Improving light use efficiency of lettuce in vertical farms by far-red and dynamic light intensities

Wenqing Jin

Propositions

1. Far-red is critical for optimal light use efficiency in a vertical farm.
(this thesis)

2. Despite vertical farming is usually praised for keeping all environmental conditions constant, dynamic variation of these conditions is needed for optimal light use efficiency.
(this thesis)

3. Successful application of Artificial Intelligence in horticulture requires horticultural knowledge.

4. High tech food production systems are the only solution to feed the growing world population.

5. A philosophy course is essential to any PhD training program.

6. Writing brings freedom.

Propositions belonging to the thesis, entitled

Improving light use efficiency of lettuce in vertical farms by far-red radiation and dynamic light intensities

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Wageningen, 16 December 2022

Improving light use efficiency of lettuce in vertical farms by far-red radiation and dynamic light intensities

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Wenqing Jin

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Chapter 1

General Introduction

1.1 Vertical farms as a food production system

Rapid urbanization, climate change, and land degradation challenge the current food supply chain (van Delden et al., 2021a). The COVID-19 pandemic made governments and the public aware of the importance of developing localized food production systems to improve the robustness of food supply. Any solution should make efficient use of increasingly scarce resources, especially land and water, and must also assure food safety. Vertical farms is a potentially feasible food production system to address these challenges, since it makes food production independent of solar light and weather conditions (SharathKumar et al., 2020; van Delden et al., 2021a). In vertical farms, crops are grown in a closed system with all light provided by lamps (no solar radiation), typically in stacks on top of each other (Fig. 1-1). This means that vertical farms makes much more efficient use of land area (Asseng et al., 2020), while maintaining a high level of hygiene through the use of barely any pesticides (SharathKumar et al., 2020; van Delden et al., 2021a). In vertical farms, all growth factors are under control. Climate is managed by a heating, ventilation, and air-conditioning system (HVAC) and crops are cultivated on well-developed soilless cultivation systems, such as aeroponics, ebb-flood systems and nutrient film techniques (Eldridge et al., 2020; SharathKumar et al., 2020; van Delden et al., 2021a). Since all processes of crop production in vertical farms can be well controlled, they are also known as plant factories with artificial light (PFAL) – a descriptive name (Kozai, 2013).

The development of vertical farms, however, has been hindered by their high energy consumption occasioned by the light provided to the crops (Kozai, 2013), even though light-emitting diodes (LED) with high efficacy (μmol photons per J electricity input) have become available (Kusuma et al., 2020). LEDs are a suitable light source for vertical farms not only due to their high energy efficacy but also their flexibility across the light spectrum, which can be matched to the plants' photoreceptive characteristics (Kusuma et al., 2020). In addition, the lower heat emissions of LEDs allow for a shorter distance between the LED modules and the canopy, saving space. LEDs need replacement only after a long time, and they can be switched on and off instantly without needing to warm up (Morrow, 2008). Overall, LEDs are facilitating a fundamental revolution in plant photobiology research (Pattison et al., 2018) and crop production in controlled-environment agriculture. However, LEDs are not the ultimate solution to the massive energy consumption of vertical farming (Graamans et al., 2018). A substantial amount of energy is still needed for lighting, and more for the HVAC system due to the heat emitted by the LEDs. Under the current circumstances, it is strongly necessary to increase the efficiency of crop light use.

Figure 1-1. Vertical farming systems in practice: a. BrightBox, Philips, Venlo, Netherlands; b. GrowWise, Philips, Eindhoven, Netherlands; c and d: tomato and lettuce cultivation trails, De Lier, Netherlands. Photo source: Priva Image Library.

1.2 Light use efficiency (LUE)

Light use efficiency (LUE) is the biomass production per unit of light. It can be defined in different ways. LUE may refer to the ratio of crop dry mass or yield of harvested product per unit of absorbed, intercepted, or incident light. In many field- or greenhouse-grown crops, dry mass production (g m^{-2}) has a linear relationship with cumulative intercepted photosynthetically active radiation (MJ m^{-2}; Arkebauer et al., 1994). In crop science, LUE (g MJ^{-1}) is usually defined as the slope of this linear relationship (e.g. Heuvelink, 1995; Lee et al., 2002). In greenhouse cultivation with supplementary lighting and vertical farms where the lamps' spectra differ from that of solar radiation, light is normally presented as photosynthetic photon flux density (PPFD) expressed in number of photons (μmol m^{-2} s^{-1} or mol m^{-2} d^{-1}) and hence LUE is expressed in g mol^{-1}.

The overall efficiency of light use in vertical farms could be improved by more careful attention to light interception and its efficient conversion to harvestable biomass. For the overall performance of vertical farms, the most relevant definition of LUE is the fresh mass of harvested product per unit of photosynthetic photon flux density (PPFD, in μmol

m^{-2} s^{-1}) incident on the canopy. In this thesis, LUE is defined as the dry mass of harvested product per unit of PPFD unless stated otherwise. The fraction of light intercepted by a crop depends on the leaf area index (LAI) and the crop architecture, which is mainly determined by the orientation, shape, and optical properties of the leaves, their internode and petiole length, as well as their planting density (Barillot et al., 2014; Chen et al., 2014a). LAI is determined by the area of individual leaves, the number of leaves per plant and planting density (number of plants per unit area). Canopy photosynthesis is dependent on PPFD, light spectrum, the direction of light and other environmental factors like CO_2 concentration and temperature (Baker, 2008). Plant dry mass mainly depends on photosynthesis and respiration which includes maintenance and growth respiration (metabolic conversion of carbohydrate into structural plant mass). Plant fresh mass is determined by plant dry mass and dry matter content (the fraction of dry mass over fresh mass). Eventually, the harvested fresh mass also depends on the harvest index (the fraction of harvestable organ mass over total plant fresh mass), which can be influenced by environmental factors like the light spectrum (Ji et al., 2019) and methods of cultivation management (Brouwer, 1983).

As long as LAI is not that high, increasing planting density results in an increased interception of PPFD which is the driver of photosynthesis. An initial high planting density might be advantageous for optimal use of cultivation space (Franklin, 2008). While a high planting density can increase biomass production per unit area, it reduces biomass per plant. Furthermore, it may also change the shape of the plants (Pierik and De Wit, 2014; Demotes-Mainard et al., 2016) and may result in etiolated leaves (Scaife et al., 1987) which affects the quality of leafy vegetables such as lettuce.

Moreover, crop growth results from photosynthetic photons emitted from the light source and absorbed by the leaves. Captured photons result in the production of photosynthetic assimilates (carbohydrates). These assimilates are partitioned to different crop organs, like roots, stem, leaves and fruits. The efficiency of all these processes and therefore also LUE is affected by crop management and environmental factors, like planting density, light intensity and spectrum, photoperiod, temperature, air humidity, carbon dioxide (CO_2) concentration, air movement, and the availability of water and nutrients.

Crops grown in the field have limited opportunities to increase LUE compared to crops grown in vertical farms or greenhouses, as only certain measures can be applied to the canopy environment such as the control of light, temperature, or CO_2. Nevertheless, growers can still affect the LUE by crop management practices such as planting density and harvesting time. In greenhouses, the environmental factors can be controlled within a certain level and the light intensity is determined completely or partly by the natural light. In a vertical farm, environmental factors can be fully controlled. Here, light is normally the only limiting factor as other resources can be supplied at relatively low cost. Therefore, LUE is expected to be higher in a vertical farm compared to in greenhouse or field cultivation.

1.3 Effect of far-red radiation on plant morphology

Far-red radiation (FR: 700-800nm) is a wavelength range of radiation just above the photosynthetically active radiation (PAR) range and most of it is reflected or transmitted by leaves, while only a small fraction is absorbed (Taiz et al., 2015). In natural circumstances under the influence of solar light only, the Red (R):FR ratio perceived by leaves decreases when vegetation is in close proximity or other leaves cast a shadow, as R is substantially absorbed but FR is mostly scattered. Therefore, perception of a lowered R:FR ratio is an early sign of the proximity of neighbouring plants (Ballaré et al., 1990; Pierik and De Wit, 2014). Plants perceive the R:FR ratio through the photoreceptor phytochrome, which exists in two photo-convertible isomers, P_r and P_{fr}, with P_{fr} being the bioactive form (Ballaré, 1999; Smith, 2000). These two isomers can transform into each other by absorbing R or FR photons and thus the R:FR ratio largely determines the equilibrium between P_{fr} and P_r in plants (Smith, 2000; Pierik and De Wit, 2014).

Shading of plants by vegetation results in an array of plant responses, as indicated by shade avoidance syndrome (Franklin, 2008; Vos et al., 2010; Bongers et al., 2014). This syndrome is characterized by elongated stems, internodes and leaves, hyponasty (leaves moving upward), accelerated flowering and relocation of resources towards reproductive structures (Franklin, 2008; Pierik and De Wit, 2014; Ballaré and Pierik, 2017). A review by Demotes-Mainard et al. (2016) concluded that a low R:FR ratio or reduced *PHYB* expression resulted in the incremental growth of stems, internodes, petioles and leaves in many plant species. Franklin and Whitelam (2005) and Bongers et al. (2018) observed enlarged petiole elongation in Arabidopsis at a lower R:FR ratio. Hyponasty of Arabidopsis leaves from the petiole base was also observed in a lowered R:FR ratio condition (Pantazopoulou et al., 2017). Kalaitzoglou et al. (2019) added supplementary FR to red and blue light (RB) in a greenhouse experiment and observed that supplementary FR resulted in a taller tomato crop. For leafy vegetables grown in vertical farms, adding FR during the photoperiod to either RB or white light (Meng and Runkle, 2019; Meng et al., 2019; Zou et al., 2019, 2021; Zhen, 2020; Zhen and Bugbee, 2020; Legendre and van Iersel, 2021) and end-of-day treatment (Zou et al., 2019) promoted leaf expansion as well as their projected canopy area which increases light interception

(Legendre and van Iersel, 2021). However, once the canopy is more or less closed, leaf expansion will not further increase light interception. Research combining different planting densities with additional FR is required, as well as an exploration of the effects underlying the growth response.

1.4 Effect of far-red radiation on photosynthesis

FR absorption by leaves is very low, as the leaf absorption rate starts to drop sharply from 685nm (McCree, 1971) and hardly any absorption is measured above 750nm (McCree, 1971; Zhen and Bugbee, 2020). Therefore, when applied alone, FR produces hardly any photosynthetic assimilation. However, this doesn't mean FR doesn't contribute at all to photosynthesis. There is a synergistic effect between FR and shorter wavelengths (400-700 nm). The quantum yield in the FR waveband can be increased by adding shorter wavelength radiation in R region, which is known as the Emerson Enhancement Effect (Emerson and Rabinowitch, 1960; Zhen and van Iersel, 2017). There are two reaction centers, photosystem I (PSI) and photosystem II (PSII), absorbing photosynthetic active photons and operating in series to transfer electrons along the electron-transport chain and carry out photochemical reactions (Baker, 2008). The peak wavelength for excitation of PSI is at 700nm and for PSII, 680nm, from which they can be named P700 and P680, respectively. Applying R and FR together will balance the excitation level of both photosystems, which improves the efficiency of photosynthesis (Zhen and van Iersel (2017), Zhen et al. (2018), Zhen (2020), and Zhen and Bugbee (2020). By adding FR to R and B or white light, the quantum yield (the number of molecules transferred by PSII divided by the number of photons absorbed by the system) of PSII was increased and non-photochemical quenching (NPQ: the excitation energy within PSII which is lost as heat) was decreased (Baker, 2008; Zhen and van Iersel, 2017; Zhen et al., 2018). In addition, for twelve C3 and two C4 species studied, Zhen and Bugbee (2020) observed a similar increment in canopy gross photosynthetic rate (the sum of instantaneous net photosynthesis rate and dark respiration) when adding the same amount of FR or PAR photons. This was further studied in lettuce, where a drop in instantaneous net canopy photosynthesis rate was observed when FR was switched off for a short period (Zhen, 2020). However, other studies came to different conclusions. Ji et al. (2019) found no P_n increase from addition of FR either to R and B or white background light in tomatoes. On the other hand, Zou et al. (2019) found an increase of P_n by temporarily switching on FR but reported that long-term FR light acclimation downregulates stomatal conductance.

The debate over the instantaneous effect of additional FR on net photosynthesis rate remains unresolved. However, it is generally agreed that in the long term photosynthetic capacity is affected by the light spectrum as plants acclimate to the growth environment (Wang et al., 2016; Ji et al., 2019). FR acclimation downregulates photosynthetic capacity through decreasing chlorophyll content (Demotes-Mainard et al., 2016).

1.5 PPFD effects on plant growth

PPFD is a significant determining factor for plant growth, as it provides the energy needed for photosynthesis. In vertical farming, where all light comes from lamps, a high PPFD requires substantial initial investment in the LED modules, and electricity consumption is a major daily operational cost (Kozai, 2013), despite the improved efficiency of LED lighting (Pattison et al., 2018; Kusuma et al., 2020). Since other growing factors, such as CO_2, temperature, relative humidity, and the supply of water and nutrients, can be well maintained in vertical farming, PPFD is typically the limiting resource for crop growth.

Instantaneous net canopy photosynthesis rate and hence biomass production positively correlates with PPFD. Crops typically show an expo-linear growth pattern, i.e., initially growth is exponential and gradually they will show linear growth (Goudriaan and Monteith, 1990). In young crops that are growing exponentially any increase in relative growth rate by, for instance, improved photosynthesis, may have an outsized effect on final plant mass. Light conditions during an early stage of crop development may determine later morphological and leaf photosynthetic characteristics due to acclimation to light (Bunce, 2000). Hence, one strategy would be to use a high PPFD to shorten the growth period and increase the land area use efficiency (crop production in weight per unit of ground or per unit of growing bench area). Several researchers have tried to identify an optimal PPFD for lettuce cultivation in vertical farms, varying from 85 to 750 µmol m^{-2} s^{-1} provided during a 16 up to 24 hours photoperiod (Fu et al., 2012; Chen et al., 2016, 2019a; Sago, 2016; Zhang et al., 2018; Cammarisano et al., 2020; Kelly et al., 2020; Pennisi et al., 2020b; Xu et al., 2020; Carotti et al., 2021). A high PPFD may increase the growth rate, however, it has been reported to reduce light use efficiency at the same time (Carotti et al., 2021) and may lead to tipburn (Sago, 2016; Xu et al., 2020). Tipburn is a physiological disorder expressed as a necrosis in the margins of young developing leaves (Xu et al., 2020), which requires labor to remove the tip-burned part manually and reduces the economic value of the lettuce. Nonetheless, a high PPFD during growth has been shown to have a positive effect on the shelf life and visual quality of leafy greens such as lettuce (Woltering and Witkowska, 2016; Min et al., 2021). In particular a high PPFD towards the end of production substantially increases the shelf life of lettuce (Min et al., 2021).

In vertical farms, PPFD is usually kept constant from transplanting until harvest. However, as PAR photon utilization increases with crop growth, keeping the incident PPFD constant might mean that the intensity is too high in the beginning and too low shortly before harvest. In the early growing period, when the leaf area index is still small, a large fraction of the light falls on the floor instead of being absorbed by the plants, and young crops with a low leaf area index show light saturation at a lower PPFD than a full-grown crop (Fisher et al., 1996). This can lead to a relatively low light use efficiency based on incident radiation. Once the canopy is closed, the plants can intercept and utilize a larger fraction of the incident light. Therefore, a PPFD that gradually increases during the growing period may lead to a higher biomass production and light use efficiency. On the other hand, providing a high PPFD during early growth may promote crop growth by means of a faster leaf area expansion, resulting in a strong increase in plant growth as the crop grows exponentially in this period (Goudriaan and Monteith, 1990). Increasing the light interception in early days will benefit subsequent canopy light interception and possibly increase the final output of biomass. Accordingly, two contrasting effects determine the final outcome of a gradual change of light intensities during the growth period of the crop on lettuce biomass production and light use efficiency.

1.6 Aim, research questions and thesis outline

The overall aim of this research is to improve the LUE of lettuce in vertical farms by investigating and explaining the effects of additional far-red on lettuce morphology and photosynthesis and exploring the effect of dynamic light intensities. Research objectives are:

1. To compare LUE among open field, greenhouse, and vertical farming lettuce cultivation, and to understand the determining underlying components.

2. To investigate the interaction between FR and planting density and to quantify the underlying components of the FR effects on growth.

3. To explore whether short-term photosynthesis efficiency is increased to the same extent by additional FR compared to adding the same PPFD.

4. To investigate the effect of a gradual increasing or decreasing PPFD compared to a constant average PPFD on lettuce biomass production.

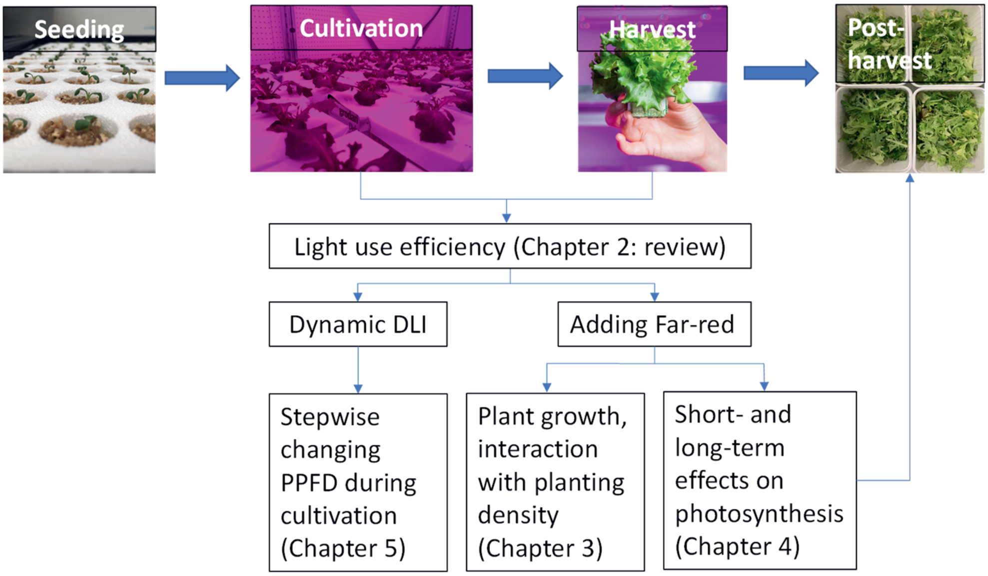

Figure 1-2. Research scheme for this thesis. Focus is on effects of treatments during lettuce cultivation on lettuce biomass and light use efficiency and to a lesser extent on post-harvest quality.

Correspondingly, I hypothesized:

1. Vertical farming's LUE is higher than that of greenhouse or field cultivation as the environment is well-controlled and PPFD is the only limiting environmental factor.

2. The addition of FR increases the partitioning to the shoot, resulting in increased biomass production because of enlarged leaf area and hence light interception. At high planting densities, additional FR has a smaller impact because at a higher planting density the fraction of light interception is high.

3. FR and PAR are equally efficient at powering photosynthesis and FR promotes growth more efficiently than PPFD, as it also encourages leaf expansion.

4. A gradually increasing PPFD during cultivation compared to a constant average PPFD improves cumulative light interception resulting in higher total dry mass and improved light use efficiency.

This thesis contains six chapters. Besides a general introduction and a general discussion, the place of the four research chapters is indicated in Figure 1-2.

Chapter 1, the present chapter, serves as the general introduction presenting the background of the thesis and the pathway to the research objectives.

As a newly developed production system, vertical farming demonstrated its potential as an alternative to greenhouse and open-field lettuce production. However, whether light is used more efficiently in vertical farms than in greenhouses or open-field farming has not been quantitatively established. A literature review was conducted in **Chapter 2**, calculating light use efficiency (LUE: lettuce shoot dry weight per incident mole of PPFD cumulatively from transplanting to harvesting) from published research. The range (boxplot), average and median LUE were compared among all three production systems and to the theoretical maximum LUE. The key factors correlating to the LUE were also collected and analyzed, including lettuce size (dry mass) at harvest, plant age, daily light integral (DLI), cumulative DLI, planting density, and the concentration of CO_2 applied during lettuce growth. An optimal production capacity of vertical farming was estimated based on a feasible LUE.

Planting density and FR both may enhance the leaf area per unit ground area and therefore increase light interception and consequently LUE. In **Chapter 3**, an experiment was conducted to explore the effects of FR at three planting densities (23, 37 and 51 plant·m^{-2}). Fresh and dry weight for shoot and root, leaf area, projected leaf area, and instantaneous net photosynthetic rate (P_n) were determined. Yield component analysis was applied to further understand the underlying reasons for a possible interaction between planting density and FR. Three use efficiencies of light were calculated: incident LUE (total dry weight at harvest per cumulative incident PPFD during growth), intercepted LUE (total dry weight at harvest per cumulative intercepted PPFD during growth), and radiation use efficiency (RUE: total dry weight at harvest per cumulative incident photon flux density in the waveband of 400-800nm).

While some authors have clearly shown that FR might act in a similar manner to PAR on photosynthesis when different wavelengths are supplied simultaneously, others did not report the effect of FR on photosynthesis. In **Chapter 4**, lettuce was grown at two PPFD levels (200 and 400 μmol·m^{-2}·s^{-1}) with 25% additional FR, 25% additional RB, or no additional radiation. The P_n of plants grown in FR-enriched and FR-absent environments was measured under growth circumstances and in a special designed system. The system consists of dimmable R, B and FR light sources and a gas exchange measuring system, by which the P_n was measured at different incident PAR and FR intensities. Growth parameters, including fresh and dry weight and leaf area, were measured to determine the possible increase in LUE when substituting part of RB with FR.

Plants intercept a larger fraction of light photons later in their development (higher LAI). Therefore, applying an increasing PAR during growth could be a means of increasing LUE, instead of the more typical constant average PAR regime. In **Chapter 5**, lettuce was grown under dynamic lighting with the same total light integral at harvest. PPFD increased, decreased, or was kept constant during the crop cycle. Growth parameters including fresh and dry weight, leaf area, and projected leaf area, LUE and shelf life were determined. A yield component analysis was conducted to break down the effect of this lighting strategy upon its underlying components.

In Chapter 6 the results of the literature review and the experimental work of the previous chapters are discussed collectively and suggestions for further research given.

Chapter 2

Light use efficiency of lettuce cultivation in vertical farms compared to greenhouse and field

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Abstract

Vertical farming is a relatively new fresh fruit and vegetable production system, where lamps (mostly light emitting diodes (LED)) are the sole light source. A high light use efficiency (LUE_{inc}), defined as shoot dry weight per incident photosynthetic photon flux density (PPFD; g·mol^{-1}) integral, is crucial for the economic viability of vertical farming. Very different values for LUE_{inc} have been reported in the literature and it is not clear whether LUE_{inc} is higher in vertical farming than in greenhouse or open field cultivation. Values of LUE_{inc} of lettuce grown in a vertical farm (53 studies), greenhouse (13 studies) and open field (8 studies) were collected from literature, as well as relevant cultivation aspects like lettuce weight at harvest, cultivation period (plant age at harvest), daily light integral, cumulative daily light integral for the whole cultivation period, planting density and CO_2 concentration. The average LUE_{inc} for lettuce grown in a vertical farm was 0.55 g mol^{-1} which was higher than 0.39 g mol^{-1} for greenhouse-grown lettuce. Both were substantially higher than for field-grown lettuce (0.23 g mol^{-1}). The maximum measured LUE_{inc} for lettuce grown in a vertical farm (1.63 g mol^{-1}) is close to the published maximum theoretical value, which ranges from 1.26 to 1.81 g mol^{-1}. Since all environmental factors can be fully controlled, vertical farming has the capability to achieve the theoretical maximum LUE_{inc}. Using the highest reported LUE_{inc} based on shoot fresh weight (44 g mol^{-1} at 200 μmol m^{-2} s^{-1} PPFD and 16 h photoperiod), it is estimated that each layer of a vertical farm can potentially produce annually up to 700 kg of lettuce per m^2 at 500 μmol m^{-2} s^{-1} of continuous light.

Key words: Indoor farming, Vertical farming, Plant factory, Light use efficiency, potential production

2.1 Introduction

Vertical farming, where plants are grown in stacked layers and lamps are the sole light source, is a production system considered as a solution for water and land scarcity, as well as a system to reduce transport distance, especially for fresh fruit and vegetables (SharathKumar, Heuvelink, & Marcelis, 2020; van Delden et al., 2021). However, compared to conventional production of fresh fruit and vegetables in open field and greenhouses, vertical farming's energy consumption is substantially higher, mainly because of electricity use for lighting (Kozai, 2013). Hence, increasing the light use efficiency is urgently needed to improve the economic feasibility of vertical farming.

Light use efficiency (LUE) can be defined in different ways. For analysing the economics or sustainability of commercial production, the marketable crop fresh weight per unit of electricity used is important. Therefore, several studies define LUE as gram of marketable fresh weight per joule electricity consumed by the lighting system (Kozai, 2013). There are many aspects affecting this efficiency, including the efficacy of the lamps (photons emitted by the lamp per Joule electricity), which is not relevant for understanding the effect of environmental factors on LUE. For that purpose LUE is often based on photosynthetically active radiation (PAR, 400-700nm), commonly indicated as photosynthetic photon flux density (PPFD), or it can be based on a wider range of photons (300-800 nm or 400-800 nm), which can be indicated as photon flux density (PFD). In physiological research, LUE is often calculated as crop dry weight per intercepted (LUE_{int}) or incident photosynthetic photons (LUE_{inc}), the latter representing the efficiency of the whole process from photon capture to biomass accumulation.

Crop growth in vertical farms results from photosynthetic photons emitted from the light source and absorbed by the leaves. The fraction of emitted photons captured by a leaf depends on the leaf area, leaf optical properties and leaf orientation. Captured photons result in photosynthetic assimilate production (carbohydrates). These assimilates are partitioned to different crop organs, like roots, stem, leaves and fruits. The efficiency of all these processes and therefore also LUE is affected by crop management and environmental factors, like planting density, light intensity and spectrum, photoperiod, temperature, air humidity, carbon dioxide (CO_2) concentration, air movement, and water and nutrient availability.

When crops are grown in the field there are limited opportunities to increase LUE compared to crops grown in vertical farms or greenhouses as there are limited measures that can be applied to the canopy environment such as controlling light, temperature or

CO_2. Nevertheless, growers can still affect the LUE by crop management practices such as planting density and harvest time. In greenhouses, the environmental factors can be controlled within a certain level and the light intensity is determined completely or partly by the natural light. In a vertical farm environmental factors can be fully controlled. Here, light is normally the only limiting factor as other resources can be supplied at relatively low cost. Therefore, LUE is expected to be closer to its potential value in a vertical farm compared to greenhouse or field cultivation. The potential LUE based on incident PPFD (gram of plant dry mass per incident mole of photosynthetic photons) has been discussed and calculated in several publications (Loomis and Williams, 1963; Bugbee and Salisbury, 1988; Zhu et al., 2010; Kozai, 2013; Pattison et al., 2018). An optimal environment for the crop, although not specifically defined, is assumed in these calculations for example an elevated CO_2 concentration. Therefore, vertical farming is expected to realize a LUE closest to the potential LUE.

The aim of this study is to quantify LUE based on incident light of lettuce grown in a vertical farm where lamps are the sole light source and compare it with LUE of lettuce grown in greenhouse or open field, based on data in scientific literature. Furthermore, we aim to analyse the relative importance of several factors determining LUE in vertical farming. We hypothesize that vertical farming's LUE based on incident light is higher than that for greenhouse or field cultivation as the environment is well-controlled and light is the only limiting environmental factor.

2.2 Materials and methods

Data acquisition, extraction, and processing

Light use efficiency (LUE_{inc}) in this paper is defined as the shoot dry weight (SDW; g m^{-2}) divided by cumulative incident PPFD (Cumulative Daily Light Integral, DLI_{cum}; mol m^{-2}). Shoot dry weight is the weight at harvest and cumulative incident PPFD is calculated from transplanting to harvesting. Publications reporting LUE or with sufficient data available to calculate LUE for lettuce grown in vertical farming, greenhouse or open field were collected. Peer-reviewed publications were searched in SCOPUS (https://scopus.com/). The keywords applied included lettuce, LUE, radiation, light intensity, PPFD, vertical farming, greenhouse, field, and open field, etc. (Supplementary S2-1). Furthermore, data from one PhD thesis was used (Both, 1995).

In addition to SDW, shoot fresh weight (SFW) and total dry weight (TDW) were also collected or calculated from the studies. When only dry weight was given, fresh weight was calculated assuming a dry matter content and vice versa (Table 2-1). When only total plant dry weight was reported shoot dry weight was calculated based on fixed fraction of shoot and vice versa (Table 2-1). In studies where plant dry and fresh weight, including SDW, SFW and/or TDW, were reported per plant, it was multiplied by the planting density to obtain values per square meter of cultivation surface.

There are a few other variables related to the calculation of DLI_{cum}. DLI_{cum} is the product of daily light integral (DLI) and the duration in days of the experiment from transplanting to harvest (plant age). When there is no transplanting between sowing and harvesting, 7 days were taken out of the calculation of plant age. When the incident PPFD (μmol m^{-2} s^{-1}) was reported, DLI was calculated by multiplying this PPFD with the photoperiod. For greenhouse, field, and a few vertical farming studies, averaged DLI over the cultivation period was reported. For greenhouse cultivations, since the cover material (mostly glass) transmits only part of the outside radiation, a fixed transmissivity was assumed (Table 2-1), when PPFD at canopy level was not reported. For greenhouse studies with supplemental light, incident light intensity from the lamps was always reported (DLI or PPFD).

When data were not reported in the text but in a figure, chart and/or plot, they were extracted using WebPlotDigitizer (Ankit Rohatgi, WebPlotDigitizer, Version 4.3., Pacifica, California, USA).

Box plots were used to present median and variation in the analysed variables for vertical farming, greenhouse, and open field. These box plots cover the whole range of data, with the end of the lower whisker being the minimum value and the end of the top whisker the maximum value, and the lower and upper section of the box represent the second and third quartile, respectively. The bar in the middle of box stands for the median.

For LUE analysis, we took the data from the treatment that resulted in the highest LUE_{inc}, when a paper contained more than one treatment. Presented data were extracted from 53 publications for vertical farming, 13 publications for greenhouse production and 8 publications for production in open field (Supplementary S2). A typical vertical farm contains several features including a multi-layer production system. However, when plants were grown in a single layer in a climate room, the data were also considered as being representative for vertical farming and, therefore, used in this study. Without further explanation, such conditions were considered as representing vertical farming.

Table 2-1. Assumptions made for the calculations when data on shoot dry weight, shoot fresh weight, or solar light inside greenhouse were not reported in the study. When only data on dry weight was given, fresh weight was calculated assuming a dry matter content of 0.04 and vice versa. When only total plant dry weight was reported shoot dry weight was calculated based on fraction shoot of 0.8 and vice versa. When only radiation outside but not inside the greenhouse was reported, a greenhouse transmissivity of 0.62 was assumed. For solar light a conversion factor of 4.6 mol per MJ was applied when solar light data was provided in MJ.

