

## Laser biostimulation of perennial ryegrass (*Lolium perenne* L.) seeds in a pilot study

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### SUMMARY

*In our pilot study, perennial ryegrass seeds were exposed to 0, 1, 2, 4, and 8 minutes of single-wavelength red laser (660 nm, 150 mW) irradiation, and were grown to 13 weeks of age. No statistically significant effects were observed in germination rate or seedling shoot length. However, the 2- and 4-minute treatments significantly increased shoot length in 13-week-old plants by 28% and 19%, respectively, compared to the control. There were no significant differences in shoot or root weight of the 13-week-old plants, though shoot dry weight was 57% to 65% higher across all treatments. The shoot-to-root dry weight ratio increased in all treatments compared to the control, with the 1-minute treatment significantly exceeding the control by 54%. The protein content of the entire seedlings was notably lower in the 1- and 2-minute treatments and higher in the 4-minute treatment than in the control, as measured by NIRS analysis. The fiber fractions examined (NDF, ADF, hemicellulose) surpassed the control values in the shoots of 13-week-old plants under all treatments, but these differences were not statistically significant.*

**Keywords:** low-power red laser, germination, shoot length, biomass, nutritional value

### ÖSSZEFOGLALÁS

*Kísérletünkben angolperje vetőmagjait 0, 1, 2, 4 és 8 perces, vörös diódalézer (660 nm, 150 mW) besugárzásnak tettük ki, majd a csíranövényeket 13 hetes korig neveltük. A csírázóképeségben vagy a csíranövények hajtáshosszában nem tapasztaltunk hatást. A 13 hetes növények hajtáshossza azonban a 2 és 4 perces kezelés esetében szignifikánsan, 28%-kal, illetve 19%-kal nagyobb volt a kontrollhoz képest. A hajtás- és gyökértömegben nem mértünk jelentős különbséget, ugyanakkor a hajtás-gyökér száraztömeg aránya minden kezelésben láthatóan nagyobb volt a kontrollhoz képest, az 1 perces kezelés esetében 54%-kal haladta azt meg. A teljes csíranövények fehérjetartalma (NIRS) szignifikánsan kisebb volt az 1 és 2 perces kezeléseknél, és nagyobb a 4 perces kezelésben. A 13 hetes növények hajtásaiban a rostfrakciók (NDF, ADF, hemicellulóz) minden kezelésben meghaladták a kontroll értékeit, de ezek a különbségek nem voltak statisztikailag igazolhatók.*

**Kulcsszavak:** kis teljesítményű vörös lézer, csírázóképeség, hajtáshossz, biomassza, takarmányozási érték

### INTRODUCTION

The term Photobiostimulation was coined by Endre Mester, who first reported the beneficial biological effects of laser light in 1967 at Semmelweis University (Budapest), based on their observations from a mouse experiment (Mester et al., 1968 cit. Serrage et al., 2019). Low-intensity or low-level laser irradiation (LILI or LLLI), also known as photobiomodulation (PBM), involves treating biological materials with coherent, monochromatic light at low optical power density. The most commonly used laser types include gas lasers, such as the He-Ne laser (632.8 nm), and semiconductor (diode) lasers, like the red diode laser (630-660 nm). Exposure to low-intensity laser light does not cause significant heating or tissue damage, but induces observable photochemical and photophysical regulatory effects in cells and tissues.

The effects of LILI on plants were first studied for agricultural implications in the late 1960s (Wilde et al., 1969). By the 1980s and 1990s, it was already used in agrotechnical applications, such as pre-sowing seed treatment (Koper, 1994; Dineev et al., 2004). Although many publications have discussed it, the exact mechanism of laser treatment remains unclear due to the diversity of variables like species, treated tissue, laser type, radiation dose, and tested parameters (Dudareva, 2024).

