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Additional far-red light improves the growth and resistance of the photosynthetic apparatus of *Lactuca sativa* L. to high-intensity light

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Abstract

The effects of additional far-red light (FRL) on the growth parameters, photosynthetic activity, and pro- or antioxidant balance of *Lactuca sativa* L. plants grown for 30 d were studied. The plants were grown under white light-emitting diodes with equal PAR intensities at red/far-red light ratios of 0.29, 0.89, and 1.67 and without FRL. Compared to the absence of the FRL, growth at a 0.29 ratio caused an increase in plant biomass and leaf area, but a decrease in PSII activity, net photosynthetic rate (P_N) per unit area, and stomatal conductance. High irradiance for 4 h at 1,000 µmol(photon) m^{-2} s^{-1} decreased PSII activity and P_N, but to the least extent in the 0.89 option. The possible pathways of the FRL's impact on the photosynthetic apparatus were analysed.

Keywords: far-red light; growth; high irradiance; *Lactuca sativa* L.; photosynthesis; red light.

Introduction

Light intensity and quality play important roles in regulating photomorphogenesis, photosynthesis, and growth processes under both normal and stressful conditions (Lanoue *et al.* 2018, Pashkovskiy *et al.* 2018, Kochetova *et al.* 2022, Paradiso and Proietti 2022). Herein, narrow spectra of LED sources are increasingly used to study the effects of light of different spectral ranges on plants, which makes it possible to regulate photosynthesis and the accumulation of various biologically active compounds (Legendre and van Iersel 2021, Vitale *et al.* 2022). However, to optimise the use of LED sources, it is important to know how light of different spectral ranges affects certain processes, such as growth, photomorphogenesis, and photosynthesis. In this case, various cellular photoreceptors, primarily receptors of UV-A and blue light (BL) – cryptochromes and phototropins and red light (RL) – phytochromes (PHY), play the key role of light sensing in light signal transduction of different spectral ranges in the photosynthetic apparatus (PA) and many other plant systems.

Phytochromes and far-red light: In higher plants, phytochrome B (PHYB) and accessory phytochromes (PHYC, PHYD, PHYE, *etc.*) are RL receptors, whereas PHYA is a far-red light (FRL) receptor (Hu *et al.* 2013,

Highlights

- Adding far-red light increased biomass and leaf area of lettuce
- Adding far-red light at a 0.29 RL/FRL ratio increased the photosynthetic rate per plant
- The photosynthetic apparatus was the most resistant to HIL at a 0.89 RL/FRL ratio

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Abbreviations: BL – blue light; Chl – chlorophyll; *E* – transpiration rate; FRL – far-red light; GL – green light; g_s – stomatal conductance; HIL –high-intensity light; MDA – malondialdehyde; PA – photosynthetic apparatus; PHY – phytochrome; P_N – net photosynthetic rate; POD – guaiacol-dependent peroxidase; RL – red light; SOD – superoxide dismutase.

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Paik and Huq 2019, Voitsekhovskaja 2019). They regulate plant growth and photomorphogenesis and induce the expression of genes encoding a number of photosynthetic proteins and enzymes of the biosynthesis of various pigments, enzymes of the Calvin cycle, particularly the key enzyme of the Calvin–Benson cycle, Rubisco (Kreslavski *et al*. 2009, Quail 2010, Carvalho *et al*. 2011). The effects induced by PHY are determined primarily by the relative content of its active form (P_{FR}). The content of the active form of phytochrome usually varies with the ratio of RL (λ_m = 660 nm) to FRL (λ_m = 730 nm) (Quail 2010, Cheng *et al*. 2021). An increase in this ratio leads to an increase in the P_{FR} content. The maximum increase in P_{FR} was observed after the irradiation of plants with RL (λ_m = 660 nm). One of the functions of PHYs is to control shade acclimation responses by reacting to changes in the relative amount of FRL in the environment (Smith and Whitelam 1997). Changing light quality by changing the RL/FRL ratio, which is achieved mainly by illuminating plants with additional FRL, affects many physiological processes (Legendre and van Iersel 2021, Tan *et al*. 2022, Kong *et al*. 2024, Li *et al*. 2024). Moreover, depending on the type of plants and growing conditions, both an increase and a decrease in various parameters characterising growth, photosynthesis, and other metabolic processes in plants, as well as the contents of various metabolites, are observed (Li and Kubota 2009, Kong *et al*. 2024, Lisina *et al*. 2024, Shmarev *et al*. 2024).

Note that a lower RL/FRL ratio can be considered as a model of a shaded environment, such as a plant canopy (Sellaro *et al.* 2010, Casal 2013). In this case, increasing the FRL affects plant morphology and photosynthesis, plant height, leaf size and angle, stomatal activity and phytohormone content (Shibuya *et al*. 2010, Hitz *et al*. 2019, Zhen *et al*. 2019, 2021; Legendre and van Iersel 2021, Tan *et al*. 2022). A low RL/FRL induces strong growth responses, known as shade avoidance responses, including stem elongation and leaf hyponasty (Meijer *et al*. 2022). However, photosynthetic responses may differ in sign, partly because the experimental conditions may not correspond to shadow conditions.

