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Research Paper

Far-red radiation management for lettuce growth: Physiological and morphological features leading to energy optimization in vertical farming

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A B S T R A C T

Recently, far-red wavelengths (FR, 700–750 nm) have been largely investigated in indoor cultivation systems due to their morphological effects on plants (e.g., leaf expansion and stem elongation), resulting also in increasing yield. This work investigated the effect of substituting part of the red (R) and blue (B) radiation with far-red radiation, while keeping constant the photon flux density, in the light spectrum for lettuce grown in a vertical farm. Lettuce (*Lactuca sativa* var. Canasta) plants were transplanted and grown in an ebb-and-flow system for 29 days. During the cycle, plants were subjected to five different light treatments: a control treatment consisting of an optimized R and B spectrum (ratio of 3; RB_3) with a photosynthetic photon flux density of 200 μmol m^{-2} s^{-1}, and four treatments in which R and B were partially replaced by 10, 30, 50 and 70 μmol m^{-2} s^{-1} of FR light, resulting in an increasing FR fraction. Biomass production and most of the morphological parameters were affected from 15 days after transplanting (DAT), while stomatal conductance from 22 DAT. Leaf greenness and specific leaf area values were influenced by the FR radiation starting from 8 DAT. At 29 DAT, substitution of an amount of R and B photons equal to 30 (RB_3–30) or 50 (RB_3–50) μmol m^{-2} s^{-1} with the same amount of FR radiation resulted in increased leaf biomass in both fresh (+49 and +47%, respectively) and dry weight (+45 and +42%, respectively). With RB_3–30, the increase was due to leaf area expansion (+103%), whereas stomatal conductance (g_s) and quantum efficiency of photosystem II (ΦPSII) did not change compared with the spectrum with only R and B. With RB_3–50, g_s and ΦPSII decreased compared with RB_3 (-27 and -6%, respectively), but the greater biomass accumulation was supported by the greater leaf expansion (+119 %). The adoption of RB_3–30 and RB_3–50 also promoted light use efficiency (+45 and +42 %, respectively), lighting energy use efficiency (+48 and +53 %, respectively) and therefore the overall energy performance of the system. The adoption of RB_3–30 and RB_3–50 is a valid strategy to increase yield for lettuce production, but further studies, also in relation to blue radiation intensity, are needed to avoid the negative effect on leaf pigmentation.

1. Introduction

In controlled environmental agriculture, light features (e.g., spectrum, intensity, photoperiod, and direction) represent key parameters to be precisely monitored and controlled since they greatly affect growth, yield, and quality traits of crops (Wong et al., 2020; Appolloni et al., 2021). In vertical farms (VFs), artificial light represents the only source for photosynthesis (Kozai and Niu, 2016; Kalantari et al., 2018; Orsini et al., 2020a). It was estimated that more than 50 % of the energy in VFs is used for the lighting system (Yokoyama, 2019), making the optimization of the lighting environment critical to their economic and environmental sustainability (Van Delden et al., 2021; Martin and Orsini, 2023; Ji et al., 2023). Accordingly, multiple efforts are underway to maximize light use efficiency (yield per unit of input used for lighting), including both the knowledge in photobiology involving light management itself (Pennisi et al., 2020; Avgoustaki and Xydis, 2021) and the management of other environmental parameters (Carotti et al., 2021). Accordingly, the fine-tuning of the light spectrum appears critical, given

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the impact it has on plants' growth (Kong et al., 2019; Wong et al., 2020).

It is well-recognized how specific wavelengths can drive photosynthesis with different levels of efficiency (Goto et al., 2014; Yang et al., 2018). The most widely used wavelengths for indoor agriculture are red (R, 600–700 nm) and blue (B, 400–500 nm) (Pennisi et al., 2019a, 2019b; Ying et al., 2020), since they match the absorption peaks of chlorophyll a and chlorophyll *b*, the main photosynthetic pigments (Lin et al., 2013). The impact that specific wavelengths have on plant morphology and architecture, thereby also affecting the amount of light intercepted by the plants, should not be underestimated (Ren et al., 2019). R and far-red radiation (FR; 700–750 nm), are perceived and absorbed by phytochrome (phy), a family of photoreceptors (named phyA-phyE), that have reversible photochromism (Smith, 2000). Phytochromes can change reversibly between two conformers: from a biologically active form "Pfr", that mainly absorbs in the region of FR radiation, to a biologically inactive form "Pr" that has the peak of absorption in the R region (Fankhauser, 2001; Quail, 2002). The Pfr form, translocated from the cytoplasm to the nucleus, triggers a downstream transduction pathway, inducing the so-called "shade avoidance syndrome" (SAS), a series of plant responses that allow to capture as much light as possible (Smith, 2000). These responses are precisely triggered when plants are in a low-light environment (where the R:FR ratio, an indicator of the degree of shade, is low), making increased light interception a necessary strategy for survival (Casal and Smith, 1989; Franklin, 2008). Indeed, plants could improve their fitness by increasing the ability to intercept light as compared to nearby plants (Dreccer et al., 2022). In different species of heliophytes, the main phenotypic response observed in the presence of a low R:FR, concerns rapid elongation of the stem and leaves, altogether with changes in the upward orientation of the leaves (Franklin, 2008; Fankhauser and Batschauer; 2016). Under shaded environment, indeed, because of the decrease of the active form of phytochrome, an accumulation of phytochrome interacting factors (PIFs) occurs (Leivar and Quail, 2011). These specific PIFs (in particular, PIF1, PIF3, PIF4, PIF5, and PIF7), in turn, activate growth-promoting targets such as those involved in the biosynthesis and transport of auxin (Carriedo et al., 2016) required for the correct activation of the SAS (Tao et al., 2008). A FR enriched environment is what occurs in a natural setting such as undergrowth or in a field with a high-density crop canopy (Tan et al., 2021) or even during a specific time of the day, with variation that are not only spatial, but also temporal (Holmes and Smith, 1977). Obviously, this specific plant response can be exploited in horticulture: the importance of the regulation of R:FR ratio in modulating plant morphology has already been highlighted, for example when pepper plant height in greenhouse was studied (Brown et al., 1995). Thanks to the introduction of the latest generation of light emitting diode (LED) lamps, it is possible to provide plants a specific amount of FR radiation, independently from the photosynthetically active radiation (PAR) (Kusuma et al., 2020; Paucek et al., 2020). In addition to studies on the morphological effects associated with FR radiation, recent research has also focused on its influence on photosynthesis (Zhen and Bugbee, 2020). Several studies reported that when a PAR-based spectrum is enriched by FR radiation, there is an increase in the photosynthetic activity, thanks to a restored balanced excitation between the two photosystems (Zhen and Van Iersel, 2017). These results have led some authors to reconsider the definition of PAR (which traditionally includes wavelengths between 400 and 700 nm), in order to include FR radiation (Zhen and Bugbee, 2020).

Lettuce (*Lactuca sativa* L.) is one of the most widely grown species in vertical farms (Orsini et al., 2020b; Ampim et al., 2022). For what concerns FR, most of the already existing literature for lettuces focuses mainly on the addition of FR radiation to red and blue or a white background spectrum, thereby increasing the total photon flux density receveid by the plant (Kusuma and Bugbee, 2023). In these conditions, some studies have already shown that FR radiation led to an increase in biomass and yield in some lettuce cultivars (Zou et al., 2019; Li et al., 2020; Jin et al., 2021). On the other hand, the presence of a higher intensity of FR radiation can increase the allocation of biomass towards the stem reducing leaf yield (Kusuma and Bugbee, 2023). Therefore, characterization of SAS from a qualitative and quantitative point of view is crucial to optimize the use of FR radiation. Different metrics have been developed to predict morphological responses triggered by phytochromes, beside the already mentioned R:FR ratio, such as the phytochrome photoequilibrium (PPE) (Morgan and Smith, 1976) and the FR fraction (Kusuma and Bugbee, 2021)

In addition to the morphological and physiological parameters, that ultimately affect the final yield of the product, the management of light radiation in VFs has also a great impact at a systemic level, influencing, for example, the energy (Orsini et al., 2020a) and water use efficiency, as demonstrated for the latter in the specific case of FR radiation for lettuce growth (Carotti et al., 2023a).

Within this context, the aims of this paper were the following: 1) firstly, the construction of dose-response curves regarding morphological and physiological parameters on lettuce grown in VF when specific amount of R and B radiation was replaced by the same amount of FR radiation, while keeping constant the optimal ratio between R and B radiation; 2) the evaluation of the growth parameters at different times during the growth cycle, to be able to identify specific stages in which plants are more sensitive to FR radiation. Energy consumption implications of the entire indoor vertical farm through FR radiation management, was also considered in this work.

2. Materials and methods

2.1. Growing conditions

The experiment has been conducted in AlmaVFarm, the experimental vertical farm located within the Department of Agricultural and Food Sciences at the University of Bologna. Seeds of lettuce (*Lactuca sativa* L., var. Canasta) were sown in plastic net-pots containing plugs of a biodegradable polymer (GROWFOAM®, Foamplant BV, Groningen, The Netherlands) and placed in an aeroponic system (Frm srl, Rovereto, TN, Italy), specifically dedicated to germination, where they remained for 14 days. In the aeroponic sectors (previously described in Carotti et al., 2023a), the pressured (70 bar) nutrient solution was nebulized to the plants' roots every 15 min for 90 s during the whole growing cycle. The nutrient solution (electrical conductivity, EC=2.3 dS m^{-1} ± 0.2, pH=6.5± 0.2) had the following composition: N—NO_3: 14 mM; N—-NH_4: 4.4 mM; P: 1.0 mM; K: 5.0 mM; S: 2.0 mM; Ca: 5.2 mM; Mg: 1.2 mM; Fe: 17.9 μM, Cu: 2.0 μM, Zn: 3.8 μM, B: 11.6 μM, Mn: 18.2 μM, Mo: 0.5 μM. Light was provided by LED lighting system (Flytech s.r.l., Belluno, Italy), emitting red and blue radiation with the specific ratio of 3:1 (RB_3; Pennisi et al., 2019b), with a PPFD of 200 μmol m^{-2} s^{-1} and a photoperiod of 16 h d^{-1}. At the second true leaf stage,14 days after sowing, plants were moved into an ebb-and-flow system, where they remained for 29 days. Plants were transplanted into 6 × 6 × 7 cm (0.20 L) pots containing peat and then placed into the stacked levels with a planting density of 153 plants m^{-2}. In the ebb-and-flow system, the nutrient solution was circulated once a day for 30 min for the first two weeks of the growing cycle (from 0 to 14 days after transplanting, DAT), and twice a day for 20 min each, for the last two weeks (from 15 DAT to end of the cycle). The nutrient solution had the same chemical features as the one used during the germination phase. The ebb and flow system consists of a closed-loop water cycle, with the drained water returning to the water tank, where a fertigator (NidoPro®, LogicSun, Cattolica, RN, Italy) checks, and if necessary, corrects pH and EC parameters before returning the water to the system (Carotti et al., 2023a). During the trial, air temperature and humidity of the vertical farm were set at 24/21 ± 1 °C day/night and 70/75 ± 10 % day/night, respectively, and additional CO_2 was supplied in order to maintain a constant concentration of 850 ppm.