Laser radiation is increasingly recognized for its effects on plant physiological processes, particularly germination and early seedling growth (Klimek-Kopyra et al., 2020). Laser parameters, such as wavelength, power density, and exposure time, can differently affect seed viability, germination rates, and subsequent seedling development (Atta et al., 2023). While low-intensity laser treatments below 0.5 Watts (500 mW) have shown significant improvements in root and shoot growth, biomass accumulation, and overall yield in various plants (Qi et al., 2025), high-dose laser irradiation has been found to inhibit germination and vigor, and increase fungal infections, especially on unprotected seed surfaces, as noted by Andreasen (2023). The currently proposed mechanism is that light energy is converted into chemical and

electrical energy within plant cells, stimulating metabolic pathways and increasing the electrical potential of biomembranes (Kornarzyński et al., 2018). Laser irradiation enhanced water uptake and shortened germination time in wheat (*Triticum* species), thereby directly impacting the early stages of seed activation (Iqbal et al., 2019). Specific laser treatments also selectively affected the fungal flora of the seeds, reducing pathogenic species while leaving nonpathogenic species unaffected (Klimek-Kopyra et al., 2020). This presents a promising opportunity for sustainable agriculture by addressing seed disinfection and growth stimulation without the use of chemicals.

## MATERIALS AND METHODS

The effects of different laser dose densities on the germination capacity of perennial ryegrass seeds, the protein content of seedlings, and the shoot length, biomass, and nutritional values of 13-week-old plants grown from treated and untreated seeds were investigated.

*Device:* The parameters of the SL150 device (*Safe Laser Trade Ltd., Hungary*) with a 3.5 cm-diameter treatment cup are shown in *Table 1*.

Table 1.

Parameters of the SL150 soft laser device

Power (mW)(1)	Beam diameter (cm)(2)	Beam area (cm <sup>2</sup> )(3)	Power density (mW/cm <sup>2</sup> )(4)	Energy (J.min <sup>-1</sup> )(5)	Dose density (J.cm <sup>-2</sup> )(6)
150	3.5	9.616	16.64	9.6	1.00

1. táblázat: Az SL 150 típusú lágylézer eszköz jellemzői  
Teljesítmény (mW)(1), Fénysugár átmérő (cm)(2), Fénysugár terület (cm<sup>2</sup>)(3), Teljesítmény sűrűség (mW/cm<sup>2</sup>)(4), Energia (J/perc)(5), Dózissűrűség (J/cm<sup>2</sup>)(6)

*Seed preparation:* Perennial ryegrass (*L. perenne*) cv. 'Gulács' seed mixture of parental seed-stocks harvested in 2017 and 2018, stored by freezing (-16 °C), and of parental seed-stock harvested in 2019 from a 5-year-old dry storage. Seed samples were cleaned with a seed blower to achieve comparable size and specific gravity, and are therefore expected to have similar germination capacity. The initial moisture content (*FOSS NIRSTM DS2500F*; 'Wheat' calibration) of the frozen-stored seeds at the start of the experiments was 11.4%, whereas that of the dry-stored seeds was 10.3% before pre-moistening to 21% humidity with distilled water over 2 days at 5 °C prior to laser treatment.

*Treatment:* 25-25 seeds were counted using a seed counter (*Pfeuffer Contador*) and then treated in the soft-laser device's treatment cup for 0, 1, 2, 4, and 8 minutes, corresponding to energy densities of 0, 1, 2, 4 and 8 J.cm<sup>-2</sup>. The distance between the samples and the light source was 1.5 cm.

*Experiments:* The germination capacity of the seed samples described above was evaluated using 25 seeds in duplicate within 18 cm diameter Petri dishes, placed on a single layer of 120 g/m<sup>2</sup> crepe paper used for seed testing, ensuring all treatments were contained within one dish. According to the Hungarian standard MSZ 6354-3 (2008), pre-cooling (2 days at 5 °C) and two assessments (on days 5 and 14) were conducted (*Table 2*).

Table 2.