Parameter	value	references
Dry matter content (ratio dry to fresh weight)	0.04	(Gent, 2014; Carotti et al., 2021)
Fraction of biomass partitioned to shoot	0.85	(De Pinheiro Henriques & Marcelis, 2000)
Greenhouse transmissivity (glass)	0.62	(Heuvelink et al., 1995)
Conversion factor for solar light (mol MJ^{-1})	4.6	(Sager and McFarlane, 1997)

2.3 Results

Several publications provide information to obtain a theoretical LUE_{inc} (g DW per incident mole of photosynthetic photons; Table 2-2). Pattison et al. (2018) calculated LUE_{inc} as the product of Fa (fraction of incident photons absorbed by the crop), QY (quantum yield; mole of carbon fixed per mole of photon absorbed), CUE (carbon use efficiency; moles of carbon incorporated into plant biomass per mole of carbon fixed), HI (harvest index; moles of carbon in edible product per mole of carbon in plant biomass) and k (mass of CH_2O (g carbohydrate) per mole of carbon in the edible product). In several papers, different values were assumed for these five parameters (Table 2-2) resulting in different values for LUE_{inc}: 1.81 g mol^{-1} for Loomis and Williams (1963), 1.64 g mol^{-1} for Bugbee and Salisbury (1988) and 1.33 g mol^{-1} for Pattison et al. (2018). Bugbee and Salisbury (1988) conservatively assumed their calculated LUE_{inc} of 1.64 g per mol incident photosynthetic photons is only possible for low-lipid plants even though it is lower than Loomis and William's 1.78. Zhu et al. (2010) calculated the minimum energy losses from solar radiation to the energy fixed within crop biomass to obtain a theoretical maximum energy fixation in the crop. They estimated for C3 species that 4.6% of solar radiation energy is fixed into the crop (E_{total}). The LUE_{inc} for Zhu et al. (2010), which was

1.26 g per incident mole of photosynthetic photons, was calculated based on the proportionality between E_{total} and LUE_{inc} from Bugbee and Salisbury (1988). Kozai (2013) calculated a theoretical maximum LUE_{inc} for vertical farming. He assumed a maximum efficiency of 10% from PAR to chemical energy in the crop (E_{PAR}) and based on the proportionality between E_{PAR} and LUE_{inc} from Bugbee and Salisbury (1988) this means a LUE_{inc} of 1.26 g mol^{-1}.

Table 2-2. Theoretical LUE (g DW per incident mole of photosynthetic photons) reported in publications. Fa (dimensionless) is the fraction of incident photons absorbed by the crop, QY is the quantum yield (mole of carbon fixed per mole of photon absorbed), CUE is the carbon use efficiency (moles of carbon incorporated into plant biomass per mole of carbon fixed), HI is the harvest index (moles of carbon in edible product per mole of carbon in plant biomass), k is the mass of CH_2O (g carbohydrate) per mole of carbon in the edible product, E_{PAR} (dimensionless) is the conversion efficiency from the energy within incident PAR to the energy fixed into the dry weight and E_{total} (dimensionless) is the conversion efficiency from the energy of incident total radiation to the energy fixed into the dry weight.

Publication	LUE_{inc}	Parameters values used in the calculations						
		Fa	QY	CUE	HI	k	E_{PAR}	E_{total}
Loomis and Williams, 1963	1.81[1]	0.90	0.10	0.67	1	30		
Bugbee and Salisbury, 1988	1.64[1]	0.95	0.077	0.75	1	30	13%	6.0%
Zhu et al., 2010	1.26[2]							4.6%
Kozai, 2013	1.26[3]						10%	
Pattison et al., 2018	1.33[1]	0.95	0.08	0.65	0.90	30		

[1] LUE_{inc} calculated as Fa x QY x CUE x HI x k

[2]Calculated from E_{total}, assuming proportional relation between E_{total} and LUE_{inc} based on Bugbee and Salisbury (1988), hence 4.6% / 6.0% x 1.64

[3]Calculated from E_{PAR}, assuming proportional relation between E_{PAR} and LUEinc based on Bugbee and Salisbury (1988), hence 10% / 13% x 1.64

The observed LUE_{inc} of crops grown in vertical farming covers a wider range than greenhouse and open field (Fig. 2-1a and 2-1b). LUE_{inc} for lettuce grown in vertical farming showed a high variability in the fourth quartile, whereas for greenhouse-grown lettuce more variation was found in the second quartile. The highest and the second highest observed LUE_{inc} for lettuce in a vertical farm based on shoot dry weight (1.63 and 1.23 g·mol^{-1}) is in the reported range of the theoretical maximum LUE_{inc} (Table 2-2) and significantly larger than the highest LUE_{inc} for greenhouse (0.77 g mol^{-1}) or open field (0.49 g mol^{-1}) lettuce production. The median LUE_{inc} of vertical farming is lower than for greenhouse cultivation, whereas for field production this is clearly lower. However, the average LUE_{inc} for lettuce production in vertical farming (0.55 g mol^{-1}) is 41% higher than for greenhouse production (0.39 g mol^{-1}) and 139% higher than for production in the open field (0.23 g mol^{-1}).

A similar pattern is observed for LUE_{inc} based on shoot fresh weight (Fig. 2-1b). The highest fresh weight LUE_{inc} was 43.6 g mol^{-1} and the highest values for greenhouse and open field were 18.5 and 12.4 g mol^{-1}, respectively. Median fresh weight LUE_{inc} for vertical farming was 9.3 g mol^{-1}, which was lower than 10.8 g mol^{-1} for greenhouse cultivation. Both were higher than the median fresh weight LUE_{inc} for open field cultivation (5.3 g mol^{-1}). However, average fresh weight LUE_{inc} for vertical farming was 11.6 g mol^{-1}, which is higher than for greenhouse or open field (9.2 g mol^{-1} and 5.9 g mol^{-1}, respectively).

Based on Carotti et al. (2021) who observed an incident LUE_{inc} in fresh weight of 44 g mol^{-1} (as observed in plants grown at 200 μmol m^{-2} s^{-1} PPFD and 16 h photoperiod), potential annual lettuce production was estimated for several combinations of photoperiod and incident PPFD (Fig. 2-2). Since the lettuce cultivated in a vertical farm is clean, total shoot weight is taken as harvestable weight. Potential annual yield was only 35 kg m^{-2} y^{-1} when grown at 50 μmol m^{-2} s^{-1} and a photoperiod of 12 h but reached up to 700 kg m^{-2} y^{-1} when grown at 500 μmol m^{-2} s^{-1} and a photoperiod of 24 h.

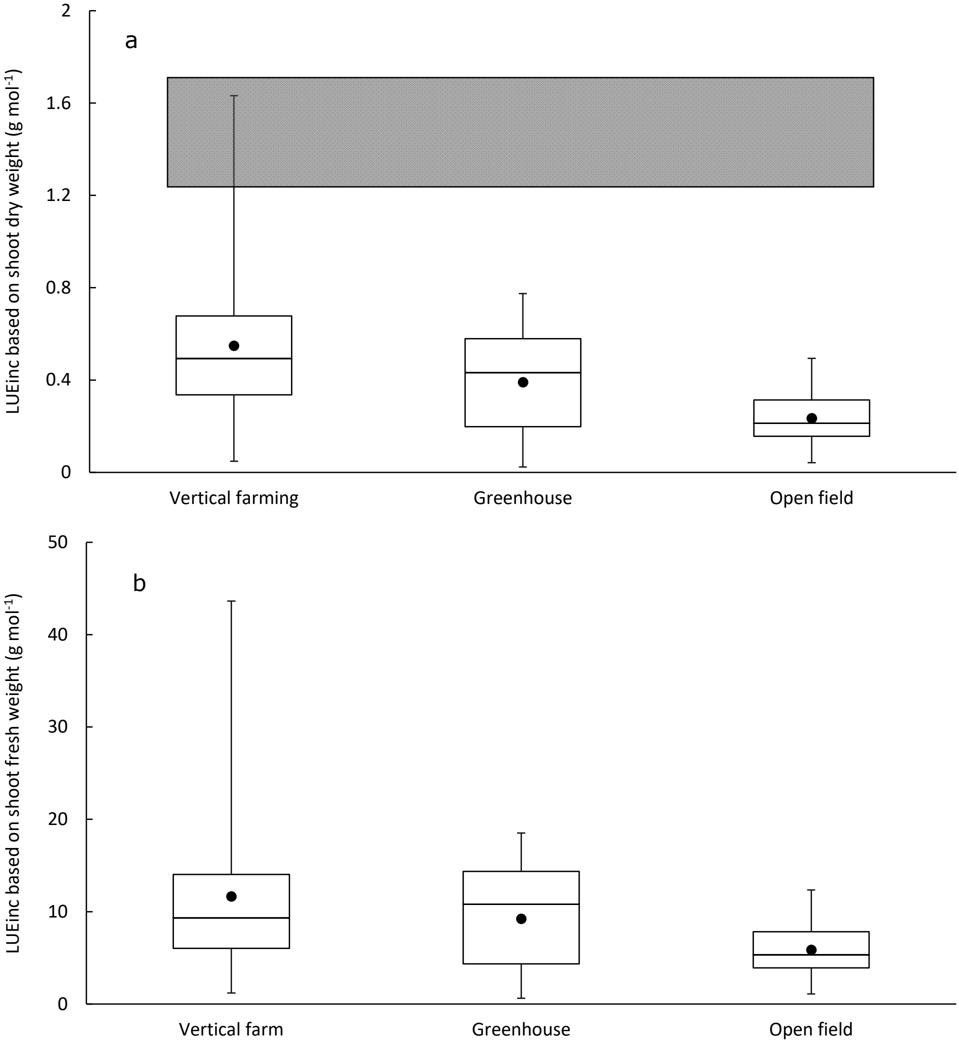

Figure 2-1. Boxplots of LUE_{inc} (g (shoot weight) mol^{-1} (cumulative incident DLI)) for vertical farming, greenhouse, and open field lettuce cultivation based on shoot dry weight (a) or shoot fresh weight (b). Data used are the highest LUE_{inc} values reported in each publication, so excluding suboptimal treatments. The black dots represent the average values. The grey area (a) represents reported theoretical maximum LUE_{inc} values (Table 2-2).

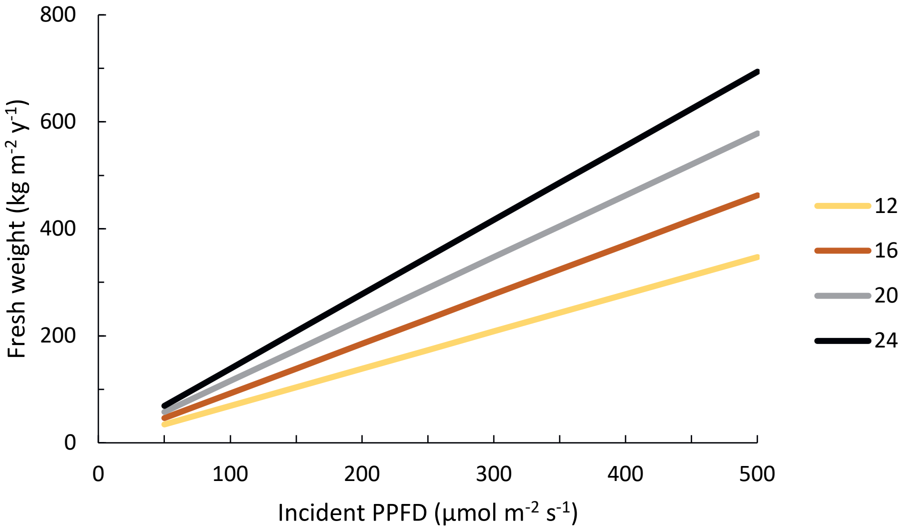

Figure 2-2. Potential annual yield of fresh lettuce production for different combinations of incident PPFD and photoperiods of 12 h d^{-1}, 16 h d^{-1}, 20 h d^{-1} and 24 h d^{-1}. The calculations of potential yield were based on the highest observed incident LUE of 44 g (shoot fresh weight) mol^{-1} (Carotti et al., 2021).

For the reported maximum LUE_{inc} values from each publication, several potentially explaining factors were compared (Fig. 2-3a-f), including shoot dry weight at harvest (plant size), plant age (the number of days from transplanting to harvesting), daily light integral (the daily incident PPFD sum), cumulative DLI (the total incident PPFD from transplanting to harvesting), planting density (the density applied at transplanting), and CO_2 concentration. The size of harvested lettuce plants differed substantially when comparing studies of vertical farming, greenhouse, and open field. In vertical farming studies the shoot dry weight (SDW) of the harvested lettuce was lower than for greenhouse-grown lettuce, which was again lower than for open field-grown lettuce (Fig. 2-3a). Lettuce in vertical farming takes a shorter growing period. Surprisingly, the growing duration in greenhouses was rather similar to open field (Fig. 2-3b). Lettuce grown on the field received the highest and vertical farming the lowest DLI (Fig. 2-3c) and cumulative light during the growing period (Fig. 2-3d). The median planting density in vertical farming was higher than for greenhouse (Fig. 2-3e) and open field Greenhouse and open field cultivation's CO_2 concentration was not different from the atmospheric concentration (Fig. 2-3f). However, elevated CO_2 concentration was often applied in vertical farming cultivation to promote plant growth.

As plants in the vertical farming studies are usually harvested as smaller plants (Fig. 2-3a) and at a younger age (Fig. 2-3b) than in greenhouse or open field, we analysed the dependence of LUE_{inc} on plant age and size. During cultivation the LUE_{inc} based on cumulative plant dry mass and cumulative PPFD integral increased strongly (Fig. 2-4). Hence the older or the bigger the plant at harvest the higher the LUE_{inc}. At the end of a recent experiment (Jin et al., 2021), LUE_{inc} averaged over the whole growing period was 0.5 $g \cdot mol^{-1}$. However, when calculated only for the last week before harvest LUE_{inc} was 1.2 $g \cdot mol^{-1}$.

Using the six variates on the y-axes of the panels in Figure 2-3, a correlation analysis was performed for all production systems combined (Supplementary S2-4 and S2-5) and vertical farming only (Supplementary S2-6 and S2-7). For all production systems combined, from the six regressors three showed a significant correlation with LUE_{inc} (DLI with r=-0.29; cumulative DLI with r=-0.35; planting density with r=0.46), while plant age and shoot dry weight at harvest and CO_2 concentration showed no significant correlation with LUE. Multiple linear regression starting with a model with all six regressors followed by backward elimination resulted in a model with three significant ($P<0.05$) regressors. Shoot dry weight at harvest, planting density and cumulative DLI together explaining 45% of the variance in LUE_{inc}. The first two regressors (shoot dry weight at harvest, planting density) positively influenced LUE_{inc} and the last regressor (cumulative DLI) negatively influenced LUE_{inc}. For vertical farming only shoot dry weight at harvest (r=0.45) and planting density (r=0.48) showed a significant correlation with LUE_{inc} (Supplementary S2-6 and S2-7). Multiple linear regression starting with a model with all six regressors followed by backward elimination resulted in a model with four significant ($P<0.05$) regressors. Shoot dry weight at harvest, planting density, daily light integral and plant age together explaining 83.0% of the variance in LUE_{inc}, the former two regressors positively influencing LUE_{inc} and the latter two negatively influencing LUE_{inc}.

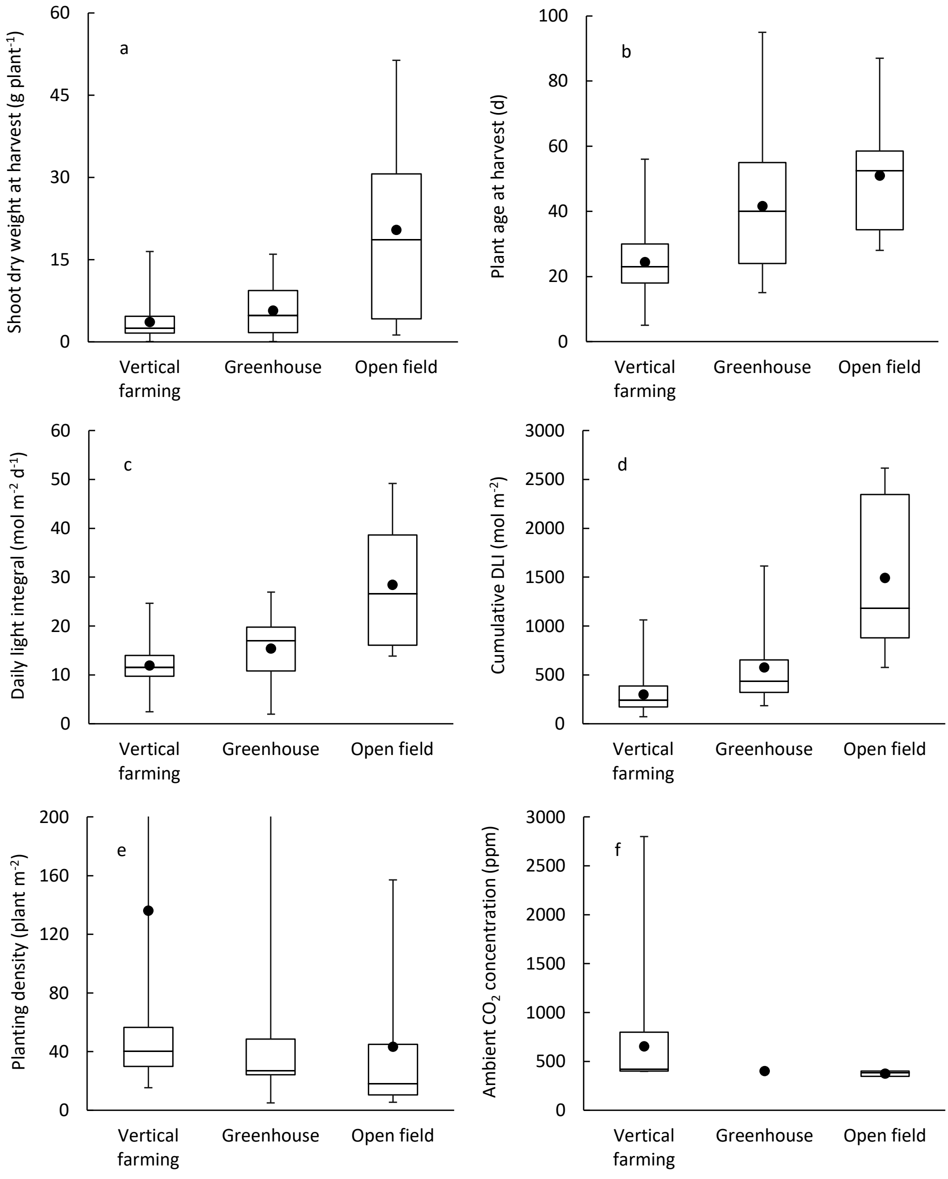

Figure 2-3. Boxplots of shoot dry weight at harvest (a), plant age (from transplanting to harvest, b), daily light integral (c), DLI_{cum} (d), planting density (e; three extremes of 700, 1000 and 1300 plants m^{-2} for vertical farming not shown, and ambient CO_2 concentration (f) for lettuce cultivation in vertical farming, greenhouse, and open field. Black dots represent the average values.

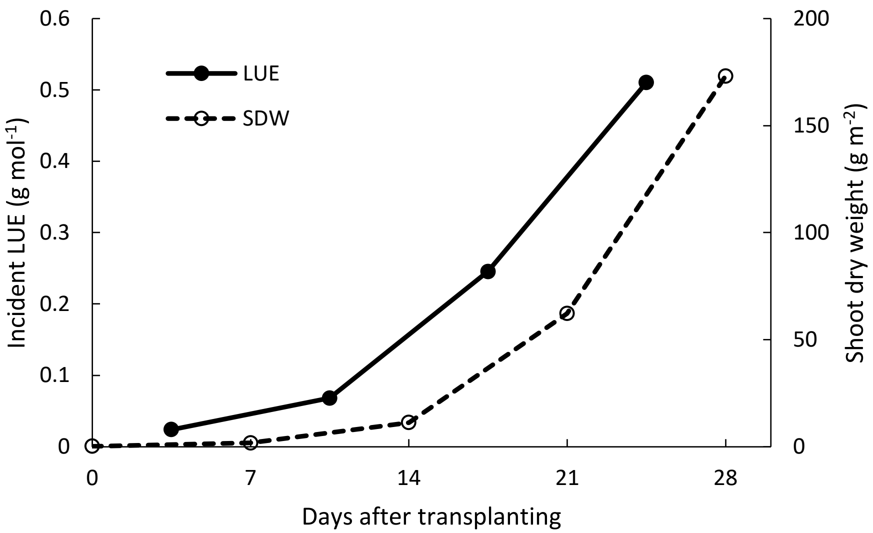

Figure 2-4: LUE_{inc} (solid line) based on cumulative incident PPFD, and lettuce shoot dry weight (dashed line; g m^{-2}) as a function of days after transplanting. Data was taken from the experiment described in Jin et al. (2021) for lettuce (Lactuca sativa cv. Expertise RZ) grown at 220 µmol m^{-2} s^{-1} red (88%) and blue (12%) LED with 45 µmol m^{-2} s^{-1} far-red and at 51 plants m^{-2}. LUE_{inc} is calculated for each 7-day interval between two destructive harvests and plotted against the middle of each interval.

2.4 Discussion

Highest observed LUE in vertical farming close to its theoretical maximum

LUE_{inc} in vertical farming can be high, and there is only a 10% gap between the highest LUE_{inc} observed in a vertical farming experiment, which is 1.63 g mol^{-1} (Pennisi et al., 2019), and the maximum theoretical value of 1.81 g mol^{-1} (Table 2-2). The majority of the theoretical LUE_{inc} values (Table 2-2) were estimated by simplifying the actual process of biomass production from photosynthetic photon absorption to the biomass accumulated in the harvestable organs with different efficiencies for these processes (Loomis and Williams, 1963; Bugbee and Salisbury, 1988; Zhu et al., 2010; Pattison et al., 2018). One exception is Kozai (2013) who calculated LUE_{inc} by applying a constant conversion factor from dry mass to chemical energy fixed in dry mass. These calculations were conducted based on a closed canopy absorbing at least 90% of the incident PPFD. A theoretical maximum LUE_{inc} is based on the assumption that the ambient environment for growing is always optimal, with ample supply of CO_2, water, and nutrients. In the actual experimentation and production, the incident PPFD between transplanting and canopy closure is not fully absorbed. The highest LUE_{inc} reported in experiments is 1.63 g mol^{-1} (Pennisi et al., 2019) and was achieved at a high planting density of 100 plants m^{-2} and by transplanting rather large plants 14 days after sowing. Therefore, the fraction light intercepted was very high from the start of the cultivation. The second highest measured LUE_{inc} is 1.23 g mol^{-1} (Carotti et al., 2021) obtained at a much lower planting density of 25 plants m^{-2}. These cases demonstrate the great potential of vertical farming to get close to the theoretical LUE_{inc}, as incident photons will have inevitably been falling on the floor instead of being utilized by crops in the early crop stage especially in the Carotti et al. (2021) case. Quicker full light interception may be obtained by adding far-red light (Meng and Runkle, 2019; Zou et al., 2019; Jin et al., 2021) or dynamically changing planting densities, i.e. gradually decreasing as the plant develops (van Delden et al., 2021). Considering that not all researchers are very good growers, that many experiments were not conducted with the aim to maximize growth and that vertical farming is relatively new, it can be expected that still quite some improvements in LUE_{inc} are possible for the LUE_{inc} data presented in literature. A higher fraction of assimilate partitioned to leaf will benefit further light interception and thus increase the biomass production, especially in the relatively short growing period. With a cultivation practice such that a high fraction of incident light is absorbed by canopy already from the start of the cultivation lettuce cultivation in vertical farming may well be able to realize the theoretical LUE_{inc} as environmental factors like temperature and CO_2 (Becker and Kläring, 2016), nutrients and water availability can be kept at optimal levels.

LUE_{inc} is largest in vertical farming followed by greenhouses and smallest in open field

The average LUE_{inc} for vertical farming (0.55 g mol^{-1}) was higher than for greenhouse-grown lettuce (0.39 g mol^{-1}). In vertical farming, when other factors become non-limiting, like nutrient and water availability and CO_2 concentration, light may become the only limiting factor, by which the LUE_{inc} can be maximized. Moreover, new cultivation practices can be easily applied in vertical farming and further improve LUE_{inc}. Elevating CO_2 concentration is another practice to promote plant growth which can be rather simply realized in the closed environment of a vertical farm (Fig. 2-3f). In greenhouses, a non-limiting root environment and a shoot environment closer to optimal than in open field cultivation can be obtained, but less optimal than in a fully-controlled vertical farm.

In addition, as solar light is the only or main light source in a greenhouse, PPFD could be close to crop photosynthetic saturation level which will result in a lower light use efficiency. Therefore, LUE_{inc} in greenhouse varies over the seasons. In summer when the PPFD is high, the LUE_{inc} will be reduced and LUE_{inc} is expected to be higher when PPFD is lower, typically in winter. Such a seasonal variation in LUE_{inc} has been observed for greenhouse cut chrysanthemum cultivation (Lee et al., 2002). Moreover, in the field or greenhouse PPFD may fluctuate rapidly during a day. As photosynthetic induction may take some time, this may lead to less photosynthesis (e.g. Kaiser et al., 2017) compared to a vertical farm where PPFD can be kept constant. Even though there are many modern techniques applied, the temperature, relative humidity and CO_2 concentration in a greenhouse cannot always be maintained at the desired level, which will negatively affect LUE_{inc} in greenhouses. Climate control is most advanced for vertical farming, less so for a greenhouse and absent for open field cultivation. Therefore, the LUE_{inc} is expected to be lowest for open field cultivation and lower for greenhouse-grown lettuce than for vertical farming.

In addition, the lettuce types and cultivars grown in vertical farming, greenhouse or open field differ because of different markets. Remarkably in the vertical farming studies the lettuces were harvested at a smaller size (Fig. 2-3a) and after a shorter growing period (Fig. 2-3b) than in greenhouse or open field, which negatively affects LUE_{inc} (Fig. 2-4). If a longer cultivation period would be adopted, the period of closed canopy would most likely represent a larger fraction of the total cultivation period leading to a higher LUE_{inc} as a closed canopy intercepts most of the incident light. Therefore, if similar lettuce types would be grown in vertical farming, greenhouse and open field, the differences in LUE_{inc} are expected to be much larger than observed now (Fig. 2-1).

A high planting density can strongly increase the light interception in a young crop. However, in practice planting density is first of all determined by lettuce type. Therefore, vertical farming, which often focuses on relatively small lettuce plants, is the system that allows for a higher planting density than others (Fig. 2-3e). As mentioned in the previous section, variable planting density, starting with a very high density when the plants are small and gradually decreasing during the crop cultivation as the plants get larger would result in consistently intercepting most of the incident light, which would increase LUE_{inc} and is most feasible to apply in a vertical farm.

Using data of lettuce experiments with a relatively long growing duration and a reasonable harvest size the potential yield per unit growing surface was calculated. At a continuous (day and night) PPFD of 500 μmol m^{-2} s^{-1} annual yield could be as high as 700 kg m^{-2} yield. This might be a too optimistic estimation ('best case scenario'), as it assumes that the LUE is maintained at high PPFD with continuous light. At higher light levels LUE of lettuce may decline or growth rate might even reduce, but these responses seem to depend on cultivar and growth conditions (Pérez-López et al., 2013; Lee et al., 2019; Viršilė et al., 2019; Pennisi et al., 2020b). A number of crops cannot stand continuous 24 hours lighting (Velez-Ramirez et al., 2011), but lettuce seems to be capable of growing under continuous light, although optimal photoperiod might be lower than 24h (Pennisi et al., 2020a).Furthermore, tipburn is often a severe problem at high growth rates (Sago, 2016). Here we ignored potential occurrence of tipburn, but it might be an important limiting factor for realizing these high growth rates. This would need further experimental testing in order to verify the estimations. On the other hand, this estimate is not even based on the highest observed LUE_{inc} (1.23 instead of 1.63 g mol^{-1} was used). Compared to a commercial Dutch greenhouse productivity (33 kg m^{-2}; (Raaphorst et al., 2019)), one layer of vertical farming could be 20 times more productive. For wheat Asseng et al. (2020) estimated yield per layer in a vertical farm to be 22-60 times higher than in the field. Considering multiple layers are applied in vertical farming, production per unit of floor area can become manyfold higher than in greenhouse or open field. However, the economical optimum yield might be different from the maximum yield.

2.5 Conclusion

The average LUE_{inc} (light use efficiency; ratio of shoot dry weight and incident PPFD integral) of lettuce was higher in vertical farming (0.55 g dry weight mol^{-1}) than for greenhouse cultivation (0.39 g mol^{-1}), which was higher than in the open field (0.23 g mol^{-1}). Since all environmental factors can be fully controlled, vertical farming has the capability to achieve the theoretical maximum LUE_{inc}. Indeed, the maximum measured LUE_{inc} for lettuce grown in vertical farming (1.63 g mol^{-1}) is close to the maximum theoretical values, ranging from 1.26 to 1.81 g mol^{-1}, which can make LUE_{inc} in vertical farming about 5 times higher compared to average production in the open field.

Supplementary

S2-1. Description of search terms for publications with vertical farming, greenhouse, and open field lettuce cultivations.

Website: https://www.scopus.com/search/form.uri?display=basic#basic

Up to date: 15-Sep-2021

Search words: {lettuce} AND {vertical farm} OR {vertical farming} OR {indoor} OR {plant factory} OR {greenhouse} OR {glasshouse} OR {field} OR {open field} OR {cultivation} OR {growth} with addition {light use efficiency} OR {lue} OR light {intensity} OR {light} OR {radiation} OR {PPFD}

The input words within SCOPUS advanced search: (TITLE-ABS-KEY (lettuce)) AND (TITLE-ABS-KEY ("vertical farm") OR TITLE-ABS-KEY ("vertical farming") OR TITLE-ABS-KEY ("plant factory") OR TITLE-ABS-KEY ("indoor")) AND (TITLE-ABS-KEY ("radiation") OR TITLE-ABS-KEY ("PPFD") OR TITLE-ABS-KEY ("lue") OR TITLE-ABS-KEY ("light use efficiency") OR TITLE-ABS-KEY ("light")) AND (TITLE-ABS-KEY ("growth") OR TITLE-ABS-KEY ("biomass") OR TITLE-ABS-KEY ("weight"))

S2-2. Publications used for the analysis in this paper

Lettuce in vertical farming

Ahmed, H. A., Yu-xin, T., & Qi-chang, Y. (2020). Lettuce plant growth and tipburn occurrence as affected by airflow using a multi-fan system in a plant factory with artificial light. *Journal of Thermal Biology*, *88*(December 2019), 102496. https://doi.org/10.1016/j.jtherbio.2019.102496

Bhuiyan, R., & van Iersel, M. W. (2021). Only Extreme Fluctuations in Light Levels Reduce Lettuce Growth Under Sole Source Lighting. *Frontiers in Plant Science*, *12*(January), 1–12. https://doi.org/10.3389/fpls.2021.619973

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Cammarisano, L., Donnison, I. S., & Robson, P. R. H. (2020). Producing Enhanced Yield and Nutritional Pigmentation in Lollo Rosso Through Manipulating the Irradiance, Duration, and Periodicity of LEDs in the Visible Region of Light. *Frontiers in Plant Science*, *11*(December), 1–10. https://doi.org/10.3389/fpls.2020.598082

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Table S2-3. Publications combined within present research with factors collected. The empty cell means the factor was not given in the corresponding paper. For all factors, CO_2 indicates CO_2 concentration in ppm, Photoperiod in hours per day, PPFD (Photosynthetic Photon Flux Density) in $\mu mol \cdot m^{-2} \cdot s^{-1}$, DLI (Daily Light Integral) in $mol \cdot m^{-2} \cdot d^{-1}$, Plant age in days from transplanting to harvesting, Cumulative DLI is the product between DLI and Plant age in mol, Density in $plant \cdot m^{-2}$, Shoot DW (Dry Weight) at harvest in $g \cdot plant^{-1}$, LUESDW in $g \cdot mol^{-1}$ based on shoot dry weight, and LUESFW in $g \cdot mol^{-1}$ based on shoot fresh weight.