2.2. Experimental design and light treatments

Five different LED light treatments were provided by LED lights from the transplant up to 29 DAT, when the final harvest was performed. Light treatments used were the following: a control treatment composed by R radiation (peak at 663 nm) and B radiation (peak at 470 nm) in a ratio of 3:1 (RB_3) and four different treatments in which 10, 30, 50 and 70 μmol m^{-2} s^{-1} of R and B radiation were substituted by the same amount of FR radiation (FR, peak at 729 nm). In all light treatments the ratio between R and B radiation was maintained constant at 3:1 (RB_3), as also the photon flux density (set at 200 μmol m^{-2} s^{-1}) and photoperiod (16 h d^{-1}). The spectral distribution was measured by a spectroradiometer (CL-500A, Konica Minolta, Chiyoda, Tokyo, Japan), while the intensity was measured with a PAR meter (Apogee Instruments, Logan, UT, USA) at the beginning of the experiment at the level of the pot, averaging the results of 60 measurements in the cultivation area. Specific characteristics of the lighting regimes are given in Table 1, while the relative spectral response is shown in Fig. 1. The phytochrome effect was expressed as FR fraction, as described by Kusuma and Bugbee (2021):

$$\text{FR fraction} = \frac{(\text{FR photon flux})}{(\text{R photon flux} + \text{FR photon flux})}$$

The FR fraction metric is used to characterize the effects of phytochromes, both with reference to the light treatment used in this work and in other works that are introduced in the discussion section of this manuscript. Each light treatment was applied with three replications, represented by three different layers of the ebb-and-flow system.

2.3. Physiological determinations

Physiological measurements were conducted at 8, 15, 22 and 29 days after transplanting (DAT). At each sampling day, 5 plants per level (15 plants per light treatment) were randomly selected from the cultivation tray. To minimize border effects, only plants grown in the central part of the tray were used. As explained in the following section, physiological measurements were followed by destructive morphological ones. Therefore, after each sampling, the outer plants were used to replace plants selected for destructive harvests in order to restore and maintain the same planting density. Measurements were only performed on plants that always remained in the central part of the cultivation tray. Stomatal conductance (g_s) was assessed using a leaf porometer (AP4, Delta-T Devices, Cambridge, United Kingdom), taking three measurements for each of the two most developed leaves. Leaf greenness (SPAD unit) was estimated using a hand-held leaf chlorophyll meter (SPAD-502, Konica Minolta, Chiyoda, Tokyo, Japan), performing three measurements for each of the two most developed leaves. At 28 DAT, the effective quantum yield efficiency of photosystem II (ΦPSII) was assessed in six plants per treatment (2 plants per layer) using a portable LI-COR 6400 (LI-COR Biosciences, Lincoln, United States). The ΦPSII, indicates the capacity of PSII to absorb photon energy, and it has been calculated with the formula (Fm'-Fs)/Fm', where Fm' is the maximum fluorescence in a leaf adapted to light and Fs is the steady-state fluorescence.

2.4. Morphological determinations

Destructive morphological measurements were performed on the same plants just after the completion of the physiological measurements. Plant height (PH), leaf length (LL) and width (LW) of the most developed leaves were measured with a ruler. Leaf area (LA) was assessed with the app Easy Leaf Area (developed by University of California, USA) and the total leaf number (LN) for each plant was counted. Leaf fresh weight (LFW) and leaf dry weight (LDW; obtained after drying the leaves for 72 h at 48 °C in an oven) were measured with a precision balance. At 29 DAT, fresh weight of stem was measured separately from the leaves. Because of the small size of the stem, the division between stem and leaves was not performed for the dry weight assessment. Leaf dry matter content (LDMC) was calculated from the ratio between LDW and LFW. Leaf water content (LWC) was calculated as the reciprocal of LDMC. Leaf area index (LAI) was calculated by dividing LA by the unit of surface occupied by plants, while specific leaf area (SLA) was calculated by dividing LA by LDW. The ratio of leaf length to leaf width (L:W) was calculated by dividing LL by LW. Average leaf dry weight (Avg LDW) was calculated by dividing LDW by LN.

Table 1
Spectral characteristics (red and blue and far-red radiation intensity; μmol m^{-2} s^{-1}), total photosynthetic photon flux density (PPFD; μmol m^{-2} s^{-1}, 400–700 nm), total photon flux density (PFD; μmol m^{-2} s^{-1}, 400–750 nm), far-red fraction (FR/R+FR) and percentage of far-red radiation in the spectrum for the five different light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50 and RB_3–70.

Light treatments	RB_3 (μmol m^{-2} s^{-1})	FR (μmol m^{-2} s^{-1})	Total PPFD 400–700 nm (μmol m^{-2} s^{-1})	Total PFD 400–750 nm (μmol m^{-2} s^{-1})	FR fraction (FR/R+FR)	FR percentage (%)
RB_3	200	0	200	200	0	0
RB_3–10	190	10	190	200	0.07	5
RB_3–30	170	30	170	200	0.2	15
RB_3–50	150	50	150	200	0.3	25
RB_3–70	130	70	130	200	0.4	35

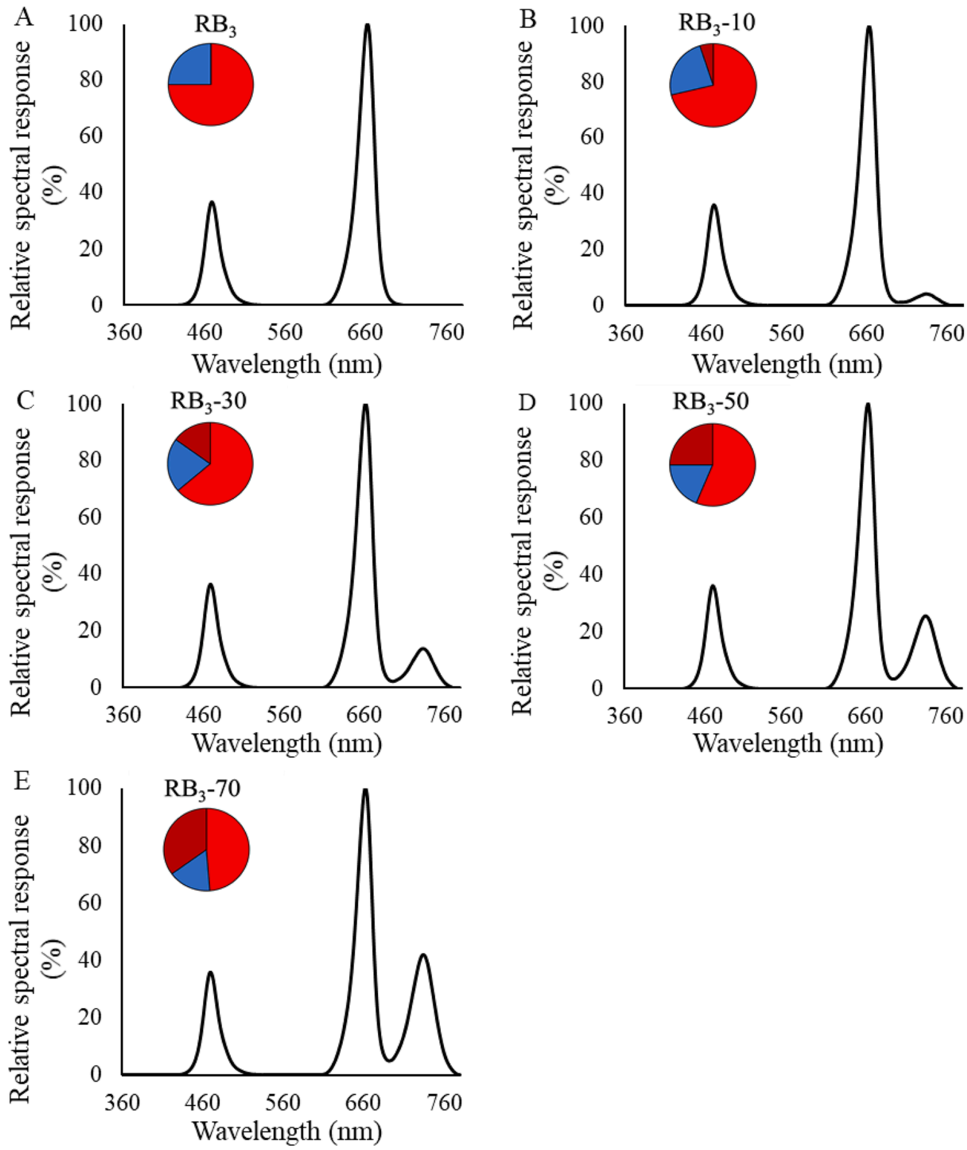

Fig. 1. Relative spectral response (%) of light treatments used in the experiment: A) RB_3; B) RB_3–10; C) RB_3–30; D) RB_3–50 and E) RB_3–70.

2.5. Growth analysis

To evaluate the effect of FR radiation at the different stages of the growth cycle relative growth rate (RGR) and net assimilation rate (NAR) were calculated. RGR was calculated according to the following formula (1) (Williams, 1946):

$$RGR = \frac{\ln LDW_2 - \ln LDW_1}{t_2 - t_1} \tag{1}$$

Where LDW_2 is the LDW at time 2 and LDW_1 is the LDW at time 1.