Parameters of the germination tests

Code of germination test(1)	Laser treatment duration (min)(2)	Dose density (J.cm <sup>-2</sup> )(3)	Seed sample(4)
L1	0-1-2-4	0-1-2-4	6 and 7 year-old seed-mix from frozen-storage, treated in a dry state(5)
L2	0-2-4-8	0-2-4-8	5 year-old seeds from dry-storage, treated after pre-moistened state(6)

2. táblázat: A csírázóképeség vizsgálatok bemutatása  
Csírázóképeség vizsgálat kódja(1), Lézerkezelés időtartama (perc)(2), Dózissűrűség (J/cm<sup>2</sup>)(3), Vetőmag(4), 6 és 7-éves, fagyasztva tárolt vetőmag, légszáraz állapotban kezelve(5), 5 éves, száraz magtárolásból származó vetőmag, előnedvesített állapotban kezelve(6)

Germination tests were performed in a plant growth chamber (*Memmert HPP750*) with a 14h/10h light/dark cycle and alternating temperatures of 18 °C (16h) / 25 °C (8h), at 80% relative humidity.

*Seedling investigations:* Seedling evaluation for abnormality was also performed at the end of the germination tests. Further investigations were conducted on the L1 test seedlings. Shoot length was measured on 10 randomly selected seedlings per

treatment. Protein content of the whole fresh seedlings was measured in 3 replicates (*FOSS NIRSTM DS2500F*; 'Grass and Alfalfa Silage' calibration).

*Cultivation and further investigations:* Twenty to twenty normal seedlings from each treatment, originating from the L1 germination test, were planted in rhizoboxes (45×22×1 cm) filled with potting soil (Agro CS; ≥75% organic matter, pH 5.5±0.5, NPK 0.1-0.01-0.03 w/w% DM) and grown outdoors for

11 weeks. Water was applied as needed, with no nutrient supplementation. At the end of the growing period, during the tillering stage of ryegrass, the roots were washed out of the soil (Fig. 1-2).

Three subsamples of five randomly selected 13-week-old plants from each treatment were prepared. The shoot length of each plant was measured. After drying the plant material at 50 °C (Heraeus T6200) to constant weight, the dry weight of both shoots and roots was determined. The nutritional values, such as protein content, ash content, neutral detergent fiber (NDF), and acid detergent fiber (ADF), were measured non-destructively in three replicates (FOSS NIRS™ model DS2500F; 'Grass and Alfalfa Silage' calibration) from the dried subsamples. Hemicellulose was calculated as the difference between NDF and ADF.

**Data analysis:** We used the Shapiro-Wilk normality test and one-way ANOVA and post-hoc Tukey test in R 4.2.2. (R Core Team, 2022) environment using R-Commander v.2.8-0 package.

**Figure 1: Open rhizobox with 13-week-old perennial ryegrass plants from the 1-minute laser seed-treatment**  
(Photo: Lepossa, 2025)



1. ábra: Egyperces lézerkezelésből származó, 13-hetes angolperje növények felnyitott rizoboxban (fotó: Lepossa, 2025)

**Figure 2: Washed plants grown from seeds exposed for 0, 1, 2, and 4 minutes**  
(Photo: Lepossa, 2025)



2. ábra: A 0, 1, 2 és 4 perces lézerkezelésből származó, mosott gyökerű angolperje növények (fotó: Lepossa, 2025)

## RESULTS AND DISCUSSION

### Germination

The laser treatment, neither in a dry-seed state nor in a pre-moistened state, did not show significant effects in the experiments (Table 3). The seeds germinated at over 88% on the 5th day and reached 92-96% by the end of the L1 test. The germination rate of pre-moistened seeds from long-term dry storage was low (22-40%) on day 5, but it reached 82-94% on day 14 in the L2 test. The seeds from the 4-minute treatment germinated at the highest rate, exceeding the control by 33% on day 5 and 12% on day 14. In the L2 experiment, the 2-minute treatment had a higher proportion of abnormal seedlings (12%) than the other treatments (2-4%).

Table 3.