Additional FRL can stimulate plant growth by increasing canopy size, primarily leaf area (Zou *et al.* 2019, Legendre and van Iersel 2021). For example, Li *et al.* (2023) reported that long-term exposure to FRL leads to an increase in the leaf area of *Brassica alboglabra*, both due to elongation and expansion. This phenomenon may be associated with an increase in the content of auxins and cytokinins under the action of the FRL (Li *et al*. 2012, 2023).

Concerning photosynthesis, several studies have reported a positive effect of decreasing the RL/FRL ratio on photosynthesis under artificial lighting conditions (Zhen and Bugbee 2020, Kong *et al*. 2024). Thus, Zhen and Bugbee (2020) recently demonstrated that FRL increases canopy photosynthesis, and this increase is as effective as the increase of PAR (region of 400–700 nm). Kong *et al*. (2024) showed that the addition of FRL to white light leads to an increase in photosynthetic activity (by 31.7%) and the carbohydrate content in the organs of grape seedlings, as well as an increase in plant height, although the number of leaves and their dry mass decreased. The positive effect of the FRL and the optimal RL/FRL ratio can also be associated with the optimisation of the photosystems and the increase in photosynthesis due to the absorption of additional FRL by PSI (Zhen and van Iersel 2017, Yang *et al*. 2020, Zhen and Bugbee 2020, Zhen *et al*. 2021). After all, the FRL is well reflected and scattered but poorly absorbed by photosynthetic pigments (Yang *et al*. 2020). Therefore, the importance of the FRL in the lower plant tiers increases. On the other hand, a decrease in the rate of photosynthesis per unit leaf area was found with a 2-fold increase in the proportion of FRL in the spectrum, but an increase in leaf area and plant biomass was observed (Shmarev *et al*. 2024).

Thus, there is good potential to improve crop productivity and photosynthesis under various light conditions by manipulating the RL/FRL ratio (Holopainen *et al*. 2018, Pierik and Ballaré 2021, Tan *et al*. 2022). However, it is difficult to draw a definitive conclusion from the results of many of these studies.

Stressful conditions and RL/FRL ratios: According to the literature and our data, changes in the proportion of FRL and the RL/FRL ratio have the greatest effect on the physiological parameters of plants under stressful conditions, such as high-intensity light (HIL) (Kreslavski *et al*. 2018) or salt stress (Cao *et al*. 2018). Under normal conditions, tomato biomass accumulation and photosynthesis are greatest at a low RL/FRL ratio of 1.2 compared with those of the control (7.4) (Cao *et al*. 2018). Under saline conditions, the maximum was detected at an RL/FRL ratio of 0.8. However, biomass accumulation and photosynthesis did not depend on this ratio in the *phyB1* mutant. Therefore, a conclusion was made about the key role of PHYB1 in the response of plants to changes in the RL/FRL ratio. HIL is one of the main stressors, especially in combination with other unfavourable environmental factors, which significantly disrupts the functioning of the PA (Didaran *et al*. 2024).

During the day, most plants are exposed to HIL to varying degrees. Moreover, PSII and CO_2 fixation systems are the most sensitive to light stress (Allakhverdiev *et al.* 1987, Aro *et al.* 1993, Murata *et al.* 2007, Didaran *et al.* 2024). In this context, identifying the effects of HIL on the rate of photosynthesis and PSII activity at different RL/FRL ratios in the spectrum is important.

In the present work, the effects of different RL/FRL ratios on growth, photosynthetic processes, the content of photosynthetic pigments and the activity of a number of antioxidant enzymes under normal conditions and after short-term HIL were studied.

Materials and methods

Materials and experimental design: Plants (*Lactuca sativa* L.) were planted in 1-L pots filled with a standard soil matrix and were watered during growth with a 2-fold diluted Hoagland nutrient solution. The plants were grown for 30 d in a thermostatically controlled chamber at a temperature of 23 ± 1°C during the day and 21 ± 1°C during the night in light from a combination of LEDs: blue (BL, maximum 447 nm, half-intensity width 17 nm), green (GL, maximum 518 nm, half-intensity width 24 nm), red (RL, maximum 655 nm, half-intensity width 21 nm), and far-red (FRL, maximum 735 nm, half-intensity width 27 nm). For comparison, four variants were used (Fig. 1): 1 – RL:BL:GL, where the FRL was practically absent (further in the text No FRL); 2 – RL:BL:GL:FRL, where the RL/FRL ratio was 0.29; 3 – RL:BL:GL:FRL, where the RL/FRL ratio was 0.89; and 4 – RL:BL:GL:FRL, where the RL/FRL ratio was 1.67. Here, the ratios of the FRL_{660nm}/FRL_{730nm} for options 0.29; 0.89 and 1.67 were 0.43, 1.23 and 2.16, respectively.

The PAR light intensity for all options was 180 µmol(photon) m^{-2} s^{-1}, and the photoperiod was 12 h. Subsequently, some plants were irradiated for 4 h with HIL from white LEDs [1,000 µmol(photon) m^{-2} s^{-1}]. The spectral characteristics of the light sources (Fig. 1) were determined *via* a "*MK350N Premium*" spectroradiometer (*Zhunan Township*, Taiwan). The eighth to twelfth fully developed leaves from the upper parts of the plants were used for analysis.

Growth parameters: Effects of changes in the RL/FRL ratio on the fresh (FM) and dry (DM) total masses of plants, shoot and root masses, and total leaf area. The fresh and dry masses of the plants and their organs were determined with an accuracy of 1 mg *via* an analytical balance (*OHAUS Pioneer PA213C*, *Ohaus Corporation*, Parsippany, NJ, USA). The total leaf area was determined *via APFill Ink&Toner Coverage Meter* software.