	CO_2	Photoperiod	PPFD	DLI	Plant age	Cumulative DLI	Density	Shoot DW at harvest	LUE_{SDW}	LUE_{SFW}
Vertical farming publications:										
(Ahmed et al., 2020)	1000	16	200	11.5	21	241.9	32	3.7	0.5	11.5
(Bhuiyan and van Iersel, 2021)	797			11.5	43	494.5	100	1.7	0.3	8.5
(Bian et al., 2016)	400	13.5	200	9.7	24	233.3	94	2.5	1.0	12.6
(Cammarisano et al., 2020)	470.5	18		19.9	30	597.3	51	1.8	0.2	1.6
(Cammarisano et al., 2021)	400	18	270	17.5	30	524.9	51	1.3	0.1	1.8
(Carotti et al., 2021)	1200	16	200	11.5	29	334.1	25	16.5	1.2	43.6
(Chen et al., 2019a)	600	16	130	7.5	40	299.5	25	4.4	0.4	9.1
(Chen et al., 2019b)	400	16	230	13.2	13	172.2	39	2.3	0.5	9.4
(Chen et al., 2021)	400	12	200	8.6	35	302.4	50	2.5	0.4	7.9
(Cope et al., 2014)	430	16	200	11.5	24	276.5	37	0.4	0.0	1.2
(Esmaili et al., 2020)	1600	12	300	13.0	40	518.4	57	8.0	0.9	21.9
(Gómez and Jiménez, 2020)	405	20	220	15.8	28	443.5	21	6.7	0.3	6.5
(Hytönen et al., 2018)	400	20	136	14.0	35	490.0	45	4.7	0.5	10.7
(Incrocci et al., 2006)	400	24	120	10.4	15	155.5	1300	0.1	0.6	13.8
(Jayalath and van Iersel, 2021)	825	16	214	12.4	28	345.9		2.5	0.9	22.5

(Jin et al., 2021)	752	18	220	14.3	28	399.2	51	3.4	0.4	7.0
(Joshi et al., 2017)	400	14	230	11.6	21	243.4	33	8.7	1.2	29.5
(Kelly et al., 2020)	377	16	150	8.6	16.5	141.9	40	1.7	0.5	13.8
(Kim et al., 2004a)	1200	18	150	9.7	21	204.1	53	2.3	0.6	9.3
(Kim et al., 2004b)	1200	18	150	9.7	14	136.1	53	0.3	0.1	2.0
(Kong et al., 2019)	400	24	247	21.3	23	490.8	43	4.0	0.3	8.7
(Kook et al., 2013)	40	18	200	13.0	28	362.9	360	0.2	0.2	4.7
(Kuno et al., 2017)	400	24	240	10.4	21	217.7	28	1.3	0.2	3.4
(Kusuma et al., 2021)	477	16	200	11.5	21	241.9	28	1.7	0.2	5.0
(Lee and Kim, 2013)	40	20	230	16.6	28	463.7	49	6.3	0.7	18.3
(Li et al., 2016)	400	16		5.8	25	144.0	32	1.5	0.3	8.4
(Meng et al., 2020)	404	20	181	13.0	27.5	357.8	48	2.9	0.4	4.2
(Meng et al., 2019)	391	20	117	8.4	20	167.9	48	2.1	0.6	8.7
(Meng and Runkle, 2019)	400	24	180	15.6	9	140.0	1372	0.1	0.5	8.5
(Morsi et al., 2022)	2800	16	330	19.0	56	1064.4	26	8.8	0.2	2.9
(Nguyen et al., 2021)	400	12	130	5.6	28	156.9	62	1.1	0.4	10.7
(Ohtake et al., 2018)	1000	24	80	6.9	25	172.5	21	6.6	0.8	17.7
(Ohtake et al., 2021)	1000	24	80	6.9	21	144.9	25	5.4	0.9	14.0
(Pennisi et al., 2020a)	450	16	250	14.4	21	302.4	100	2.7	0.9	22.5
(Pennisi et al., 2019a)	450	16	215	12.4	14	173.4	100	2.8	1.6	26.3
(Pennisi et al., 2020b)	450	16	250	14.4	21	302.4	100	3.3	1.1	20.2
(Pennisi et al., 2019b)	450	16	215	12.4	14	173.4	100	2.0	1.1	22.9
(Rouphael et al., 2019)	390	12	210	9.1	19	172.4	16	2.1	0.2	4.6
(Saengtharatip et al., 2021)	400	14	200	10.1	38	383.0	33	12.7	1.1	27.4

(Spalholz et al., 2020)	704	18	199	12.9	15	193.2	711	0.0	0.1	3.1
(Tosti et al., 2018)	400	14	300	15.1	31	468.7	952	0.2	0.5	12.3
(Touliatos et al., 2016)	400	16	428	24.6	35	862.7	50	10.6	0.6	15.4
(Vaštakaite-Kairien et al., 2021)	400	18	250	16.2	5	81.0	403	0.2	0.8	8.5
(Voutsinos et al., 2021)	400	12	310	13.4	29	388.4	30	8.8	0.7	16.9
(Wang et al., 2016)	400	12	150	6.5	35	226.8	25	5.1	0.6	5.8
(Xu et al., 2020)	1500	16	150	8.6	12	198.7	34	3.4	0.6	13.1
(Yan et al., 2019)	400	14		10.1	20	201.6	39	1.7	0.4	8.1
(Yi et al., 2021)	400	16	43	2.5	30	74.3	23	1.6	0.5	12.1
(Zhang et al., 2015)	1000	16	221	12.7	31	394.0	37	6.8	0.6	16.0
(Zhang et al., 2018)	800	12	150	6.5	18	116.6	22	1.3	0.2	4.9
(Zou et al., 2021)	420	16	200	11.5	15	172.8	39	2.3	0.5	13.1
(Zou et al., 2019)	400	16	200	11.5	15	172.8	39	3.2	0.7	11.5
(Zou et al., 2020)	400	16	210	12.1	15	181.4	33	1.9	0.3	8.7
Greenhouse publications:										
(Both, 1995)	400			22.0	24	528.0	35	9.7	0.6	14.3
(De Pinheiro Henriques and Marcelis, 2000)	400			10.8	57	326.4	27	9.4	0.8	18.5
(Ioslovich et al., 2002)	400			6.7	48	321.6	3200	0.0	0.4	10.8
(Hassanien and Li, 2017)	400			26.9	60	1616.1	5	16.0	0.1	1.3
(Hernandez et al., 2020)	400			17.0	55	935.0	24.3	7.6	0.2	4.9
(Hooks et al., 2021)	400			12.3	15	184.6	16.7	2.7	0.2	4.3
(McCall and Willumsen, 1999)	400			2.0	95	191.0	20	4.8	0.6	12.6
(Meng et al., 2020)	400	16		17.6	28	652.2	48	1.7	0.1	1.9

Vertical farming has the

(Miller et al., 2020)	400	10	331	11.9	40	476.0	43	0.3	0.0	0.6
(Tani et al., 2014)	400			19.8	22	435.6	100	2.5	0.6	14.3
(Weaver and van Iersel, 2020)	400	21	225	17.0	22	374.0	224	0.7	0.4	11.2
(Wheeler et al., 1993)	400			26.0	46	1196.5	25	10.4	0.2	5.4
(Zhang et al., 2019)	400			10.2	36	367.2	26	8.1	0.6	14.5
Field publications:										
(Conversa and Elia, 2019)	400				57	2616.0	11	51.4	0.2	5.4
(Elamri et al., 2018)	400			42.8	57	2439.6	11	25.5	0.1	2.9
(Ilić et al., 2017)	400		654	16.5	35	577.2	8	11.8	0.2	4.2
(Krizek, 1998)	400			34.5	32.5	951.7	157	1.3	0.2	5.2
(Rabeendran et al., 2000)	400			49.2	28	1377.2	25	2.4	0.0	1.1
(Schaffer, 1989)	400		160	13.9	48	665.3	5	38.3	0.3	7.8
(Tei et al., 1996)	400			15.7	63	987.4	102	4.8	0.5	12.3
(Wurr and Fellows, 1984)	400			26.6	87.03	2314.5	26	28.1	0.3	7.9

Table S2-4. Correlation (r) and *p* probability value (two-sided t-test with null-hypothesis r=0) for six regressors from Figure 3 making use of data for all production systems (except for 10 entries with planting densities above 100 plants m^{-2}). LUE_{inc} stands for light use efficiency based on cumulative incident photosynthetic photon flux density and shoot dry weight (g mol^{-1}). Plant age stands for the duration (number of days) from transplanting to harvesting (d). SDW at harvest means shoot dry weight at harvest (g $plant^{-1}$). DLI presents daily light integral (mol d^{-1}). DLI_{cum} is the cumulative DLI through duration from transplanting to harvesting (mol). CO_2 stands for CO_2 concentration applied in the duration from transplanting to harvest (ppm). Density is planting density from transplanting to harvest (plants m^{-2}).

Correlation (r)

LUE_{inc}	-						
Plant age	-0.1358	-					
SDW at harvest	0.0807	0.4312	-				
DLI_{cum}	-0.3541	0.5087	0.4009	-			
Density	0.4591	-0.1864	-0.3678	-0.0979	-		
DLI	-0.2897	0.0958	0.1580	0.8272	0.0302	-	
CO_2	0.0451	0.0506	0.0602	0.0736	-0.0805	-0.0556	-
	LUE_{inc}	Plant age	SDW at harvest	DLI_{cum}	Density	DLI	CO_2

p probability

LUE_{inc}	-						
Plant age	0.3276	-					
SDW at harvest	0.5620	0.0011	-				
DLI_{cum}	0.0086	<0.001	0.0027	-			
Density	<0.001	0.1772	0.0062	0.4811	-		
DLI	0.0336	0.4907	0.2540	<0.001	0.8283	-	
CO_2	0.7460	0.7164	0.6654	0.5970	0.5626	0.6897	-
	LUE_{inc}	Plant age	SDW at harvest	DLI_{cum}	Density	DLI	CO_2

Figure S2-5. Scatter plots for three regressors in the linear model for density (a), DLI (b) and cumulative DLI (c) selected by backward elimination using all vertical farming data with planting densities up to 100 plants m^{-2}. LUEinc_SDW stands for light use efficiency based on cumulative incident photosynthetic photon flux density and shoot dry weight (g mol^{-1}). Density is planting density in the duration from transplanting to harvesting (plants m^{-2}). DLI presents daily light integral (mol d^{-1}). DLI_{cum} is the cumulative DLI from transplanting to harvesting (mol).

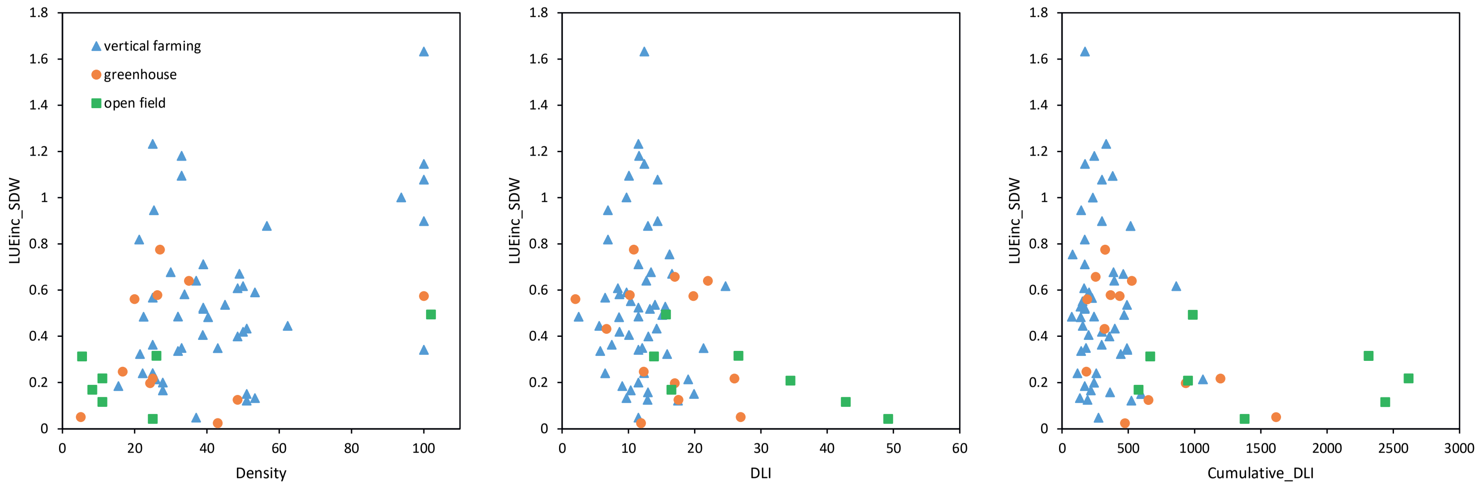

Figure S2-6. Scatter plots for both regressors in the linear model for Shoot dry weight at harvest (a) and density (b) selected by backward elimination using all vertical farming data with planting densities up to 100 plants m^{-2}. LUEinc_SDW stands for light use efficiency based on cumulative incident photosynthetic photon flux density and shoot dry weight (g mol^{-1}). Shoot_DW_at_harvest means shoot dry weight at harvest (g $plant^{-1}$). Density is planting density from transplanting to harvesting (plants m^{-2}).

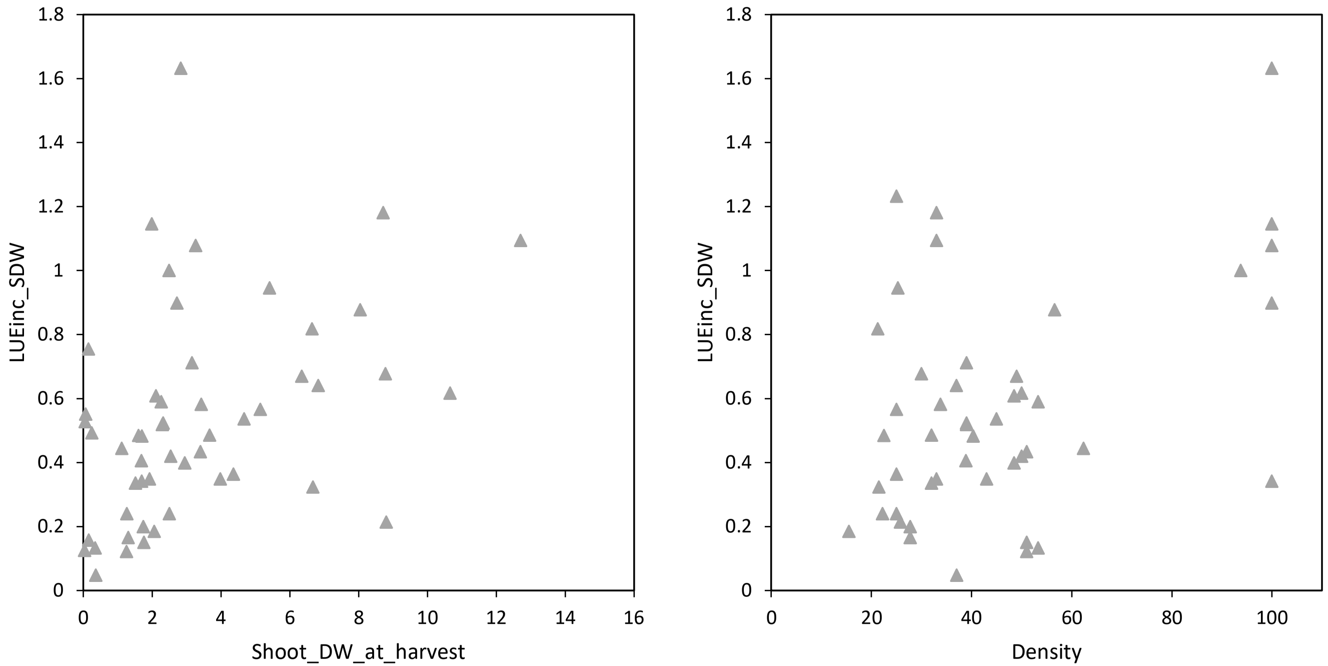

Table S2-7. Correlation and *p* probability value (two-sided t-test with null-hypothesis r=0) for six regressors in Figure 3 making use of data for vertical farming (except for 6 entries with planting densities above 100 plants m^{-2}). LUE_{inc} stands for light use efficiency based on cumulative incident photosynthetic photon flux density and shoot dry weight (g mol^{-1}). Plant age stands for the duration (number of days) from transplanting to harvesting (d). SDW at harvest means shoot dry weight at harvest (g $plant^{-1}$). DLI presents daily light integral (mol d^{-1}). DLI_{cum} is the cumulative DLI through duration from transplanting to harvesting (mol). CO_2 stands for CO_2 concentration applied in the duration from transplanting to harvest (ppm). Density is planting density in the duration from transplanting to harvesting (plants m^{-2}).

Correlation (r)

LUE_{inc}	-						
Plant age	-0.1184	-					
SDW at harvest	0.4516	0.4953	-				
DLI_{cum}	-0.1362	0.7638	0.4757	-			
Density	0.4814	-0.0909	-0.2254	0.0580	-		
DLI	-0.0482	0.2700	0.2704	0.7934	0.2613	-	
CO_2	-0.0179	0.3686	0.3361	0.4241	-0.1466	0.1084	-
	LUE_{inc}	Plant age	SDW at harvest	DLI_{cum}	Density	DLI	CO_2

p probability

LUE_{inc}	-						
Plant age	0.4494	-					
SDW at harvest	0.0024	<0.001	-				
DLI_{cum}	0.3839	<0.001	0.0013	-			
Density	0.0011	0.5619	0.1462	0.7117	-		
DLI	0.7589	0.0799	0.0794	<0.001	0.0906	-	
CO_2	0.9093	0.0150	0.0275	0.0046	0.3483	0.4888	-
	LUE_{inc}	Plant age	SDW at harvest	DLI_{cum}	Density	DLI	CO_2

Chapter 3

Adding Far-red to red-blue LED light promotes yield of lettuce at different planting densities

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Abstract

The economic viability and energy use of vertical farms strongly depend on the efficiency of the use of light. Increasing far-red radiation (FR, 700-800 nm) relative to photosynthetically active radiation (PAR, 400-700 nm) may induce shade avoidance responses including stem elongation and leaf expansion which would benefit light interception and FR might even be photosynthetic active when used in combination with PAR. The aim of this study is to investigate the interaction between FR and planting density and to quantify the underlying components of the FR effects on growth. Lettuce (*Lactuca sativa* cv. Expertise RZ) was grown in a climate chamber under two FR treatments (0 or 52 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and three planting densities (23, 37, 51 $plants \cdot m^{-2}$). Photosynthetically active radiation (PAR) of 89% red and 11% blue was kept at 218 $\mu mol\ m^{-2}\ s^{-1}$. Adding FR increased plant dry weight after 4 weeks by 46%-77% (largest effect at lowest planting density) and leaf area by 58%-75% (largest effect at middle planting density). Radiation use efficiency (RUE: plant dry weight per unit of incident radiation, 400-800nm) increased by 17%-42% and incident light use efficiency (LUE_{inc}: plant dry weight per unit of incident PAR, 400-700nm) increased by 46%-77% by adding FR; the largest FR effects were observed at the lowest planting density. Intercepted light use efficiency (LUE_{int}: plant dry weight per unit of intercepted PAR) increased by FR(8%-23%). Neither specific leaf area nor net leaf photosynthetic rate was influenced by FR. We conclude that supplemental FR increased plant biomass production mainly by faster leaf area expansion, which increased light interception. The effects of FR on plant dry weight are stronger at low than at high planting density. Additionally, an increased LUE_{int} may contribute to the increased biomass production.

Key words: Vertical farm, LED, Far-red, Lettuce, Light use efficiency, Yield component analysis.

3.1 Introduction

Vertical farming is a relatively new plant production system, where plants are grown without solar light in many layers above each other. Plants receive light from lamps (usually Light-Emitting Diodes, LEDs) and all growth conditions can be fully controlled. This production system scores high on sustainability since crops can be grown without the use of pesticides, without nutrient emission, and high water and land use efficiencies (SharathKumar et al., 2020). However, the energy consumption is high, especially for lighting. Therefore, there is an urgent need for increased light use efficiency.

Light use efficiency (LUE) can be defined in several ways. For overall performance of vertical farming, the fresh yield of harvested product per unit of emitted light by light source is the most relevant definition. The efficiency of the lighting may also refer to the ratio between plant dry weight and total photon flux incident on the canopy which is called Radiation Use Efficiency (RUE, $g \cdot mol^{-1}$), or the ratio between plant dry weight and total photosynthetic photon flux intercepted by the canopy which is called intercepted Light Use Efficiency (LUE_{int}, $g \cdot mol^{-1}$). RUE is directly connected to the energy use efficiency (Pennisi et al., 2020b) and LUE_{int} indicates the efficiency of the plants transforming intercepted photons into biomass

Far-red radiation (FR, 700-800 nm) is relatively little absorbed by leaves and mostly reflected or transmitted (Taiz et al., 2015). In nature where sun is the sole light source, the ratio between red (R) and FR (R:FR ratio) perceived by leaves decreases when vegetation proximity or shading by leaves occurs. R:FR ratio determines the equilibrium of Pfr and Pr in plant (Pierik and De Wit, 2014). Pr and Pfr are two photo-convertible isomers of phytochrome which could transform to each other by absorbing R or FR (Demotes-Mainard et al., 2016b). A rebalanced equilibrium by lowered R:FR ratio induces Shade Avoidance Syndrome (SAS), which includes responses such as increased stem length and/or leaf elongation, leaf moving upward (hyponasty), a higher fraction of assimilate partitioning to stem and/or increased specific leaf area (Franklin, 2008; Vos et al., 2010; Bongers et al., 2014).

As the application of light-emitting diodes (LEDs) expanded in the past decade, several studies on FR have been conducted for further understanding its effect on crop growth. Park and Runkle (2017) reported 28-50% shoot dry weight increase by adding 16-64 $\mu mol \cdot m^{-2} \cdot s^{-1}$ FR on top of 128 $\mu mol \cdot m^{-2} \cdot s^{-1}$ R and 32 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue in geranium and snapdragon. Zou et al. (2019) observed a 49% leaf area increase and 39% biomass production increase by addition of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ FR during the whole photoperiod in lettuce with the background 200 μmol·m^{-2}·s^{-1} R and B (R:B = 7:1). Thus, adding FR is a possible approach to increase plant light interception and biomass production.

Planting density affects R:FR ratio as well, since R will be mostly absorbed by plants but FR only to a small extent. A lowered R:FR ratio will be perceived by plants in a higher planting density. In addition, adding FR to photosynthetically active radiation (PAR, 400-700nm) may increase the efficiency of photosystem II electron transport and thus increase the net instantaneous photosynthesis rate (Zhen and van Iersel 2017; Zou et al. 2019). Some authors even proposed to consider a part of FR (700-750 nm) as PAR (Zhen and Bugbee, 2020) when it is applied in combination with PAR such as R and B, although some others did not find an increment in instantaneous net photosynthesis rate when plants acclimated to FR-enriched light were compared with plants under light without FR (Zhang et al. 2019; Ji et al. 2019). Although several studies on the effect of FR on lettuce growth have been conducted (Meng and Runkle, 2019; Zou et al., 2019) a study quantifying the contribution of underlying components on FR improved crop growth is lacking.

Yield component analysis has been used to quantify contributions of underlying components of yield in several studies (Higashide and Heuvelink, 2009; Li et al., 2014; Ji et al., 2019). The aim of this study is to investigate the interaction between FR and planting density and to quantify the underlying components of the FR effects on growth. We hypothesize that FR addition increases the partitioning to the shoot, resulting in an increased biomass production by enlarged leaf area and hence light interception. We expected that the effects on light interception are in particular of importance when plants are widely spaced. For testing this hypothesis, a climate room experiment was conducted with lettuce applying two levels of FR at three planting densities.

3.2 Materials and Methods

Plant material and experimental setup

Lettuce (*Lactuca sativa* cv. Expertise RZ) was grown in a climate room with 6 compartments divided by white plastic screens (treatment distribution see Supplementary Table S1). Seeds were sown in 108-cell plug trays filled with a mix of peat and perlite (Lentse Potgrond, Horticoop, the Netherlands). Germination procedure involved 2 days in dark followed by 5 days in light at 18h light/6h dark with a light intensity of 132 ± 1.5 μmol m^{-2} s^{-1} provided by red (R) and blue (B) LEDs (89%R and

11%B) (GreenPower LED production module, 2nd generation, Philips). Seven days after sowing, seedlings with 2 cotyledons were transplanted to individual pots (9 cm x 9 cm x 10 cm, L x W x H) filled by expanded clay grid (4-8 mm; Jongkind hydrocorns, the Netherlands) and were grown for 28 days. Light and planting density treatments started at the same time. Pots were always in 1.5-2.0 cm layer of nutrient solution. Nutrient solution (electrical conductivity (EC) 2.3 dS·m^{-1} and pH 5.8), containing 0.38 mM NH_4^+, 8.82 mM K^+, 4.22 mM Ca^{2+}, 1.15 mM Mg^{2+}, 12.92 mM NO_3^-, 1.53 mM Cl^-, 1.53 mM SO_4^{2-}, 0.12 mM HCO_3^-, 1.53 mM $H_2PO_4^-$, 0.38 mM SiO_3^{2-}, 30.67 μM Fe^{3+}, 3.83 μM Mn^{2+}, 3.83 μM Zn^{2+}, 38.33 μM B, 0.77 μM Cu^{2+} and 0.38 μM Mo, was applied from the second day after transplanting. Nutrient solution was completely renewed twice a week to keep EC, composition and pH stable. During the whole cultivating period, temperature and relative humidity (RH) were maintained at 22±0.0ºC and 75±0.1% for photoperiod and 20±0.0°C and 79±0.2% for dark period, respectively. CO2 concentration was kept at 752±6.2ppm. These data are average with standard errors of means of three blocks (replications in time).

Light and planting density treatments

Two far-red (FR) treatments (with FR and without FR: RB+FR and RB, respectively) in combination with three planting densities (23 (low), 37 (middle) and 51 (high) plants m^{-2}) were applied. PAR was 218 ± 0.5 μmol m^{-2} s^{-1} and 219 ± 1.5 μmol m^{-2} s^{-1} (89%R and 11%B, GreenPower LED production module, 2nd generation, Philips) for treatment with and without FR, respectively. In the treatment with FR the FR intensity (700-800nm) was 52 ± 0.2 μmol m^{-2} s^{-1} provided by GreenPower LED production module, Philips (Fig. 3-1). These intensities of R, B and FR resulted in Phytochrome stationary state (PSS) of 0.83 (RB+FR) and 0.88 (RB) as calculated by procedure of Sager et al. (1988). The choice for light intensity, photoperiod and red blue ratio of the light was based on what is commonly used in vertical farms. The FR level was chosen such that a distinct effect on plant growth could be expected, but not so high that it would never be realistic for a vertical farm. Light measurements were done at pot height using a quantum sensor (LI-COR, 6400XT Lincoln, United States) and with a spectroradiometer (Apogee Instruments model SS-110, Utah, United States). In each of the three blocks the light intensity was measured at 24 locations per plot. The presented average values and their standard errors were based on three block per treatment.

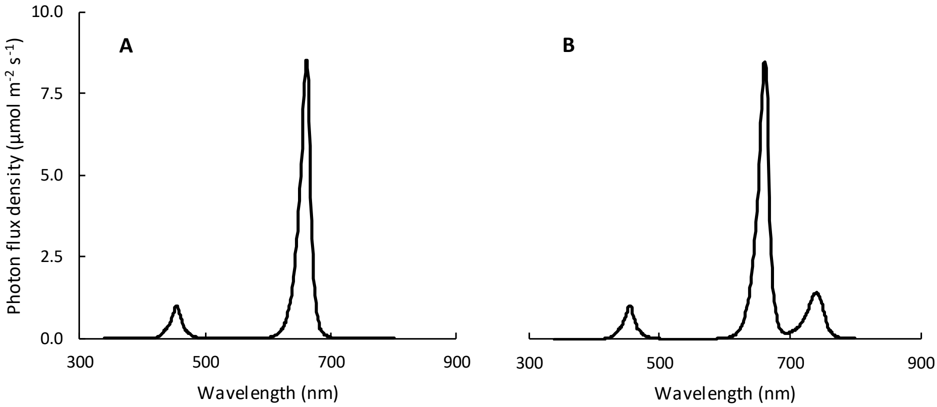

Figure 3-1. *Spectral distribution of the two light treatments: A) Without far-red (RB); B) With far-red (RB+FR). Spectra were recorded and averaged on 21 locations along the cultivated area at pot level, measured by a spectroradiometer.*

Plants were distributed equidistantly following a chess board pattern. The outer row of plants in each plot was considered as border plants and not used for measurements. After each destructive harvest plants were relocated to keep the original planting density.

Biomass and leaf net photosynthesis rate

Destructive measurements were conducted at 0, 7, 14, 21 and 28 days after transplanting (DAT). Individual plant pictures from the top were taken before destructive measurement for estimation of projected leaf area (PLA) at 14, 21 and 28 DAT. Leaf area was measured by a leaf area meter (LI-3100 Area Meter, Li-Cor, Lincoln, United States). Fresh and dry weight (forced air oven at 105°C for 24 h) of shoot and root were determined. As the stem of this cultivar was extremely small, leaf dry weight was considered to be equal to the shoot dry weight.

At 20 DAT, leaf net photosynthesis rate was measured with a portable gas exchange system (LI-6400; LI-COR, Lincoln, United States) using a transparent cuvette under the growing condition (incident light intensity: 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with 90% R and 10% B, 22°C for the temperature, 75% for relative humidity and CO_2 concentration for 700ppm). Measurements were performed on fully expanded and unshaded leaves.

Light interception and use efficiency of light

Floor coverage fraction was calculated based on individual plant projected leaf area and planting density. Daily floor coverage fraction was calculated by linear interpolation between measurement days at 14, 21 and 28 DAT. Floor coverage fraction at 0 DAT was assumed to be zero. Daily light interception was calculated as the product of incident light intensity and floor coverage fraction at that day. For these calculations the incident light intensity was measured before start of the experiment at half the final height of the plants. Considering the small height of the lettuce plants, this is a reasonable estimate of the average light intensity.

Radiation use efficiency (RUE) was calculated by dividing plant total dry weight by the cumulative incident radiation, including PAR and FR (400-800 nm), at canopy top level. Incident light use efficiency (LUE_{inc}) was calculated as the ratio between plant total dry weight and cumulative incident PAR (400-700 nm). Intercepted light use efficiency (LUE_{int}) was calculated as the ratio between plant total dry weight and cumulative intercepted PAR.

Yield component analysis

Treatment effects can be analyzed by breaking down fresh weight into underlying components (Fig. 3-2). In this analysis, leaf fresh weight (FW_{leaf}) is the product of leaf dry weight (DW_{leaf}) and the fresh - dry leaf weight ratio (FW_{leaf}/DW_{leaf}). Leaf dry weight is the product of total dry weight (DW_{plant}) and fraction of biomass partitioning to leaf (Leaf:Plant). Canopy intercepted photosynthetic photon flux density (PPFD) (I_{int}), which is the cumulative PPFD interception during the whole cultivating period (0-28 DAT), and the dry weight production per unit intercepted PPFD (LUE_{int}) determine the total dry weight. Canopy intercepted PPFD was calculated based on projected leaf area which is determined by leaf area per plant ($\overline{LA}$) and plant openness defined as the ratio between projected leaf area and leaf area ($\overline{PLA/LA}$). Leaf dry weight ($\overline{LW}$) and specific leaf area ($\overline{SLA}$) determine the leaf area. The $\overline{LA}$, $\overline{PLA/LA}$, $\overline{SLA}$ and $\overline{LW}$ were averaged over 14, 21 and 28 DAT representing the average levels of all parameters during the whole cultivating period (0-28 DAT).