NAR, similarly to RGR, was calculated according to the following formula (2) (Vernon and Allison, 1963):

$$NAR = \frac{LDW_2 - LDW_1}{t_2 - t_1}\,\frac{\ln LA_2 - \ln LA_1}{LA_2 - LA_1} \tag{2}$$

Where LA_2 is the LA at time 2 and LA_1 is the LA at time 1. Considering that destructive measurements have been performed every 7 days (at 8, 15, 22 and 29 DAT), Δt was always equal to 7.

To evalute the effect of FR radiation at the final harvest, in relation to the growth at the beginning of the growth cycle, specifically at 8 DAT, RGR was calculated according to the formula (3):

$$RGR = \frac{\ln LDW_{29} - \ln LDW_8}{t_{29} - t_8} \tag{3}$$

and NAR was calculated according to the formula (4):

$$NAR = \frac{LDW_{29} - LDW_8}{t_{29} - t_8}\,\frac{\ln LA_{29} - \ln LA_8}{LA_{29} - LA_8} \tag{4}$$

Where LDW_{29} and LDW_8 are LDW at 29 and 8 DAT, and LA_{29} and LA_8 are LA at 29 and 8 DAT, respectively.

2.6. Light and energy use assessment

LED-related electricity consumption (considering different lighting conditions as described in Table 1) was monitored through the use of energy meters. In the same way, the consumption of other system components (HVAC system and cultivation facilities, e.g., water pumps and fertigation units) was monitored for the entire growth period. Light use efficiency (LUE) was calculated as the ratio of grams of LDW to mol of light (400–750 nm) emitted by the lighting systems, and lighting energy efficiency (L-EUE) as the ratio of grams of LFW to the energy used by the lighting systems (kWh). Energy use efficiency (EUE) was calculated as the ratio of g of LFW to the energy used by all the components of the system (LED lighting system, HVAC system and cultivation facilities).

2.7. Color determinations

At 28 DAT, leaf coloration was assessed by using a portable colorimeter (Chroma Meter CR-400, Minolta, Tokyo, Japan), performing three measurements for each of the two most developed leaves of each plant. The colorimeter was calibrated to a standard white reflective plate and the following index were analyzed: L*, a* and b*. L* indicates the degree of lightness of leaves, ranging from 0 (=black) to 100 (=white), while a* indicates the level of greenness and redness ranging from −60 and +60, respectively. The parameter b* indicates the level of blueness and yellowness ranging from −60 and +60, respectively. The hue angle was calculated to evaluate redness (0°), yellowness (90°), greenness (180°) and blueness (270°), using the formula: $h^{\circ} = \tan^{-1}\left(\frac{b^*}{a^*}\right)$ (Owen and Lopez, 2015).

2.8. Statistical analysis

Data were analyzed by using a one-way ANOVA for each day of measurement using SPSS statistic. Significant differences between light treatments were tested by Tukey test at 95 % confidence. For PH, LA, L:W, SLA and ΦPSII a linear regression analysis (α=0.05) was performed. A regression analysis was also performed with LUE and L-EUE to check for significance of LAI, L:W, PH and SLA (α=0.05) at the final harvest and with SLA and leaf greenness (α=0.05) at 8, 15, 22 and 29 DAT.

3. Results

3.1. Plant growth, leaf dry matter content and growth indexes

Leaf fresh weight (LFW) and leaf dry weight (LDW) were affected by the applied lighting regimes, and, therefore, by the amount of FR radiation, starting from 15 DAT (Fig. 2**A** and Fig. 2**B**). In fact, no effect of FR radiation was reported at 8 DAT. For both LFW and LDW, no statistically significant differences were observed between RB_3–10 and control at any stage of the growth cycle (Fig. 2**A** and Fig. 2**B**). At 15 DAT, the replacement of 30 and 50 µmol m^{-2} s^{-1} (FR fraction of 0.2 and 0.3, respectively) resulted in an increase in LFW by 47 and 45 % as compared to RB_3 (6.56 g $plant^{-1}$) (Fig. 2**A**). At 22 and 29 DAT, LFW increased with RB_3–30, RB_3–50 and RB_3–70 as compared with the control. Indeed, at 22 DAT, an increase ranging 35 to 54 % was observed as compared to the control and, at 29 DAT, the increase ranged 47 to 64 %, reaching 28.22 g $plant^{-1}$ with the treatment RB_3–70 (Fig. 2**A**). LDW showed a different trend compared to LFW. At 15 DAT RB_3–30 and RB_3–50 showed higher values (between +36 and 41 %) as compared to RB_3–10 and RB_3–70 (Fig. 2**B**). At 22, DAT RB_3–50 reported a higher value compared to RB_3 with an increase of +40 %, while at 29 DAT both RB_3–30 and RB_3–50 increased LDW, compared to RB_3, with the increase ranging 42 to 45 % (Fig. 2**B**).

Table 2
Leaf dry matter content (LDMC;%) of plants at 8, 15, 22 and 29 DAT grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 0, 10, 30, 50 and 70 µmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3 and 0.4, as shown in Table 1. Each value is the mean of three replicates, each with 5 replicate plants. Data ± standard errors are reported. Different letters indicate significant differences, according to the ANOVA test and the Tukey post-hoc test for mean separation with $p \leq 0.05$.

	Light treatment	8 DAT	15 DAT	22 DAT	29 DAT
Leaf dry matter content (%)	RB_3	5.94 ±0.16 a	5.53 ±0.29 a	7.35 ±0.31 a	8.40 ±0.16 a
	RB_3–10	5.50 ±0.22 ab	4.73 ±0.20 ab	5.93 ±0.27 ab	6.83 ±0.24 ab
	RB_3–30	5.61 ±0.20 ab	4.75 ±0.19 ab	6.10 ±0.26 ab	8.17 ±0.24 ab
	RB_3–50	4.83 ±0.23 b	4.93 ±0.15 ab	6.62 ±0.22 ab	8.20 ±0.35 ab
	RB_3–70	4.85 ±0.16 b	3.95 ±0.15 b	5.63 ±0.27 b	6.63 ±0.28 b

Leaf dry matter content (LDMC) was found to be negatively affected by the increase of FR fraction replacing the red and blue one (Table 2). Throughout the growing cycle, the highest values were reported in plants grown with RB_3. Significant reductions were observed with RB_3–70 in all stages of the growing cycle, and at 8 DAT also with RB_3–50 (Table 2).

Influence of the light spectrum on the relative growth rate (RGR) was detected at 15, 22 and 29 DAT (Table 3). At 15 DAT, plants grown with RB_3–30 and RB_3–50 had a higher RGR as compared to the control and RB_3–10. At 22 DAT, a significant increase was reported in plants grown with RB_3–70, compared to RB_3, RB_3–10 and RB_3–30. At the final harvest (29 DAT), the use of RB_3–30 and RB_3–70 lead to an increase in RGR, compared to RB_3 and RB_3–50. Comparing the different harvests, the greatest influence of FR radiation on RGR was reported at 29 DAT (Table 3). Net assimilation rate (NAR) was higher in plants grown with R and B during the entire growth cycle and in plants grown with RB_3–30 at 29 DAT (Table 3). At 15 DAT, the RB_3–70 treatment was the only one that statistically differed from the control, while at 22 DAT the effect of FR was greater and all FR treatments decreased NAR compared to RB_3. At 29 DAT, treatments RB_3–50 decreased NAR, compared to RB_3 and RB_3–30. The greatest influence of FR radiation on NAR was reported at 22 and 29 DAT (Table 3).

3.2. Plant and leaf morphology

Plant height (PH), leaf area (LA), ratio between leaf length and width

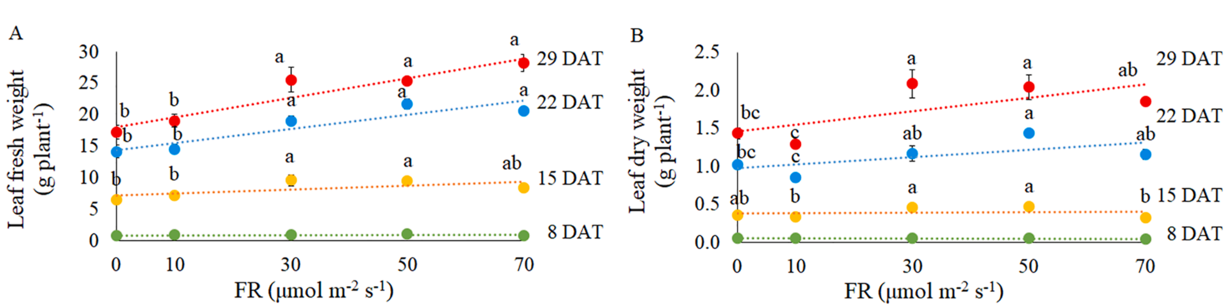

Fig. 2. A) Leaf fresh weight (LFW; g $plant^{-1}$) and B) leaf dry weight (LDW, g $plant^{-1}$) of plants at 8, 15, 22 and 29 DAT grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 0, 10, 30, 50 and 70 µmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3 and 0.4, as shown in Table 1. Each value is the mean of three replicates, each with 5 replicates plants. Vertical bars represent standard errors. Different letters indicate significant differences, according to the ANOVA test and the Tukey post-hoc test for mean separation with $p \leq 0.05$.

Table 3
Relative growth rate (RGR; g day^{-1}) and net assimilation rate (NAR; mg cm^{-2} day^{-1}) (according to the formula (1) and (2)) of plants at 15, 22 and 29 DAT grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 0, 10, 30, 50, and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3, and 0.4, as shown in Table 1. Each value is the mean of three replicates, each with 5 replicates plants. Data ± standard errors are reported. Different letters indicate significant differences, according to the ANOVA test and the Tukey post-hoc test for mean separation with $p \leq 0.05$.