Chlorophyll fluorescence: Fluorescence parameters were estimated based on the JIP-test by the fluorimeter *Monitoring Pen MP 100* (*Photon Systems Instruments*, Czech Republic), as well as with PAM fluorometry.

PAM-fluorometry: Induction fluorescence curves were measured with a *JUNIOR-PAM* fluorimeter (*Walz*, Effeltrich, Germany). The intensity of the measuring light (blue LEDs, maximum = 474 nm, 10 min] was 0.5 µmol(photon) m^{-2} s^{-1}, that of the actinic light was 125 µmol(photon) m^{-2} s^{-1}, and that of the saturating light was 6,000 µmol(photon) m^{-2} s^{-1}. To calculate F_0, F_v, F_m, $Y_{(II)}$, F_v/F_m and NPQ, the *WinControl-3 v. 3.32* program (*Walz*, Effeltrich, Germany) was used. Here, $Y_{(II)} = (F_m' - F_t)/F_m'$ and F_v/F_m – effective and maximum photochemical quantum yields of PSII; NPQ = $(F_m - F_m')/F_m'$ – nonphotochemical quenching; F_m and F_m' – maximum levels of chlorophyll fluorescence under conditions of adaptation to darkness and light; $F_v = F_m - F_0$ – photoinduced change in fluorescence; F_t – stationary level of Chl fluorescence; and F_0 – initial level of Chl fluorescence (Kramer *et al.* 2004, Kreslavski *et al.* 2014, Hou *et al.* 2017).

JIP-test: OJIP curves were measured under illumination with blue light (455 nm) with an intensity of 3,000 µmol(photon) m^{-2} s^{-1} for 1 s. Dark time before irradiation was 15 min. Based on the obtained OJIP induction curves, the fluorescence parameters F_v/F_m, DI_0/RC, and PI_{ABS} were calculated. F_v/F_m is the maximum photochemical quantum yield of PSII, where F_v is variable fluorescence defined as the difference between the maximum (F_m) and minimum (F_0) Chl fluorescence. Here, $DI_0/RC = (ABS/RC) - (TR_0/RC)$ – the amount of energy dissipated predominantly into heat by the reaction centre of PSII, and $PI_{ABS} = (ABS/RC) \times (F_v/F_0) \times [ET_0/(TR_0 - ET_0)]$ – the PSII performance index. ABS/RC is the flux of absorbed energy per active reaction centre, TR_0/RC is the maximum energy flow absorbed by all PSII reaction centres and used for primary charge separation in the PSII reaction centre, and ET_0 is the electron flux from Q_A to Q_B (Goltsev *et al.* 2016).

Pigments: The contents of photosynthetic pigments [Chl (*a*+*b*) and carotenoids] were measured in 96% ethanol extracts *via* the Lichtenthaler (1987) method. The pigment content was determined as µg g^{-1}(FM).

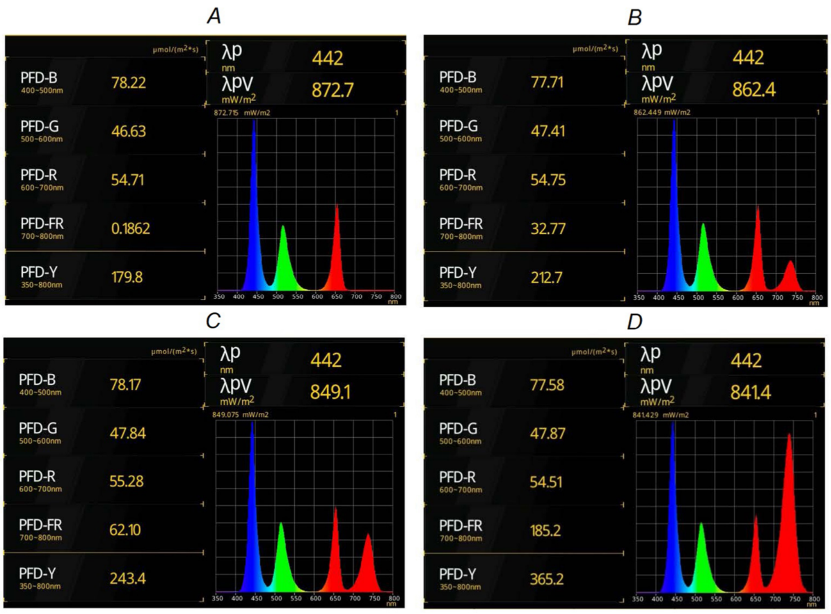

Fig. 1. Emission spectra of the LEDs used in the experiments. (*A*) No FRL; (*B*) RL/FRL ratio = 1.67; (*C*) RL/FRL ratio = 0.89; (*D*) RL/FRL ratio = 0.29. FRL – far-red light; RL – red light.

The contents of anthocyanins were determined spectrophotometrically (Liu *et al*. 2018). The leaf mass (0.10–0.15 g) per sample was ground in liquid nitrogen and incubated in 600 µL of extraction buffer (methanol containing 1% HCl) in an ultrasonic bath for 15 min, and then overnight at 4°C in the dark. Furthermore, the samples were centrifuged for 5 min at 10,000 × *g*. Then, the supernatant was transferred to a 2-mL centrifuge tube, 400 µL of water and 400 µL of chloroform were added to each sample and vortexed, followed by centrifugation at 10,000 × *g* and 4°C for 5 min. The absorbance of the supernatant was determined at wavelengths of 530 (A_{530}) and 657 (A_{657}) nm. The anthocyanin content was calculated as ($A_{530} - 0.33 \times A_{657}$) g^{-1}(FM).

