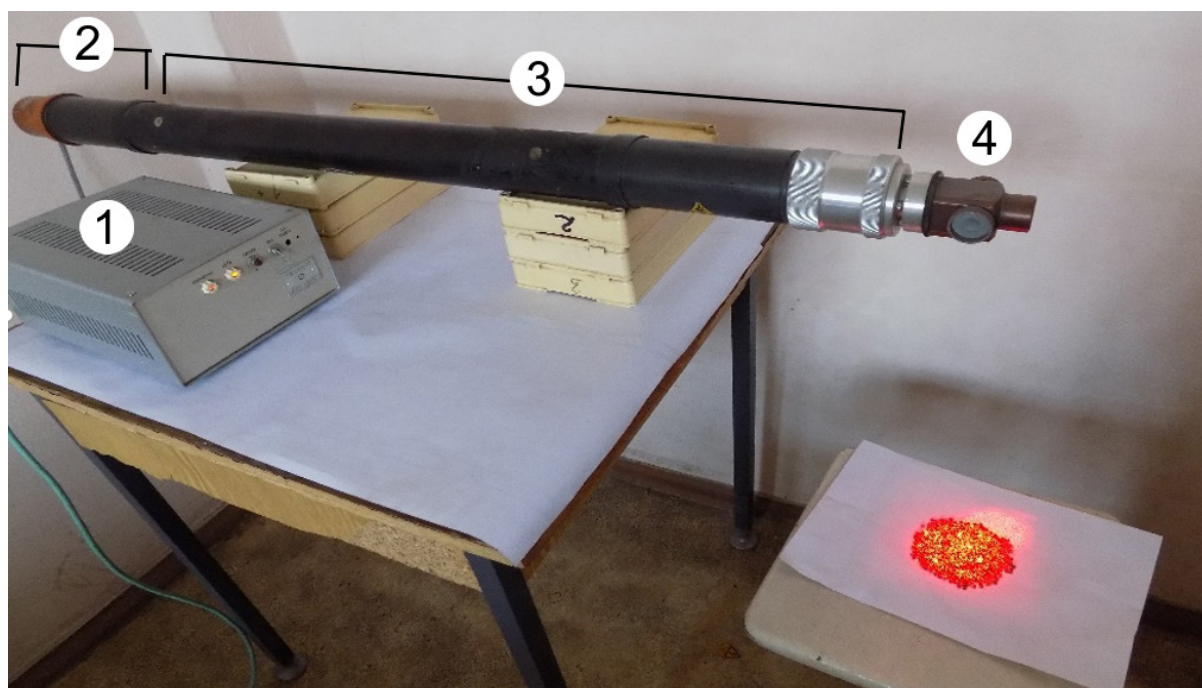


Figure 2. The light treatment device used in the study

1 – power supply module; 2 – module for a cooling and cleaning illuminator; 3 – module for generating optical radiation with a lens system and a low-intensive coherent light source (wavelength of 632.8 nm); 4 – optical hinge with an illuminator

Table 2. Sowing qualities of Scots pine and sugar beet seed samples before LCR-activation

Samples	Energy		Germination	
	(%)	percentage to the control	(%)	percentage to the control
<i>Pinus sylvestris</i> L.				
CTRL P	82	100	96	100
D	80	97.6	94	97.9
L	78	95.1	96	100
LD	88	107.3	96	100
<i>Beta vulgaris</i> L.				
CTRL B	85	100	91	100

CTRL P – bulk; D – dark; L –light; LD – light-dark; CTRL B – control

Table 3. Parameters of Scots pine and sugar beet germinant biomass before LCR-activation; mean values followed by different letters are statistically different

Samples	Germinant height (mean \pm SD, cm)	Germinant mass (mean \pm SD, 10^{-3} g)
<i>Pinus sylvestris</i> L.		
CTRL P	3.55 ^a \pm 0.99	31 ^a \pm 8
D	3.35 ^a \pm 0.78	27 ^a \pm 6
L	3.87 ^b \pm 0.65	32 ^a \pm 6
LD	3.58 ^a \pm 0.78	30 ^a \pm 7
<i>Beta vulgaris</i> L.		
CTRL B	3.45 ^a \pm 0.62	28 ^a \pm 5

CTRL P – bulk; D – dark; L –light; LD – light-dark; CTRL B – control

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Table 4. Sowing qualities of LCR-activated Scots pine and sugar beet seeds

LCR-Exposition (min)	Spectrometric		Energy		Germination	
	group	(%)	percentage to the control	(%)	percentage to the control	
<i>Pinus sylvestris</i> L.						
Control P		82	100	96	100	
1	D	82	100	93	96.8	
	L	85	103.7	96	100	
	LD	84	102.4	94	97.9	
2	D	80	97.6	90	93.8	
	L	79	96.3	92	95.8	
	LD	86	104.9	92	95.8	
3	D	76	92.7	90	93.8	
	L	74	90.2	96	100	
	LD	80	97.6	90	93.8	
5	D	75	91.5	82	85.4	
	L	71	86.6	84	87.5	
	LD	78	95.1	84	87.5	
10	D	72	87.8	84	87.5	
	L	70	85.4	85	88.5	
	LD	76	92.7	86	89.6	
15	D	64	78.0	80	83.3	
	L	62	75.6	81	84.4	
	LD	70	85.4	82	85.4	
<i>Beta vulgaris</i> L.						
Control B		85	100	91	100	
1		86	101.2	89	97.8	
2		86	101.2	92	101.1	
3		76	89.4	83	91.2	
5		83	97.6	89	97.8	
10		70	82.4	79	86.8	
15		86	101.2	90	98.9	