Germination in L1 and L2 experiments

Experiment(1)	Treatment (min)(2)	5 <sup>th</sup> day mean germination (%) (3)		14 <sup>th</sup> day mean germination (%) (4)	
		normal	abnormal	normal	abnormal
L1	0	96	94	2	
	1	90	94	2	
	2	96	96	2	
	4	88	92	2	
L2	0	30	84	4	
	2	38	82	12	
	4	40	94	2	
	8	22	90	2	

3. táblázat: Csírázókéesség az L1 és L2 kísérletben  
Kísérlet(1), Kezelés (perc)(2), Csírázási erély az 5. napon (%) (3), Csírázókéesség a 14. napon (%) (4)

### Shoot length

Shoot length of perennial ryegrass seedlings ranged from 26 to 84 mm. The mean values were similar across the treatments: 62, 67, 62, and 65 mm, in order of treatment duration. Treatments 1 and 4 had longer shoots by 7 and 5%, but there was no significant effect of any treatment (Fig. 3a).

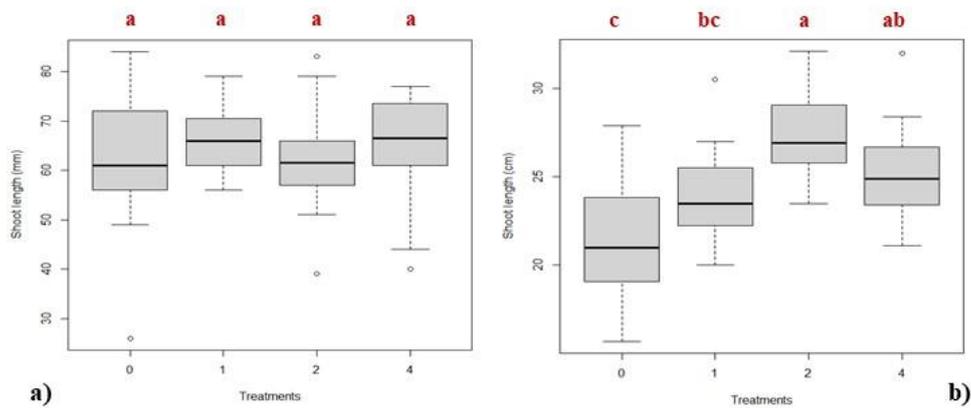
Compared to the control, 13-week-old plants had significantly longer shoots in the 2- and 4-minute treatments by 28% and 19%, respectively. Additionally, plants in the 2-minute treatment also exhibited significantly longer shoots than those in the 1-minute treatment ( $p < 0.01$ ) (Fig. 3b).

Moždzeń et al. (2020) found a stimulating effect on the morphological and physiological properties of *Triticale* plants grown from seeds irradiated with He-Ne laser light, and on leaf chlorophyll content, for a three-hour duration, whereas 24-hour-long radiation significantly inhibited seedling elongation.

### Shoot and root biomass

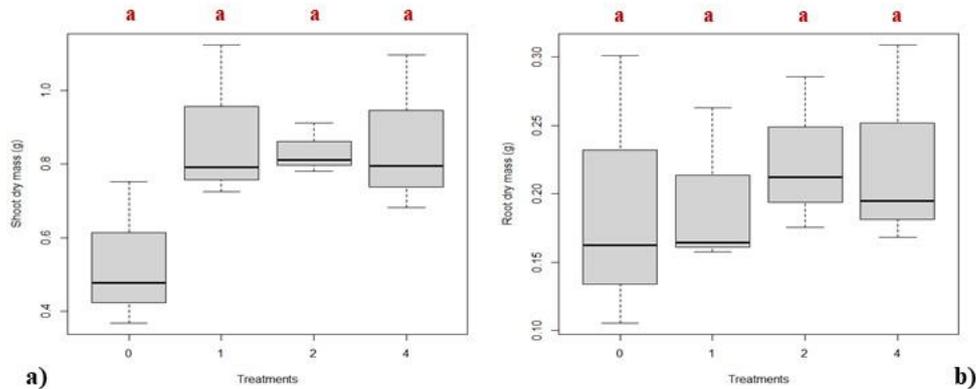
The 13-week-old plants showed a non-significant but noticeable difference in shoot weight compared to the control across all treatments, ranging from 57% to 65% (Fig. 4a, b). There was no significant difference in root weight either.