Leaf gas exchange: Photosynthetic (P_N) and transpiration (E) rates and leaf stomatal conductance (g_s) were measured by an *LCPro+* portable gas-exchange analyser (*ADC BioScientific Ltd.*, UK) in an open system at a temperature of 21 ± 0.5°C, a CO_2 concentration of 430 ± 10 µmol, and a relative humidity of 70–80%. A light intensity of 200 µmol(photon) m^{-2} s^{-1} was used for measuring CO_2 gas exchange. Photosynthetic and transpiration rates and stomatal conductance were recorded for 6–8 min.

Based on the data of total leaf area and the value of P_N per unit area determined in the experiments, we calculated the approximate rate of net photosynthesis for the whole plant.

Malondialdehyde and antioxidant enzyme activity: The degree of lipid peroxidation was evaluated by measuring the content of malondialdehyde (MDA) according to Zhang *et al*. (2007), and the method of analysis and measurement of the content of MDA was described in detail in a previous study (Kreslavski *et al*. 2025). A *Genesis 10 UV* spectrophotometer (*Spectronic Unicam*, USA) was used to evaluate the MDA concentration.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to Gupta *et al*. (1993) with small modifications. The inhibition of the photochemical reduction of nitroblue tetrazolium at 560 nm was studied. The activity of guaiacol-dependent peroxidase (POD, EC 1.11.1.7) was evaluated *via* the method described in Maehly and Chance (1954). Enzyme activity was determined by evaluating the increase in absorbance at 470 nm [ε = 26.6 mM^{-1} cm^{-1}] compared with that of the control sample without the enzyme.

Statistics: Eight to twelve plants (n) were used for each spectral option. Growth parameters were measured for at least ten lettuce plants in each treatment group. The fluorescence parameters and photosynthetic pigment contents of no less than ten leaf discs with a diameter of 1 cm taken from different plants were measured. *SigmaPlot 12.0* (*Systat Software*, *Inc.*, San Jose, CA, USA) was used for statistical data processing and plotting, as well as for one-way analysis of variance (*ANOVA* followed by *Duncan*'s method). Different letters indicate statistically significant differences between tested experimental variants at $p<0.05$. The data in the tables and graphs are presented as the means ± SE.

Results

Growth parameters: The maximum values of the fresh and dry masses of the above-ground part and roots and the plant as a whole, as well as the total leaf area, were found in the variant with excess FRL (the ratio of RL/FRL = 0.29) (Table 1) compared with the plants without FRL. For the 1.67 and 0.89 options, intermediate values of these parameters were observed. The number of developed leaves was the greatest in the option without FRL. The average fresh mass of cuttings from leaves with a diameter of 1 cm was 17.3 ± 1 mg and 14 ± 0.7 mg for variants without FRL and with a ratio of 0.29, respectively (data not shown).

Photosynthetic activity: Initially, the maximum values of PI_{ABS} and F_v/F_m, reflecting the activity of PSII, were observed in the variants with a higher proportion of RL. At the same time, with a decrease in the proportion of RL, these values decreased slightly but increased at a ratio of RL/FRL = 0.29 (Fig. 2*A*,*B*). After irradiation with HIL, all these parameters significantly reduced. Moreover, the greatest decrease was observed in the variants with a predominance of RL, and the lowest decrease was

Table 1. Effects of changes in the RL/FRL ratios on total biomass, shoot and root biomass, total leaf area, and the number of developed leaves per plant (N). The data are presented as the means ± SE. *Different letters* indicate statistically significant differences ($p<0.05$), $n = 10$.

Parameter/treatment	No FRL	1.67	0.89	0.29
Total fresh biomass [mg]	$3,852 \pm 190^{b}$	$4,154 \pm 211^{ab}$	$4,326 \pm 293^{ab}$	$4,810 \pm 263^{a}$
Total dry biomass [mg]	333 ± 20^{b}	386 ± 23^{ab}	440 ± 18^{ab}	447 ± 27^{a}
Shoot fresh biomass [mg]	$3,510 \pm 204^{b}$	$3,772 \pm 223^{ab}$	$3,912 \pm 230^{ab}$	$4,351 \pm 255^{a}$
Shoot dry biomass [mg]	306 ± 15^{b}	353 ± 16^{ab}	402 ± 12^{a}	408 ± 24^{a}
Root fresh biomass [mg]	342 ± 17^{b}	382 ± 30^{ab}	414 ± 34^{ab}	435 ± 26^{a}
Root dry biomass [mg]	27 ± 2^{b}	33 ± 4^{ab}	38 ± 3^{a}	39 ± 4^{a}
N	8 ± 1	6 ± 1	6 ± 1	6 ± 1
Total leaf area [dm^2]	2.04 ± 0.16^{b}	2.44 ± 0.14^{ab}	2.76 ± 0.22^{ab}	3.08 ± 0.17^{a}
Photosynthesis per plant [µmol(CO_2) s^{-1}]	0.112 ± 0.008^{b}	0.151 ± 0.011^{a}	0.121 ± 0.009^{ab}	0.142 ± 0.008^{a}