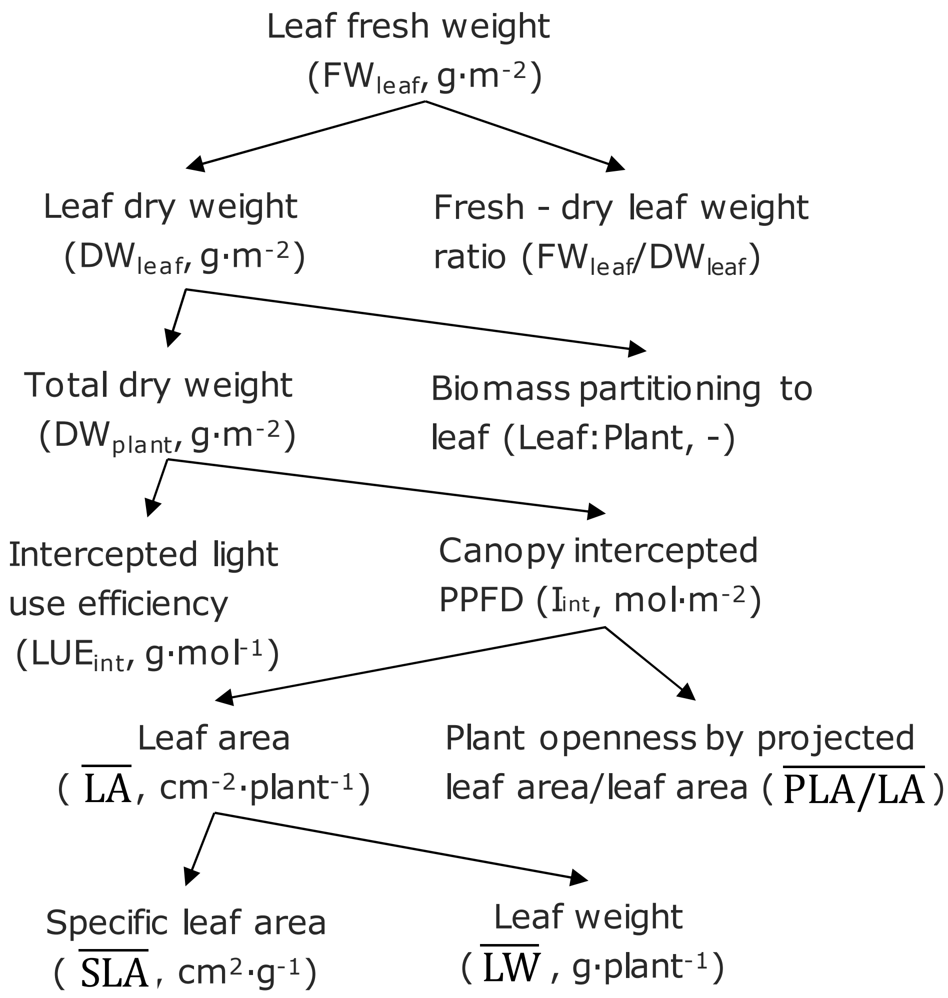

Figure 3-2. *Leaf fresh weight separated into underlying components. Abbreviation and unit are given in between brackets.*

Statistical set-up and analysis

A randomized complete block design was applied. The experiment was repeated three times, with repetitions in time representing three blocks (n=3). At 28 DAT for high planting density and no additional FR only data from two blocks were used. The third block gave an extreme outlier for leaf:root ratio, 15 instead of 4 to 6, therefore these measurements were not included in the final analysis. There were 4-6 replicate plants per block for each destructive measurement and 3 for photosynthesis. For each block, a new randomization of the light treatments positions was done. Analysis of variance was used to determine treatment effects using Genstat software (18th edition, United Kingdom). Normality of the residuals was tested using the Shapiro-Wilk test, and equal variances was assumed as this could not be tested with only 3 repetitions. Mean separation was done with Fisher´s Protected LSD test (P=0.05 or P=0.10). In each repetition the measurements were based on 3-6 replicate plants, as indicated in the description of the measurements.

FR effects were tested for each planting density separately using a one-way ANOVA in component analysis. Since for such a test the total number of experimental units was only six, a level of significance of 0.10 was applied as is normal in such cases (Ott and Longnecker, 2010). FR effects were also tested together with planting density using a two-way ANOVA in other figures and results with the level of significance of 0.05.

3.3 Results

Biomass, leaf area, leaf:root ratio, photosynthesis rate, LUE_{inc}, LUE_{int}, RUE and SLA

At all three planting densities, plant dry weight and leaf area were higher when FR was added (Fig. 3-3). Neither plant dry weight nor leaf area per plant was affected by planting density when no FR was present. Dry weight per plant in the presence of FR was lower at higher planting density. The effects of FR on plant dry weight and leaf area were smaller at higher planting density. Adding FR increased plant dry weight after 4 weeks by 46%-77% (largest effect at lowest planting density) and leaf area by 58%-75% (largest effect at middle planting density).

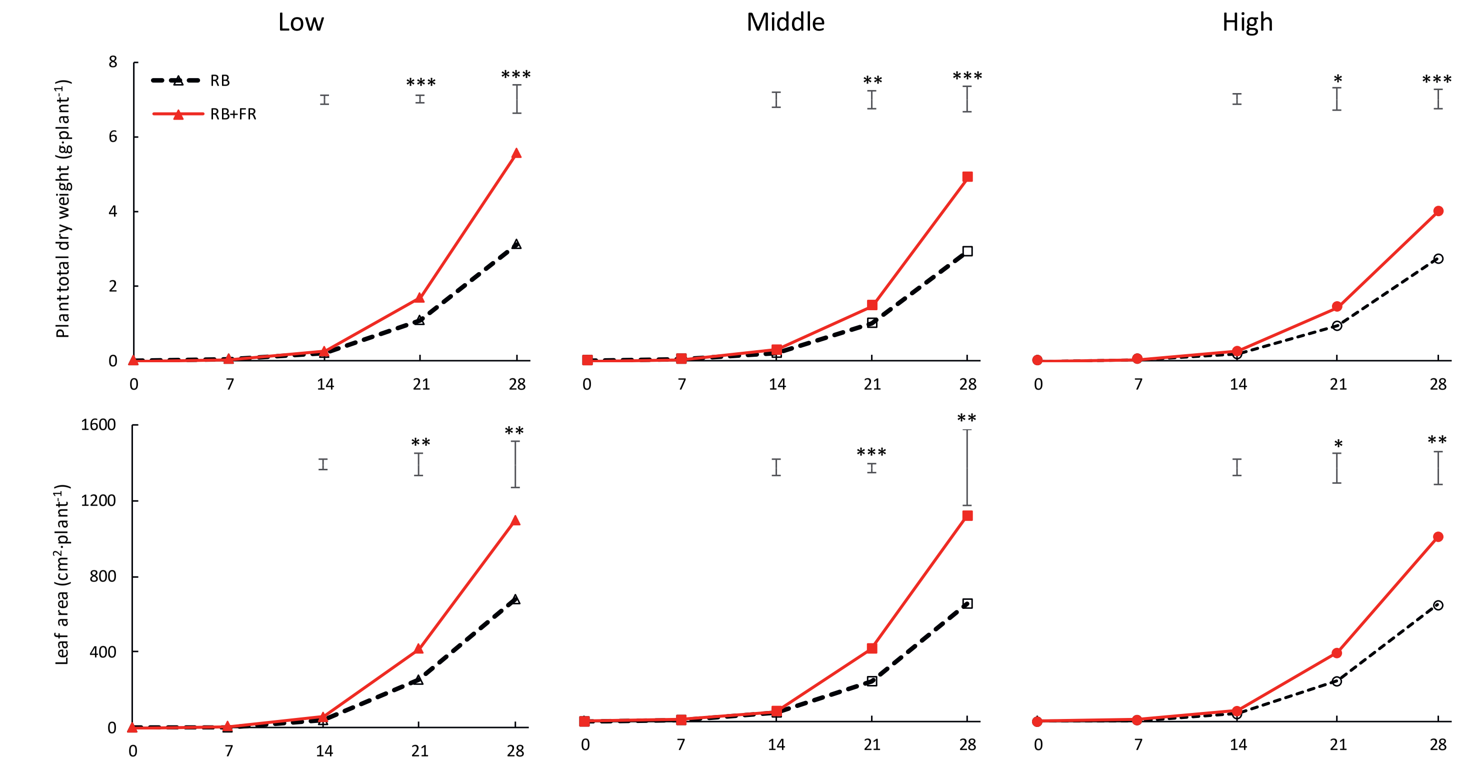

Figure 3-3. *Time course of total dry weight of lettuce plants (g·plant^{-1}, upper layer) and plant leaf area (cm^2·plant^{-1}, lower layer) when grown without (RB) or with (RB+FR) 52 μmol m^{-2} s^{-1} FR intensity, at three planting densities (low, middle, and high, being 23, 37 and 51 plants·m^{-2}). Solid lines represent RB+FR treatment and dashed lines indicate RB treatment. Bars on top of each day represent least significant difference. * indicates significant effect of FR. *$P<0.10$, **$P<0.05$ and ***$P<0.01$ Data are means of 3 blocks (n=3) each with 4-6 replicate plants.*

Leaf:root ratio increased during plant development. FR increased leaf:root ratio significantly at 14 and 21 DAT (Fig. 3-4). Planting density did not significantly affect leaf:root ratio.

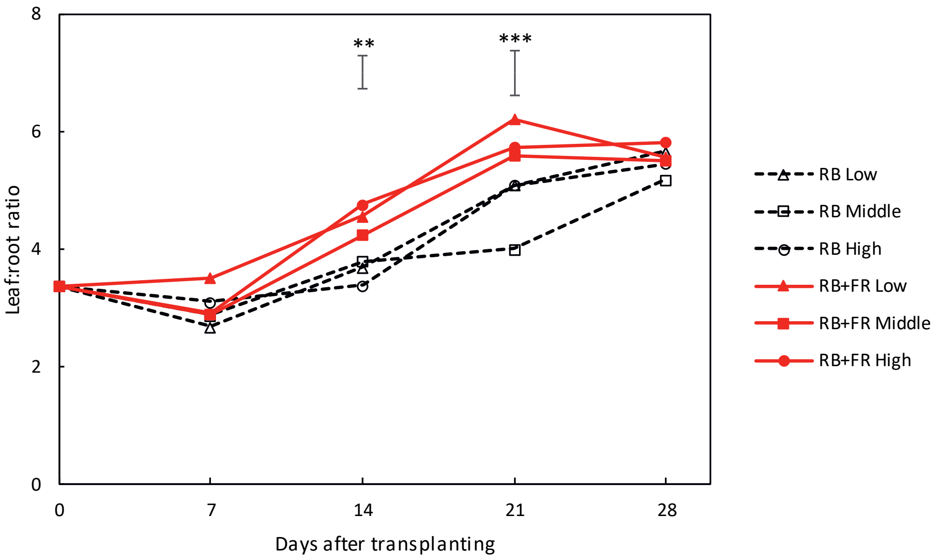

Figure 3-4. *Pattern of leaf:root ratio over time for lettuce plants grown with or without FR at three planting densities (low, middle and high, being 23, 37 and 51 plants·m^{-2}). Solid lines represent RB+FR treatment and dashed lines indicate RBtreatment. Bars on top of each day represent least significant difference. * indicates significant FR effect **P<0.05 and ***P<0.01. Data are means of 2 (n=2) or 3 blocks (n=3) each with 4-6 replicate plants.*

Canopy intercepted PPFD increased with time (Fig. 3-5) which was related to the increase in leaf area. Intercepted PPFD was larger for plants grown with FR compared to plants grown without FR, at all three planting densities.

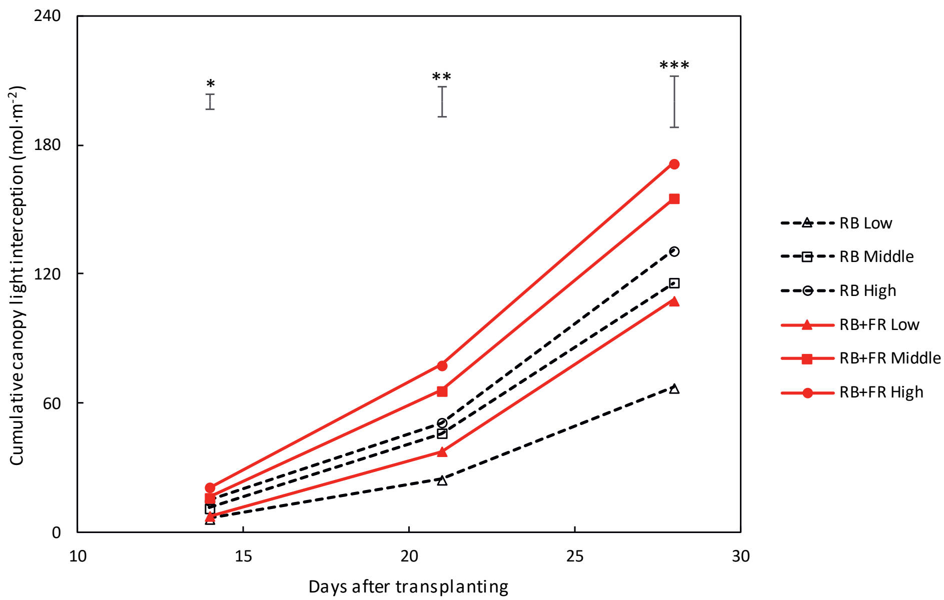

Figure 3-5. *Intercepted PPFD of lettuce canopy grown at three planting densities (low, middle and high, being 23, 37 and 51 plants·m^{-2}). Solid lines represent with FR treatment (52 μmol m^{-2} s^{-1} FR) and dashed lines indicate treatment without FR. Light was cumulated from 14 to 28 DAT. Bars on top of each day represent least significant difference. * indicates significant FR effect. *$P<0.10$, **$P<0.05$ and ***$P<0.01$ Data are means of 2 blocks (n=2) each with 4-6 replicate plants.*

FR significantly increased incident light use efficiency (LUE_{inc}, Fig. 3-6A) and radiation use efficiency (RUE, Fig. 3-6B) at all three planting densities. Radiation use efficiency (RUE: plant dry weight per unit of incident radiation, 400-800 nm) increased by 17%-42% and incident light use efficiency (LUE_{inc}: plant dry weight per unit of incident PAR, 400-700 nm) increased by 46%-77% by FR; the largest FR effects were observed at the lowest planting density. Intercepted light use efficiency (LUE_{int}: plant dry weight per unit of intercepted PAR) also increased by FR, but to a lesser extent (8%-23%) (Fig 3-6C).

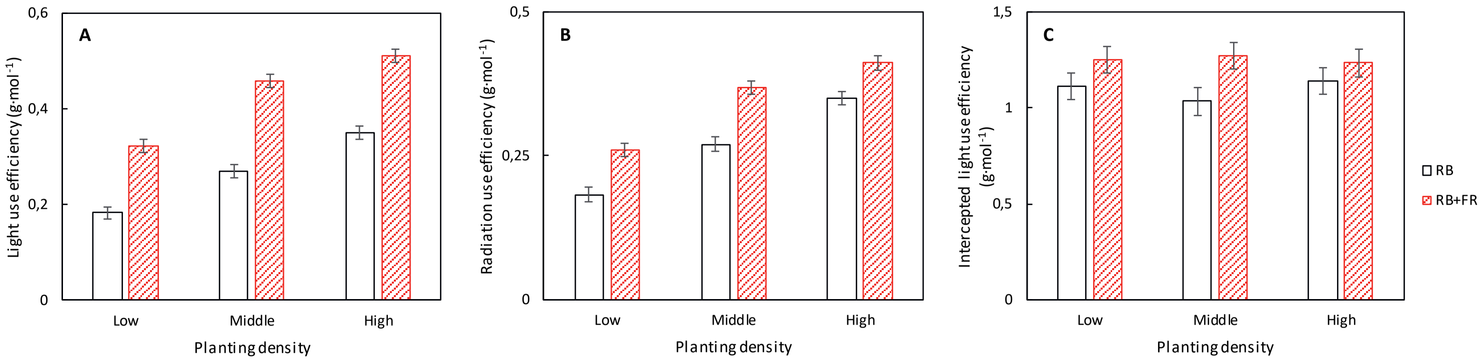

Figure 3-6. *Incident light use efficiency (A. LUE_{inc} which is plant dry weight per unit of incident PAR), radiation use efficiency (B. RUE which is plant dry weight per unit of incident radiation including PAR and FR) and intercepted light use efficiency (C. LUE_{int} which is the plant dry weight per unit of canopy intercepted PPFD) of lettuce plants grown at 3 planting densities (23, 37 and 51 plants m^{-2}), with (52 μmol m^{-2} s^{-1}) and without FR at 28 DAT. Error bars indicate standard errors of means. None of these 3 parameters showed a significant interaction between FR and planting density ($P>0.25$). For incident light use efficiency (A) and radiation use efficiency (B) effects of both FR (LSD= 0.024 and LSD = 0.022, respectively, n=3) and planting density (LSD= 0.030 and LSD = 0.027, respectively, n=2) were significant ($P<0.001$). For intercepted light use efficiency (C) planting density effect was not significant ($P=0.87$) and FR effect was significant ($P=0.043$; LSD=0.15; n=2). Data are means of 2 (n=2) or 3 blocks (n=3) each with 4-6 replicate plants.*

No difference of specific leaf area (SLA) among treatments was observed at 14 and 21 DAT (Fig 3-7). At 28 DAT, SLA was significantly affected by planting densities but not by FR (Fig 3-7). Similarly, the increment in SLA during the final cultivating week, from 21 to 28 DAT, was significantly different among planting densities and not affected by FR (not shown).

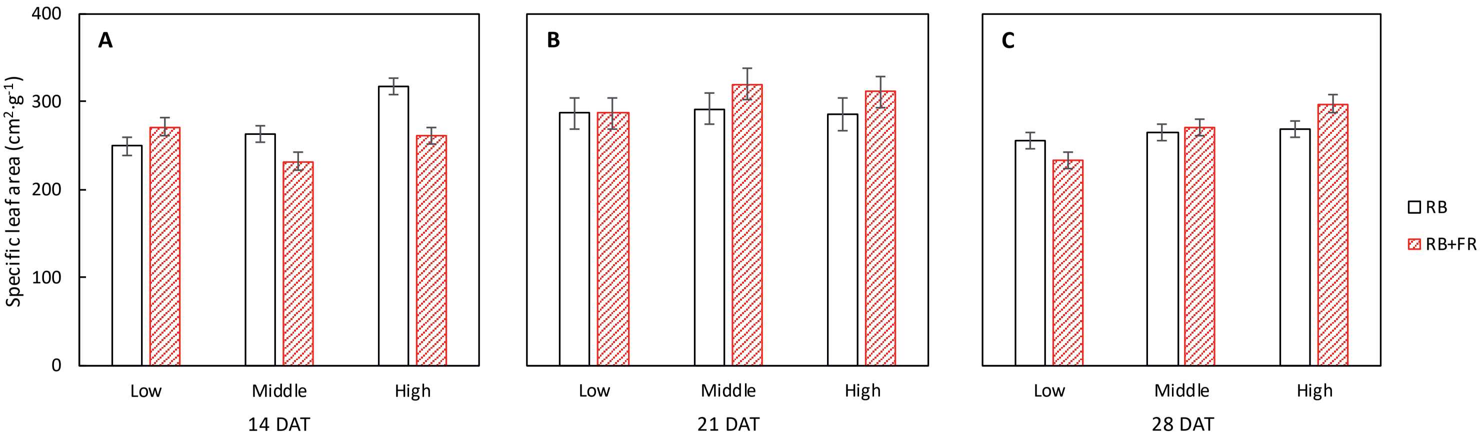

Figure 3-7. *Specific leaf area (SLA) of lettuce plants grown at 3 planting densities (23, 37 and 51 plants m^{-2}), with (52 µmol m^{-2} s^{-1}) and without FR at 14 (A), 21 (B) and 28 DAT (C). Error bars indicate standard errors of means. For 14 DAT, a significant interaction between FR and planting density was observed (P=0.027; n=2). For 21 DAT, no significant interaction (P=0.70), effect of planting density (P=0.59) or effect of FR (P=0.26) was found. For 28 DAT, there was a significant interaction (P=0.055; n=3). Data are means of 2 (n=2) or 3 blocks (n=3) each with 4-6 replicate plants.*

Yield component analysis

FR increased leaf fresh weight (FW_{leaf}) for all planting densities by 42%-61%. This was the result of increased leaf dry weight (DW_{leaf}) and not a higher fresh - dry weight ratio (FW_{leaf}/DW_{leaf}); this ratio actually was lower at RB+FR at the low planting density. FR increased DW_{plant} by 46-77% which increase was mainly due to a higher canopy intercepted PPFD (I_{int}) which increased by 29-64% and a to a smaller extent (8-23%) by higher intercepted light use efficiency (LUE_{int}). The higher I_{int} was caused by an increased average leaf area (LA_{plant}) by 58-67%, rather than plant openness (PLA/LA) which varied little between treatments with and without FR. FR increased overall biomass partitioning to leaf (Fig. 3-4) which led to a higher leaf area with a relative constant specific leaf area (SLA). The overall reasoning based on the component analysis (Fig. 3-8) was supported by the correlation analysis (Supplementary Table S3-2).

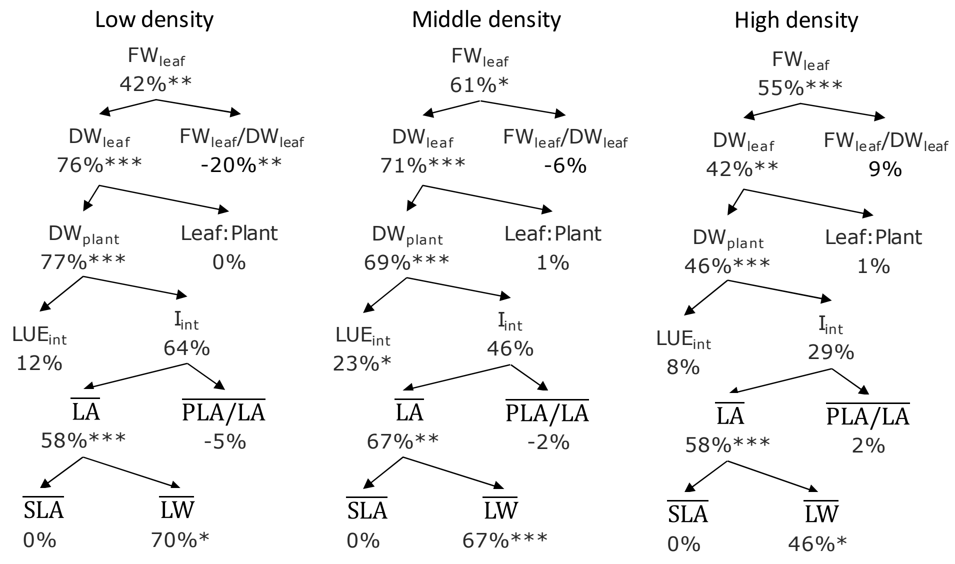

Figure 3-8. Effect of adding FR on top of red and blue at 3 planting densities. Percentages are the RB+FR increment on top of RB. Abbreviations within schemes are: FW_{leaf} (leaf fresh weight), DW_{leaf} (leaf dry weight), FW_{leaf}/DW_{leaf} (leaf fresh - dry weight ratio), DW_{plant} (plant total dry weight), Leaf:Plant (ratio leaf dry weight in total plant), LUE_{int} (intercepted Light Use Efficiency), I_{int} (canopy intercepted PPFD), $\overline{LA}$ (plant leaf area), $\overline{PLA/LA}$ (projected leaf area and leaf area ratio), $\overline{SLA}$ (specific leaf area) and $\overline{LW}$ (leaf weight). The $\overline{LA}$, $\overline{PLA/LA}$, $\overline{SLA}$ and $\overline{LW}$ are all averaged values over DAT 14, 21 and 28 representing

*cumulative values during the whole cultivating period (0-28 DAT). * P<0.10, ** P<0.05 and ***P<0.01. Data are means of 2 (n=2) or 3 blocks (n=3) each with 4-6 replicate plants.*

3.4 Discussion

Higher efficiencies of the photochemistry of photosystem II (PSII) and I (PSI), which are maximumly excited at 680 and 700 nm, respectively, contribute to a higher photosynthesis rate (Baker, 2008). Due to Emerson enhancement effect the PSII efficiency might be increased by adding FR, hence the net photosynthetic rate increases in short term (Emerson et al., 1957). Zou et al. (2019) observed a 7%-10% immediate increment in net photosynthesis rate by adding FR on top of plants acclimated to environments with and without FR. However, due to a lower chlorophyll and total nitrogen content as well as lower leaf absorbance, FR acclimated plant's photosynthetic capacity decreased in the long-term (Zou et al., 2019; Ji et al., 2019). In the present study we did not find an effect of FR on leaf net photosynthesis rate, 9.8 ± 0.2 μmol (CO_2) m^{-2} s^{-1}in average (Supplementary Table S3-3), when measured under the light conditions the plants were grown at 20 DAT which resulted in similar results as reported by Ji et al. (2019) and Zhang et al. (2019) in tomato. There is a possible cancelling out of a positive instantaneous effect on net photosynthesis rate (Emerson enhancement effect) and lowered chlorophyll content per unit leaf area by FR-enriched environment acclimation. Plants acclimate to the growing light environment by adapting photochemistry system under RB or RB+FR conditions to utilize absorbed photons efficiently (Walters 2005; Zhen et al, 2019). As shown by Kalaitzoglou et al. (2019) and Ji et al. (2019) in tomato, on the long run, the effect of FR on plant growth via affecting leaf photosynthesis rate is limited. The significant higher biomass production is rather due to a substantial increment of photosynthetic leaf area by adding FR (Fig. 3-3).

Adding FR on top of red and blue increased plant fresh weight significantly at all three planting densities (Fig. 3-3, 3-8). This resulted from a higher total plant dry weight (DW_{plant}) as well as leaf dry weight (DW_{leaf}) in agreement with previous studies (Meng et al., 2019; Zou et al., 2019). The significantly higher DW_{leaf} when FR was added was due to a substantially higher leaf area (Fig. 3-3). Several papers (Franklin 2008; Vos et al. 2010; Bongers et al. 2014) have reviewed the effect of lowered R:FR which typically happens in vegetation proximity where Red (R) photons were mainly absorbed and thus the ratio between R and FR decreases. Lowering R:FR may result in more expansion of leaf area but not increases the leaf number (Supplementary Table S3-3). In our experiment an increase in expansion of leaf area by FR resulted in a cumulatively advantage in intercepting a much higher fraction of incident light resulting in an increase in plant dry weight (Fig. 3-3, 3-8) at all planting densities. The more rapid expansion of leaf area resulted in a larger fraction of floor cover, and consequently a higher light interception for the RB+FR treatment (Fig. 3-5). The incident light use efficiency (LUE_{inc}: plant dry weight per unit of cumulative incident PPFD) was consequently increased by adding FR. Radiation use efficiency (RUE: plant dry weight per unit of cumulative incident PFD) was also improved due to the strong increase in radiation interception by the enhanced leaf area expansion, which is in agreement with the lettuce experiment of Zou et al. (2019). The intercepted light use efficiency (LUE_{int}: plant dry weight per unit of canopy intercepted PPFD) was significantly increased by FR but no planting density effect was observed. Which was in line with the results of instantaneous net photosynthesis rate increase when adding FR on top of R and B (Zou et al. 2019). The effects of FR on plant dry weight were stronger at low planting density, which could be explained by the fact that at low planting density the light interception is lower and therefore an increase in light interception will have a larger effect on plant growth. Surprisingly this stronger effect at low planting density was not observed for fresh weight, as the ratio of fresh to dry weight was strongly reduced at low planting density. Unexpectedly, this ratio did not decrease by FR at high planting density. In basil plants the fresh to dry ratio was also reduced by FR (Larsen et al., 2020).A higher fraction of biomass partitioning to the shoot is one of the effect of lowered R:FR (Vos et al., 2010; Bongers et al., 2014). In case of lettuce which has only a very small stem, it is the leaf that benefits from this. The increase of leaf:root ratio under FR suggests the relative sink strength of leaves had increased compared to that of the root (Marcelis 1996). Specific leaf area (SLA) has often been found to increase by additional FR or a lowered R:FR which normally happen in vegetation proximity (Ballaré and Pierik, 2017), however, not in current research (Fig. 3-8). Therefore, a higher fraction of biomass partitioned to the leaf resulted in a larger leaf area.

Considering that FR resulted in a higher biomass partitioning to the shoot, a higher leaf area, and improved light interception, the data suggest that adding FR on top of PAR is likely more efficient for dry weight production than adding same intensity PAR. The radiation use efficiency was indeed higher for plants grown with additional FR compared to no FR. It would be worthwhile to grow plants with and without FR, but with same total radiation, in order to test the addition of FR is more efficient in promoting growth than addition of extra PAR.

3.5 Conclusion

Our results demonstrate that adding FR on top of red and blue light increased lettuce fresh and dry weight significantly at three planting densities. The effects on dry weight were strongest at low planting density. The increased plant growth by adding FR was caused by a higher light interception by an enlarged leaf area resulting from a higher biomass partitioning to shoot, rather than from a higher leaf photosynthesis rate or specific leaf area. FR increased incident light use efficiency and radiation use efficiency, while it increased intercepted light use efficiency to a lesser extent.

Supplementary

Table S3-1. Treatments distribution in the climate chamber (Droevendaalsesteeg 1, 6708 PB, Radix, Wageningen University & Research, Wageningen, the Netherlands) in 3 replicates.

Replicate	Side	Upper layer	Lower layer
1	South	RB+FR High and Middle density	RB+FR Low density
	North	RB Low density	RB High and Middle density
2	South	RB High and Middle density	RB Low density
	North	RB+FR High and Middle density	RB+FR Low density
3	South	RB Low density	RB High and Middle density
	North	RB+FR High and Middle density	RB+FR Low density

Table S3-2. Correlation analysis output for all components in component analysis (Fig. 3, 8) and some LUE_{inc}, LWR, Pn and RUE. FW_{leaf}: leaf fresh weight DW_{leaf}: leaf dry weight; FW_{leaf}/DW_{leaf}: fresh - dry leaf weight ratio; DW_{plant}: total plant dry weight; Leaf:Plant: fraction of biomass partitioning to leaf; I_{int}: canop intercepted photosynthetic photon flux density (PPFD) which is the cumulative PPFD interception during the whole cultivating period (0-28 DAT); LUE_{int}: th dry weight production per unit of canopy intercepted PPFD; RUE: plant total dry weight per cumulative incident radiation including PAR and FR; LUE_{inc}: plar total dry weight per unit of cumulative incident PAR; $\overline{LA}$: leaf area per plant; $\overline{PLA/LA}$: plant openness defined as the ratio between projected leaf area and lea area; $\overline{LW}$: leaf dry weight; $\overline{SLA}$: specific leaf area. The $\overline{LA}$, $\overline{PLA/LA}$, $\overline{SLA}$ and $\overline{LW}$ were averaged over 14, 21 and 28 DAT representing the average levels of a parameters during the whole cultivating period (0-28 DAT) (n=3 for components numbered 1-11 and n=2 for components numbered 12-15). Correlation leve in bold indicates a significant correlation. *P<0.10 and **P<0.05.