Figure 3: Shoot length of seedlings (a) (n=20) and 13-week-old (b) perennial ryegrass plants (n=15)



3. ábra: Csíranövények (a) (n=20) és 13-hetes angolperje növények (b) (n=15) hajtáshossza

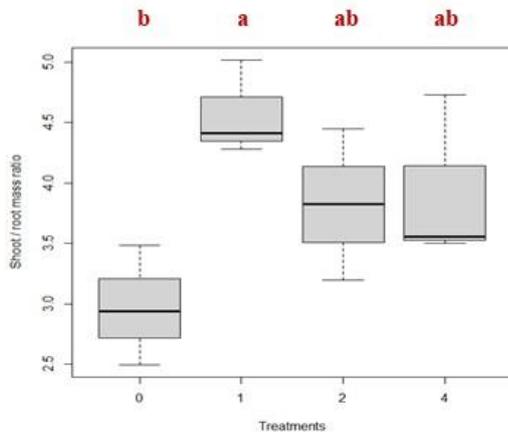
Figure 4: Dry weight of shoots (a) and roots (b) of five 13-week-old plants (n=3)



4. ábra: 13-hetes növények hajtás (a) és gyökér (b) száraz tömege (n=3)

The shoot-to-root dry weight ratio ranged from 2.5 to 5.02, with mean values of 2.97, 4.57, 3.82, and 3.93 across the treatment times. The 1-minute treatment significantly exceeded the control by 54% ( $p < 0.05$ ) (Fig. 5).

Figure 5: Shoot-to-root dry weight ratio of five 13-week-old plants (n=3)



5. ábra: 13-hetes növények hajtás-gyökér száraztömeg aránya (n=3)

The treatment had no significant impact on the germination of perennial ryegrass seeds at doses of 1-8 minutes, whether applied to a dry or pre-moistened state. The seven-year-old seed lot stored frozen at  $-16^{\circ}\text{C}$  maintained its original germination capacity. Laser treatment did not influence shoot length in seedlings grown from dry-treated seeds.

Although the laser treatments we applied did not significantly affect germination rate, we observed significant effects on shoot length and the shoot-to-root dry weight ratio in the 13-week-old perennial ryegrass plants. In their laser biostimulation experiments of Nadimi et al. (2022), neither the 100 mW single-wavelength red laser (659 nm), nor the dual-wavelength (DW) green/infrared laser (531 and 810 nm in the ratio of 10:1) showed significant effects on wheat germination, but the 10 min DW laser treatment shortened the mean germination time (14.3%), increased the germination speed (15.2%), germination rate index (15.2%), number of roots (31.8%) and the hypocotyl length (60.9%) significantly compared to the control. Samiya et al. (2020) reported statistically significant effects of both red diode laser (630 nm, semi-coherent red beams, 1 mW max. power, 20 min) and green laser (532 nm, 5 mW, 2 min) irradiation on wheat germination, the red laser treatment enhanced by 25%, while the green

laser treatment decreased the germination by 6.25% compared to the untreated control. The potential of laser biostimulation to reverse the adverse effects of poor crop storage was demonstrated on flaxseeds by Nadimi et al. (2022).