observed with the 0.89 option. Therefore, the resistance of PSII to HIL, if judged by the values of the PI_{ABS} and F_v/F_m, was the lowest with an excess of RL. At 1.67, after irradiation with HIL, the PI_{ABS} value decreased by about 3.5 times, while in the 0.89 option by about 1.6 times, and at 0.29 and without FRL by 2 times and 4 times, respectively (Fig. 2*B*). Thus, the most HIL-resistant PSII was in plants grown at the 0.89 ratio (Table 2), which was close to this ratio under greenhouse conditions on sunny days at midday (Holmes and Smith 1977). The initial value of DI_0/RC was the lowest in the plants grown without FRL. This trend persisted after irradiation with HIL (Fig. 2*C*). Moreover, the value of DI_0/RC as a result of HIL increased the most noticeably in the 1.67 option. In the other variants, the changes were smaller.

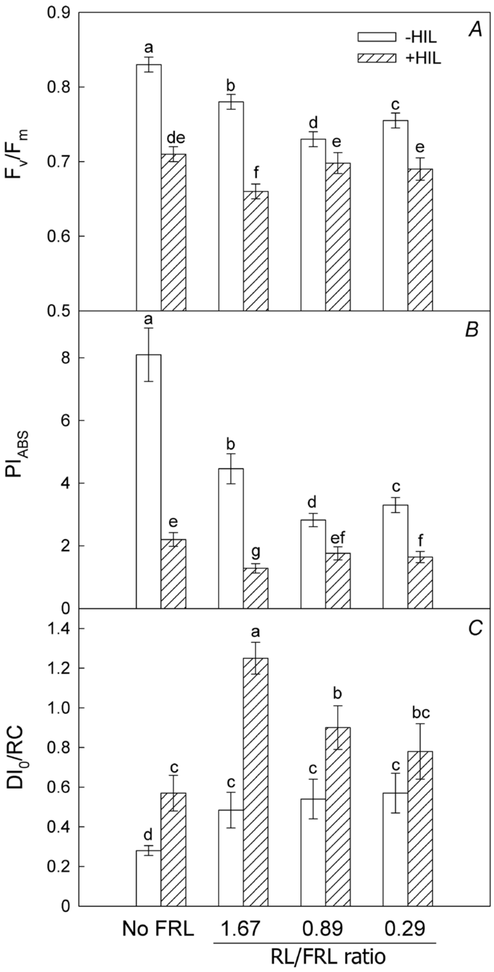

Fig. 2. Effects of changes in the RL/FRL ratios on fluorescence parameters calculated based on the JIP-test: (*A*) F_v/F_m, (*B*) PI_{ABS}, (*C*) DI_0/RC. Here, HIL is high-intensity light [4 h, 1,000 μmol(photon) m^{-2} s^{-1}]. The data are presented as the means ± SE. *Different letters* indicate statistically significant differences ($p<0.05$), $n = 7$. Here, PI_{ABS} is the PSII performance index, F_v/F_m is the PSII maximal photochemical quantum yield, and DI_0/RC is the dissipated energy flux by a reaction centre.

For the values determined *via* PAM fluorimetry, the abovementioned trends were generally preserved (Table 1S, *supplement*). For example, the $Y_{(II)}$ values were the greatest in the variants with a high proportion of RL, and with a decrease in the proportion of RL, these values decreased. The minimum change in the $Y_{(II)}$ value after exposure to HIL was 0.89 ratio. The NPQ values changed little after exposure to HIL, but the ratio of 1.67 was an exception. In this case, the NPQ value increased significantly. Compared with those of the other options, the P_N values before HIL irradiation were the highest in the option without the FRL and at a 1.67 ratio (Fig. 3*A*, Table 2). However, according to our approximate calculations per plant, photosynthesis in the 0.29 and 1.67 variants [0.142 and 0.151 μmol(CO_2) s^{-1}, respectively] was approximately 1.3 times greater than that in the plants without FRL [0.112 μmol(CO_2) s^{-1}] (Table 1). After irradiation, all the P_N values decreased, but the decrease was the smallest for the 0.89 option. Here, the highest value of the P_N per plant was also observed for the 1.67 and 0.29 options (data not shown). The values of E (Fig. 3*B*) and g_s (Fig. 3*C*) were the highest in the option without the FRL and at a 1.67 ratio, and after irradiation, the highest values of g_s and E were observed in the 1.67 option.

Pro- and antioxidant balance: SOD activity was the lowest at the 0.29 RL/FRL ratio both before and after irradiation (Fig. 4*A*). A difference between other options was practically absent. The POD activity (Fig. 4*B*) and the MDA content (Fig. 4*C*) after irradiation increased in all options, and before it, the MDA values were the highest at a 1.67 RL/FRL ratio and in the variant without an additional FRL. POD activity was the highest at the 0.89 ratio and in the variant without the FRL.

Table 2. Optimal RL/FRL ratios for different plant parameters. PSII resistance to HIL was calculated as the ratio of PI_{ABS} after HIL irradiation to PI_{ABS} before HIL irradiation.