	Light treatment	15 DAT	22 DAT	29 DAT
Relative growth rate (g day^{-1})	RB_3	0.27 ±0.01 b	0.15 ±0.01 bc	0.05 ±0.01 c
	RB_3–10	0.27 ±0.01 b	0.13 ±0.00 c	0.06 ±0.00 bc
	RB_3–30	0.31 ±0.01 a	0.14 ±0.01 c	0.08 ±0.01 a
	RB_3–50	0.31 ±0.01 a	0.16 ±0.00 ab	0.05 ±0.01 c
	RB_3–70	0.29 ±0.02 ab	0.18 ±0.01 a	0.07 ±0.00 ab
Net assimilation rate (mg cm^{-2} day^{-1})	RB_3	0.67± 0.04 a	0.57± 0.04 a	0.25 ±0.03 ab
	RB_3–10	0.60± 0.03 a	0.40± 0.05 b	0.22 ±0.02 bc
	RB_3–30	0.65± 0.03 a	0.38 ±0.04 b	0.30 ±0.03 a
	RB_3–50	0.61± 0.02 a	0.44± 0.03 b	0.16 ±0.04 c
	RB_3–70	0.44 ±0.03 b	0.36± 0.04 b	0.19 ±0.01 bc

(L:W) and specific leaf area (SLA) showed a high correlation with FR fraction at 29 DAT with a R^2 of 0.89 (p-value≤ 0.001), 0.92 (p-value≤ 0.001), 0.71 (p-value≤ 0.001) and 0.71 (p-value≤ 0.001), respectively (Fig. 3). While LN did not show any significant differences in response to the light spectrum used (Fig. 6), PH and LA significantly increased starting from 30 μmol m^{-2} s^{-1} of FR radiation in the spectrum (Fig. 3**A** and Fig. 3**B**). For PH the increase ranged 48 to 121% as compared to the control (8.0 cm plant^{-1}), reaching an average value of 17.8 cm plant^{-1} with RB_3–70 (Fig. 3**A**). For LA, that reported averaged value of 251.42 cm^2 plant^{-1} for control plants, the increase ranged 103 to 159%, reaching the value of 651.77 cm^2 plant^{-1} with RB_3–70 (Fig. 3**B**). L:W significantly increased from 50 μmol m^{-2} s^{-1} of FR radiation, with an increase of +33–34% with RB_3–50 and RB_3–70 (Fig. 3**C**). SLA increased starting from 10 μmol m^{-2} s^{-1} of FR radiation in the spectrum, reaching

Table 4
Stomatal conductance (g_s; mmol m^{-2} s^{-1}) and leaf greenness (SPAD unit) of lettuces at 8, 15, 22, and 29 DAT grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 0, 10, 30, 50, and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3, and 0.4, as shown in Table 1. Each value is the mean of three replicates, each with 5 replicates plants. Data ± standard errors are reported. Different letters indicate significant differences, according to the ANOVA test and the Tukey post-hoc test for mean separation with $p \leq 0.05$.

	Light treatment	8 DAT	15 DAT	22 DAT	29 DAT
Stomatal conductance (mmol m^{-2} s^{-1})	RB3	332.7 ± 4.7 a	367.5 ± 34.3 a	253.9 ± 10.6 a	205.1 ± 18.7 a
	RB3–10	320.9 ± 34.3 a	341.8 ± 49.7 a	254.5 ± 7.7 a	161.2 ± 6.7 ab
	RB3–30	315.6 ± 19.2 a	390.7 ± 54.3 a	220.1 ± 5.5 ab	167.1 ± 17.2 ab
	RB3–50	303.4 ± 10.0 a	378.2 ± 12.8 a	211.7 ± 10.1 ab	148.7 ± 8.4 bc
	RB3–70	303.8 ± 12.8 a	316.1 ± 27.1 a	173.1 ± 21.3 b	95.1 ± 4.9 c
Leaf greenness (SPAD unit)	RB3	23.4 ± 0.3 a	28.7 ± 0.1 a	28.5 ± 0.3 a	24.0 ± 0.5 a
	RB3–10	24.1 ± 0.4 a	26.3 ± 0.3 ab	26.8 ± 0.4 b	23.6 ± 0.1 a
	RB3–30	22.8 ± 0.2 ab	26.0 ± 0.2 abc	24.2 ± 0.3 c	21.4 ± 0.4 b
	RB3–50	20.8 ± 0.3 b	23.9 ± 0.5 bc	23.4 ± 0.1 c	19.1 ± 0.2 b
	RB3–70	19.9 ± 0.7 b	23.1 ± 0.6 c	23.9 ± 0.1 c	19.5 ± 0.4 b

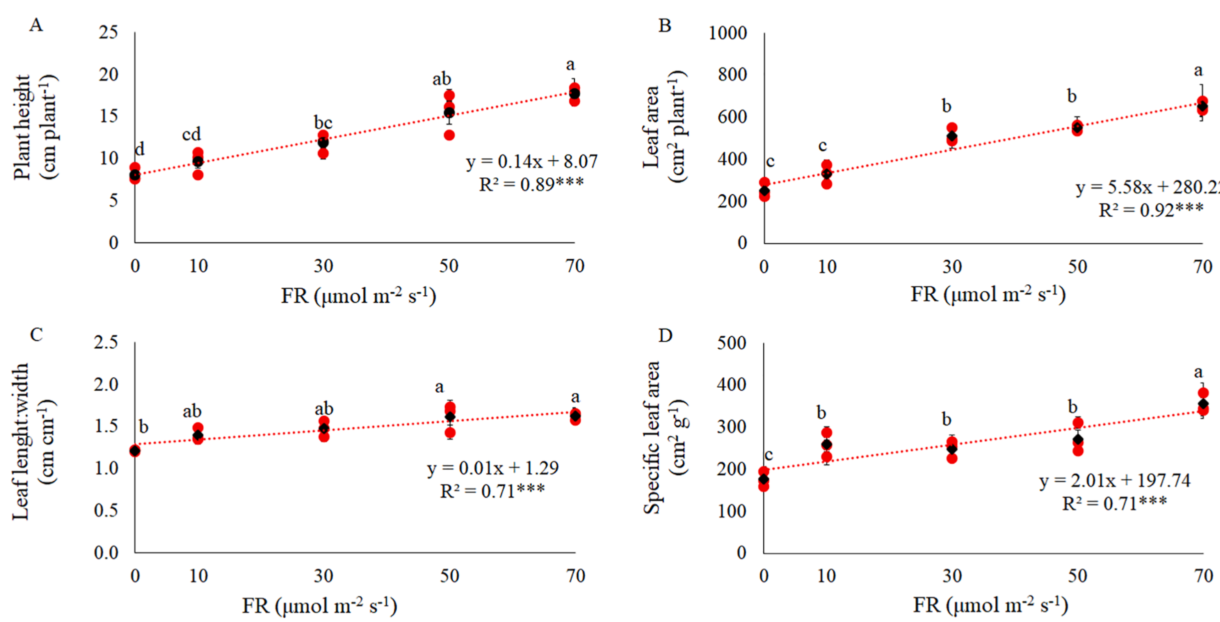

Fig. 3. A) Plant height (PH; cm plant^{-1}), B) leaf area (LA; cm^2 plant^{-1}), C) leaf length:width (L:W; cm^{-1}) and D) specific leaf area (SLA; cm^2 g^{-1}) of lettuces at 29 DAT grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 0, 10, 30, 50 and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3 and 0.4, as shown in Table 1. Red circles represent values as the mean of one replicate ($n = 3$), each with 5 replicates plants. Vertical bars represent standard errors. Equations, coefficients of determination (R^2), and p-values are given for linear relationships (α =0.05). *** indicates significance at $p \leq 0.001$. Black rhombuses represent the average values for the treatment, each with 3 replicates. Vertical bars represent standard errors. Different letters indicate significant differences, according to the ANOVA test and the Tukey post-hoc test for mean separation with $p \leq 0.05$.

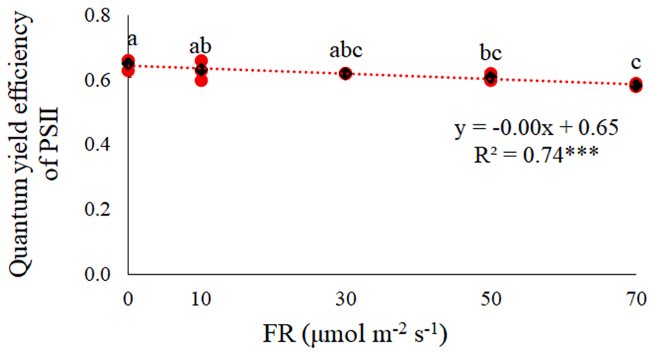

Fig. 4. Quantum yield efficiency of photosystem II (ΦPSII) of lettuces at 28 DAT grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, and RB_3–70. The light treatments contained 0, 10, 30, 50, and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3, and 0.4, as shown in Table 1. Red circles represent values as the mean of one replicate (n = 3), each with 2 replicate plants. Vertical bars represent standard errors. Equation, coefficient of determination (R^2), and p-value are given for linear relationships (α =0.05). *** indicates significance at $p \leq 0.001$. Black rhombuses represent the average values for the treatment, each with 3 replicates. Vertical bars represent standard errors. Different letters indicate significant differences, according to the ANOVA test and the Tukey post-hoc test for mean separation with $p \leq 0.05$.

the highest values with 70 μmol m^{-2} s^{-1} of FR radiation (+102%) (Fig. 3**D**).

At the final harvest (29 DAT), the allocation of fresh biomass partitioned to the stem compared to the entire shoot (stem + leaves) was not affected by the amount of far-red radiation in the spectrum (data not shown).

3.3. Physiological parameters: stomatal conductance, relative chlorophyll content, quantum efficiency of photosystem II

Stomatal conductance (g_s) was affected by the adopted lighting regimes from 22 DAT. At 22 DAT, a significant decrease was observed in plants grown with RB_3–70 (173.1 mmol m^{-2} s^{-1}) as compared to control plants (253.9 mmol m^{-2} s^{-1}) (Table 4). At 29 DAT, the highest value was reported with RB_3 (205.1 mmol m^{-2} s^{-1}) and a significant decrease (−27 and −54%, respectively) was reported under RB_3–50 and RB_3–70 (Table 4). The values of leaf greenness (SPAD units) decreased as FR radiation increased within the spectrum, with higher values always associated with RB_3 (ranging 23.4 to 28.7 SPAD unit according to the DAT) (Table 4). A decrease was reported with RB_3–50 and RB_3–70 at 8 and 15 DAT, while at 22 DAT the decrease started with the treatment RB_3–10 and at 29 DAT with RB_3–30 (Table 4).

The quantum yield efficiency of photosystem II (ΦPSII) showed a linear correlation with the amount of FR photon flux density in the spectrum (R^2=0.74, p-value≤ 0.001) (Fig. 4). Plants grown with RB_3–50 and RB_3–70 reported a significant decrease of −6 and −10 %, respectively, as compared to the control treatment (Fig. 4).