DW_{leaf}	1	-														
DW_{plant}	2	**1.00****	-													
FW_{leaf}_DW_{leaf}	3	0.16	0.18	-												
$\overline{LA}$	4	**0.52****	**0.54****	-0.33	-											
Leaf_Plant	5	0.17	0.09	-0.21	-0.16	-										
Leaf_root	6	-0.03	-0.02	0.10	-0.24	-0.07	-									
Pn	7	0.24	0.22	-0.34	0.35	0.29	-0.19	-								
RUE	8	**0.96****	**0.96****	0.25	0.30	0.16	0.07	0.12	-							
$\overline{SLA}$	9	**0.46***	**0.45***	0.07	0.04	0.26	-0.15	0.19	**0.49****	-						
LUE_{inc}	10	**1.00****	**1.00****	0.18	**0.54****	0.08	-0.03	0.21	**0.96****	**0.45***	-					
FW_{leaf}	11	**0.93****	**0.94****	**0.49****	0.36	0.07	-0.01	0.05	**0.93****	**0.46***	**0.94****	-				
I_{int}	12	**0.86****	**0.86****	-0.07	0.43	0.14	0.33	0.11	**0.86****	**0.70****	**0.85****	**0.70****	-			
LUE_{int}	13	0.31	0.33	0.49	0.38	-0.11	**-0.69****	-0.06	0.23	0.18	0.35	0.48	-0.19	-		
$\overline{PLA/LA}$	14	0.08	0.07	**-0.59****	0.00	0.10	**0.53***	0.19	0.09	0.15	0.04	-0.18	**0.52***	**-0.85****	-	
$\overline{LW}$	15	**0.99****	**0.99****	0.25	**0.53***	0.14	0.01	0.03	**0.95****	**0.81****	**0.99****	**0.95****	**0.85****	0.33	0.04	-
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Table S3-3. Actual values the plant growth components shown in Figure xx, averaged over 3 blocks. Values followed by different letters are significantly different according to Student's protected LSD test at P=0.05. When interaction between FR treatment and plant density was significant, letters refer to comparison of the six interaction means. When interaction was not significant, letters refer to comparing the 3 averages for plant density and the 2 averages for FR treatment separately.

Leaf Fresh Weight (g·plant^{-1})	Light treatment		
Planting density	RB	RB+FR	Mean
Low	40.7	57.6	49.1a
Middle	36.6	59.0	47.8a
High	35.5	55.0	45.2a
Mean	37.6a	57.3b	
Leaf Dry Weight (g·plant^{-1})	Light treatment		
Planting density	RB	RB+FR	Mean
Low	2.66a	4.70d	3.68
Middle	2.43a	4.16c	3.30
High	2.40a	3.40b	2.90
Mean	2.50	4.08	
Leaf Fresh Weight/Leaf Dry Weight (-)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	15.3bc	12.3a	13.8
Middle	15.0bc	14.1b	14.6
High	14.8bc	16.1c	15.5
Mean	15.1	14.2	
Plant Dry Weight (g·plant^{-1})	Light treatment		
Planting density	RB	RB+FR	Mean
Low	3.14a	5.54d	4.34
Middle	2.91a	4.91c	3.91
High	2.73a	3.98b	3.36
Mean	2.92	4.81	
Leaf Dry Weight/Plant Dry Weight (-)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	0.85	0.85	0.85a
Middle	0.84	0.85	0.84a
High	0.88	0.85	0.86a
Mean	0.85a	0.85a	

Intercepted light use efficiency ($g \cdot mol^{-1}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	1.11	1.25	1.18a
Middle	1.03	1.27	1.15a
High	1.14	1.23	1.19a
Mean	1.10a	1.25b	
Canopy intercepted PPFD at 14 DAT ($mol \cdot m^{-2}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	6.68	7.76	7.22a
Middle	11.63	16.39	14.01b
High	14.94	21.06	18.00c
Mean	11.08a	15.07a	
Canopy intercepted PPFD at 21 DAT ($mol \cdot m^{-2}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	24.9	37.7	31.3a
Middle	46.2	65.9	56.1b
High	51.1	77.9	64.5c
Mean	40.8a	60.5b	
Canopy intercepted PPFD at 28 DAT ($mol \cdot m^{-2}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	67.6	108.0	87.8a
Middle	116.1	155.1	135.6b
High	130.8	171.3	151.1c
Mean	104.8a	144.8b	
Leaf area ($cm^2 \cdot plant^{-1}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	177.5	280.6	229.1a
Middle	169.2	283.2	226.2b
High	157.8	249.6	203.7c
Mean	168.2a	271.1b	
Projected leaf area/Leaf area (-)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	0.48	0.46	0.47a
Middle	0.53	0.52	0.53a

High	0.49	0.50	0.49a
Mean	0.50a	0.49a	
Specific leaf area ($cm^2 \cdot g^{-1}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	294	295	294a
Middle	305	323	314b
High	333	336	334c
Mean	311a	318a	
Leaf weight ($g \cdot plant^{-1}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	2.67a	4.69d	3.68
Middle	2.43a	4.16c	3.29
High	2.40a	3.34b	2.90
Mean	2.50	4.08	
Instantaneous net photosynthesis rate ($\mu mol(CO_2) \cdot m^2 \cdot s^{-1}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	9.7	9.9	9.8a
Middle	9.5	10.0	9.8a
High	9.7	9.9	9.8a
Mean	9.6a	9.9a	
Leaf number ($leaves \cdot plant^{-1}$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	18.8	20.5	19.7a
Middle	19.6	20.5	20.1a
High	18.1	18.5	18.3a
Mean	18.9a	19.8a	
Incremental SLA from 14 to 21 DAT ($(DW_{leaf21} - DW_{leaf14})/(LA_{21} - LA_{14})$)	Light treatment		
Planting density	RB	RB+FR	Mean
Low	271	298	284a
Middle	262	341	301a
High	282	312	297a
Mean	272a	317a	
Incremental SLA from 21 to 28 DAT	Light treatment		

$(DW_{leaf28} - DW_{leaf21})/(LA_{28} - LA_{21})$			
Planting density	RB	RB+FR	Mean
Low	242	210	226a
Middle	257	249	253b
High	262	293	277c
Mean	254a	251a	

Plant openness (PLA/LA) averaged 14, 21 and 28 DAT	Light treatment		
Planting density	RB	RB+FR	Mean
Low	0.48	0.46	0.47a
Middle	0.53	0.52	0.53a
High	0.49	0.50	0.49a
Mean	0.50a	0.49a	

Chapter 4

Far-red light is photosynthetic active, but not to the same extent as PAR light

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(to be submitted)

Abstract

Recently far-red light (FR) in the range 700-750nm has been claimed to have similar photosynthesis efficiency as photosynthetically active radiation (PAR: 400-700nm), when supplied in combination with PAR. The objective of this study was to investigate if adding FR (700-750nm) to PAR light (400-700nm) is equally efficient in promoting photosynthesis as adding PAR light. Besides quantifying the instantaneous effects of FR on photosynthesis we aimed to study if plants acclimate to FR and whether this results in a different photosynthetic response to FR. Finally, we aimed to quantify if the radiation use efficiency of plants is higher for plants grown under a mixture of PAR and FR or only PAR. Lettuce plants were grown in a climate chamber at two levels of PPFD (200 and 400 μmol m^{-2} s^{-1}), and at each PPFD level 25% of PAR or FR was added. In all treatments response curves of leaf photosynthesis to additional FR and PAR were determined. Furthermore, based on growth analyses, light and radiation use efficiencies of the plants were determined. Results showed that adding FR on top of red and blue light (RB) increased net photosynthesis rate distinctly, but this increase was to a smaller extent than the increase due to adding RB with the same intensity. At 200 μmol m^{-2} s^{-1} PPFD, the increase in P_n due to adding 50% FR was 39% of the increase due to adding 50% RB, while at 400 μmol m^{-2} s^{-1} PPFD, this increase in P_n by adding 50% FR was 45% of the increase due to adding 50% RB. Hence, when FR is combined with PAR light it is photosynthetically active but not with similar efficiency. Plants grown under FR also had lowered chlorophyll a and b content as well as lower light-saturated photosynthesis rate at all levels of background PPFD – indicating acclimation. Both substituting PAR by FR or adding FR to PAR increased light use efficiency based on incident and intercepted PPFD. Substituting PAR by FR also increased radiation use efficiency.

Key words:

Far-red, Emerson enhancement effect, quantum yield, net photosynthesis rate, light use efficiency

4.1 Introduction

The sun emits a large range of electromagnetic radiation, a large part of which is used by plants to fuel their growth and shape their development. Far-red (FR: 700-800 nm) light has a wavelength just above what is typically defined as photosynthetically active radiation (PAR: 400-700 nm). Adding FR to PAR may have two routes to improve biomass production. Additional FR promotes leaf expansion and improves biomass production (Kalaitzoglou et al., 2019; Meng et al., 2019; Zou et al., 2019; Spalholz et al., 2020; Jin et al., 2021; Kong and Nemali, 2021; Legendre and van Iersel, 2021). The increased leaf expansion benefits the interception of photosynthetic photon flux density (PPFD) of the canopy and hence promotes plant growth (Kalaitzoglou et al 2019; Jin et al., 2021).

The other route to improve biomass production by additional FR is through improvement of photosynthesis efficiency. Leaf light absorptance of FR in the range 700-750nm is low (Emerson and Lewis, 1943), while hardly any absorptance is measured in the 750-800 nm range (McCree, 1971; Inada, 1976; Zhen and Bugbee, 2020). Even though FR barely contributes to photosynthesis when applied alone, together with shorter wavelengths (600-685 nm) FR powers photosynthesis efficiently and more strongly than the sum of its parts, a phenomenon named the Emerson Enhancement Effect (Emerson and Rabinowitch, 1960). Photosystem I (PSI) and photosystem II (PSII) operate in series to transfer electrons along the photosynthetic electron transport chain and carry out photochemical reactions. The peak wavelength for excitation of PSI is at 700 nm and at 680 nm for PSII. Therefore, applying PAR or FR alone causes imbalanced excitation between the two photosystems, reducing photosynthesis efficiency.

Several studies (Zhen and van Iersel, 2017; Zhen, 2020; Zhen and Bugbee, 2020) have shown that adding FR on top of PAR increases photosynthesis efficiency. Additional FR in the range 700-750nm was proposed identically photosynthesis efficient as PAR (400-700nm) (Zhen and van Iersel, 2017; Zhen, 2020; Zhen and Bugbee, 2020). However, Jin et al. (2021) measuring the P_n of lettuce leaves with a transparent chamber under growing light, found no difference between treatments at the same background PPFD with and without additional FR, while Ji et al. (2019) reported alike findings on tomato. In addition, Jin et al. (2021) estimated the cumulative PPFD interception during lettuce growth and reported the dry mass production efficiency by addition FR. Results showed that the additional FR improved the light use efficiency based on PPFD interception (dry mass per unit of cumulative intercepted PPFD) . Therefore, whether additional FR increases photosynthesis rate and dry mass production efficiency is still under debate.

Short term effects of light spectrum may be different to long term effects due to acclimation of the plants to the spectrum. Acclimation to FR has been shown to downregulate photosynthetic capacity by decreasing the chlorophyll content per leaf area (Demotes-Mainard et al., 2016a). For example, acclimation to a lowered R:FR ratio led to higher PSII/PSI ratio, decreased Chl a/b ratio, and higher light harvesting complex II (LHCII) concentration per PSII core (Heraut-Bron et al., 1999; Hogewoning et al., 2012), which partly restored the excitation balance between both photosystems. However, a question that remains – and which, in the light of the recent massive interest in FR effects on photosynthesis efficiency is gaining importance – is whether acclimation to FR also improves the photosynthesis efficiency while measuring with FR.

The objective of this study is to investigate if adding far-red (700-750nm) to PAR light (400-700nm) is equally efficient in promoting photosynthesis as adding PAR light. Besides quantifying the instantaneous effects of far-red on photosynthesis we aim to study if plants acclimate to far-red and whether this results in a different photosynthetic response to far-red. Finally, we aim to quantify if the radiation use efficiency of plants is higher for plants grown under a mixture of PAR and FR or only PAR. Lettuce plants were grown in a climate chamber at two levels of PPFD (200 and 400 μmol m^{-2} s^{-1}), and at each PPFD level 25% of PAR or FR was added. In all treatments response curves of leaf photosynthesis to additional far-red and PAR were determined. Furthermore, based on growth analyses, light and radiation use efficiencies of the plants were determined.

4.2 Materials and Methods

Growth conditions and plant material

Lettuce (*Lactuca sativa* cv. Expertise RZ) was grown in a climate chamber in three subsequent growing cycles. Six compartments were divided by white plastic screens to create six treatments. Seeds were sowed in watered stonewool plugs with 240-cell trays (Grodan, Roermond, Netherlands) and stored in darkness at 4°C for two days, and then allowed to germinate for seven days at a photoperiod of 18 h light with a light intensity of 142.1 ± 1.3 μmol m^{-2} s^{-1} provided by red (R) and blue (B) LEDs (88% R and 12% B) (GreenPower LED production module, Philips, Eindhoven, the Netherlands). Then, seedlings with two cotyledons were transplanted to individual stonewool blocks (7 x 7 x 7 cm; Grodan) and grown for 28 days at a density of 146 plants m^{-2}. Plants of the outer rows per treatment were considered as border plants and not used for measurements. The photoperiod was 18 h, temperature was 21.9 ± 0.1 °C, relative humidity was 74.9 ± 0.2%

(day) and 78.9 ± 0.0 % (night) and CO_2 concentration ([CO_2]) was 760.0 ± 4.2 ppm (day) and 730.0 ± 0.9 ppm (night). The stonewool blocks were always in a 1.0-1.5 cm deep layer of nutrient solution. The nutrient solution (electrical conductivity: 2.3 $dS \cdot m^{-1}$; pH: 5.8) contained 0.38 mM NH_4^+, 8.82 mM K^+, 4.22 mM Ca^{2+}, 1.15 mM Mg^{2+}, 12.92 mM NO_3^-, 1.53 mM Cl^-, 1.53 mM SO_4^{2-}, 0.12 mM HCO_3^-, 1.53 mM $H_2PO_4^-$, 0.38 mM SiO_3^{2-}, 30.67 µM Fe^{3+}, 3.83 µM Mn^{2+}, 3.83 µM Zn^{2+}, 38.33 µM B, 0.77 µM Cu^{2+} and 0.38 µM Mo, and was applied from the second day after transplanting onwards. It was completely renewed three times a week to keep EC, composition, and pH stable.

Treatments

Six light treatments were applied (Table 4-1). Compared to a reference treatment of 200 µmol m^{-2} s^{-1} of red and blue (RB) light (namely 200RB) there was a treatment with additional 50 µmol m^{-2} s^{-1} of RB light (namely 250RB) or 50 µmol m^{-2} s^{-1} of FR light (namely 250FR). The fourth to sixth treatments were similar, except that all intensities were doubled (400RB, 500RB and 500FR, therefore). Photosynthetic photon flux, consisting of 88% Red (R) and 12% Blue (B), was supplied by LED modules, in treatments 200RB, 250RB and 250FR (GreenPower LED production modules; Philips), and in treatments 400RB, 500RB, 500FR (GreenPower LED Toplighting modules; Philips). Far-red (FR: 700-800nm) in treatment 250FR and 500FR were supplied by LED (GreenPower LED production modules; Philips). PPFD was measured by a quantum sensor (LI-250A; Li-Cor Biosciences, Lincoln, NE, USA) at canopy height. FR intensity was determined by a spectroradiometer (SS-110; Apogee Instruments, Logan, UT, USA) at canopy height. Light spectrum of all treatments is given in Fig. S4-1 (Supplementary S4-1).

Plant growth measurement

Lettuce shoot fresh (FW) and dry weight (DW) as well as leaf area (LA) were determined at harvest (28 days after transplanting, DAT) on eight plants per treatment and growing cycle. Shoot DW was determined after drying by forced air in an oven at 70 °C for 2 h, followed by 105 °C for 24 h. LA was measured using a leaf area meter (LI-3100 Area Meter; Li-Cor Biosciences).

Light use efficiency is the ratio between biomass production and light integral from transplanting to harvesting. Three types of light use efficiency (LUE) were calculated:

Radiation use efficiency (RUE) was defined as shoot DW, divided by the sum of incident PFD. LUE_{inc} was shoot DW per cumulative incident PPFD, whereas LUE_{int} was shoot DW per cumulative intercepted PPFD. Cumulative PPFD interception was the sum of daily PPFD interception, which was the product of daily incident PPFD and floor coverage fraction at that day. Pictures were taken from the top of the canopy at 0, 7, 14, 17, 21, 24, and 28 DAT to determine floor coverage fraction. Pictures were taken at a fixed position from the canopy with a ruler for reference. Canopy projected leaf area was extracted by program ImageJ (IOCI, University of Wisconsin-Madison, Madison, WI, USA). The daily floor coverage fraction was calculated by linear interpolation between measurements.

Table 4-1. *Overview of six light treatments with their photosynthesis photon flux density (PPFD), far-red (FR) intensity and photon flux density (PFD). PFD consists of red (R), blue (B) and FR intensity. Light spectrum of all treatments is given in Fig. S1 (Supplementary S1). Data are the averages of three growing cycles, together with the standard error of means (SEM; n=3). The average value per growing cycle and treatment was determined from 18 measuring spots scattered in the growing area.*

Treatment	PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	FR intensity ($\mu mol\ m^{-2}\ s^{-1}$)	PFD ($\mu mol\ m^{-2}\ s^{-1}$)
200RB	201±1.2	2.5±0.0	203.5±1.2
250RB	247±1.0	0.9±0.0	248±1.0
250FR	200±2.0	52.0±5.0	252±7.0
400RB	398±11.3	2.2±0.1	400.0±11.4
500RB	500±3.7	1.9±0.1	502±3.7
500FR	404±3.4	106.0 ±5.6	510±9.0

Response curves measurement

Light and CO_2 response curves were measured between 21 and 28 DAT in the 2nd and 3rd growing cycles. Per cycle and treatment, four plants were randomly selected, and measurements were conducted on a fully expanded and unshaded flat leaf, using the Li-Cor 6800 photosynthesis system (Li-Cor Biosciences) with the multiphase flash fluorometer (Li-Cor Part No. 6800-01A) and on a 2 cm^2 leaf area. All plants were dark-adapted at 22 °C air temperature for 20 minutes prior to the measurement for measurement of maximum chlorophyll *a* fluorescence (F_m).

The light response curve of photosynthesis was conducted under 400 ppm [CO_2], and the leaves were adapted to 1500 $\mu mol\ m^{-2}\ s^{-1}$ with 90% R and 10% B for 15 minutes, until reference H_2O, reference CO_2 and stomatal conductance (g_s) reached a steady state (the slopes of these three curves were smaller than 0.01). Net photosynthesis rate (P_n) was measured under steps of light intensity at 1500, 1250, 1000, 800, 600, 400, 200, 100, 50, and 0 μmol $m^{-2}s^{-1}$. The CO_2 response curve was conducted under 1200 μmol m^{-2} s^{-1} with 90% R and 10% B. Leaves were adapted to 400 ppm CO_2 for 15 minutes, until reference H_2O, reference CO_2 and g_s reach steady state. P_n was measured at steps of 400, 300, 200, 100, 50, 0, 400, 600, 800, 1000, and 1200 ppm [CO_2]. Data was recorded by an automatic program for both light responses curve and CO_2 response curve. At every level of light intensity and CO_2 concentration, P_n was measured when reference H_2O, reference CO_2 and stomatal conductance reach steady state (the slope < 0.1). To eliminate potential leaf damage effects on the measurement, the Fv/Fm was determined by a portable photosynthesis system (Li-Cor 6400; Li-Cor Biosciences) together with photosynthesis rate measurement. The measurements on leaves where Fv/Fm was between 0.7-0.8 were accepted for the following data analysis. Gas exchange rates were corrected in the case of leaves not fully covering the cuvette area by the fraction of leaf coverage fraction. The cuvette clumping area was labelled and being checked by a spared cuvette gasket after measurements. Pictures were taken to calculate the covering area fraction when clumped leaf area was smaller than cuvette area. Gas exchange rates were corrected by the fraction proportionally.

Light response curve data were fitted by a non-rectangular hyperbola (Ögren and Evans, 1993), to obtain α (maximum quantum yield, mol CO_2 mol^{-1} photons), P_{max} (Light-saturated photosynthesis rate, μmol CO_2 m^{-2} (leaf area) s^{-1}), and R_d (day respiration, μmol CO_2 m^{-2} s^{-1}). CO_2 response curve data were fitted using the excel tool provided by Sharkey (2016) to obtain V_{cmax} (the maximum carboxylation rate of Rubisco), J_{1200} (maximum rate of electron transport at 1200 μmol m^{-2} s^{-1} light intensity) and TPU (maximum rate of triose phosphate use).

Quantum yield measurement

Three to four plants were randomly selected in 2nd and 3rd growing cycles for detailed quantum yield measurements that were conducted with and without FR. A separate compartment within the growing chamber was built to conduct the quantum yield measurement. The compartment contained individually dimmable light sources for R, B, and FR, a spectroradiometer (SS-110; Apogee Instruments), and a gas exchange measurement system (Li-6400; Li-Cor Biosciences) with a clear-top chamber (Li-6400; LI-COR). The transmissivity of the clear-top chamber was measured by a spectroradiometer (SS-110; Apogee Instruments) within the 400-800 nm range. The clear-top chamber was fixed at the position named measuring spot, and a second measuring spot was named the reference spot. A linear relationship of light intensities between the measuring spot and the reference spot, measured by two spectroradiometers (SS-110; Apogee Instruments) was established, to identify the photon flux density at the measuring spot during gas exchange measurements. R (peak at 639 nm), B (peak at 448 nm), and FR (peak at 734 nm) intensities were dimmed and confirmed separately (for spectrum, see Supplementary file: Fig. S4-2). Environmental factors within the clear-top chamber were: 22 ± 0.3°C air temperature, 75 ± 5% relative humidity, 1200 ppm [CO_2], and 500 μmol s^{-1} flow rate. One fully expanded unshaded leaf was selected from every sampled plant. To eliminate the possibility of shading, surrounding leaves close to the measuring leaf were removed. The measurement was corrected by fraction of leave coverage in the chamber in case leaf area was <6 cm^2.

Per plant, four protocols were used. Per protocol, the leaf was exposed to either of two background light intensities (200 and 400 μmol m^{-2} s^{-1} RB), which were each supplemented with four intensities of additional FR or RB light (0%, 12.5%, 25%, and 50% of background photon flux density), resulting in four separate response curves with each four intensities. Within a measurement, the leaf was first exposed to 200 μmol m^{-2} s^{-1} RB light until reference H_2O, reference CO_2 and stomatal conductance (g_s) reached steady-state (slope < 0.1). Then, three intensities of FR were added, after which this procedure was repeated with three steps of additional RB. Then, background RB was increased to 400 m^{-2} s^{-1}, and three steps of FR and PPF were added separately, as previously described. At each step, the leaf was given two minutes for P_n to stabilize. Gas exchange data were recorded 7 times with 5 seconds interval, and the resulting values were later averaged. Quantum yield is the slope of P_n measured with additional 0%, 12.5%, 25%, and 50% RB or FR at each background photon flux density level (200 and 400 μmol m^{-2} s^{-1} RB) and the corresponding PFD. Based on leaf light absorption, quantum yield based on incident light (QY_{inc}) and on absorbed light (QY_{abs}) were calculated. The absorption was the integral of the product between leaf absorption rate and incident light intensity per wavelength (400-800 nm) and per growing cycle.

Chlorophyll and carotenoid concentrations and leaf optical properties measurement

Three lettuce plants were randomly sampled at harvest (28 DAT) per treatment and growing cycle. One fully expanded unshaded leaf was selected per plant, and three leaf discs (total area: 9.42 cm^2) were collected. Fresh weight of these three discs were measured and immersed in liquid nitration sequent. Samples were stored at -80 °C.

Chl and car concentrations were measured based on the protocol described by Wellburn (1994). Leaf discs were pooled in a glass bottle with 3 mL N,N-Dimethyl formamide (DMF, >=99.9% ACS reagent), and kept in the -20 °C freezer for 14 days until complete discoloration of leaf discs. Absorbance measurements were conducted in a UV-visible spectrophotometer (Genesys 150, Thermo Fisher Scientific, Massachusetts, USA). Absorbance (A) was measured at 663.8, 646.8, and 480.0 nm. Chl a, Chl b and total carotenoids (cars) were determined using the following equations:

$$Chl\ a = 12 * A_{663.8} - 3.11 * A_{646.8}$$

$$Chl\ b = 20.78 * A_{646.8} - 4.88 * A_{663.8}$$

$$Total\ carotenoids = \frac{(1000 * A_{480} - 1.12 * Chl\ a - 34.07 * Chl\ b)}{245}$$

Leaf light transmittance and reflectance were measured by a laboratory-built system consisting of two integrating spheres as described by Taylor et al. (2019), but with the white LED being replaced by a halogen light source. The absorbance was calculated as incident light minus the sum of transmitted and reflected light.

Statistical analysis

A randomized complete block design with three blocks (growing cycles) was applied. For each block, a new randomization of the light treatment positions was done. Analysis of variance (ANOVA) was used to determine treatment effects, using Genstat (18^{th} edition, VSN International, Hempstead, UK). Homogeneity of variance of the residuals was assumed and normality of the residuals was shown at P=0.05 with the Shapiro-Wilk test. A two-way ANOVA was conducted with as factors the basic PPFD (200 and 400 µmol m^{-2} s^{-1}) and additional light (non-addition, 25% added PPF, and 25% added FR) during cultivation. RUE, LUE_{inc}, LUE_{int}, Chl (a + b), total carotenoids, and Chl a:b were analyzed using all three blocks (n=3). Photosynthesis parameters alpha, Pmax, Vcmax, J, TPU, QY_{in} and QY_{abs} were analyzed for two blocks (n=2) as these were not determined in the first growing cycle. A four factorial analysis was conducted (GENSTAT, REML variance components analysis; only main effects and 2-way interactions were considered) to examine the effects of background PPFD and additional RB or FR during growth and background PPFD and additional RB or FR during measurement on incident and absorbed quantum yield. F-tests were conducted at P=0.05 followed by mean separation according to Fisher's Protected LSD test (P=0.05).

4.3 Results

Photosynthesis traits and pigment concentrations

Additional FR (comparing 250FR to 200RB and 450FR to 400RB) did not affect maximum carboxylation rate of Rubisco (Vcmax, Fig. 4-1a), maximum rate of triose phosphate use (TPU, Fig. 4-1b), maximum rate of electron transport at 1200 µmol m^{-2} s^{-1} light intensity (J_{1200}, Fig. 4-1c), light-saturated photosynthesis rate (Amax, Fig. 4-3d), maximum quantum yield (α, Fig. 4-1e) day respiration (Rd, Fig. 4-1f) at 200 µmol m^{-2} s^{-1} background PPFD level, but decreased Vcmax, TPU, J_{1200}, and Amax at 400 µmol m^{-2} s^{-1} background PPFD level (Fig. 4-1a-f). PAR substitution by FR (comparing to 250RB and 500RB at each PPFD background level) decreased Vcmax, TPU, J_{1200}, Amax, α, and Rd at both background PPFD levels (Fig. 4-1a-f). Chlorophyll a+b (Chl a+b, g), chlorophyll a:b (Chl a:b, h), and carotenoid content (i) were lowered by FR at both background PPFD levels (Fig. 4-1g-i).

Quantum yield based on incident and absorbed PFD

Quantum yield is the slope of P_n measured with additional 0%, 12.5%, 25%, and 50% RB or FR over two background photon flux density levels (200 and 400 µmol m^{-2} s^{-1} RB) and the corresponding PFD (400-800 nm). Quantum yield is calculated either based on incident (QY_{inc}) or absorbed PFD (QY_{abs}), which was measured and calculated under four different measuring schemes (Fig. 4-2). Neither QY_{inc} nor QY_{abs} were affected by growth conditions, including the presence or absence of FR in the growth light. QY_{inc} were lower when FR was added during measurement, and QY_{inc} was clearly depressed at higher measurement PPFD (Fig. 4-2a). When taking the differences of absorbed light into account (FR is less strongly absorbed than PAR), QY_{abs} were higher when FR was added during the measurement (Fig. 4-2b).

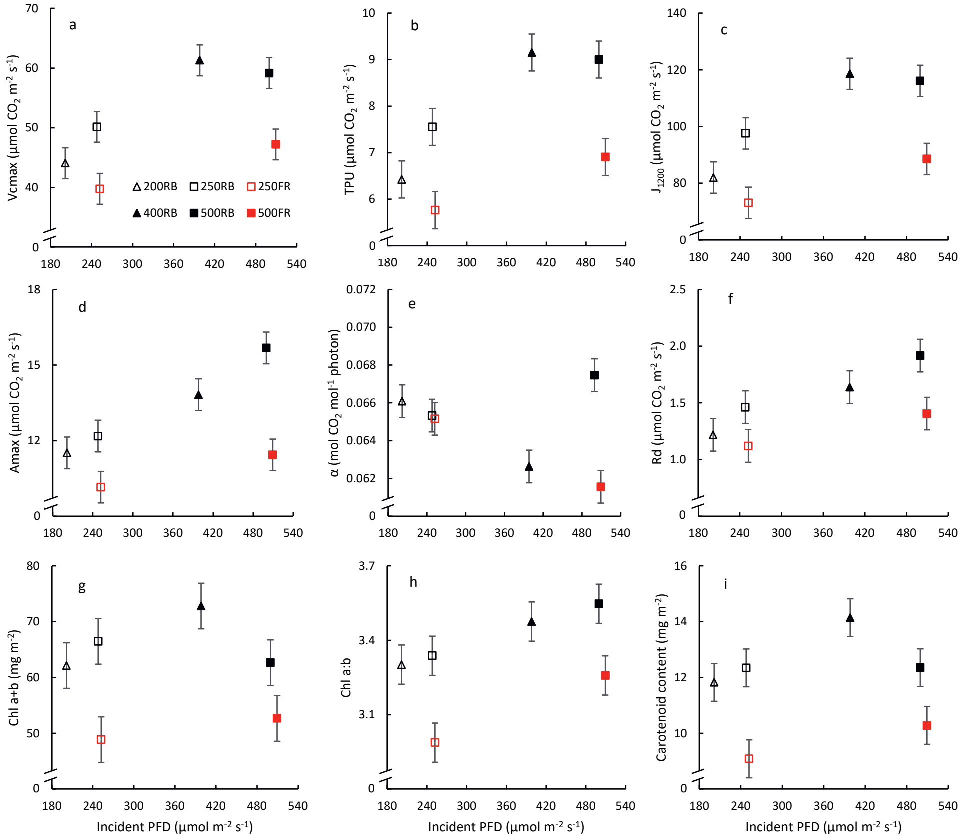

Figure 4-1. Photosynthesis traits and pigment concentrations. The maximum carboxylation rate of Rubisco (Vcmax, a), maximum rate of triose phosphate use (TPU, b), maximum rate of electron transport at 1200 µmol m^{-2} s^{-1} light intensity (J_{1200}, c), light-saturated photosynthesis rate (Amax, d), maximum quantum yield (α, e), day respiration (Rd, f), Chl a+b (g), and Chl a:b (h), and carotenoid content (i). Black symbols refer to plants grown under RB light without FR, while red symbols refer to plants grown under RB light with 25% additional FR. Photosynthesis measurements were conducted under red (90%) and blue (10%) light given by multiphase flash fluorometer (Li-Cor 6800 part No. 6800-01A). Symbols show means ± standard error of means. The two-way ANOVA was done based on two blocks with three to four plants for each block (n=2).