Parameter/treatment	Optimum ratio before HIL	Optimum ratio after HIL
Total biomass	0.29	0.29
Net photosynthetic rate per leaf area	1.67	1.67
Net photosynthetic rate per plant	1.67; 0.29	1.67; 0.29
Stomatal conductance	No FRL; 1.67	1.67
PSII resistance to HIL	0.89	-
Chl content	0.29; 0.89	0.29; 0.89
Carotenoid content	0.89	0.89

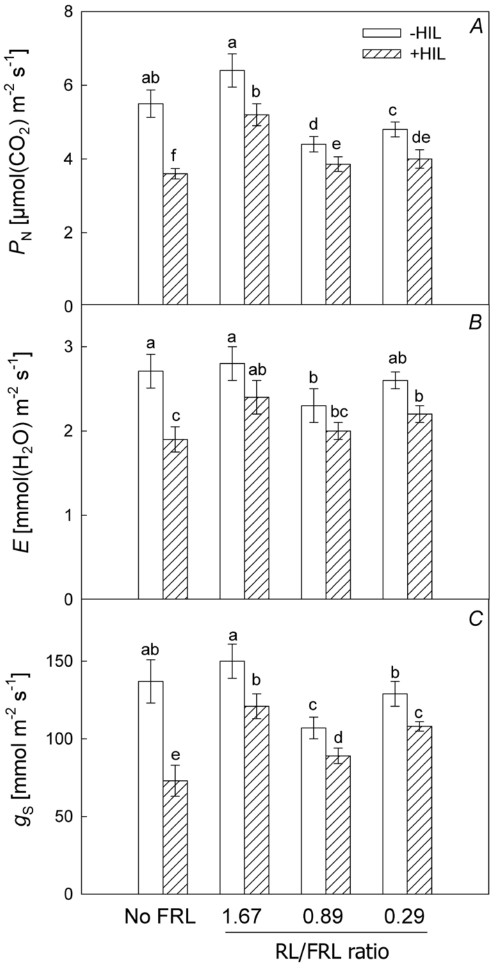

Fig. 3. Influence of changes in the RL/FRL ratios on the net photosynthetic rate (P_N) (*A*), transpiration rate (*E*) (*B*), and stomatal conductance (g_s) (*C*). P_N was measured at 200 µmol(CO_2) m^{-2} s^{-1}. The data are presented as the means ± SE. *Different letters* indicate statistically significant differences (p<0.05), n = 4.

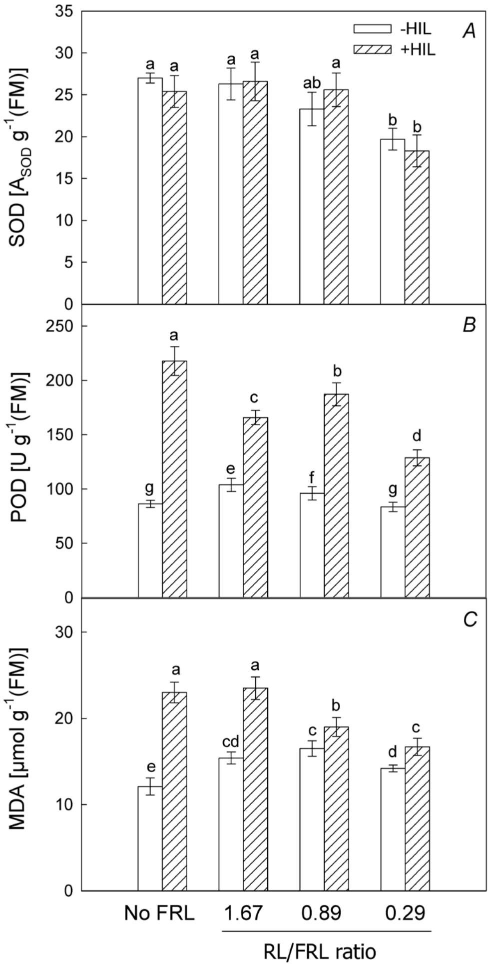

Fig. 4. Effects of changes in the RL/FRL ratio on enzyme activity: superoxide dismutase (SOD) activity (*A*), guaiacol-dependent peroxidase (POD) activity (*B*), and malondialdehyde (MDA) content (*C*). 1 A_{SOD} = the amount of enzyme capable of reducing the observed absorbance of samples by 50% compared to a solution without the enzyme. 1 U = the amount of enzyme that oxidises 1 µmol of H_2O_2 min^{-1}. The data are presented as the means ± SE. *Different letters* indicate statistically significant differences (p<0.05), n = 3.

Pigments: Initially, and after HIL irradiation, the lowest contents of Chl (*a*+*b*) (Fig. 5*A*) and carotenoids (Fig. 5*B*) occurred at a 1.67 ratio, and the highest content of Chl (*a*+*b*) occurred in the variants with ratios of 0.89 and 0.29, and carotenoids in the variant with a 0.89 ratio (Table 2). After irradiation, the content of pigments essentially did not decline in the variants with ratios of 0.89 and 0.29, whereas the other variants demonstrated slight decreases in the content of pigments. The highest contents of anthocyanins were found in the absence of the FRL both before and after irradiation, and the lowest content before irradiation occurred at a 0.29 ratio (Fig. 5*C*).