D – dark; L –light; LD – light-dark

the germination energy of Scots pine seeds, while compared to CTRL P, seeds of the LD spectrometric group germinate 7.3% and more uniformly, while those of the L group grow 5.1% more slowly. The germination of pine seeds is slightly higher than the germination of beet seeds, and the values of the germination of the spectrometric groups D, L, LD are almost levelled with CTRL P.

Germinants obtained from the Scots pine seeds of the spectrometric group L showed the highest height with the smallest standard deviation (SD) among the spectrometric groups (Table 3). At the same time, the mass of the L germinants statistically slightly exceeds the mass of the CTRL

P germinants. The division of Scots pine seeds into spectrometric groups reduces the spread of SD values compared to CTRL P samples. Beet seeds have the lowest germinant height and weight, which is statistically slightly different from all other groups.

The results of determining the seeding qualities of seeds and parameters of the germinant biomass after LCR-activation are shown in Table 4 and 5, respectively.

For pine seeds with increased exposure, as well as for beet seeds, the germination energy decreases from 82 to 62% at 1 min and 15 min of LCR exposure, and the germination rate decreases from 93 to 81%. The lowest germination is demonstrated by the seeds

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Table 5. Parameters of the biomass of seedlings obtained from LCR-activated seeds of Scots pine and sugar beet; mean values followed by different letters are statistically different

LCR-Exposition (min)	Spectrometric group	Germinant height (mean ± SD; cm)	Germinant mass (mean ± SD, 10 ⁻³ g)
<i>Pinus sylvestris</i> L.			
Control P		3.55 ^a ± 0.99	31 ^a ± 8
1	D	3.13 ^a ± 0.66	28 ^a ± 9
	L	2.94 ^b ± 0.69	27 ^a ± 6
	LD	2.81 ^b ± 0.61	23 ^b ± 6
2	D	3.23 ^a ± 0.84	26 ^a ± 9
	L	2.90 ^b ± 0.46	22 ^b ± 4
	LD	3.09 ^b ± 0.75	23 ^b ± 6
3	D	3.41 ^a ± 0.58	22 ^b ± 4
	L	3.39 ^a ± 0.47	24 ^b ± 4
	LD	3.01 ^b ± 0.58	20 ^b ± 8
5	D	3.00 ^b ± 0.59	21 ^b ± 6
	L	3.31 ^a ± 0.49	24 ^a ± 5
	LD	3.49 ^a ± 0.53	24 ^a ± 4
10	D	4.39 ^c ± 0.53	33 ^a ± 6
	L	4.91 ^c ± 0.48	35 ^a ± 6
	LD	4.7 ^c ± 0.59	36 ^a ± 9
15	D	3.40 ^a ± 0.67	39 ^c ± 11
	L	4.31 ^c ± 0.54	41 ^c ± 6
	LD	3.94 ^a ± 0.77	47 ^c ± 16
<i>Beta vulgaris</i> L.			
Control B		3.45 ^a ± 0.62	28 ^a ± 5
1		3.02 ^b ± 0.62	24 ^a ± 7
2		3.08 ^b ± 0.66	24 ^a ± 6
3		3.25 ^a ± 0.53	20 ^b ± 4
5		3.32 ^a ± 0.53	23 ^a ± 4
10		4.57 ^c ± 0.55	35 ^c ± 5
15		4.12 ^c ± 0.78	42 ^c ± 9

D – dark; L –light; LD – light-dark

of the common pine of the spectrometric group L processed for 15 min, and beet seeds processed for 10 min. With the exposure of beet seeds to LCR, the germination energy changes in a wavelike manner with increasing exposure, reaching maxima at 5 and 15 min and minima at 3 and 10 min. A slight excess of the values compared to CTRL B is observed at exposures of 1, 2, and 15 min. The energy of beet seeds is lower (by 13.2%) relative to CTRL B at an exposure for 10 min, at other timings it is within the control group.