Table 5
Light use efficiency (LUE; g LDW mol^{-1}) and lighting energy use efficiency (L-EUE; g LFW kWh^{-1}) of lettuces at 8, 15, 22, and 29 DAT grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 0, 10, 30, 50 and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3 and 0.4, as shown in Table 1. Data ± standard errors are reported. Different letters indicate significant differences, according to the ANOVA test and the Tukey post-hoc test for mean separation with $p \leq 0.05$.

	Light treatment	8 DAT	15 DAT	22 DAT	29 DAT
Light Use Efficiency (g LDW mol^{-1})	RB_3	0.09 ± 0.05 a	0.32 ± 0.02 ab	0.62 ± 0.01 bc	0.66 ± 0.03 bc
	RB_3–10	0.09 ± 0.05 a	0.30 ± 0.01 b	0.52 ± 0.02 c	0.59 ± 0.01 c
	RB_3–30	0.09 ± 0.05 a	0.41 ± 0.04 a	0.70 ± 0.06 b	0.96 ± 0.08 a
	RB_3–50	0.09 ± 0.05 a	0.41 ± 0.02 a	0.87 ± 0.02 a	0.94 ± 0.07 a
	RB_3–70	0.07 ± 0.04 a	0.29 ± 0.01 b	0.70 ± 0.02 b	0.85 ± 0.02 ab
Lighting Energy Use Efficiency (g LFW kWh^{-1})	RB_3	6.51 ± 0.40 a	24.73 ± 1.35 c	36.42 ± 2.55 d	32.18 ± 2.16 b
	RB_3–10	7.19 ± 0.22 a	28.10 ± 1.72 bc	40.27 ± 0.48 cd	36.43 ± 2.07 b
	RB_3–30	6.71 ± 0.51 a	36.29 ± 3.25 a	46.70 ± 1.80 bc	47.78 ± 3.72 a
	RB_3–50	8.36 ± 0.38 a	35.80 ± 1.56 a	55.49 ± 1.61 a	49.21 ± 2.18 a
	RB_3–70	6.60 ± 0.64 a	32.44 ± 1.32 abc	53.19 ± 0.62 ab	53.75 ± 2.46 a

3.4. Light and lighting energy use efficiency

Light use efficiency (g LDW mol^{-1}) was affected from 15 DAT by the introduction of FR radiation in the spectrum (Table 5). As shown in Table 5, significant differences compared to the control RB_3 emerged with RB_3–30 at 22 DAT, and with RB_3–30 and RB_3–50 at 29 DAT. At the final harvest (29 DAT), the increase ranged 42 to 45 %. Lighting energy use efficiency (g LFW kWh^{-1}) also varied from 15 DAT based on the FR radiation in the spectrum, with an increase at 15 DAT with RB_3–30 and RB_3–50 (+45–47 %), at 22 DAT with RB_3–30, RB_3–50 and RB_3–70 (+28–52 %) and the same at 29 DAT (+48–67 %) (Table 5).

There was a positive and significant correlation between leaf area index (LAI) and LUE (R^2=0.52, p-value≤0.01) and L-EUE (R^2=0.92, p-value≤0.001) at 29 DAT (Fig. 5**A**). A positive and significant correlation has been also observed between L-EUE and L:W (R^2=0.63, p-value≤0.001; Fig. 5**B**), PH (R^2=0.77, p-value≤0.001; Fig. 5**C**) and specific leaf area (R^2=0.52, p-value≤0.01; Fig. 5**D**) at 29 DAT.

Fig. 6 represents the variation in yield, morphological and physiological parameters and finally on light use efficieny at 29 DAT, compared to the control RB_3, with the use of light treatments RB_3–30, RB_3–50 and RB_3–70. LFW differed from the control with all the light treatments used. With 30 μmol m^{-2} s^{-1} of FR radiation (FR fraction of 0.2), leaf water content (LWC) was not affected, while LDW increased by +45%, followed by an increase in RGR by +13%. Considering the components of RGR, NAR did not change (the same was found for the measured physiological components, ΦPSII and g_s), while SLA increased by 41%. The increase in SLA was the result of a large increment in both LA (+103%) and average leaf dry weight (Avg LDW) (+45%), leading overall to a significant increase in LUE (Fig. 6**A**). With 50 μmol m^{-2} s^{-1} of FR radiation (FR fraction of 0.3), as for the previous case, LFW increased by +47 %, as a consequence in an increase in LDW (+42 %). The increase in RGR (+10 %), was the result of an increase in SLA (+55 %), while NAR decreased compared to the control (−22 %). LA and Avg LDW increased by +119 and 60 %, respectively (while no differences have found for LN). What emerges from the picture is that 50 μmol m^{-2} s^{-1} of FR radiation had a negative impact on the physiological components (ΦPSII was reduced by −6% and g_s by −27%), but the increase in LA and SLA contributed to an increase in LUE compared to the control, RB_3 (Fig. 6**B**). With 70 μmol m^{-2} s^{-1} of FR radiation, LFW increased by +64%. In this case, while the increase in LDW (+29%) was not significant, LWC increased by +21%. RGR increased by +15% and SLA increased by +102%, as a consequence of an increase in Avg LDW (+52 %) and a considerable increase in LA (+159 %). However, the greater reduction in NAR (−36 %), and ΦPSII (−10 %) and g_s (−54 %), avoided increasing LDW (and thus LUE) compared to the control (Fig. 6**C**).

The energy consumption of the entire vertical farm is represented with the partition between the various components: LED artificial lighting system (51 %); climate control system (47 %), and cultivation facilities (2 %) (Fig. 7**A**). The adoption of RB_3–30, RB_3–50 and RB_3–70

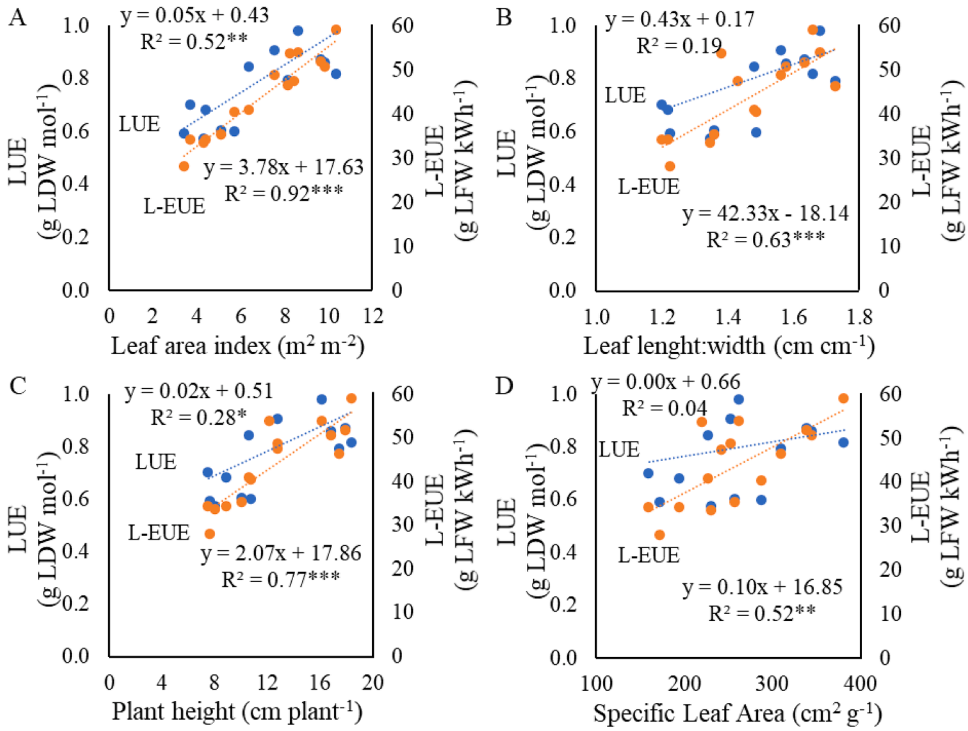

Fig. 5. Relation between light energy use efficiency (LUE; g LDW mol^{-1}) and lighting energy use efficiency (L-EUE; g LFW kWh^{-1}) with A) leaf area index (m^2 m^{-2}); B) leaf length to width ratio (cm cm^{-1}); C) plant height (cm plant^{-1}) and D) specific leaf area (cm^2 g^{-1}). Each point represents the result of LUE or L-EUE of lettuces at the final harvest (29 DAT) grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, and RB_3–70. The light treatments contained 0, 10, 30, 50, and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3, and 0.4, as shown in Table 1. Each point is the mean of 5 replicate plants. Equations, coefficients of determination (R^2), and p-values are given for linear relationships (α =0.05). *** indicates significance at $p \leq 0.001$, ** indicates significance at $p \leq 0.01$ and * indicates significance at $p \leq 0.05$.

(FR fraction of 0.2, 0.3 and 0.4, respectively) significantly improved the energy use efficiency of the entire system, reaching an increase ranging 49 to 65 % (values between 21.0 and 23.3 g FW kWh^{-1}) compared to the control, 14.1 g FW kWh^{-1} (Fig. 7**B**).

3.5. Color determinations

Leaf pigmentation was evaluated before the final harvest (at 28 DAT) through the assessment of the L*, a* and b* values and the hue angle h°. Plants were largely affected by the applied light treatments (Fig. 8 and Fig.9). Plants grown under RB_3 showed the lowest value of L* (38.07), which significantly increased with the increase of FR radiation in the spectrum, reaching the maximum level under RB_3–70 (64.04) (Fig. 8). The b* values were the lowest with RB_3 (18.39) and they increased with all the light treatments with FR radiation, reaching the highest value with RB_3–50 and RB_3–70 (44.29 and 47.13, respectively). The highest redness of leaves (a*) was found with RB_3 (3.57) and it decreased from 10 μmol m^{-2} s^{-1} of FR, decreasing as the FR radiation in the spectrum increased up to RB_3–70. Plants grown with this treatment showed a sharp decline, reaching the value of −24.10 with RB_3–70. Compared to the control, the hue angle values (h°) increased from 10 μmol m^{-2} s^{-1} of FR radiation and remained constant as FR increased in the spectrum (Fig. 8).

4. Discussion

Modulating spectral characteristics during cultivation has an impact on growth, resource use efficiency and product quality. Moreover, in controlled environment agriculture, radiation can be managed in a timely and precise manner, giving the opportunity to consider, within artificial lighting management techniques, the time factor dimension (Meng and Runkle, 2020). In this experiment, all parameters analyzed proved to be sensitive to the FR fraction in the spectrum and showed a dependency from the phase of the growth cycle.