Discussion

The ratio of RL to FRL significantly affects many processes of plant growth and development, and the activity of the photosynthetic apparatus. Under photoculture conditions, a change in the RL/FRL ratio is often achieved by adding FRL to the emission spectrum of the light source and is accompanied by an increase in plant biomass and leaf area (Yang *et al.* 2020). In some cases, an increase in the accumulation of plant biomass is not detected with an increase in the proportion of FRL in the emission spectrum. Thus, when lettuce was grown for a long time under white light with the addition of FRL, there was no increase in the accumulation of raw or dry biomass, although there was an increase in the leaf surface area (He *et al.* 2021).

In our work, the greater accumulation of biomass by plants when irradiated with light at a 0.29 ratio was the result of a high total leaf area rather than the rate of photosynthesis per unit of leaf surface. The leaf area of plants with a 0.29 RL/FRL ratio was 1.5 times greater than the leaf area of plants without FRL (Table 1). Moreover, the total biomass under the 0.29 option was more than

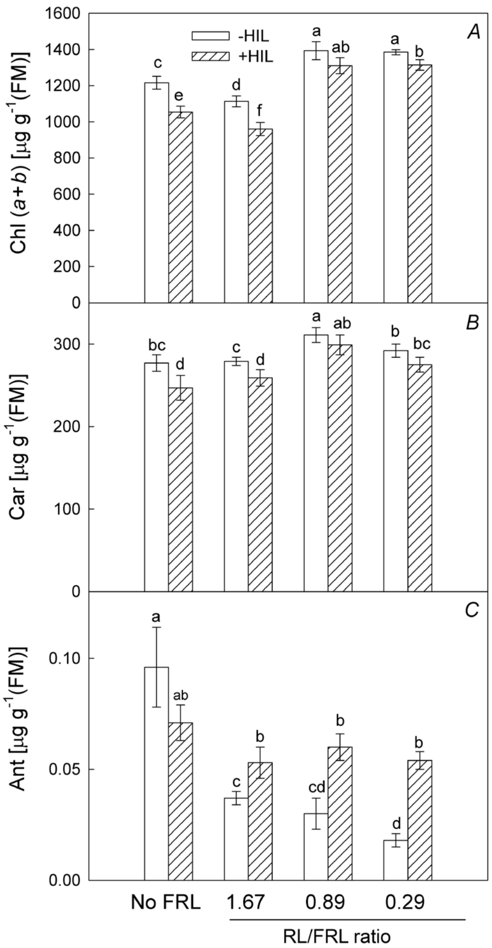

Fig. 5. Effects of changes in the RL/FRL ratios on the Chl (*a*+*b*) (*A*), carotenoid (*B*), and anthocyanin (*C*) contents. The data are presented as the means ± SE. *Different letters* indicate statistically significant differences ($p<0.05$), $n = 3$.

1.2 times greater, which is consistent with data (not shown) indicating a lower specific leaf density with an increased proportion of FRL in the emission spectrum.

Similar results were obtained in previous work (Shmarev *et al*. 2024), where the increased leaf area, rather than the rate of photosynthesis, was decisive in the accumulation of lettuce biomass, with an increase in the FRL/RL ratio in the spectrum almost twofold. Another reason for the increased biomass of the plants at low RL/FRL ratios is an increase in the net photosynthetic rate and, as a result, an increase in the content of carbon metabolites. This effect was found in grapevine leaves with an increase in the proportion of FRL in the spectrum (Kong *et al*. 2024). However, such an effect would be difficult to expect in our case since the value of P_N decreased at the 0.29 ratio. In addition, a decrease in the rate of photosynthesis per unit area may be due to a decrease in g_s (Fig. 3) and, as a result, a decrease in CO_2 intake.

Thus, the effect of the FRL on photosynthesis of the whole plant depended on the opposite effects: a lower rate of photosynthesis per unit leaf area but an increase in the total leaf area and photosynthesis per plant. As a result, in terms of one plant, the P_N in the variant with a 0.29 ratio was noticeably greater than that in the variant without FRL. The exclusion of the FRL from the emission spectrum led to an increase in the rate of photosynthesis per unit leaf surface compared with that of the other variants, which may be due to the higher stomatal conductivity, as well as the resulting higher activity of photochemical processes (PI_{ABS} and F_v/F_m values) (Figs. 2, 3).

It is known that the FRL preferentially excites PSI and, in many cases, enhances its photochemical efficiency when it is combined with light over-exciting PSII (Zhen *et al*. 2019). Therefore, under certain conditions, the effect of FRL can manifest itself not only in increasing the leaf area and total plant biomass, which is primarily due to the effect of FRL on the phytochrome system, but also in enhancing photosynthesis through increased photosynthetic electron transport. However, we did not observe increased PSII activity, with FRL predominating. In contrast, in a previous work (Shmarev *et al*. 2024), an increase in the FRL fraction in the spectrum and, accordingly, the FRL/RL ratio from 0.57 to 1.14 led to an increase in the PSII activity of 30-d-old lettuce plants. The effect of the FRL on PSII apparently depends on the spectral composition of the light used during cultivation and its intensity, which differed between our work and the aforementioned work. An increase in the rate of photosynthesis at low light intensity by improving the photochemical efficiency of photosystems *via* the example of soybean plants was obtained in the work of Yang *et al*. (2020). It was found that a low RL/FRL ratio (0.42) at a sufficiently high FRL intensity (the ratio of the flux density of photosynthetically active radiation to the FRL flux density was 1.8) can increase the photosynthetic assimilation of CO_2 at low light intensity by improving the photochemical efficiency of photosystems. Moreover, the low RL/FRL ratio (0.42) significantly increased not only the total biomass and leaf area but also the starch and sucrose contents, chlorophyll content, net photosynthetic rate, and PSII quantum yield compared with those of the normal RL/FRL ratio (1.3) at the same light intensity.