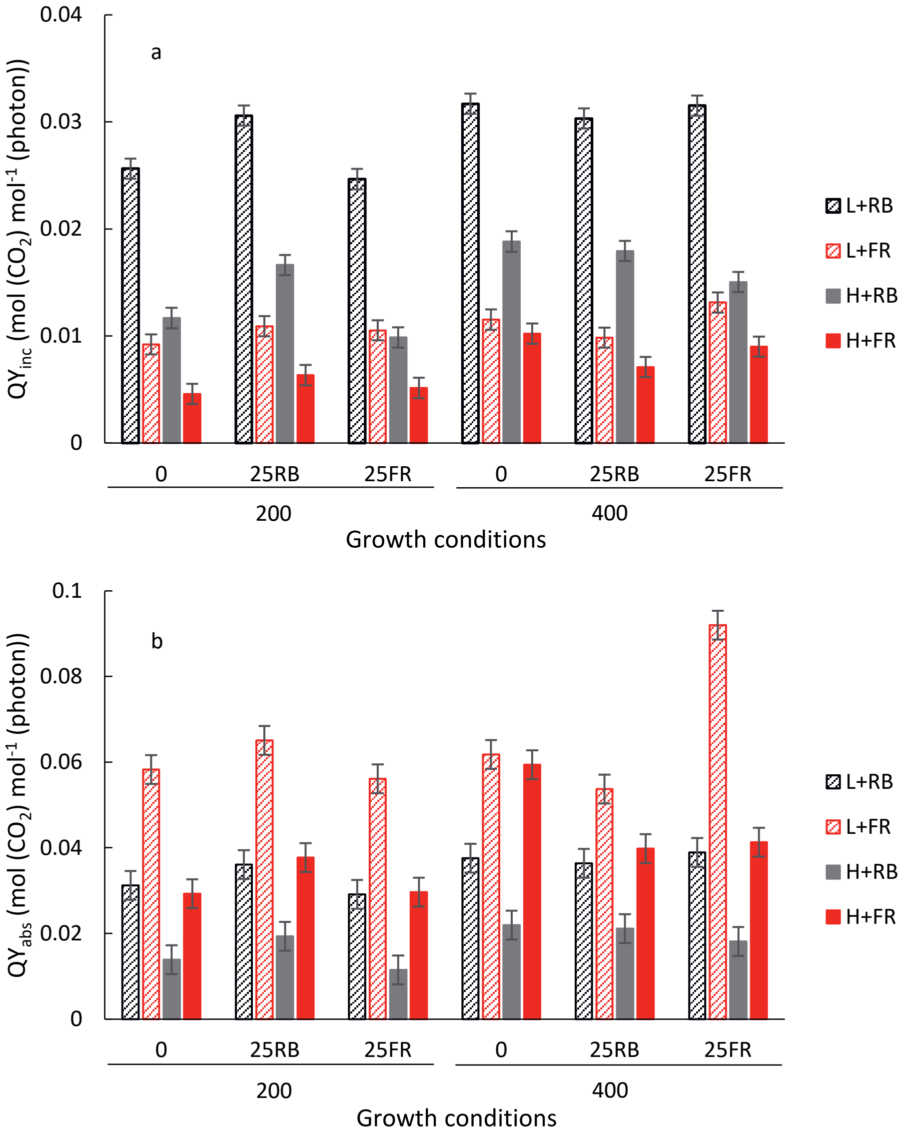

Figure 4-2. Quantum yield based on incident photon flux density (PFD: 400-800nm) (QY_{inc}; a) and absorbed PFD (QY_{abs}; b) based on response curves to RB or FR added to low (200 μmol m^{-2} s^{-1}) or high (400 μmol m^{-2} s^{-1}) RB intensities. Quantum yield is the slope of P_n measured with additional 0%, 12.5%, 25%, and 50% RB or FR over two background photon flux density levels (200 and 400 μmol m^{-2} s^{-1} RB). Abbreviations in legend represents: L indicates the background PPFD is 200 μmol m^{-2} s^{-1}, and H is 400 μmol m^{-2} s^{-1}, with additional RB (+RB) and FR (+FR). Error bars show the standard error of means from four factorial analysis.

Net photosynthesis rate measurements with additional FR and RB

Adding RB and FR, by 12.5%, 25% and 50% of the background PPFD, both increased instantaneous net photosynthesis rate (P_n) (Fig. 4-3). However, the increment by adding additional FR is less than adding additional RB at 25% and 50% additional level. At 200 μmol m^{-2} s^{-1} PPFD, the increase in P_n due to adding 12.5%, 25% and 50% FR were 58%, 48% and 39% of the increase due to adding 12.5%, 25% and 50% RB, respectively, while at 400 μmol m^{-2} s^{-1} PPFD, this increase in P_n by adding 12.5%, 25% and 50% FR were 64%, 54% and 45% of the increase due to adding 12.5%, 25% and 50% RB.

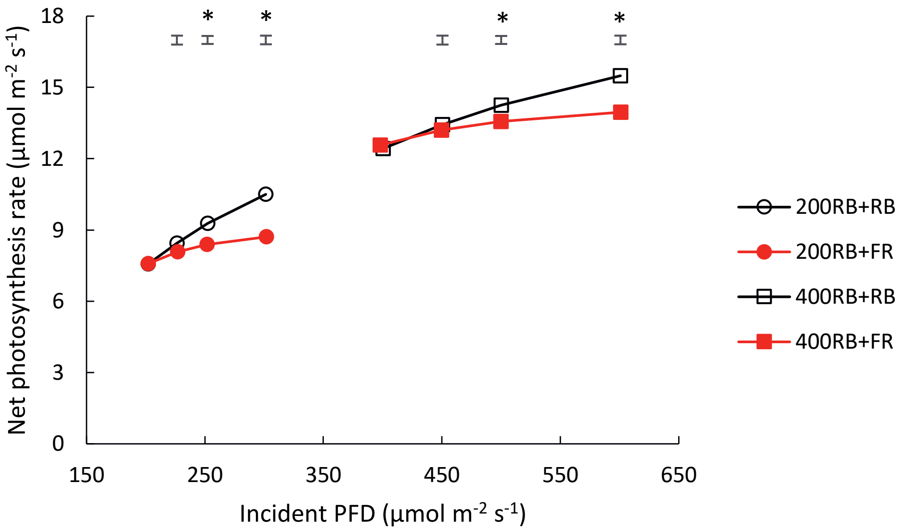

Figure 4-3. Light response curve of photosynthesis (PFD: 400-800nm). Hollow black circles and squares represent PAR without FR, filled red circles and squares represent a mixture of background PAR (either 200 or 400 μmol m^{-2} s^{-1}, namely 200RB and 400RB, respectively) with additional RB (namely +RB) and FR (namely +FR). Each point represents the average value from all six grow light treatments. Bars on top represent least significant differences of means (5% level).

Lettuce biomass, leaf area, and specific leaf area

Both additional FR (adding 50 μmol m^{-2} s^{-1} FR to 200 μmol m^{-2} s^{-1} RB or adding 100 μmol m^{-2} s^{-1} FR to 400 μmol m^{-2} s^{-1}RB)) and partial PAR substitution by FR (replacing 50 μmol m^{-2} s^{-1} out of 250 µmol m^{-2} s^{-1} RB or replacing 100 µmol m^{-2} s^{-1} out of 400 µmol m^{-2} s^{-1} RB) increased lettuce shoot fresh weight and dry weight and leaf area, but not specific leaf area (Fig. 4-4a-d). Fresh and dry weight of the plant increased with increasing PPFD (range 200-500 µmol m^{-2} s^{-1}), although there was no significant difference between 200 and 250 µmol m^{-2} s^{-1} (Fig. 4-4a-b). Leaf area was not significantly affected by PFD when there was no FR but increased with increasing PFD if FR was increased due to additional FR (Fig. 4-4c). Specific leaf area (SLA) decreased with increasing PFD but was higher when there was FR in the spectrum (Fig. 4-4d).

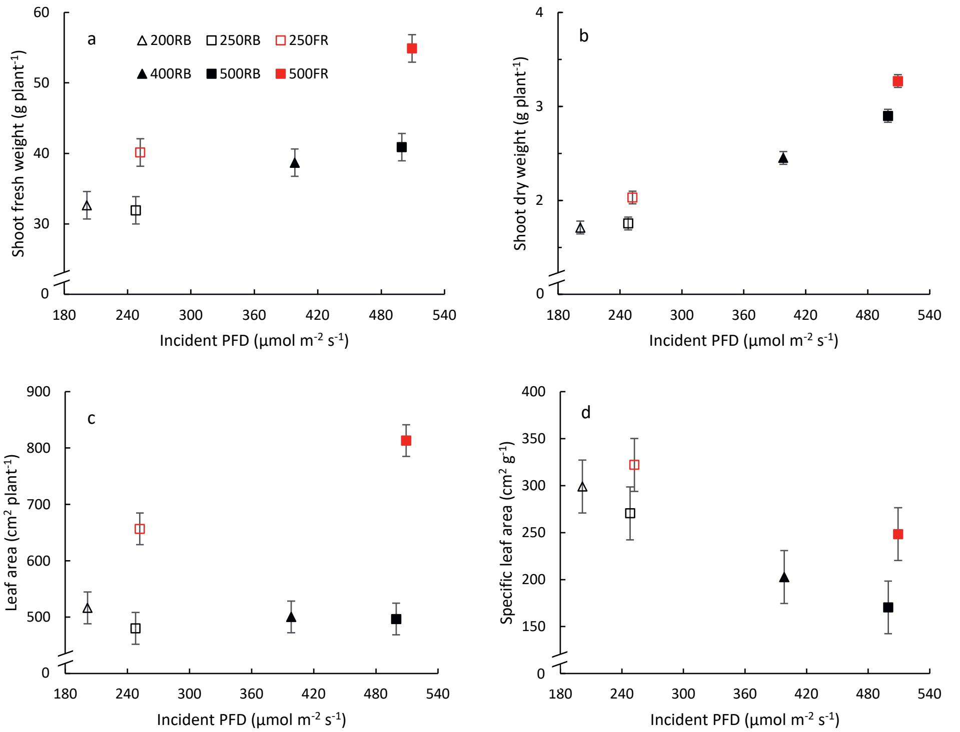

Figure 4-4. Lettuce shoot fresh weight (a), shoot dry weight (b), leaf area (c), and specific leaf area (d) at harvest (28 DAT). PFD is photon flux density (400-800 nm). Symbols show means ± standard error of means. Black symbols refer to plants grown under RB light without FR, while red symbols refer to plants grown under RB light with 25% additional FR. The two-way ANOVA was done based on three blocks (n=3), and eight plants per block.

Radiation and light use efficiency

Light use efficiency (shoot dry weight per unit of incident, $LUE_{inc,}$ or intercepted PPFD, LUE_{int}) and radiation use efficiency (RUE, shoot dry weight per unit of PFD) decreased with increasing PFD, when PFD was increased by increasing the PPFD. Additional FR increased both LUE_{inc} and LUE_{int} but did not significantly affect the RUE (comparing 250FR with 200RB and comparing 500FR with 400RB). Substituting PAR partly by FR increased LUE_{inc}, LUE_{int} and RUE (comparing 250FR with 250 RB and comparing 500RF with 500 RB).

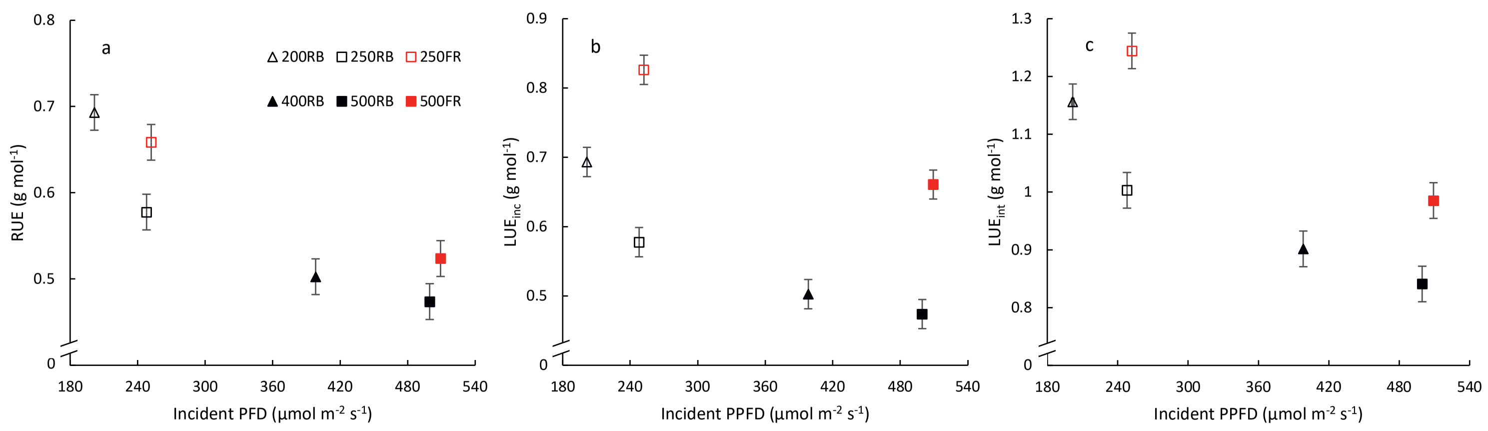

Figure 4-5. Radiation use efficiency (RUE: shoot dry weight per cumulative incident PFD (400-800 nm)) based on incident photon flux density (PFD) integral during growth (RUE, a). Light use efficiency based on cumulative incident photosynthetic photon flux density (PPFD) during growth (LUE_{inc}: shoot dry weight per cumulative incident PPFD (400-700nm), b) and based on cumulatively intercepted PPFD (LUE_{int}: shoot dry weight per cumulative intercepted PPFD (400-700nm), c). Black symbols refer to plants grown under RB light without FR, while red symbols refer to plants grown under RB light with 25% additional FR. Symbols show means ± standard error of means.

4.4 Discussion

Applying far-red (FR) over photosynthetically active radiation (PAR) improving light interception and biomass production (Kalaitzoglou et al., 2019; Jin et al., 2021; Legendre and van Iersel, 2021), and enhances photochemistry efficiency (Emerson and Lewis, 1943; Zhen and van Iersel, 2017; Zhen and Bugbee, 2020). In the present study, FR effects on biomass production and light use efficiency were explored, and whether FR is identically efficient in improving net photosynthesis rate as PAR and whether this depends on acclimation of plants to FR.

FR increased photosynthesis rate less strongly than RB

Adding FR over PAR can increase net photosynthesis rate (P_n) per leaf area as reported by (Zhen and van Iersel, 2017; and Zou et al., 2019). Present research found similar effects that additional FR increases P_n at 200 and 400 μmol m^{-2} s^{-1} background PPFDs. (Fig. 4-3). However, the P_n increments by additional FR reduced as FR intensity increased. This is in line with Zhen and van Iersel (2017), who applied six FR photon fluxes from 0 to 90 μmol m^{-2} s^{-1} over 200 μmol m^{-2} s^{-1} RB on lettuce leaf and found P_n increased asymptotically with increasing FR photon flux. Since adding FR is to rebalance the excitation levels of PSI and PSII, the P_n incremental effect by additional FR is associated with the PAR level and get saturated when FR starts to overexcite PSII. Adding RB increased P_n in a linear tendency (Fig. 4-3). Therefore, 12.5% additional FR and RB increase P_n similarly at each basic PPFD level. While adding relative higher fractions, namely 25% and 50%, additional FR increases P_n less strongly than RB. This is in agreement with Zou et al. (2019) that the 7-10% increment of P_n by adding 50 μmol m^{-2} s^{-1} FR on top of 200 μmol m^{-2} s^{-1} RB is not proportional to the additional FR intensity (~25%).

Zhen and Bugbee (2020) explored the Emerson enhancement effect on greenhouse grown lettuce at canopy level in either low light (200-400 μmol m^{-2} s^{-1}) and high light (400-600 μmol m^{-2} s^{-1}) and at leaf level with 400-460 μmol m^{-2} s^{-1} including PAR and FR. For canopy level, additional FR was added in both low and high light circumstances with 10%-35% of the background light and results demonstrated similar increment of P_{gross} (defined as the sum of net photosynthesis rate and dark respiration rate) over additional white (PAR) light, for the lettuce with different leaf area index. For leaf level, approximate 15% additional FR (~60 μmol m^{-2} s^{-1}) was added on 400 μmol m^{-2} s^{-1} white (PAR) background light and comparable net photosynthesis rate increase was observed by adding white (PAR) light in the same intensity. Present study had alike result of the leaf level observation, as 12.5% and 15% additional FR are comparable and both results were given in the manner of P_n. However, present study concluded differently with the observation of Zhen and Bugbee (2020) for the canopy level and their further proposition regarding FR waveband within 700-750 nm has identical effect on improving P_n as PAR.

The FR spectrums applied in present and Zhen and Bugbee (2020) studies had same peak at 735 nm. FR above 752 nm has no effect on enhancing photochemical efficiency due to the low photon energy and absorption to use by PSI (Wah Soon Chow et al., 1990; Zhen et al., 2018). The waveband of FR applied within present study consisted 7% in the range of 750-800 nm of total FR (comparison of FR applied in Zhen and Bugbee (2020) and present research is given in Fig. S4-4 (Supplementary S4-4)). Hence, the spectrum of FR cannot be the explanation of P_n differences between additional RB and FR, e.g. a 21% lowered P_n by adding ~100 μmol m^{-2} s^{-1} FR than adding RB with same intensity over 200 μmol m^{-2} s^{-1} RB. In addition, to eliminate any possible FR effects on g_s (or any other factor influencing rate of CO_2 diffusion into the leaf), the FR vs. RB light response curves were measured under 1200 ppm CO_2 concentration. Therefore, whether FR should be considered as PAR remains discussion, but there is an agreement on the synergistic effect between RB and FR restoring the electron transport efficiency to maximize the CO_2 assimilation rate up to some extent.

The acclimation of FR did not improve the short-term photosynthesis efficiency

Leaves tune their photosynthesis system to the spectrum of their growth environment. Leaves acclimated to FR were reported to have decreased photosynthetic capacity and CO_2 fixation efficiency per leaf area on tomato (Ji et al., 2019; Kalaitzoglou et al., 2019), cucumber (Hogewoning et al., 2012), and lettuce (Zou et al., 2019; Kong and Nemali, 2021), when being measured under R and B light source, which was confirmed in our research (Fig. 4-1a-f). Zou et al. (2019) proposed the downregulation of photosynthetic apparatuses, like Amax and α, were due to the lowered stomatal conductance (g_s) under FR enrich growing light. Further explanations can be deduced from present results (Fig. 4-1a-c) as Vcmax, J and TPU are independent from g_s. Zhen and Bugbee (2020) suggested the stomatal response to FR is most likely a response to increase in photosynthetic rate, instead of FR effect. The other reason is postulated due to the lowered chlorophyll content. Chlorophyll content is positively correlated with leaf light absorption (Evans and Poorter, 2001). FR declined leaf chlorophyll concentration and chlorophyll a:b ratio, as abovementioned studies reported, which present research in line with (Fig. 4-1g-i). There are contradict effects, between Emerson Enhancement Effect and lowered photosynthetic capacity, on the FR-acclimated leaf for the photosynthesis efficiency per leaf area.

Quantum yield was defined as the slope of Pn and either incident (QY_{inc}) or absorbed photon flux density (PFD: 400-800nm; QY_{abs}). It is a relative more comprehensive metric to understand short-term photosynthesis efficiency than individual Pn measurement at any PFD level. Leaves grown under different conditions demonstrated similar QY_{inc} at the same measuring condition (Fig. 4-2a). While per measuring condition, adding RB had clearly higher QY_{inc} than adding FR (Fig. 4-2a), regardless the growth conditions. However, an opposite effect was observed that adding FR had clearly higher QY_{abs} than adding RB (Fig. 4-2b). The absorption rate of RB at the applied spectrum (Supplementary file: Fig. S4-2) among all treatments is ~85% and ~20% for FR (Supplementary file: Fig. S4-3). The improved effect of absorbed FR suggested the different mechanism between PAR and FR photons for photosynthesis enhancement: the PAR photon increased the number of photosynthetic photons but the FR photon improves the photochemical efficiency (Zhen and Bugbee, 2020).

FR promoted biomass production, but did not lead to the highest LUE in all circumstances

Several researchers studied the effect of FR on lettuce grown in vertical farming conditions with constant radiation level, temperature, relative humidity and CO_2 concentration (Meng and Runkle, 2019; Zou et al., 2019, 2021; Zhen, 2020; Jin et al., 2021; Legendre and van Iersel, 2021). The added FR on top of PAR promoted leaf expansion, which benefits the plant intercepting higher fraction of incident light, which is in line with our current findings (Fig. 4-2b-c). Moreover, the light use efficiency based on incident radiation was increased by FR, even with end-of-day FR added only (Meng and Runkle, 2019; Zou et al., 2019; Legendre and van Iersel, 2021), which is also in line with our current findings (Fig. 4-5b). In addition FR increased light use efficiency based on intercepted PPFD (i.e. shoot dry mass divided by cumulative intercepted PPFD), which is in agreement with Jin et al. (2021). In addition, the efficiencies of light use, either on incident or intercepted PPFD basis, decreased with increasing of PPFD (Fig. 4-5b-c).

While Legendre and van Iersel (2021) found that the radiation use efficiency (RUE), defined as the shoot dry weight per incident photon flux density including RB and FR, was higher when FR was added to PAR light and it is in agreement with present research that RUE was improved by FR substitution (Fig. 4-5a). However, there was no significant effect on RUE by additional FR (Fig. 4-5a). Jin et al. (2021) quantified the effect of additional FR on RUE at three planting densities (23, 37 and 51 plant m^{-2}). They found that the highest increment of RUE due to adding FR, occurred at the lowest planting density (RUE + 42%), and the lowest increase in RUE (+17%) at the highest planting density. This could be explained by the fact that the benefits of the FR-enhanced light interception for crop photosynthesis are smaller at high plant density, because at high plant density the light interception even in the absence of FR is high. Considering the findings of Jin et al. (2021) that effects of FR on RUE gets smaller at higher plant density, it may be not so surprising that no effect was found in our present study as we used a high plant density of 146 plants per m^2. This high plant density was chosen in order that we could focus on effects of FR on photosynthesis rather than light interception. In addition, extra RB increased the net photosynthesis rate higher than the identical intensity of FR (Fig. 4-3). Photochemistry efficiency was increased by additional RB more than FR as well (Fig. 4-2a-b). The FR treatments within each level of background PPFD did not have the highest RUE. The biomass production improvement by additional FR drops while the planting density increases. In the high planting density, FR promoted a lower fraction of biomass increment than the fraction of added photon flux.

4.5. Conclusions

Adding far-red light (FR) on top of red and blue light (RB) increased net photosynthesis rate. However, additional RB increased net photosynthesis rate 34-158% more than additional FR with same intensity on top of 200-400 μmol m^{-2} s^{-1} RB. At 200 μmol m^{-2} s^{-1} PPFD, the increase in P_n due to adding 50% FR was 39% of the increase due to adding 50% RB, while at 400 μmol m^{-2} s^{-1} PPFD, this increase in P_n by adding 50% FR was 45% of the increase due to adding 50% RB. Results suggested FR is not identically efficient on improving net photosynthesis rate as RB. Though plants acclimated to FR given during the growth by lowering some photosynthesis apparatus like chlorophyll a and b content and light-saturated photosynthesis rate, the FR acclimation did not improve the quantum yield for the plants measured by additional RB or FR, either grown in low or high background PPFD levels. Both substituted and additional FR on top of RB increased lettuce biomass production and light use efficiencies based on incident and intercepted PPFD. Radiation use efficiency was increased by substituted FR but not additional FR (the latter being explained by an overall decline in RUE with increasing PFD).

Supplementary

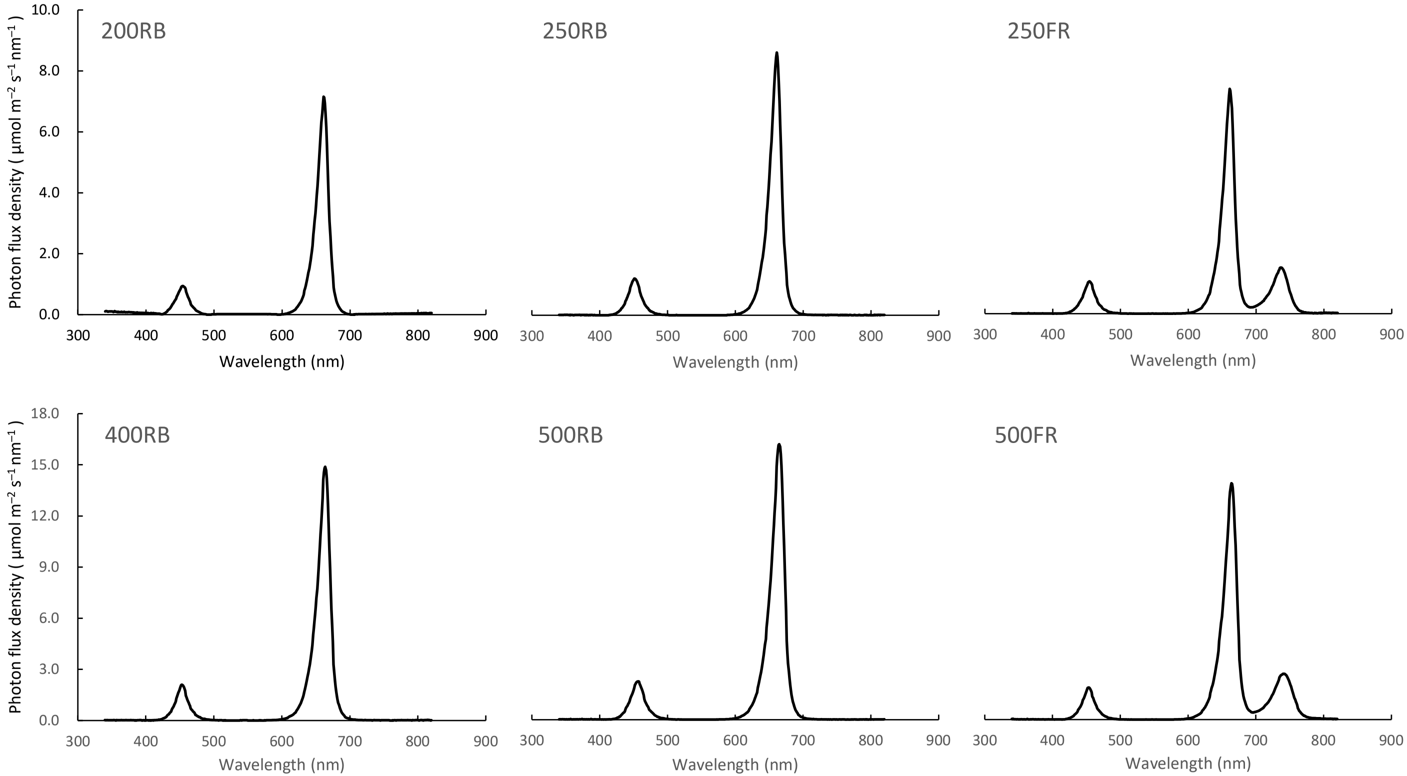

Figure S4-1. Spectral distribution of lamps in all treatments. The spectra were measured at canopy level, photon flux density at every wavelength corresponds to the average of 18 locations per treatment.

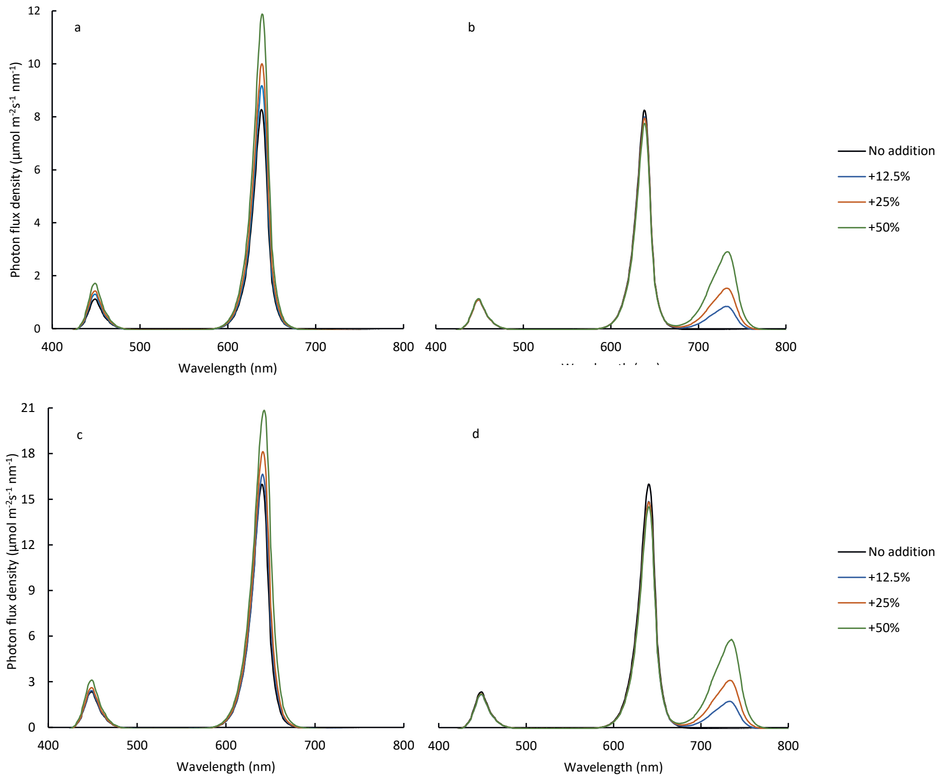

Figure S4-2. Spectral distribution of all measuring light. (a) was the spectra distribution of adding 4 levels of RB light to 200RB background light; (b) was the spectra distribution of adding 4 levels FR light to 200RB background light; (c) was the spectra distribution of adding 4 levels of RB light to 400RB background light; (d) was the spectra distribution of adding 4 levels of FR light to 400RB background light. The photon flux density is the average of 2 spectral distribution of cycle 2 and cycle 3. Legend number was the intensity of adding light (µmol m-2s-1). The peak of Red, Blue located at 448nm and 638nm in all figures, the peak of FR light located at 734nm in figure (c) and (d).