4.1. At the final harvest, far-red replacing red and blue radiation promotes biomass accumulation up to an optimum

The present research demonstrated that in a controlled environment, when environmental parameters are not limiting, LFW of lettuces increased linearly with increasing FR radiation in the spectrum. Indeed, at the final harvest, increasing the FR fraction (up to 0.4, which is equivalent to 70 μmol m^{-2} s^{-1} of FR radiation replacing the same amount of R and B) while maintaining constant the PFD, resulted in higher plants LFW (up to 64%) (Fig. 2**A**). In the case of LDW, the relationship was not linear, and a specific optimum was identified at 30 and 50 μmol m^{-2} s^{-1} (0.2 and 0.3 of FR fraction) (Fig. 2**B**). As a result, plants grown with the highest FR fraction (RB_3–70) reported a decrease in leaf dry matter content (LDMC) (Table 2). The increase in LFW and LDW with the addition of FR radiation to a shorter wavelength spectrum has been reported in various researches. Zou et al. (2019), adding 50 μmol m^{-2} s^{-1} of FR radiation to 200 μmol m^{-2} s^{-1} of PAR for the cultivation of lettuce cv. Tiberious, reported an increase by 53% and 39% for LFW and LDW, respectively. An increase in LFW and LDW was also found when the same experimental treatment was applied only at the end of the day for 1 hour (Zou et al., 2021). Jin et al. (2021), using a spectrum with 218 μmol m^{-2} s^{-1} of R and B radiation for the cultivation of green lettuce cv. Expertize RZ, observed an increase in LFW and LDW of 61 and 77% respectively, when 52 μmol m^{-2} s^{-1} of FR radiation were added to the background spectrum. Compared to these works, our results showed that replacing part of R and B radiation with the same amount of FR radiation (with a FR fraction ≥ 0.2, corresponding to 30 μmol m^{-2} s^{-1}), thus keeping the PDF constant, also has a positive effect on biomass accumulation. The effect of FR in promoting biomass accumulation

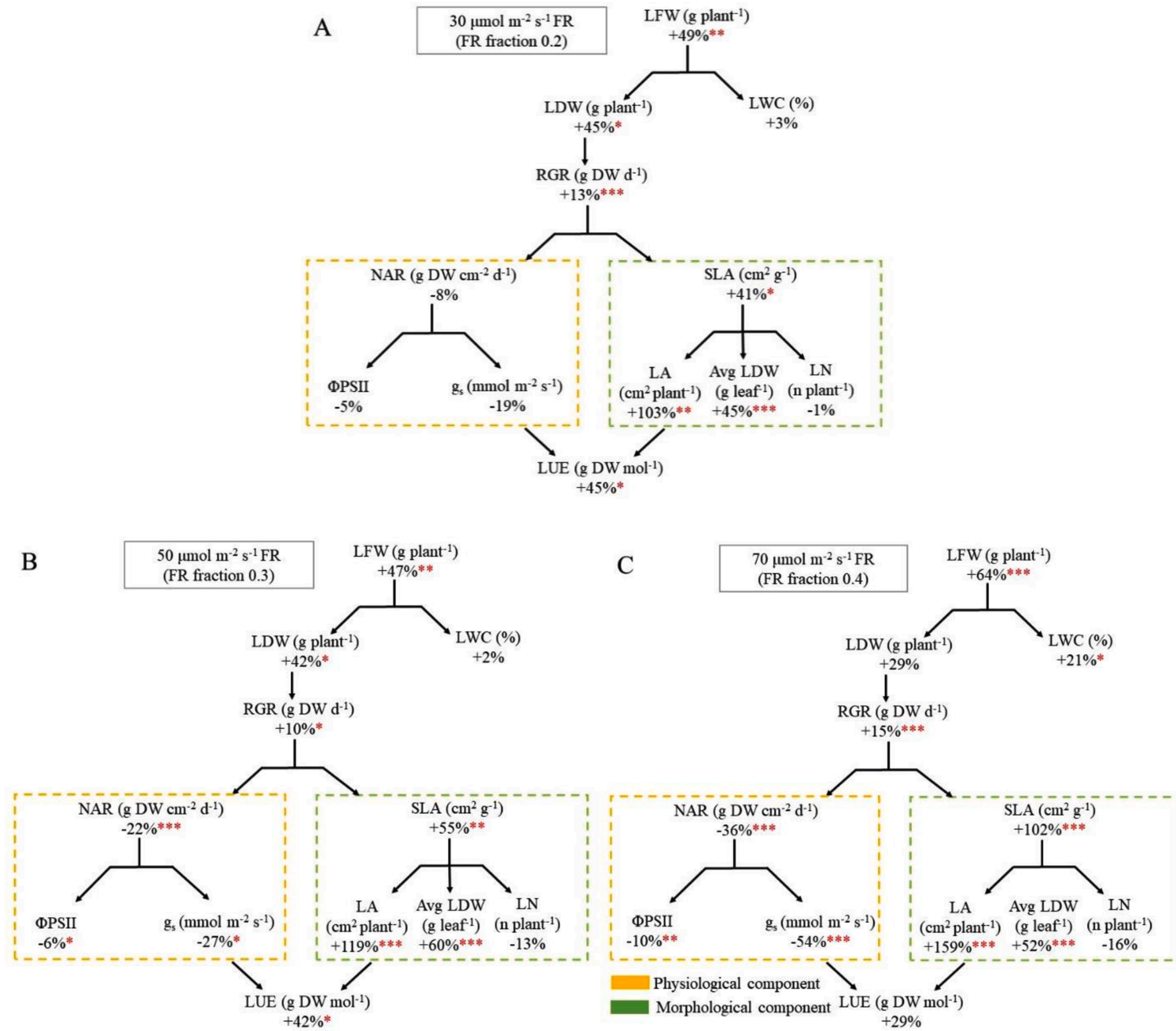

Fig. 6. Physiological (yellow) and morphological (green) parameters of lettuces, which ultimately influence light use efficiency, at 29 DAT grown with the different experimental light treatments: A) 30, B) 50 and C) 70 µmol m^{-2} s^{-1} of FR radiation substituting the same amount or R and B radiation (corresponding to 0.2, 0.3 and 0.4 of FR fraction). The percentage indicates the increase or decrease compared to the control treatment, RB_3. The asterisks indicate the presence of significant differences as compared to RB_3, according to the ANOVA test for $p \leq 0.05$ (*) or $p \leq 0.01$ (**) and $p \leq 0.001$ (***). LFW= leaf fresh weight (g $plant^{-1}$); LDW= (g $plant^{-1}$); LWC=leaf water content (%); RGR= relative growth rate (g dry weight day^{-1}) (according to formula (3)); NAR= net assimilation rate (g dry weight cm^{-2} day^{-1}) (according to formula (4)); ΦPSII= quantum yield efficiency of photosystem II; g_s= stomata conductance (mmol m^{-2} s^{-1}); SLA= specific leaf area (cm^2 g^{-1}); LA=leaf area (cm^2 $plant^{-1}$); Avg LDW = average leaf dry weight (g $leaf^{-1}$); LN= leaf number (n $plant^{-1}$); LUE=light use efficiency (g dry weight mol^{-1} light).

found in this work, was also supported by the raise in the relative growth rate (RGR) that increased at the final harvest, with at least 30 µmol m^{-2} s^{-1} of FR radiation (Fig. 6). An increase in the RGR has previously been reported for lettuce seedlings grown with 50 µmol m^{-2} s^{-1} of FR radiation, substituting 50 µmol m^{-2} s^{-1} of RB_3, compared to seedlings grown with R and B radiation only (Carotti et al., 2023b).

As reported by Zhen and van Iersel (2017), when FR radiation was added to R and B or a warm-white background, there was an increase in the quantum yield efficiency of photosystem II (PSII) and a consequent enhancement in net photosynthetic rate. The efficiency of photochemistry can indeed be enhanced by the faster re-oxidation of plastiquinones, the intermediate electron transporter between PSI and PSII, that occurs in presence of FR radiation. In presence of high light conditions, the reaction centers of PSII undergo a series of mechanism to dissipate excess energy (Tikkanen and Aro, 2012). Since FR light mainly excited PSI, the reoxided-plastoquinones can accept electrons from excited PSII and make PSII more rapidly available for new reactions (Zhen and van Iersel, 2017). In this experiment, where FR replaced R and B radiation, ΦPSII was not affected up to 30 µmol m^{-2} s^{-1} of FR radiation, indicating that, even if PPFD decreases, the presence of FR radiation was able to compensate this reduction (Fig. 4). However, this result was not observed with an amount of FR radiation greater than 30 µmol m^{-2} s^{-1} (specifically RB_3–50 and RB_3–70), which resulted in a decrease of ΦPSII (Fig. 4). The observed decrease could be related to the low PPFD used with RB_3–50 and RB_3–70 (150 and 130 µmol m^{-2} s^{-1}, respectively) that does not saturate PSII. It has been shown that by using high light intensity between 189 and 794 µmol m^{-2} s^{-1}, the quantum yield efficiency of PSII decrease linearly as PPFD increases (Elkins and Van Iersel, 2020), but with lower light intensity, the functioning of the PSII could be impaired (Ghorbanzadeh et al., 2021), as may have occurred in the case

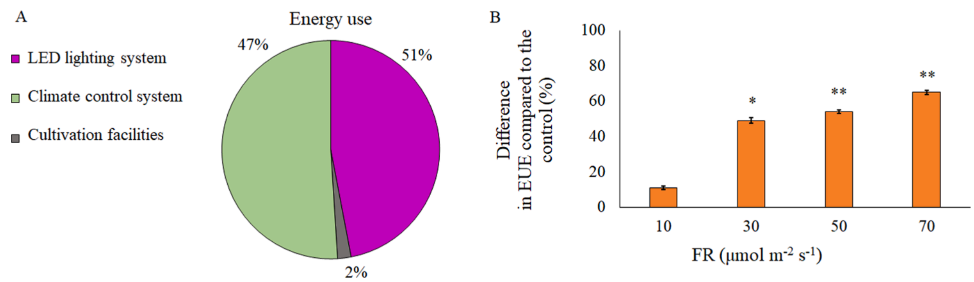

Fig. 7. A) Energy use (kWh^{-1} used during the 29 days of growth cycle) in AlmaVFarm partitioned by the different component of the system: LED lighting system, HVAC climate control system, and cultivation facilities; B) Percentage difference (%) in energy use efficiency (EUE, g LFW kWh^{-1}), compared to the control, considering the contribution of all components in the vertical farm (lighting system, HVAC system, cultivation facilities), of lettuces at the final harvest grown with the four LED light treatments with the addition of FR light used in the experiment: RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 10, 30, 50, and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0.07, 0.2, 0.3, and 0.4, as shown in Table 1. For the energy use, the contribution of all components in the vertical farm (lighting system, HVAC system, cultivation facilities), was considered. Each value is the mean of three replicates, each with 5 replicates plants. Data ± standard errors are reported. The asterisks indicate the presence of significant differences according to the ANOVA test, for $p \leq 0.05$ (*) or $p \leq 0.01$ (**).