Apparently, much of the influence of the FRL on various parameters depends on the plant variety and the conditions of their cultivation, particularly the ratios of RL/FRL and PAR/FRL, as well as the intensity of the FRL and light under which the plants are grown, as well as the tier of leaves in the crown of a plant (Yang *et al*. 2020, Legendre and van Iersel 2021, Kong *et al*. 2024, Li *et al*. 2024).

As can already be seen from the above data, the reactions of plants and the photosynthetic apparatus to the action of FRL largely depend on the ratio of RL/FRL, as well as PAR/FRL. However, much less is known about the effects of stress factors on plants adapted to different RL/FRL ratios (Cao *et al*. 2018, Shmarev *et al*. 2024). Thus, Cao *et al*. (2018) compared the effects of different RL/FRL ratios (0.8, 1.2, and 7.4) on the growth and salt resistance of tomato plants and suggested that growing plants with a relatively low RL/FRL value (0.8) could improve tomato salinity tolerance and that an RL/FRL ratio equal to 1.2 was the most optimal for the accumulation of total dry biomass and photosynthesis under normal conditions. In contrast, at the RL/FRL ratio of 7.4, the net photosynthetic rate was the lowest under all the tested conditions. Thus, during salinisation, the optimal RL/FRL ratio for growth and photosynthesis shifted from 1.2 to 0.8.

It is important to note that the maximum PSII activity (PI_{ABS}) of the initial plants before irradiation was found in the plants grown without an additional FRL, and the photosynthetic rate reached a maximum at a ratio of 1.67. In addition, the value of P_N in the No FRL option was greater than that in the 0.29 and 0.89 options. After the plants were irradiated, the lowest value of P_N was found in the No FRL option, and the highest P_N value was also observed at a 1.67 ratio (Table 2), which corresponds to the elevated value of g_s.

Thus, as a result of the HIL, the optimal ratio shifts towards an increased proportion of the FRL, as in previous work (Cao *et al*. 2018). An additional FRL is likely important under stress and for improving photochemical processes, possibly by enhancing cyclic electron transport (Zhen and Bugbee 2020). This is consistent with the fact that in our experiments, with an increased proportion of FRL (0.89) in the radiation spectrum, PA resistance to HIL was the highest (Table 2), as reflected by a smaller decrease in the net photosynthetic rate and the PSII performance index (PI_{ABS}). The reason for the increased resistance of PA at a high FRL may be the preservation of a high g_s value after exposure to HIL (Figs. 2, 3). Additionally, under stress, the better adaptation to HIL at ratios of 0.89 and 0.29 can be partly due to the increased content of carotenoids and anthocyanins, which can serve as an optical light-absorbing filter and take part in PA protection as antioxidants (Didaran *et al*. 2024). This finding is consistent with previous data (Li and Kubota 2009), which showed that FRL in the emission spectrum can affect the contents of lettuce pigments, such as anthocyanins, carotenoids, and chlorophyll. Additionally, at a 0.89 ratio, a contribution to increased resistance is possibly due to increased POD activity (Fig. 4). At a ratio of 1.67, a possible protective mechanism may be, as observed in our experiments, increased dissipation of absorbed light energy into heat, characterised by DI_0/RC and NPQ values (Didaran *et al*. 2024).

It seems that the impact of FRL on photosynthesis depends on light and other conditions. Thus, Li *et al.* (2024) studied the impact of additional FRL on lettuce and showed that plants exposed to light with a low RL/FRL ratio demonstrated increased light interception and whole-plant photosynthesis. However, at the low RL/FRL, maximum Rubisco carboxylation rate and maximum electron transport rate were reduced, indicating that additional FRL lowered photosynthetic capacity in lettuce. Based on the analysis of photosynthetic parameters and plant morphological changes, the authors suggested that the positive effects of a low RL/FRL ratio on plant morphological changes outweighed the negative impacts on photosynthetic parameters.

Conclusion: A change in the RL/FRL ratio affects the growth, photomorphogenesis, photosynthetic activity, and pro-/antioxidant balance of lettuce plants, as well as their resistance to HIL. The highest shoot and root dry mass and fresh mass were found with a predominance of FRL (RL/FRL ratio = 0.29), which seems to be due mainly to the increased P_N per plant at this ratio (Table 2). The decrease in photosynthesis, found in the presence of an additional FRL compared with the initial version without the FRL, probably occurs due to reduced g_s and PI_{ABS} values, as well as the presence of thinner leaves. However, our results imply that the morphological changes caused by the FRL increased the efficiency of light capture and photosynthesis of the entire plant, thereby increasing the biomass of the lettuce.

The optimal choice of this ratio by providing plants with additional FRL has a pronounced positive effect, which has been shown in many studies (Cao *et al*. 2018, Yang *et al*. 2020, Legendre and van Iersel 2021, Zhen *et al*. 2021, Kong *et al*. 2024, Shmarev *et al*. 2024). However, for a particular plant species, the effect depends on the intensity of the FRL, the FRL/PAR ratio, and the light conditions in which the plants may be located. Therefore, further research is needed to better identify the ways in which FRL has a positive effect on growth and photosynthesis.

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