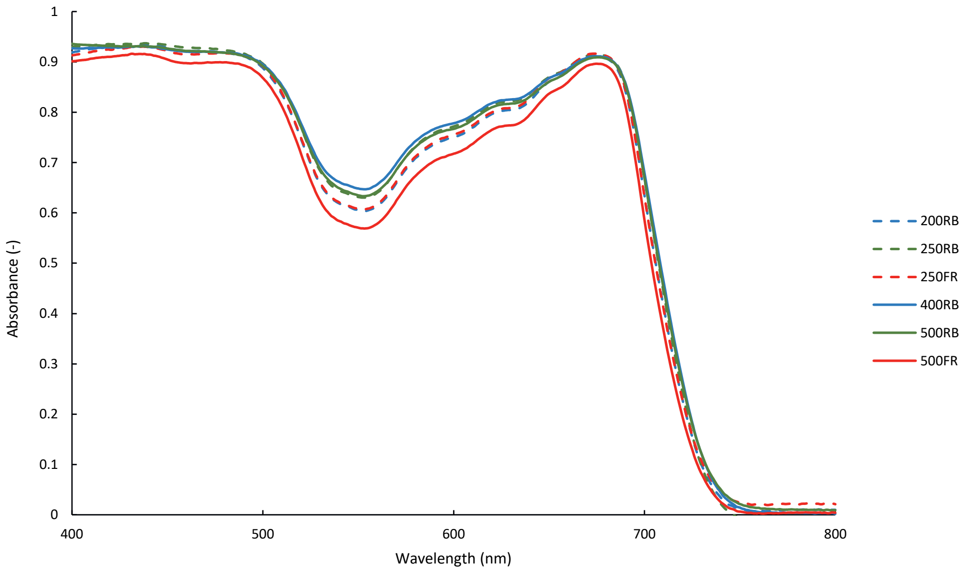

Figure S4-3. The effect of 6 growing light on leaf light absorptance. Data were the means of two blocks (n=2) both with three-five replicate plants.

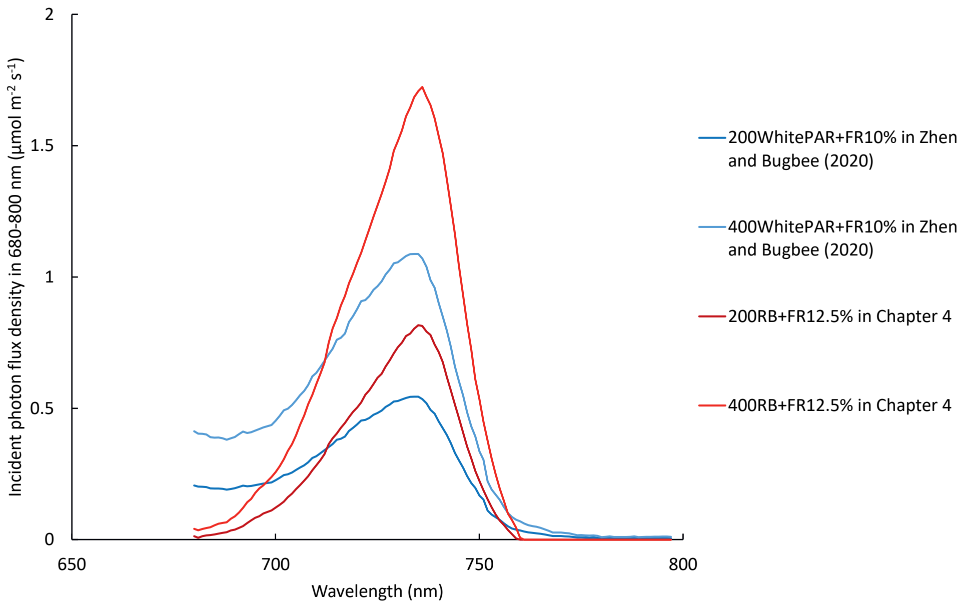

Figure S4-4. The applied far-red (FR) comparison in waveband, radiation peak and intensity between Zhen and Bugbee (2020) and present Chapter.

Chapter 5

Gradually increasing light intensity during the growth period increases dry weight production compared to constant or gradually decreasing light intensity in lettuce

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(submitted)

Abstract

The objective of this research was to investigate the effects of gradually increasing or decreasing photosynthetic photon flux density (PPFD) during cultivation compared to a constant PPFD on biomass production. Lettuce plants (*Lactuca sativa* L. 'Expertise') were grown in climate rooms in which every three days the PPFD was increased by 16 μmol m^{-2} s^{-1} (from 140 to 300 μmol m^{-2} s^{-1} from day 0 to 30), decreased (from 300 to 140 μmol m^{-2} s^{-1}), or kept constant (221 μmol m^{-2} s^{-1}), while the total light integral at the end of the cultivation period (30 d) was the same for all three treatments. Gradually increasing PPFD resulted in a 16 or 12% increase in total plant dry weight compared to treatments with decreasing or constant PPFD, respectively. This increase was explained by a higher light interception, because most of the light was provided when leaf area index was high and because of a higher specific leaf area in the early crop phase. Although light use efficiency (LUE) based on incident PPFD was highest when PPFD gradually increased, the LUE based on intercepted PPFD was highest when PPFD gradually decreased during cultivation. Despite the higher shoot dry weight when PPFD gradually increased, shoot fresh weight was not significantly affected by the light treatments. This difference in response between fresh and dry weight resulted from a higher shoot dry matter content when PPFD gradually increased. Our results show that gradually increasing PPFD had a positive effect on dry weight accumulation, increased dry matter content but did not affect shelf life.

Key words: vertical farming, dynamic light intensity, lettuce, light use efficiency, shelf-life

5.1 Introduction

Vertical farming is a relatively new food production system where crops are cultivated in stacked layers above each other and all the growing light is provided by lamps (Kozai and Niu, 2019; SharathKumar et al., 2020; van Delden et al., 2021b). It is a closed system by which all environmental factors, including temperature, relative humidity, and CO_2 concentration, can be well-controlled and the resources, such as water and nutrients can be fully recycled. Vertical farming scores high in water, land and nutrient use efficiency while use of pesticides can be avoided (Van Delden et al., 2021). However, the high energy consumption is a bottleneck for further development of vertical farming. The lighting system takes a significant amount of the total energy consumption (Van Delden et al., 2021). Thus, it is urgent to improve light use efficiency, i.e. the amount of fresh produce produced per unit of incident light.

Compared to a number of other horticultural crops such as tomato and strawberry, lettuce requires less space and a shorter cultivating period. Furthermore, lettuce has a higher harvest index (Pattison et al., 2018). These factors may explain why lettuce is at present the most grown crop in vertical farms. Light intensities used for lettuce growth in research and commercial production, are largely within 150 to 250 μmol m^{-2} s^{-1} range, and up to 700 μmol m^{-2} s^{-1} in some extreme experimental cases (Zou et al., 2019; Carotti et al., 2021; Jin et al., 2021). The photosynthetic photon flux density (PPFD, 400-700nm) is usually kept constant throughout the cultivation period from transplanting until harvest. However, keeping the incident PPFD constant might mean that the intensity is too high in the beginning and too low shortly before harvest. In the early growing period, when the leaf area index is still small, a large fraction of the light falls on the floor instead of being absorbed by the plants. This can lead to a relatively low light use efficiency based on incident light. Once the canopy is closed, the plants can intercept and utilize a larger fraction of the incident light. Therefore, a PPFD gradually increasing during the cultivation period may lead to a higher biomass production and light use efficiency. On the other hand, providing a high PPFD during early growth may promote crop growth by a faster leaf area expansion, resulting in a strong increase in plant growth as the crop grows exponentially in this period (Goudriaan and Monteith, 1990). Increasing the light interception in early days will benefit the canopy light interception afterwards and possibly increase the final biomass production. So, there are two contrasting effects that determine the final effect of a gradual change in light intensity during the cultivation period of the crop on biomass production and light use efficiency.

In addition to the effect on biomass production, a high PPFD during growth has also been shown to have a positive effect on shelf-life and visual quality of leafy greens such as lettuce (Woltering and Witkowska, 2016; Min et al., 2021). In particular a high PPFD at the end of the cultivation substantially increased shelf life of lettuce (Min et al., 2021).

Although many studies have investigated effects of PPFD (e.g., Pennisi et al., 2020; Carotti et al., 2021), effects of gradually changing the PPFD during the cultivation cycle, while keeping total light sum constant have hardly been studied. The objective of this research was to investigate the effects of gradually increasing or decreasing PPFD during cultivation compared to constant average PPFD on biomass production. We hypothesised that gradually increasing PPFD will maximize the light interception, produce more total dry weight and improve light use efficiency. An experiment was conducted with lettuce in climate rooms in which every three days the daily light integral was increased, decreased, or kept constant during the cultivation period while the total light integral at the end of the cultivation was the same for all three treatments. All other growth conditions were kept constant and equal for all three treatments. Yield component analysis (Higashide and Heuvelink, 2009; Ji et al., 2019) was used to quantify contributions of underlying components of the effects of treatments on the biomass production. Also shelf life was determined.

5.2 Materials and methods

Plant material and growth conditions

Lettuce (*Lactuca sativa* L. cv. Expertise, Rijk Zwaan, the Netherlands) was grown in a climate room with 6 compartments divided by white plastic screens. Seeds were sown in stone wool plugs in 240-cell trays (Grodan, Roermond, the Netherlands). Germination procedure involved 2 days in dark followed by 5 days in light at 18h light/6h dark with a PPFD of 145 ± 1.3 μmol m^{-2} s^{-1} provided by red (R) and blue (B) LEDs (88% R and 12% B) (GreenPower LED research module, and GreenPower LED production module, 2nd generation, Philips, the Netherlands). Seven days after sowing, plugs with seedlings having 2 cotyledons were transplanted to individual stone wool blocks (7 cm x 7 cm x 7 cm, L x W x H, Grodan, Roermond, the Netherlands) and were grown for 30 days at a planting density of 51 plants m^{-2}. Plants were distributed equidistantly following a chess board pattern. Plants in the outer rows in each plot were considered as border plants and not used for measurements. After each destructive harvest plants were relocated to keep the original planting density.

The stone wool blocks were always in 1.0-1.5 cm layer of nutrient solution. Nutrient solution (electrical conductivity (EC) 2.3 dS·m^{-1} and pH 5.8), containing 0.38 mM NH_4^+, 8.82 mM K^+, 4.22 mM Ca^{2+}, 1.15 mM Mg^{2+}, 12.92 mM NO_3^-, 1.53 mM Cl^-, 1.53 mM SO_4^{2-}, 0.12 mM HCO_3^-, 1.53 mM $H_2PO_4^-$, 0.38 mM SiO_3^{2-}, 30.67 µM Fe^{3+}, 3.83 µM Mn^{2+}, 3.83 µM Zn^{2+}, 38.33 µM B, 0.77 µM Cu^{2+} and 0.38 µM Mo, was applied from the second day after transplanting. Nutrient solution was completely renewed twice a week to keep EC, composition, and pH stable. Average temperature, and relative humidity (RH) was 22.0±0.0ºC and 74.6±0.3% during the photoperiod and 19.9±0.0°C and 79.5±0.2% during the dark period, respectively. CO2 concentration was kept at 752±12.1ppm.

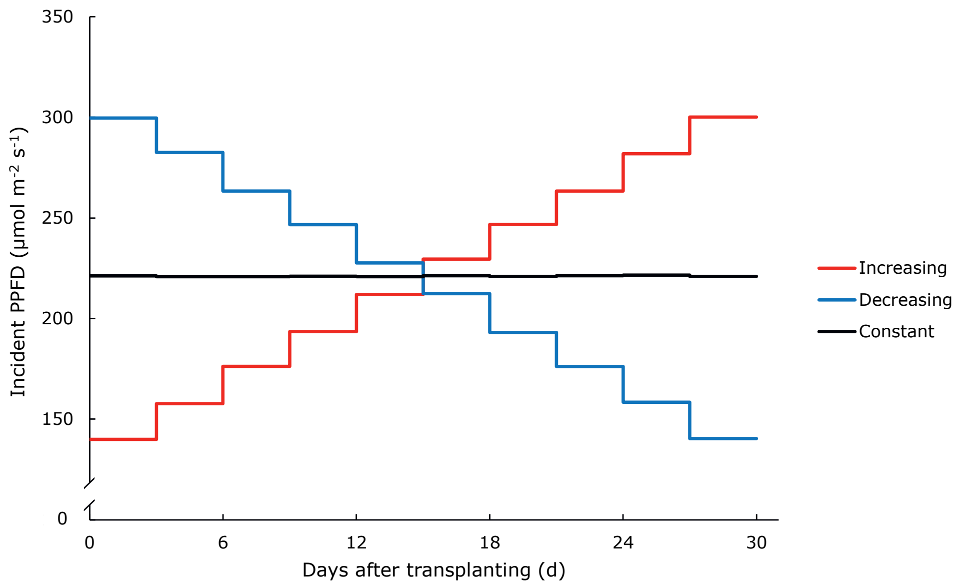

Figure 1. time course of the incident photosynthetic photon flux density (PPFD: 400-700nm) during the growth period for treatments with constant, increasing, and decreasing light intensities. In all treatments the intensity averaged over the whole cultivation period was the same.

Light treatments

During the 30 days growth period, the incident light contained R (600-700nm), B (400-500nm) and Far-Red (FR: 700-800nm). For all treatment levels and repetitions, the R and B intensity ratio was kept at 7.4±0.0 and R and FR intensity ratio was kept 5.2±0.1

(GreenPower LED research module, and GreenPower LED production module, 2nd generation, Philips, the Netherlands). Light treatments started at the day of transplanting. Three treatments (increasing, decreasing and constant PPFD) were applied through the 30 days cultivation period. The Increasing (I) and Decreasing (D) treatments were realized by step wise increasing and decreasing incident PPFD by 16 μmol m^{-2} s^{-1} every 3 days (Fig. 5-1). Incident PPFD at canopy level was kept constant at 221 μmol m^{-2} s^{-1} throughout cultivation for the Constant (C) treatment (Fig. 5-1). Incident photosynthetically photon flux density (PPFD) integral at the end of the cultivation was 428±1.0, 428±0.5 and 430±1.8 mol m^{-2} for I, D and C treatments, respectively. Light measurements were performed at canopy height using a quantum sensor (LI-250A, LI-COR, Lincoln, United States) for PPFD and a spectroradiometer (SS-110, Apogee Instruments, Utah, United States) for spectrum. The average PPFD was determined at 24 locations per treatment in each repetition.

Measurement of growth parameters

Destructive measurements were conducted at 0, 6, 12, 18, 24, and 30 days after transplanting (DAT). Pictures from above the canopy were taken (iPhone 7, Apple, Cupertino, CA, USA) before destructive measurement for estimation of canopy projected leaf area at 12, 18, 24, and 30 DAT. Pictures were taken at a fixed position from the canopy and a ruler was put next to the plants. Canopy projected leaf area were extracted by program ImageJ (IOCI, University of Wisconsin-Madison, Madison, WI, USA). Leaf area was determined using a leaf area meter (LI-3100 Area Meter, Li-Cor, Lincoln, United States). Fresh and dry weight (forced air oven at 105°C for 24 h) of shoot and root were determined. As the stem of this cultivar was extremely small, leaf dry weight was considered to be equal to the shoot dry weight. From 18 DAT onwards the lettuce root dry weight was determined by oven drying of the stone wool before and after the experiment; the difference was estimated to be the root dry weight. At 0, 6, and 12 DAT, when the lettuce roots were relatively small, the root dry weight was considered to be 15% of total dry weight, being the average fraction determined at 18 DAT.

Floor coverage fraction was calculated based on plant projected leaf area with 51 plant m^{-2} planting density. Daily floor coverage fraction was calculated by linear interpolation between measurement days at 6, 12, 18, 24 and 30 DAT. Floor coverage fraction at 0 DAT was assumed to be zero while at 6 DAT it was calculated from leaf area as there was no mutual shading of leaves. Daily light interception was calculated as the product of incident PPFD at top of canopy and floor coverage fraction at that day.

Incident light use efficiency (LUE_{inc}) was calculated as the ratio between plant total dry weight and cumulative incident PAR (400-700 nm) at canopy level. Intercepted light use efficiency (LUE_{int}) was calculated as the ratio between plant total dry weight and cumulative intercepted PAR.

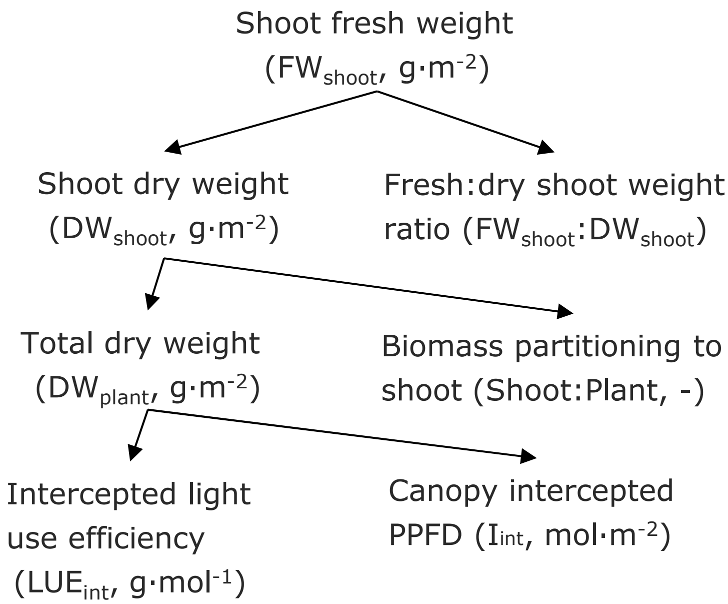

Figure 5-2. Shoot fresh weight separated into underlying components. Abbreviations and units are given in between brackets.

Yield component analysis

Treatment effects on fresh yield can be analysed by breaking down shoot fresh weight into underlying components (Fig. 5-2). In this analysis, shoot fresh weight (FW_{shoot}) is the product of shoot dry weight (DW_{shoot}) and the fresh:dry shoot weight ratio (FW_{shoot}:DW_{shoot}). Shoot dry weight is the product of total plant dry weight (DW_{plant}) and fraction of biomass partitioned to the shoot (DW_{shoot}/DW_{plant}). Intercepted PPFD (I_{int}), which is the cumulative PPFD interception during the whole cultivating period (0-30 DAT), multiplied by the dry weight production per unit intercepted PPFD (LUE_{int}) determines the total plant dry weight.

Overall visual quality and shelf life

For determining overall visual quality at the end of the cultivation stored as fresh cut lettuce at 12°C in darkness. Three lettuces were cut in squares of 2x2 cm and stored in two plastic boxes (18 L × 13 W × 6.5 H cm) per treatment per repetition. Two pieces of wet filter paper were placed underneath to keep the moisture in the box. In the lids nine holes were made with a 1 mm syringe needle to avoid a built up of CO_2. The scoring of overall visual quality was carried out according to Min *et al.* (2021). In brief, the evaluation of the overall visual quality was done by three assessors at room temperature. At each time point for the two boxes from each treatment and each repetition were taken out of storage and scored from 1-9, (i.e. 9 being the best and 1 the worst). The fresh cut lettuce was evaluated based on parameters such as colour change (yellowing, browning, and pinking), crispness and overall decay. The acceptance limit was set at 6, when the score fell below the limit the box had reached end of shelf life. The boxes were scored on day 0, 5, 10, and 15 after harvest.

Statistical setup and analysis

A randomized complete block design was applied. The experiment was repeated three times (blocks) and each time contained two repetitions representing three blocks and a total of six repetitions (n=6). There were three sampling plants per treatment per repetition for each destructive measurement. At DAT 6, no destructive harvest was done for the 3rd block (repetition 5 and 6) and nine plants were sampled at final harvest of this 3rd block. Overall visual quality and shelf life tests were done at 1st and 2nd blocks (1st to 4th repetitions). For each block, a new randomization of the light treatments positions was done in the climate chamber. Analysis of variance was used to determine treatment effects using Genstat software (18th edition, United Kingdom). Treatment effects were tested at $\alpha=0.05$ and assumptions for normality and homogeneity of residuals were tested with Bartlett's test and Shapiro-Wilk test, respectively and all accepted ($P > 0.05$). Mean separation was done with Fisher´s Protected LSD test ($P = 0.05$).

5.3 Results

Biomass production, leaf area, and dry matter content

During each 6-day period the rate of shoot dry weight growth increased with increasing PPFD of that specific period (Fig. 5-3a). Similarly, the rate of shoot fresh weight growth increased with increasing PPFD during the initial period of the cultivation cycle (Fig. 5-3c, inserted panel). However, during the last two periods of the cultivation cycle the rate of fresh weight growth was hardly affected by the PPFD. Growth rates during the final periods were much larger than during the initial periods. Therefore, effects of last periods on final shoot weight were largest. Consequently, whether PPFD decreased, increased or was constant during the cultivation had no substantial effect on the final fresh weight at the end of the cultivation cycle (Fig. 5-3d). However, final shoot dry weight was 16% and 12% higher when PPFD increased during cultivation, compared to decreasing and constant light treatments, respectively (Fig. 5-3b), and the final plant dry weight (shoot+root) was 19% and 13% higher than decreasing and constant light treatments, respectively.

A higher PPFD promoted leaf area expansion rate during the initial periods (Fig. 5-3e, inserted panel), even though the differences were limited. However, an effect of PPFD on leaf area expansion rate was not observed during the final period. Cumulatively, plants in decreasing light treatment had the highest leaf area during the first part of the growth cycle; at the end of the experiment leaf area seemed to be 9-12 % higher than in the other two treatments, but this difference was not statistically significant anymore (Fig. 5-3f). The specific leaf area (SLA) decreased with an increase in PPFD of the 6-days period preceding the measurements (Fig. 5-4a). At the end of the experiment the decreasing light treatment had 46% larger specific leaf area (SLA) than increasing treatment. The dry matter content (DMC) decreased during cultivation while at each sampling point it increased with an increase in PPFD of the 6 days period preceding the measurements, except for the first sampling point at day 12 (Fig. 5-4b).

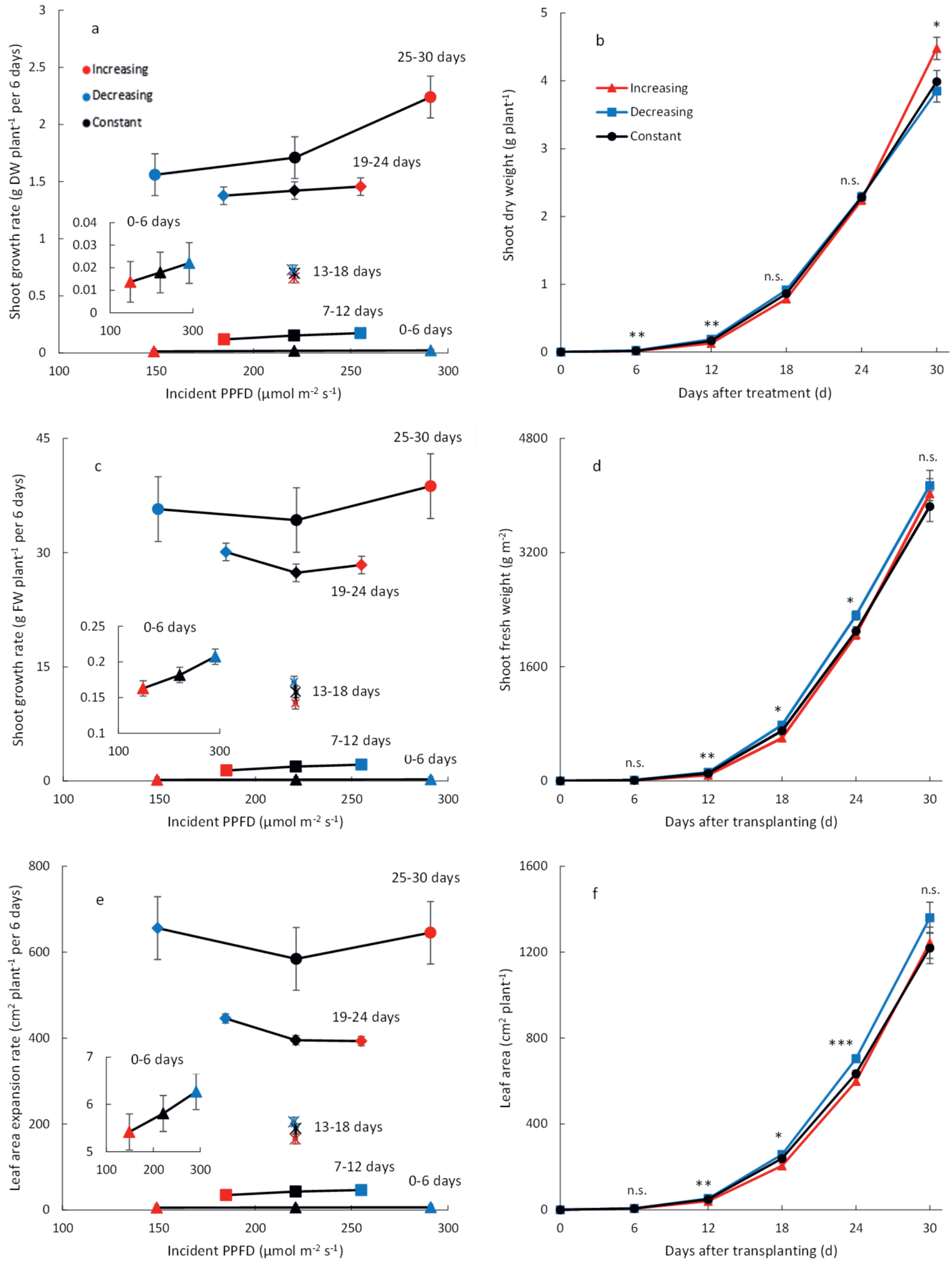

Figure 5-3. The effects of constant PPFD versus gradually increasing or decreasing PPFD throughout the cultivation period on dry (a) and fresh (c) shoot growth rate, and leaf area expansion rate (e) for each 6-days sampling interval and the cumulative shoot dry weight (b), shoot fresh weight (d), and leaf area (f) at different sampling point. Data are the means of four (DAT 6; n=4) or six repetitions (n=6). Error bars at each point represent standard error

*of means. * indicates significant effect of light treatment at *P=0.05, **P=0.01 and ***P=0.001. n.s. stands for not significant. Inset shows an enlargement of the first interval.*

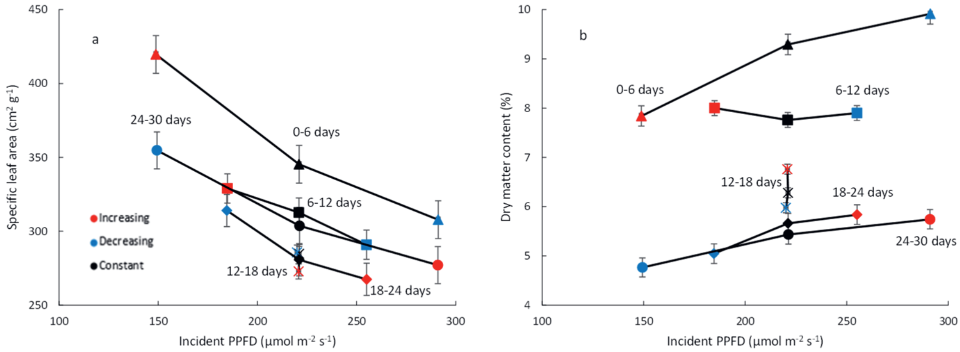

Figure 5-4. The effects of constant PPFD versus gradually increasing or decreasing PPFD throughout the cultivation period on specific leaf area (a) and dry matter content (b) for each 6-days sampling interval. Data are the means of four (DAT 6; n=4) or six repetitions (n=6). Error bars at each point represent standard error of means.

Floor coverage fraction, cumulative PPFD interception, and light use efficiency based on incident and intercepted PPFD

Even though there were differences in leaf area at each sampling point (Fig. 5-3f), there were only small differences in floor coverage fraction among treatments during growth (Fig. 5-5a). Assuming proportionality between floor coverage fraction and fraction light intercepted, the fraction of light intercepted differed only slightly among treatments for each specific date. Therefore, the increasing light treatment intercepted more PPFD than constant and decreasing light treatments (Fig. 5-5b), as for this light treatment a higher incident PPFD coincided with the cultivation period with high floor coverage. Correspondingly, intercepted PPFD was lowest for the decreasing light treatment, as for this treatment a low incident PPFD coincided with the period with high floor coverage.

Decreasing light treatment resulted in the highest light use efficiency (LUE) based on intercepted PPFD (ratio plant dry weight:cumulative intercepted PPFD) being 1.23 mol g^{-1}, which was 11.2% higher compared to the increasing light treatment and 10.6% higher compared to the constant light treatment (Fig. 5-6A). However, as the increasing light treatment intercepted most light (Fig. 5-5b), it had the highest LUE based on incident

PPFD (ratio plant dry weight:cumulative incident PPFD). LUE based on incident PPFD was 0.62 mol g^{-1} for the increasing light treatment, which was 19% higher compared to the decreasing light treatment and 13% higher compared to the constant light treatment (Fig. 5-6B).