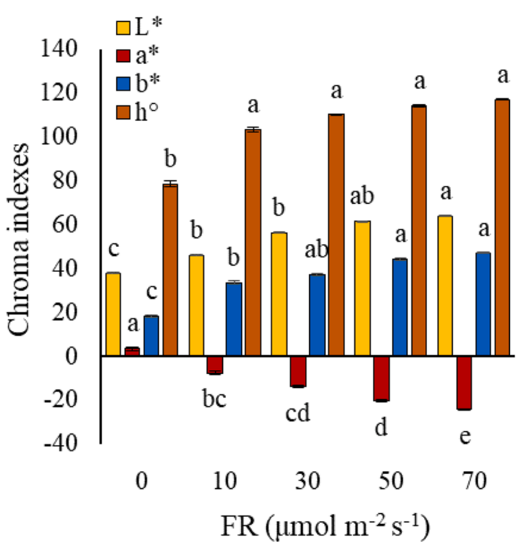

Fig. 8. L*, a* (greenness and redness), b* (blueness and yellowness) values and h° (hue angle) of plants at 28 DAT grown under five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 0, 10, 30, 50 and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3 and 0.4, as shown in Table 1. Each value is the mean of three replicates, each with 2 replicates plants. Vertical bars represent standard errors. Different letters indicate significant differences, according to the ANOVA test and the Tukey post-hoc test for mean separation with $p \leq 0.05$;.

of the two highest FR treatments used in this study. Moreover, as reported by Zhen and Bugbee (2020), only a certain amount of FR radiation is required to balance the excitation between the two photosystems, and this work highlights the threshold (30 μmol m^{-2} s^{-1} of FR radiation, corresponding to 0.2 of FR fraction) beyond which replacing R and B radiation with FR is no longer an optimal strategy from the perspective of photosynthetic performance and biomass accumulation, even if the ratio between R and B is kept constant at the optimal level. Similarly, at the final harvest, lower values of net assimilation rate (NAR) were reported with RB_3–50 and RB_3–70, meaning that the results of the carbon gain and carbon losses expressed per unit of leaf area (Poorter and Remkes, 1990) decreased with the increase of FR radiation in the spectrum (Fig. 6). These results are supported by a similar trend found for g_s, a key component in the control of photosynthesis and transpiration. At 29 DAT, when FR radiation was ≥ 50 μmol m^{-2} s^{-1}, g_s decreased (Table 4). The role of B light in regulating the opening of stomata is a well-known phenomenon. Pennisi et al. (2019a) demonstrated how by increasing the RB ratio, g_s decreases. In this experiment the RB ratio remained constant (RB=3), thus an effective role of FR radiation (with both a quantitative and temporal effect) in stomatal closure can be hypothesized. Even if the role of FR in stomatal opening has not been fully elucidated, some authors have identified from studies in *Arabidopsis*, that FR radiation, through a regulatory pathway triggered by phytochromes, inhibits stomatal opening activated by R and B radiation (Talbott et al., 2003). However, more recent studies show that stomatal activity in response to R and FR is not due to a direct effect of phytochromes, but to their effect on leaf growth and stomata development, highlighting an indirect effect of these family of photoreceptor on stomata opening (Weraduwage et al., 2022). Regardless of the molecular mechanism activated, considering the results of our study, it can be assumed that the decrease in the stomatal activity may be the result of: 1) a decrease in the absolute value of B radiation; 2) an effective antagonistic activity operated by FR radiation, activated with a FR fraction ≥ 0.3. The second hypothesis is also validated by results of similar research in which FR was added to R and B spectrum. For example, in the work of Zou et al. (2019), 50 μmol m^{-2} s^{-1} of FR was added to 200 μmol m^{-2} s^{-1} of PAR (for 16 h) causing a subsequent decrease in g_s. In any case, the results of the present work showed the threshold level of FR radiation, above which a reduction in stomatal activity is triggered compared to a spectrum with only R and B radiation. Leaf greenness (SPAD value) at 29 DAT was lower in plants grown with a FR fraction ≥ 0.2, as compared to the control (Table 4). This response to FR radiation shows a trend that appears to be established among various works: a reduced chlorophyll content was observed in lettuce and kale grown with a spectrum in which FR radiation was present (Meng et al., 2019) and a negative correlation, in three different lettuce cultivars, was found between the chlorophyll content index and the intensity of supplementary FR radiation (Liu and van Iersel, 2022).

For most of the parameters analyzed in this work (both morphological and physiological, except for chroma index (as described in Section 4.4) and specific leaf area at the final harvest), 10 μmol m^{-2} s^{-1} (0.07 of FR fraction) were not enough to trigger a response (positive or negative) compared to plants grown with R and B radiation only. Zou et al. (2021), building a dose-response curve in the presence of FR radiation, have found that 15 μmol m^{-2} s^{-1} of FR radiation were already enough to enhance differences concerning LFW, LDW, and the amount of dry matter partitioning to the leaves. This shows how the FR response is specific and strictly dependent on the presence (or not) of the PAR and to its specific intensity in the background spectrum. Conclusion also

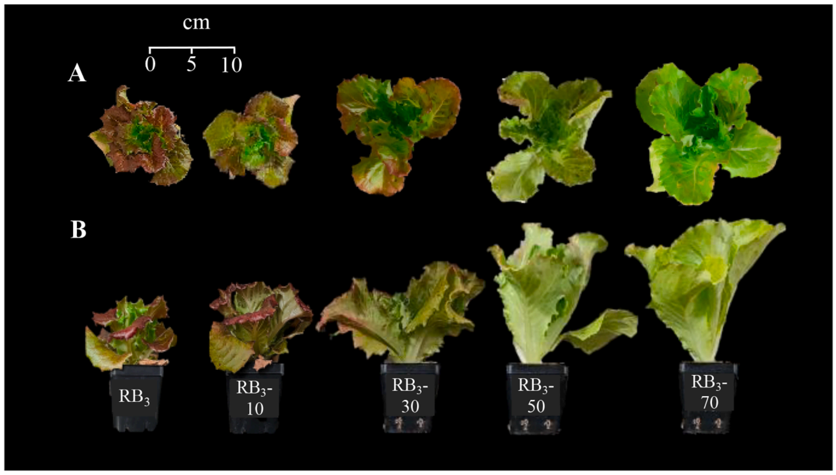

Fig. 9. A) Top view and B) front view of lettuces at 29 DAT grown with the five light treatments, namely RB_3, RB_3–10, RB_3–30, RB_3–50, RB_3–70. The light treatments contained 0, 10, 30, 50 and 70 μmol m^{-2} s^{-1} of far-red radiation, respectively, resulting in a far-red fraction of 0, 0.07, 0.2, 0.3 and 0.4, as shown in Table 1.

supported by Meng and Runkle (2019) who reported that, when 30 μmol m^{-2} s^{-1} of FR radiation were added to a background of R and B radiation, shoot fresh and dry weight increased (both in lettuce and basil), but with a magnitude that was greater when the amount of R radiation in the spectrum was higher.

4.2. Leaf expansion increased linearly as the amount of FR radiation substituting red and blue light augmented, thus fostering light interception

The variation in the morphological architecture in the presence of different FR fractions is one of the distinctive features of a plant in which shade avoidance syndrome (SAS) has been triggered. In vertical farms, this is a parameter to be precisely monitored as both a quality parameter and a central point for intercepting light radiation.

According to the results of our experiments, at the final harvest (29 DAT), plants grown with at least 30 μmol m^{-2} s^{-1} of FR radiation substituting R and B radiation (RB_3–30), were significantly higher and had greater leaf area, without reducing the number of leaves (Fig. 3**A** and 3**B**). Increase in plant height and leaf area are well described symptoms in presence of SAS, as shown for the leaf area of different seedlings of ornamental plants (Park and Runkle, 2018), lettuce seedlings (Carotti et al., 2023b) as well in mature lettuce (Zou et al., 2021), and in tomato plants for plant height (Kalaitzoglou et al., 2019). Based on our results, plants grown with RB_3–50 and RB_3–70, exhibited also narrower leaves, with an increase in L:W (Fig. 3**C**). In head lettuces, L:W has an effect on head formation, which is delayed when a high FR fraction is present in the spectrum (Van De Velde et al., 2023). While this would normally constitute an issue in traditional lettuce cultivation, when production targets the baby leaf sector, as common in vertical farms, the associated effects are negligible from a product quality perspective. According to Kong and Nemali (2021), the addition of FR radiation to a spectrum with R and B radiation, independently from the ratio between the two wavelengths, decreased the leaf number in romaine lettuce. However, Meng and Runkle (2019) highlighted a different response in leaf number in two different lettuce cultivars. This means that the development of leaf primordia in the presence of FR radiation can be cultivar-dependent. The results of this work showed that, for Canasta lettuce, keeping the same PFD and altering the spectral conditions by adding FR radiation, did not influence the number of leaves per plant. Besides, even at the highest FR fraction, the biomass distribution between leaves and stem did not vary, thus avoiding an effect that could negatively impact the amount of salable product. At the final harvest, specific leaf area was also found to be positively correlated with the fraction of FR in the spectrum, leading to the growth of plants with a significant thinner lamina when cultivated with 10, 30, 50 and 70 μmol m^{-2} s^{-1} of FR radiation in the spectrum (Fig. 3**D**). Considering that new leaves are formed from the meristem shading part of the mature leaves (Fraser et al., 2016), the morphology of the individual leaf (e.g., a more expanded leaf, with a greater leaf surface) and the height of plant can facilitate light interception and therefore enhance light use efficiency.