#### Nutritional values

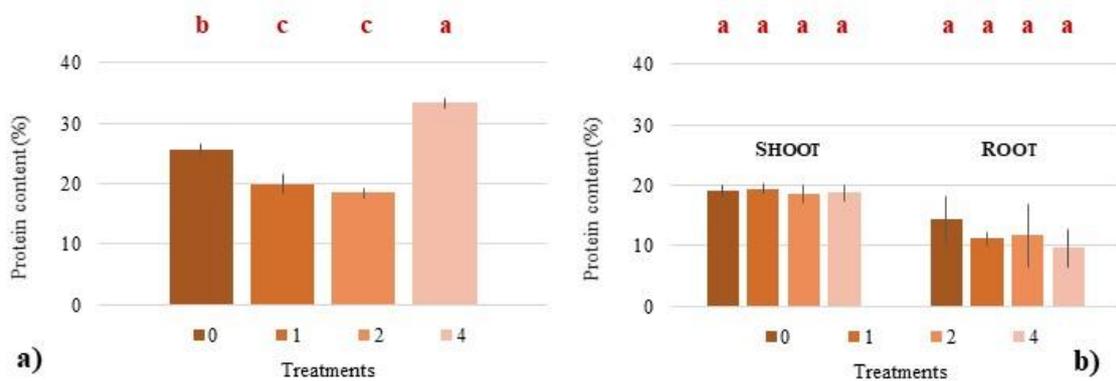
##### Protein content (NIRS)

The protein content of whole fresh seedlings ranged from 18 to 34 percent of dry matter.

Significantly higher protein levels were found in plants treated for 4 minutes compared to the control and those subjected to 1- and 2-minute laser treatments. The protein content in the 1- and 2-minute treatment plants was significantly ( $p < 0.05$ ) lower than in the control (Fig. 6a).

The protein content of the shoots in the 13-week-old plants ranged from 17% to 20% and was similar across treatments, whereas in the roots it decreased by 18-33% compared to the control. Despite this, there was no significant difference among the treatments (Fig. 6b).

Figure 6: Protein content on a dry-matter basis of whole fresh seedlings (a) and the 13-week-old plants (b)



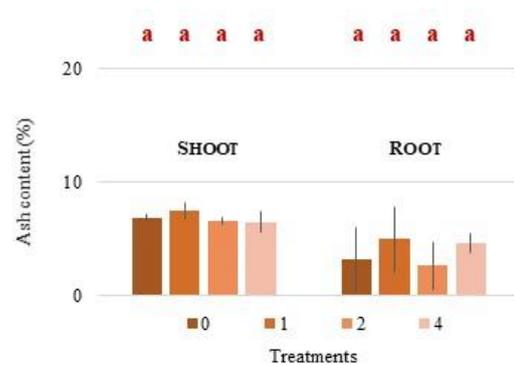
6. ábra: Csíranövények (a) és 13-hetes növények (b) szárazanyag százalékában kifejezett fehérje tartalma

In our study, the overall decrease in protein content observed at 1–2 J cm<sup>-2</sup> treatments, followed by a significant increase at 4 J cm<sup>-2</sup>, matches the biphasic dose-response relationship often reported, especially in medical low-level laser therapy (Huang et al., 2011). Our results suggest that in two-week-old seedlings, protein metabolism and nitrogen assimilation are suppressed at lower doses but boosted at moderate doses within a potential photobiomodulation window of 3–6 J cm<sup>-2</sup>. Similar dose-dependent responses were reported by Aslam et al. (2022) in their He-Ne laser (630 nm) treatment of two wheat varieties. They found that 2 min of laser priming of imbibed seeds was most effective at protecting the plants from drought stress later, whereas prolonged treatment significantly inhibited growth.

##### Ash and fiber contents (NIRS)

Ash content was up to 8% in shoots and roots of 13-week-old plants, and was more variable in roots across treatments (Fig. 7).