The highest values of the average parameters of the germinant height and weight in both beet seeds and pine seeds are observed at a 10-min exposure. For the spectrometric group L of Scots pine

seeds the height is 4.91 ± 0.48 cm, and the mass is 35 ± 6 mg. Statistically significantly lower than the CTRL P of Scots pine is the germinant height for samples 1L, 1LD, 2L, 2LD, 3LD, 5D, as well as the germinant weight in groups 1LD, 2L, 2LD, 3LD, 5D, where the number indicates the exposure time in minutes, and the letter indicates the spectrometric groups according to Table 1. The germinant height for samples 10D, 10LD, 10L, 15L, as well as the germinant weight in groups 15D, 15L, 15LD are statistically significantly higher than the indicators of the Scots pine CTRL P group. Relative to the beet germinants of the control group, the germinants obtained from seeds with LCR treatment at an exposure for 10 and 15 min are sta-

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tistically significantly taller in height, and the germinants obtained from seeds with LCR treatment at an exposure for 10 and 15 min are heavier by weight. The values of the germinant height at 1 and 2-min exposure, and the values of the germinant mass at 3-min exposure are statistically significantly lower compared to CTRL B.

DISCUSSION

The light energy applied by the laser to seed is converted into photochemical energy and stored until it is going to be consumed during germination and subsequent growth and development of plants (Chen et al. 2005). According to Chen et al. (2005), the helium-neon laser increases enzyme activity and accelerates enzyme-mediated reactions due to the electromagnetic field and thermal energy, which affect the molecules in the cell and enhance their biological activity. This leads to an increase in the initial seed energy during germination (Chen et al. 2005).

Moskvin (2008) found those local thermodynamic disturbances that cause a chain of changes in calcium-dependent physiological reactions, act as the primary active factor when plant organs and tissues are exposed to LCR. However, the direction of these reactions may be different, since it is influenced by the dose, localization of laser exposure and the initial state of the plant organism itself (Moskvin 2008).

Studies conducted on seeds of agricultural crops also showed a positive effect of laser radiation on their sowing qualities: the germination rate and drought resistance of plants (wheat) (Hernandez-Aguilar et al. 2009; Qiu et al. 2013); yield up to 12% and increased growth of the vegetative mass of peas (Podlesna et al. 2015); the size of maize cobs increased (Srećković et al. 2014); dry and raw hypocotyl mass in seedlings and the root system of bean plants (Podlešny et al. 2012).

Since 1980, several researches with laser radiation have been conducted on sugar beet. Preliminary laser treatment of sugar beet seeds with their subsequent soaking in a solution of trace elements on average for 3 years increased the yield of root crops by 62.1–70.0% and sugar content by 0.6–2.2% (Gnilomedov, Kalugina 1984). After exposure of sugar beet seeds to laser, it was possible to isolate 2 diploid forms that exceed the initial materials by 1.8–2.5% in sugar content over

two generations, with the same or slightly higher weight of root crops (Plokhii, Matsutsina 1985). Other researchers have also noted an increase in the yield of up to 13% and sugar content of sugar beet (Brizhansky 2015). More recent studies (Podvigina, Nechaeva 2019b; Podvigina et al. 2019) of the effects of laser radiation on seeds of various fractions (4.5–5.5 mm; 3.5–4.5 mm; less than 3.5 mm) and sugar beet hybrids (RMS-127, Ramosa, Cascade, RMS-120, RMS-121) proved the stimulating effect of photoactivation: seed germination energy increased by 3.0%, germination by 4.0%. A significant increase in the seedling height and germinant mass was determined when processing seeds of 3.5–4.5 mm fraction with a laser with an exposure for 30–180 s. It was found that the optimal time of seed exposure to LCR to increase the germination energy is 1 min for seeds of the 4.5–5.5 mm fraction and 2 min for seeds of the 3.5–4.5 mm fraction (Podvigina et al. 2019). Increasing the exposure to 10 min and the multiplicity of treatment (4-fold) with a laser stimulated the growth and development of seedlings – the average length of seedlings increased by 31%, the germinant mass increased by 12% relative to the control (Podvigina et al. 2019). When seeds were stored for a longer period of time, the laser treatment had a decreasing effect on these indicators (Podvigina, Nechaeva 2019a).

The effect of red photons with a wavelength of 400–800 nm on the seeds of Scots pine described by Tillberg (1992) indicates the sensitivity of the seeds to this light when they come out of the resting state. However, it is not possible to give a common denominator for comparing that study and the present one, since the mechanism of the coherent laser radiation formation is fundamentally different, and the wavelength is clearly fixed at 632.8 nm.

The results of irradiation by low-intensive coherent light compared to the control indicate the weak effect of this pretreatment on seed performance, but also the need for more comprehensive studies in the future. This will contribute to the improvement of existing designs of the mobile optoelectronic seed grader (Albekov et al. 2018; Novikov et al. 2021) in terms of implementing rapid analysis, separation and biophysical activation of seeds in one device, allowing reducing time and energy costs during seed processing.

CONCLUSION

(i) The maximum values resulted from 10-minute exposure time for sugar beet germinant height and biomass and Scots pine germinant height, and from 15-minute exposure time for Scots pine biomass.

(ii) Low-intensive coherent seed irradiation has the same and positive effect on germinant growth of both Scots pine and sugar beet.

(iii) Biophysical activation of Scots pine seeds by low-intensity coherent radiation at an exposure time of 10–15 minutes increases the germinant biomass by 15–17%, and seedling biomass from the light fraction of seeds by 18–24%.

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