For an analysis of environmental and economic sustainability, it is meaningful to denote light use efficiency as grams of LFW per kilowatt used by the system, namely lighting energy use efficiency (L-EUE). In this sense, this parameter can provide an indication of how the specific lighting conditions applied (e.g., spectral composition) affect the sustainability of the system (Orsini et al., 2020a). From a physiological point of view, it is instead more interesting to consider light use efficiency (LUE) as the ratio between LDW and the total photon flux provided to the canopy (Jin et al., 2021). Our results showed that, at the final harvest, L-EUE and LUE were positively correlated with leaf area index (LAI) values (Fig. 5**A**), that increased with the raise of the FR fraction in the spectrum. These results reinforce the hypothesis that the integration of FR radiation could facilitate light interception, particularly when LAI is high, with the overlap between leaves becoming greater. It is in fact reported that optimal values of LAI, in terms of light interception, correspond to 3–4 (Van Delden et al., 2021), where in the specific case of lettuce, a LAI of 4.4 correspond to the 95 % of ground cover (Tei, 1996). According to the results of this study, by substituting part of the R and B radiation with FR, keeping constant the PFD, it is possible to achieve higher LUE and L-EUE values even with values of LAI much greater than 4. In this study, a constant planting density of 153 plants m^{-2}, similar to the average density used in vertical farming (Jin et al., 2022), was used to simulate conditions that could be used by growers. In addition, L-EUE also showed a positive correlation with L:W, PH and SLA. Overall, these results show that the specific morphology induced by the activation of the SAS, and thus by an environment with high FR fraction, have a large influence on how plants use the light in an indoor environment (without limiting growing factors), also suggesting a sustainability functions of phytochrome, besides their physiological and ecological ones already described.

Considering that FR radiation has generally been excluded from the definition of PAR, much work has been done to distinguish the contribution of the morphological and physiological components to plant growth. According to Legendre and van Iersel (2021), enriching the spectrum with FR radiation has firstly an effect on morphology, resulting in leaves expansion that led to a larger leaf area and a larger canopy size that ultimately increases the amount of intercepted light and therefore the growth. Jin et al. (2021) reported that the increase in biomass production in presence of supplemental FR radiation (+ 52 μmol m^{-2}

s^{-1} of FR radiation) was mainly the result of faster leaf area expansion, that led to a greater light interception. In the case of the latter work was highlighted how the contribution of increased leaf area was significantly higher than the influence of specific leaf area and net photosynthetic rate. According to the results of this work, the increase in biomass up to 30 μmol m^{-2} s^{-1} of FR radiation in the spectrum (FR fraction of 0.2), was supported by g_s and ΦPSII and by a great enlargement of LA (Fig. 6**A**). When FR radiation increased (in the case of this experiment up to 50 μmol m^{-2} s^{-1}, FR fraction of 0.3), ΦPSII and g_s decreased, and the growth was only sustained by an increase in leaf expansion (Fig. 6**B**). Indeed, an enlargement in leaf area is known to counteract a reduction in the photosynthetic rate (Kim et al., 2004).

A large portion (51 %) of the energy consumed in the experimental vertical farm where the experiments took place is used for LED artificial lighting (similar results were reported by Yokoyama, 2019, while a higher contribution of LED lighting was found by Martin and Molin, 2019) and light quality management, in addition to the primary effect on morphology and physiology, has an impact on the overall energy use efficiency (EUE). This research shows a possible optimization of EUE in a vertical farm by the modulation of the spectral properties. According to our results, modulating the FR fraction in the spectrum in a range between 0.2 to 0.4 can largely increase EUE, compared to a spectrum with R and B only (Fig. 7**B**). The substitution of part of R and B radiation with FR radiation represents a possible strategy to optimize the energy use and, therefore, increase the sustainability of the system. This can be achieved through the management of phytochrome effects, by increasing the amount of fresh products produced per unit of electricity.

4.3. Influence of FR radiation can be detected throught the entire growth cycle

The results of this work showed that in the early stages of plant development after transplanting (8 DAT), LFW and LDW were not affected by the presence of FR radiation (Fig. 2A and B**)**, but the FR radiation effect occurred as of 15 DAT. Comparing the results of the RGR at the various stages of the growth cycle (Table 3), it appeared that the influence of FR radiation was already effective at 15 DAT, and it was greater at the end of the cycle, between 22 and 29 DAT, when the absolute values of the RGR decreased. This result therefore supports the already presented hypothesis that the FR radiation in the spectrum is especially beneficial when the leaf area index is high (Fig. 5**A**). These results also agreed with the work of Legendre and Van Iersel (2021), who reports a greater effect on FR radiation later in the growth cycle, probably as a consequence of a cumulative effect. Nevertheless, the use of a FR radiation between 0.2 and 0.3 led to an increase in LUE and L-EUE already at 15 DAT (Table 5), confirming the relevance of enriching the spectrum with FR radiation already from this stage of the growth cycle. Similar results were also shown by Van De Velde et al. (2023) who reported an increase in LUE in the presence of FR radiation as early as 14 DAT. At 29 DAT, no differences were reported in LDW between RB_3 and RB_3–70. However, RGR at the end of the growth cycle (calculated according to formula 3), reported an increase by +15 % (Fig. 6**C**). This is probably due to the fact that, at 8 DAT, LDW of plants grown with RB_3–70 decreased by 19 % compared with RB_3 (although no significant differences emerged, probably due to sample variability), while with all the other treatments the change compared with RB_3 was between −3 and +4 %. The greatest influence of FR radiation on NAR was found at 22 and 29 DAT (Table 3); similarly g_s resulted to be influenced by the FR fraction from 22 DAT (Table 4), suggesting that in terms of photosynthetic performances, plants in the early stage of growth are less sensitive to high FR fraction. These results also indicate a cumulative effect of FR radiation on these parameters, especially with the use of RB_3–50 and RB_3–70 treatments (corresponding to 0.3 and 0.4 of FR fraction).

Leaf greenness and SLA values (indicator of leaf thickness) were one of the parameters that have already been affected from the first days after transplanting. As early as 8 DAT, increasing FR intensity in the spectrum decreased leaf greenness (Table 4) and increased SLA, thus reducing leaf thickness (data not shown). Considering that a correlation was found between the two parameters at all destructive harvests (**Figure S1**), the results suggest that the decrease in leaf greenness could be due to a thinner leaf, thus leading to a decrease in chlorophyll content per leaf area. Variation in SLA resulted in a trade-off between larger leaf area thus more leaf area surface for light interception, but lower specific leaf nitrogen (Legendre and Van Iersel, 2021). This specific trade off could be the reason why no correlation between increased SLA and LUE emerged in our work (Fig. 5**D**). In any case, the activation of chlorophyll degradation mechanisms in the presence of FR radiation cannot be excluded and a more in-depth analysis of chlorophyll content and gene expression would be advisable in future researches.

4.4. Replacing red and blue with far-red radiation negatively affects leaf pigmentation at the final harvest

Product quality, and primarily coloration and pigmentation, is a parameter that largely influences consumers perception and choices in several crops, as well as in leafy greens (Hoppu et al., 2021; Sharma et al., 2021). The results of this study showed how the level of pigmentation was affected by the amount of FR radiation in the spectrum (Fig. 9**A** and 9**B**). The increase in FR led to an increase in lightness, in yellowness and in hue angle values. The latter differed from the others since it increased already from 10 μmol m^{-2} s^{-1}, compared to RB_3, but did not increase further by increasing FR fraction, indicating the presence of a saturation mechanism (Fig. 8). Overall, this trend, for red leafy greens, means a general increase in yellow leaf color and a change in color from red to green (Pennisi et al., 2021), trend also confirmed by the SPAD values. Red pigmentation in lettuce is due to anthocyanins, a subgroup of flavonoids, therefore, greater pigmentation may also reflect a higher content of antioxidant compounds (Kim et al., 2016). A possible role of B light, in activating the phenylpropanoid biosynthetic pathway (that lead also to the biosynthesis of anthocyanins), was formerly identified in various works (Ouzounis et al., 2015; Taulavuori et al., 2016). He et al. (2021), using a R and white spectrum (with a ratio of 2:3) for red lettuce growth (*Lactuca sativa* "Red Butter") with an additional 50 μmol m^{-2} s^{-1} of FR radiation, observed the lightest color, an increase in yellowness and an increase in lightness, compared to the control spectrum with only R and white. This shows how the presence of FR radiation, regardless of whether it is in addition or in substitution to the PAR, decreases the content of pigments in lettuce leaves. Moreover, our results showed that replacing at least 10 μmol m^{-2} s^{-1} of R and B radiation with FR (FR fraction of 0.07) was already sufficient to adversely affect the visual quality of the product. Therefore, studying the interaction with B radiation, also used in a dynamic regime, is critical to maintain the benefits of integrating FR radiation into the spectrum without negatively impacting the quality.

5. Conclusion

Replacing part of the red and blue with far-red radiation had significant effects on growth, architecture and physiology of lettuce plants grown in a vertical farm. At the final harvest, both leaf fresh weight and leaf dry weight of lettuces increased with the inclusion of 30 or 50 μmol m^{-2} s^{-1} of far-red light photons (far-red fraction of 0.2 and 0.3, respectively). A far-red fraction of 0.2 allowed the same performances in terms of stomatal activity and quantum efficiency of PSII of red and blue photons, and at the same time promoted plant morphology features (e.g., leaf expansion). With a far-red fraction of 0.3 the increase observed in growth was only due to the morphological component. However, with both light treatements, it was possible to increase the light use efficiency, the lighting energy use efficiency and finally the energy use efficiency of the entire system. Regarding the time factor, the influence of far-red radiation was reported from 15 DAT until the end of the cycle. At the final harvest, pigmentation decreased from a far-red fraction of 0.07. Therefore, more studies will be needed to consider how quality parameters are influenced by the presence of FR during the growth cycle, to reach an increase in yield and improvement of the overall energy use efficiency, without negatively affecting the quality of the final product.

CRediT authorship contribution statement

Laura Carotti: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Alessandro Pistillo:** Investigation, Conceptualization. **Ilaria Zauli:** Methodology, Investigation. **Giuseppina Pennisi:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation, Conceptualization. **Michael Martin:** Writing – review & editing, Supervision. **Giorgio Gianquinto:** Writing – review & editing, Supervision. **Francesco Orsini:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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