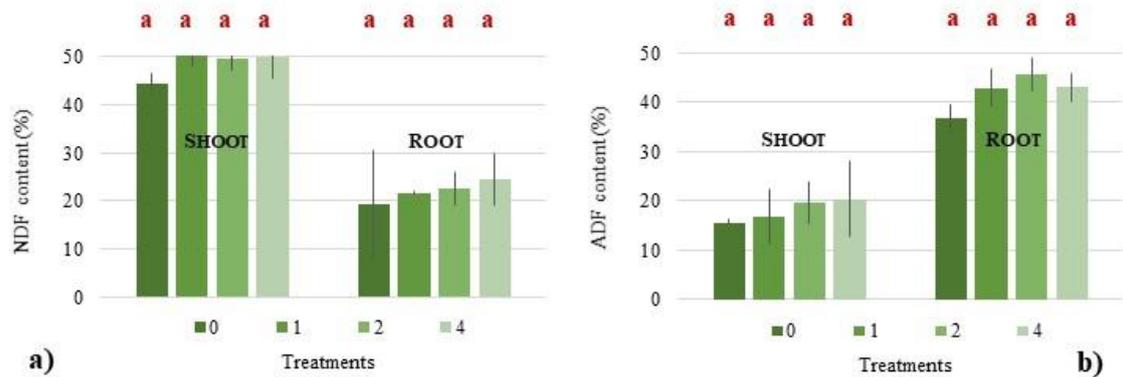
Figure 7: Ash content on a dry-matter basis of the 13-week-old plants



7. ábra: 13-hetes növények szárazanyag százalékában kifejezett hamu tartalma

The shoots had higher NDF content and lower ADF content compared to the roots. The average NDF content in the shoots ranged from 45% to 50% on a dry-matter basis, with the ADF fraction making up 37% to 43%. There was no significant difference between treatments, although laser treatment values exceeded control values for both fiber fractions in both shoot and root parts (Fig. 8a, b).

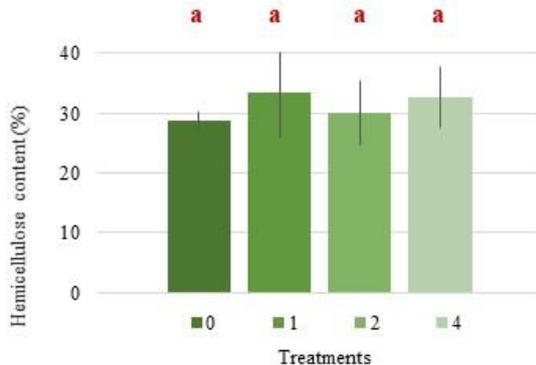
Figure 8: NDF (a) and ADF (b) content on a dry-matter basis of the 13-week-old plants



8. ábra: 13-hetes növények szárazanyag százalékában kifejezett NDF (a) és ADF (b) tartalma a hajtásban és gyökérben

Hemicellulose (HC), an easily digestible type of cell wall fiber important for feeding, is typically found in the cell walls of younger plants or plant tissues (Orosz, 2021), and the increase in its concentration in our experiment can be explained by the intensive formation of new tissues. The HC in the shoot dry matter ranged from 26 to 41%. The average content was slightly higher in plants grown from laser-treated seeds (5-16%) than in control plants, but the difference was not statistically significant (Fig. 9).

Figure 9: Hemicellulose content in the dry matter in the shoots of 13-week-old plants



9. ábra: Hemicellulóz tartalom a 13-hetes növények hajtásának szárazanyagában

## CONCLUSIONS

Our pilot experiment was conducted within the typical photobiomodulation dose range (0.1–10 J·cm<sup>-2</sup>), commonly used in many plant and biomedical studies. Although the present study did not find a significant effect of LILI on perennial ryegrass seed germination, results on subsequent development, such as shoot length and shoot-to-root dry weight ratio, suggest that future studies should build on these findings. Of the nutritional parameters measured, only the protein content of whole fresh seedlings showed a notable difference: it was significantly lower in the 1- and 2-minute treatments and significantly higher in the 4-minute treatment than in the control. This may be linked to the biphasic dose-response commonly seen in low-level laser (light) therapy (LLLT) in medical studies. Germination tests with larger seed samples from additional plant species may reveal statistically significant effects of red laser irradiation at the doses used. In cross-pollinated species like the perennial ryegrass, individual differences between plants from the same seed lot – even when it is a seed mixture from a single parent stock – can influence results. Therefore, the effects of treatments can be more reliably validated when experiments include large, as homogeneous as possible, seed or plant samples and multiple replicates.

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