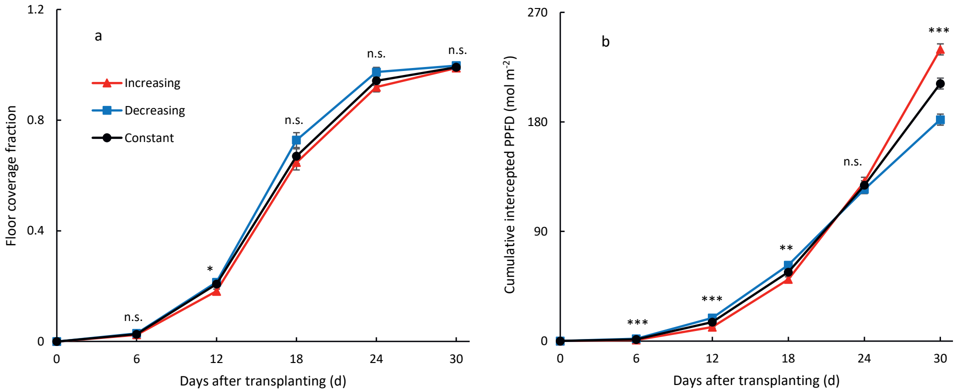

*Figure 5-5. Time course of floor coverage fraction (a) and cumulative intercepted PPFD (b) at different sampling points. Data are the means of four (DAT 6; n=4) or six repetitions (n=6). Error bars at e112,ach point represent standard error of means. * indicates significant effect of light treatment at *P=0.05, **P=0.01 and ***P=0.001. n.s. stands for not significant.*

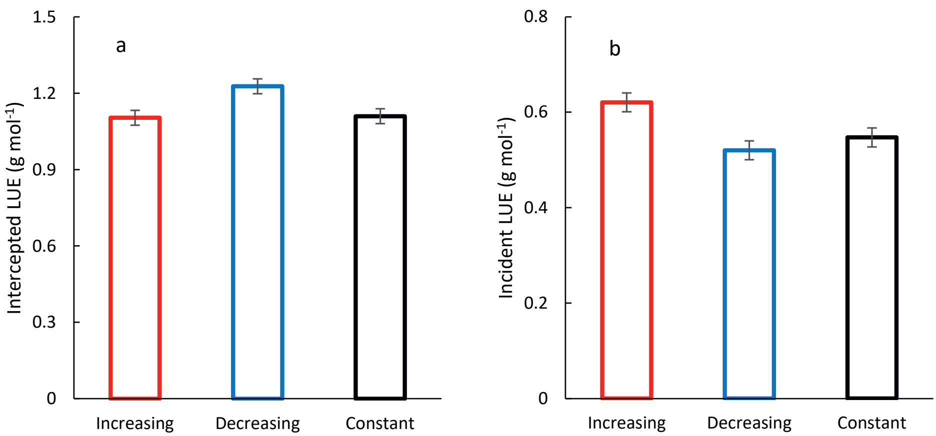

Figure 5-6. (A) Intercepted light use efficiency (LUE_{in}, ratio plant dry weight: cumulative intercepted PPFD), and (B) incident light use efficiency (LUE_{inc}, ratio plant dry weight: cumulative incident PPFD) of lettuce plants grown at increasing, decreasing or constant PPFD during 30 days of cultivation. Data are the means of six repetitions (n=6). Error bars indicate standard errors of means. Different letters indicate significant differences between treatments (P<0.01)

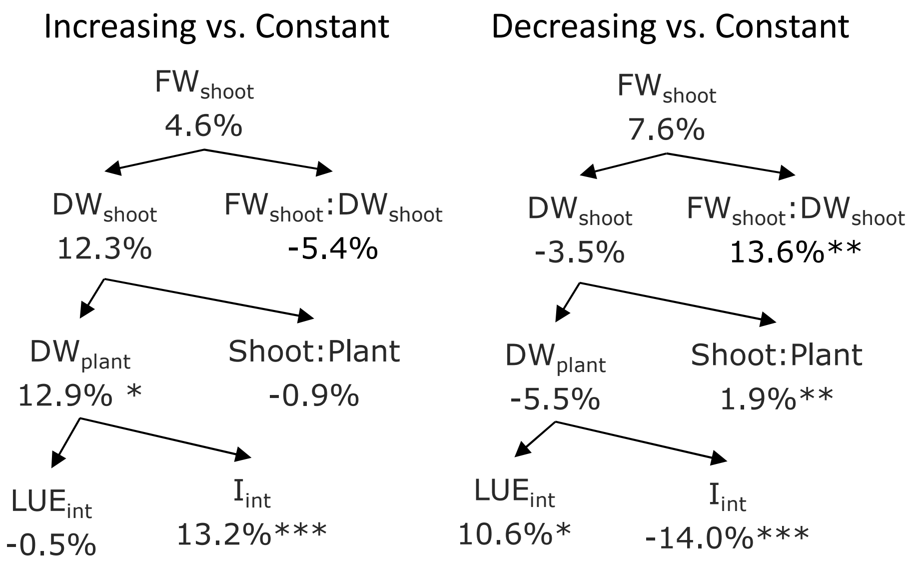

*Figure 5-7. Effect of gradually increasing (I) or decreasing (D) PPFD compared to constant PPFD (C) during cultivation. Percentages indicate the difference between increasing and constant light treatment (left) or between decreasing and constant light increment (right). Abbreviations: FW_{leaf} (leaf fresh weight), DW_{leaf} (leaf dry weight), FW_{leaf}/DW_{leaf} (leaf fresh - dry weight ratio), DW_{plant} (plant dry weight), Leaf:Plant (fraction leaf dry weight in total plant dry weight), LUE_{int} (intercepted Light Use Efficiency), and I_{int} (canopy intercepted PPFD integral). * P=0.05, ** P=0.01 and ***P=0.001. The statistical analysis was conducted based on all treatments for each parameter. Data are the means of six repetitions (n=6).*

Overall visual quality and shelf life

At harvest all leaves were crisp and green. During storage overall visual quality of the fresh cut lettuce declined due to browning and pinking at cut edges and yellowing (Fig 5-8). Overall visual quality at harvest and post harvest, hence shelf life, were not significantly affected by the light treatments.

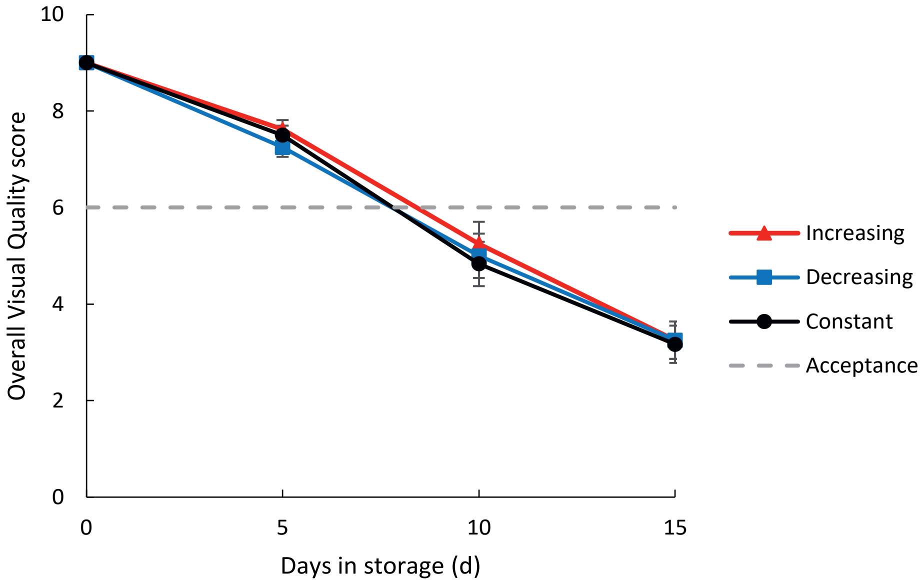

Figure 5-8. Time course of overall visual quality scores in 12°C storage. Error bars at each point represent standard error of means. Treatment effects were not significant (P=0.05). Data are the means of four repetitions (n=4)

5.4 Discussion

Gradually increasing PPFD resulted in highest LUE_{inc} due to highest light interception

A gradual increase in PPFD during cultivation resulted in a higher LUE_{inc} (LUE based on incident PPFD) compared to a constant PPFD or a gradual decreasing PPFD, when total light integral over the whole cultivation period was kept the same (Fig. 5-6b). The main reason for this higher LUE_{inc} was the increased light interception (Fig. 5-5b). A gradually increasing PPFD means that the high PPFD coincides with high fraction of light intercepted at the end of the growing period. In the treatment with gradually decreasing PPFD, the high intensities were provided when the plants were small and consequently a lot of light was not absorbed by plants but lost on the floor.

In line with our results, Yamada et al. (2000) who grew sweet potato seedlings for 15 days under stepwise increasing light (100, 200 followed by 300 μmol m^{-2} s^{-1}) or constant PPFD (200 μmol m^{-2} s^{-1}), concluded that the increasing light treatment produced 10% more biomass.

Specific leaf area (SLA) is an important factor contributing to light interception and light distribution in the canopy. SLA continuously acclimated to the PPFD of the preceding days, being lower the higher the PPFD (Fig. 5-4a). A decrease in SLA with increasing PPFD or daily light integral is in line with other studies (e.g. Poorter et al., 2019; Carotti et al., 2021; Ghorbanzadeh et al., 2021). Consequently, SLA was initially higher in the treatment with increasing PPFD, compared to constant or decreasing PPFD (Fig. 5-4a). In the early crop stages an increase in SLA, hence "thinner" leaves, resulted in a higher fraction light intercepted, when comparing at the same leaf weight. At a later stage of growth, the effect of SLA on canopy photosynthesis became probably small due to the fact that a large fraction of incident light was already intercepted (Fig. 5-4b). Taken together, the acclimation of SLA to PPFD contributed to a higher LUE_{inc} for the treatment with increasing PPFD, compared to treatments were PPFD was constant or gradually decreased during the growth period.

The observed effects likely depend on the planting density as well as the range of light intensities used. Here we used a relatively high planting density of 51 plants per m^2. When a lower planting density would have been applied, probably a larger effect of the gradual changes in PPFD would have been observed, as with a lower planting density initially a larger fraction of light is not intercepted by the leaves. As an alternative to increasing PPFD, which increases the amount of light intercepted per plant during the cultivation, a grower with a hydroponic system might apply a system where he keeps PPFD constant but varies plant spacing. This would mean staring with a very high planting density, which then is gradually decreased during cultivation. The magnitude of the treatment effects likely depends also on the range of intensities. In the present study the intensity ranged from 140 to 300 μmol m^{-2} s^{-1}. When the range would be very small, smaller effects are expected. Likewise, when the range of light intensities would be much larger the difference in biomass between increasing and decreasing PPFD is likely to be larger, but then it might be that the constant light treatment might perform better as effects on growth may saturate at high light intensities (e.g. Pennisi et al. 2020). Our experiments were performed with one variety, it might be interesting to verify if similar effects would be observed with other cultivars.

Gradually decreasing PPFD resulted in the highest LUE_{int}

In contrast to LUE_{inc}, the LUE_{int} (LUE based on intercepted PPFD) was highest in the treatment where the PPFD gradually decreased during cultivation (Fig. 5-6). The main reason for this effect is that the LUE_{int} slightly decreased with increasing PPFD and that the LUEint was highest in the later growth stages of the plants (Supplementary Fig. S5-1) This makes that the later growth stages have a dominating effect on the overall response of LUE_{int} to the light treatments. In the treatment with gradually decreasing PPFD, PPFD was lowest during the last growth stages and therefore also the LUE_{int} calculated over the whole cultivation cycle was highest.

Although a gradual increase in PPFD during cultivation resulted in the highest shoot dry weight at harvest, shoot fresh weight did not significantly differ whether PPFD was constant, decreased or increased during cultivation (while in all cases the light integral was the same; Fig. 5-3d). This is in line with results of Xu et al. (2020) who varied the PPFD during the last 12 days of cultivation while keeping light integral constant and did not find an effect on final fresh weight. The difference we found in responses between fresh and dry weight was related to differences in dry matter content (ratio between dry and fresh weight). At most time points the dry matter content increased with increasing PPFD during the preceding days (Fig. 5-4b). This effect of PPFD on dry matter content agrees well with the finding of Min et al. (2021) in lettuce leaves and of Marcelis (1993) in cucumber leaves and fruits. Therefore, even though the rate of dry weight growth at later stage was substantially higher in the treatment with gradually increasing PPFD compared to the treatment with a gradually declining PPFD, the fresh weight growth rate showed only a slight increase. Consequently, the final fresh weight was not significantly affected by the distribution of the light over the cultivation period.

Shelf life was not affected by increasing or decreasing light treatment

Min et al. (2021) observed that an increase in PPFD at the end of the production improved overall visual quality which correlated with an increase in carbohydrates and total ascorbic acid. However, in the present study no differences in overall visual quality and shelf life among treatments were found. During the last three days of our experiment PPFD ranged from 140 to 300 μmol m^{-2} s^{-1}. The strong effects on shelf life found by Min et al. (2021) occurred when differences in PPFD shortly before harvest were a bit larger than in the present study (210 versus 50 and 470 versus 210 µmol m^{-2} s^{-1}) during the last 6 days before harvest). The higher dry matter content found in the treatment where PPFD gradually increased is often assumed to be positive for a longer shelf life (Min et al., 2021).

The positive effects on dry matter content found in this study and the positive effects on shelf life, vitamin C and carbohydrates found by Min et al. (2021), suggest that when stronger changes in PPFD would have been applied than in the present experiment more positive effects could have been found. Hence, lettuce quality can potentially be improved by lighting strategies where PPFD gradually increases during cultivation. However, a high PPFD might increase the risk of tipburn (Xu et al., 2020).

5.5 Conclusion

Gradually increasing PPFD during the cultivation of lettuce, while total light integral is kept constant, improves light use efficiency based on incident PPFD (dry weight production per unit of incident light) by 16 or 12% compared to treatments with decreasing or constant PPFD, respectively. However, the light use efficiency based on intercepted PPFD was highest when PPFD gradually decreased during cultivation. When changing PPFD during cultivation specific leaf area and dry matter content continuously acclimate to the prevailing PPFD. Despite the increase in final shoot dry weight by the treatment where PPFD gradually increased, final shoot fresh weight was not significantly affected. Our results show that gradually increasing PPFD had a positive effect on biomass accumulation, increased dry matter content but did not affect shelf life.

Supplementary

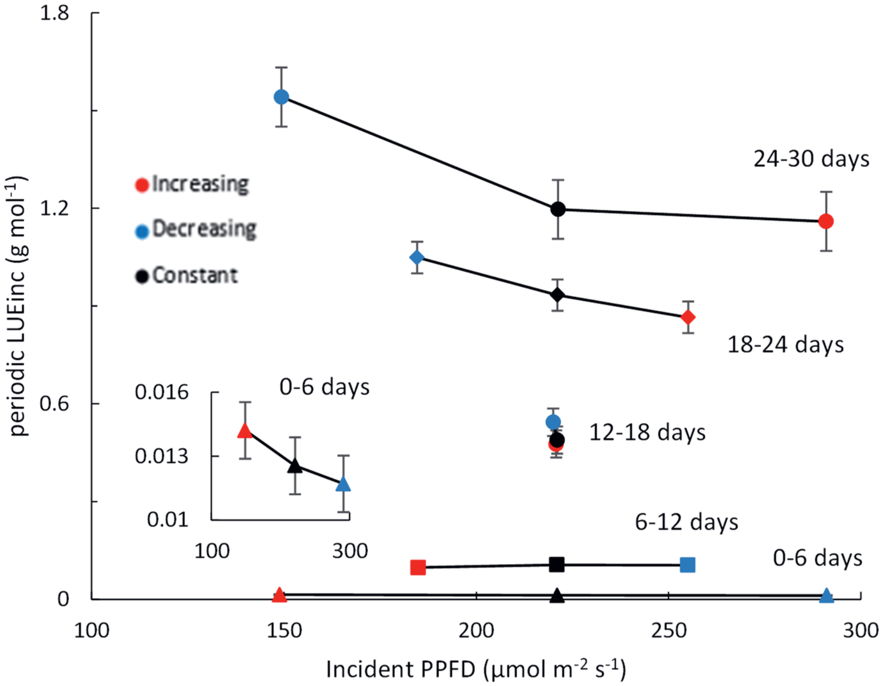

Figure S5-1. The effects of constant PPFD versus gradually increasing or decreasing PPFD throughout the cultivation period on periodic incident LUE (LUE_{inc}: shoot dry weight growth rate per cumulative incident PPFD in each 6-days sampling interval). Data are the means of four (DAT 6; n=4) or six repetitions (n=6). Error bars at each point represent standard error of means. Inset shows an enlargement of the first interval.

Chapter 6

General Discussion

The aim of this thesis is to increase the light use efficiency (LUE) of lettuce grown in vertical farms. First, a comparison of LUE, expressed as gram of shoot weight (both fresh and dry were analysed) per mole of cumulative incident photosynthetic photon flux density (PPFD)) was conducted among three lettuce production systems: vertical farming, greenhouse, and open field (**Chapter 2**). The maximum LUE of vertical farming was substantially higher than greenhouse and open field and close to the maximum theoretical values. The average LUE of vertical farming was higher than for the other two systems. Correlation analysis using data from all three production systems or for vertical farming alone showed that the shoot dry weight at harvest and planting density are significant factors which explained 83% of the variation in LUE based on the analysis of all three production systems and 45% of the LUE explained by cumulative DLI, planting density, and DLI for vertical farming alone. Either a larger harvesting shoot dry weight or a higher planting density increased LUE. In **Chapter 3** an experiment was conducted to analyse far-red (FR) effect at different planting densities. Additional FR over red (R) and blue (B) promoted the fresh and dry weight of lettuce at all three planting densities. The FR effect on dry weight was greatest at the lowest planting density. Additional FR increased lettuce leaf area expansion and made the crop intercept more of the incident photosynthetic photon flux. Consequently, FR increased LUE as well as radiation use efficiency, the latter including FR radiation. However, additional FR increased neither the instantaneous net photosynthesis rate under growth conditions, nor the light use efficiency based on PPFD interception (gram of shoot dry weight per mole of cumulative intercepted PPFD). Therefore, an experiment was conducted to explore whether the instantaneous photosynthesis rate and quantum yield of lettuce grown under FR-absent or FR-enriched environments, at two PPFD levels, would be different when measured with or without additional FR (**Chapter 4**). Lettuce grown under a FR-enriched environment did not show a higher quantum yield when measured with an additional FR compared with lettuce when measured with additional red and blue (RB), whether grown in low or high background PPFD. Adding FR on top of RB increased instantaneous net photosynthesis rate but to a smaller extent than adding RB with the same intensity. In **Chapter 5**, a different approach to increase LUE was tested by gradually increasing or decreasing PPFD during the cultivation cycle, compared to the constant PPFD commonly applied during the cultivation cycle. The cumulative incident PPFD was kept the same among all treatments. The increasing PPFD treatment produced the highest shoot dry weight, because more PAR was applied in the later phase when the fraction of PPFD intercepted by the crop is higher. Thus, a gradually increasing PPFD resulted in the highest LUE (based on shoot dry weight). However, neither shoot fresh weight nor shelf life were significantly affected.

In this general discussion, I first discuss whether FR improved LUE, taking all research within this thesis and related literature into consideration. Thereafter, I discuss whether the effects of FR depend on PPFD. Thirdly, based on my own experimental observations and study of the relevant literature, I discuss alternative approaches to increase LUE in vertical farming. Lastly, suggestions for further research and the perspectives for vertical farming are addressed.

6.1 Light spectra in vertical farms

Crop biomass production is the result of a series of physiological processes including light interception, photosynthesis, respiration, and biomass partitioning. Research within this thesis focused on how light interception and photosynthesis (and to some extent biomass partitioning and respiration) improved LUE by manipulation of the light spectrum and light intensity. The LUE improvement within present research is based on the common practice of applying light-emitting diodes (LED) modules to vertical farming, which emit photons between 400 and 700nm, without FR.

Far-red

Far-red (FR) can increase LUE in two ways: (1) promoting leaf expansion resulting in more PPFD interception, and (2) increasing photochemical efficiency. Adding FR to PAR which contains R lowers the R:FR ratio leading to crop morphological and physiological acclimation. Improved lettuce leaf expansion is a well-known morphological effect induced by lowered R:FR ratio (Park and Runkle, 2017; Mickens et al., 2018; Meng and Runkle, 2019; Meng et al., 2019; Zou et al., 2019; Jayalath and van Iersel, 2021; Kong and Nemali, 2021; Legendre and van Iersel, 2021; as well as Chapters 3 and 4). For rosette crops like lettuce, leaf expansion enlarged the projected leaf area. The increment of projected leaf area improves light interception, as radiation in vertical farming mostly comes from above. The effects of additional FR will be limited when the planting density is high, as projected leaf area will in any case be high at a high planting density. Three planting densities, 23, 37 and 51 plant m^{-2}, were investigated in Chapter 3, and the investigation revealed differences in canopy-intercepted PPFD integral with and without additional FR under the same intensity of background PPFD. The treatment with additional FR increased canopy-intercepted PPFD at the same planting density. This increment was lower at a higher planting density: 60%, 34% and 31% for 23, 37 and

51 plant m^{-2}, respectively. The radiation use efficiency (RUE: dry mass production per unit of cumulative incident radiation 400-800nm) was higher when additional FR was applied. A planting density of 146 plant m^{-2} was applied in Chapter 4, which is about 3 times higher than the highest planting density (51 plant m^{-2}) in Chapter 3. The benefits of intercepting a higher fraction of incident light when adding FR were very limited at such high planting density. Under a high planting density, the fraction of the floor covered by the canopy nears 100% in a shorter amount of time even without additional FR (Fig. 6-1). The RUE showed no differences in similar light condition with ~200 µmol m^{-2} s^{-1} R (88%) and B (12%) with ~50 µmol m^{-2} s^{-1} FR.

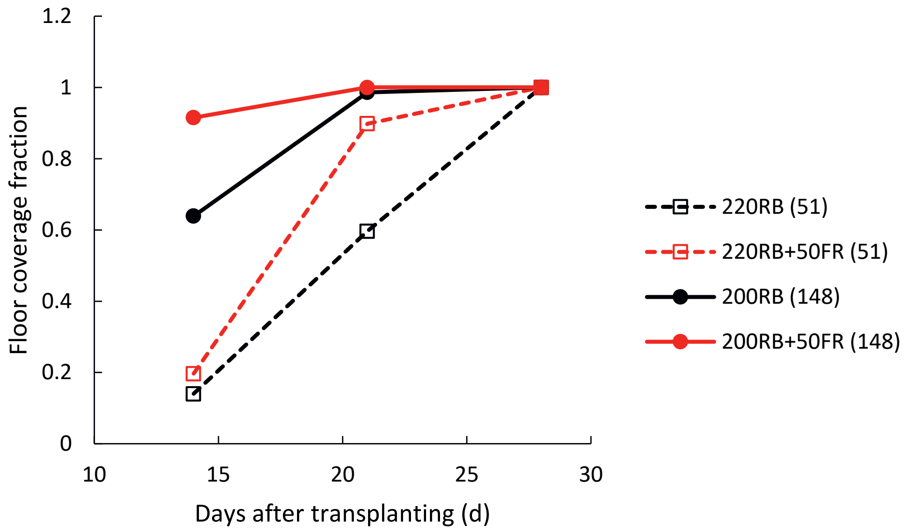

Figure 6-1. Floor coverage fraction (canopy projection area per growing area) comparison between treatments from Chapters 3 and 4 at 14, 21 and 28 days after transplanting. Planting density for each treatment is given within the bracket in the legend. 220RB represents ~220 µmol m^{-2} s^{-1} red and blue light applied in Chapter 3; 50FR represents ~50 µmol m^{-2} s^{-1} far-red light applied in Chapters 3 and 4; 200RB represents ~200 µmol m^{-2} s^{-1} red and blue light applied in Chapter 4. For the light spectrum and intensities, see Chapters 3 and 4.

The R:FR ratio of unshaded natural sunlight is ~1.2, which may slightly vary depending on the definition of R and FR (Kusuma and Bugbee, 2020). Therefore from an ecological perspective light with a R:FR ratio below 1.2 means shading by vegetation (Poorter et al., 2019). However, in vertical farming where light is coming from lamps, a 1.2 R:FR ratio is hardly ever applied and a much higher R:FR ratio commonly occurs. Often no FR at all is present (e.g., in Chapters 3 and 4). A 'lowered' R:FR ratio in vertical farming studies therefore means a drop from an infinite value which contains a negligible fraction of FR radiation to a FR addition treatment with a R:FR ratio of 4~6. A reduced R:FR ratio in vertical farming engages with a different R:FR range than in common shade avoidance research in ecology and therefore shade avoidance literature cannot be directly applied to this situation. Furthermore, shade avoidance is usually related to a combination of low PPFD and low R:FR, whereas in vertical farming R:FR can be reduced without changing PPFD level. For example, leaf area expansion is not a typical shade avoidance response under a lowered R:FR ratio (Kong and Nemali, 2021), while a positive effect of low R:FR is often seen in vertical farms (Chapters 3 and 4). Thus, it can be debated whether plant responses to FR in vertical farming studies where incident R:FR is usually much higher than 1.2 should be classified into shade avoidance syndrome, as is also discussed by Kalaitzoglou et al. (2019).

Another metric widely used in FR effect research is phytochrome photostationary state (PSS, or PPE which is the abbreviation of phytochrome photoequilibrium). PPS is calculated as the fraction of active phytochrome (P_{fr}) over total phytochrome (P_{total}) where $P_{total} = P_{fr} + P_r$. P_{fr} and P_r, as previously mentioned, are two intra-convertible states of phytochrome; P_{fr} is the FR absorbing state and P_r is the R absorbing state (Sager et al., 1988). A low R:FR ratio leads to a low PSS, and a lowered PSS correlates with shade avoidance responses such as stem elongation (Kalaitzoglou et al., 2019; Kusuma and Bugbee, 2020). Unlike R:FR ratio which focuses on R and FR radiation, PSS is affected by other wavelengths as well, such as B. The PSS of sunlight is ~0.73 (Sager et al., 1988). In most research on vertical farming only higher PSS values occur while in ecological research only lower values are investigated.

Additionally, it is worth mentioning that in most studies the reported R:FR ratio or PSS refers to the ratio of the light just above the canopy. These metrics do not exactly identify the light condition at the phytochrome photoreceptor, as incident light falling on the top layer of each leaf will be scattered within the leaf cross-section (Kusuma and Bugbee, 2020, 2021). The phytochrome photoreceptor at the cellular level perceives a substantial drop of R:FR ratio by spectral distortion while photons are transmitting, reflecting, and being absorbed within the leaf. Therefore, the actual R:FR ratio perceived by the phytochrome photoreceptor is different from the incident value.

A higher fraction of biomass partitioning to the shoot is one of the effects of lowered R:FR (Vos et al., 2010; Bongers et al., 2014). In the case of lettuce, which has only a very small stem, it is the leaf that benefits. The increase of leaf:root ratio under FR suggests the relative sink strength of leaves had increased compared to that of the root. Zou et al. (2019) reported that specific leaf area (SLA) was increased by additional FR or a lowered

R:FR ratio. However, a higher SLA by addition of FR was not observed in all treatments of Chapter 3, which is in agreement with Legendre and van Iersel (2021) and the meta-analysis conducted by Poorter et al. (2012). Therefore, a higher fraction of biomass partitioned to the leaf resulted in a larger leaf area and hence an increased light interception and biomass production.

Additional FR increases photochemical efficiency by improving electron transport efficiency. In the absence of FR, excitation of photosystem I (PSI) and II (PSII) is unbalanced, as PSI can be excited maximally at 700 nm and the peak of excitation for PSII is at 680nm (Baker, 2008). The radiation peak of R from current used LED modules in vertical farming is ~660nm, which contains limited photons at 700nm or above. Therefore, though FR photons can only limitedly drive photosynthesis directly, additional FR alongside PAR is capable of rebalancing the excitation levels of PSI and PSII and thus increasing the photochemical efficiency of electron transport (Zhen and van Iersel, 2017; Zhen and Bugbee, 2020), as the Emerson Enhancement Effect revealed (Emerson et al., 1957; Emerson and Rabinowitch, 1960). This is the fundamental hypothesis of a series of studies which also proposed that FR should be included in the definition of PAR, as additional FR promoted instantaneous photosynthesis at a similar intensity to PAR (Zhen and van Iersel, 2017; Zhen et al., 2018; Zhen and Bugbee, 2020). The effect of FR on the instantaneous photosynthesis rate was deduced from a gas exchange measurement system including FR modules for canopy and single leaf. Hence, from these instant measurement studies it was concluded that photosynthetic photons are utilized more efficiently when there is additional FR. However, looking at cumulative biomass produced, the LUE based on PPFD interception was not increased by the addition of FR for all treatments in Chapter 3. LUE based on PPFD interception of 37 plant m^{-2} treatment was improved by additional FR but not the 23 and 51 plant m^{-2} treatments. Legendre and van Iersel (2021) and Chapter 3 took different approaches in order to estimate canopy and crop daily PAR interception throughout the growth period. Legendre and van Iersel (2021) calculated the daily projected canopy area by log transforming and applying a quadratic equation, while Chapter 3 applied linear interpolation to estimate the daily projected leaf area. Legendre and van Iersel (2021) concluded that the additional FR is more effective at producing biomass than PAR. These diverse conclusions require further research which may involve 3D crop architecture modelling to simulate PPFD interception.

In addition, previous research also showed that crop acclimation to FR led to a decline in leaf chlorophyll content per leaf mass and per leaf area (Zou et al., 2019). A reduced chlorophyll content lowers the assimilation capacity per unit of leaf area. Ji et al. (2019)

and Kalaitzoglou et al. (2019) proposed that tomato plants were influenced by a trade-off effect, the positive effect of additional FR being canceled out by the lowered photosynthetic capacity, which prevented an increase in the instantaneous assimilation rate from being observed. In Chapter 4, a similar result was found with respect to lettuce: plants grown in a FR-enriched environment had a lower chlorophyll content, and there was no higher rate of instantaneous assimilation, net or gross, when measuring either with or without additional FR. Chapter 4 thus agrees with Zou et al. (2021) that net photosynthetic rates are similar between FR-absent and FR-enriched lettuce treatments at ~200 μmol m^{-2} s^{-1} and that FR-acclimated lettuce has a lower rate under high PPFD (~1500 μmol m^{-2} s^{-1}).The measurements at these two PPFD levels both included additional FR.

Red, Blue, Green, and Ultra-violet

Red (R), defined by the range of 600-700nm, was the first color provided by LEDs (~660nm) in controlled environment agriculture, which was originally combined with fluorescent lamps in vertical farming (Bula et al., 1991; Morrow, 2008). Shortly thereafter, following the development of the high-output blue LED (Nakamura et al., 1994), the first commercial horticultural LED modules were made of a mixture of R and B (Kusuma et al., 2020). Combining R and B was considered more efficient as they correspond to chlorophyll absorption peaks (McCree, 1971; Inada, 1976). When R is applied alone, there are negative effects such as a lowering of photosynthetic capacity and of the maximum quantum yield of chlorophyll fluorescence, as well as unresponsive stomatal conductance by R; the effect was named 'red light syndrome' (Trouwborst et al., 2016; Miao et al., 2019). Even though it has the highest quantum yield (McCree, 1972; Inada, 1976), the monochromatic R does not always lead to the highest biomass production (Yanagi et al., 1996; Yorio et al., 2001; Meng and Runkle, 2019). Therefore, R is mostly applied in combination with other spectra. Its application alongside FR has been extensively discussed above (section 6.1.1). The other is with blue (B).

B is typically defined as radiation between 400 and 500 nm (Pattison et al., 2018; Kusuma et al., 2020). The average photosynthetic efficiency of B within 400-500 nm is ~20% less than the one of R in 600-700 nm which makes B the second most photosynthetically efficient light quality (McCree, 1971, 1972; Kusuma et al., 2020). B is known to affect plant architecture, leaf and photosynthetic characteristics, and the content of antioxidants (Hogewoning et al., 2010; Kook et al., 2013; Pennisi et al., 2019; Kalaitzoglou et al., 2021; Kong and Nemali, 2021). These effects are regulated by several blue-light receptors, like cryptochrome 1 and 2, phototropin and phytochrome A (Lin, 2000). When applied monochromatically, B elongated the stem and enlarged the leaf area (Spalholz et al., 2020), which was similar to the effects of a low R:FR ratio. However, when applied together with R or white light (PAR), a higher proportion of B reduced crop height, leaf area and thus biomass production (Dougher and Bugbee, 2001; Lee and Kim, 2013; Pennisi et al., 2019; Kusuma et al., 2021). An increasing R:B ratio increases stomatal size but reduces stomatal density and stomatal conductance (Pennisi et al., 2019). In addition, B's increase of antioxidant capacity and its development of compact morphology is associated with more effective control of gray mold (*Botrytis cinerea*) in lettuce (Kook et al., 2013).

Green light (G: 500-600 nm) was considered to have a lower photosynthetic efficiency when compared with other radiations within PAR (McCree, 1971; Inada, 1976). As a result, its application to vertical farming is relatively new. Some studies have claimed that G can improve biomass production (Kim et al., 2004; Liu et al., 2017; Li et al., 2020). At first, G was proposed to increase the efficiency of photosynthesis (Terashima et al., 2009; Johkan et al., 2012; Liu et al., 2017). Terashima et al. (2009) found that G increases leaf photosynthesis rate more than R and B at high PPFD. Secondly, once the upper layer of canopy has been saturated with R and B, then the less-absorbed G penetrates deeper into the canopy (Brodersen and Vogelmann, 2010; Zhen and Bugbee, 2020). Hence, substituting G for R, B, orange and/or yellow increases biomass production (Snowden et al., 2016; Kusuma et al., 2020). However, there is no perceptible improvement when G is substituted for FR (Li et al., 2020). It has been suggested that G induces shade avoidance responses similar to a lowered R:FR ratio, producing, for example, a longer stem in lettuce and tomato plants (Snowden et al., 2016). In addition, adding G to R and B improves human perception which facilitates the manual operation and inspection of vertical farms.

Ultraviolet (UV) radiation is a range of radiation between 100-400nm and is divided into three categories: UVA (315-400nm), UVB (280-315nm) and UVC (100-280nm). As the Planck-Einstein relation reveals, the energy carried by a photon is inversely proportional to its wavelength. Therefore the photons in the UVC range are energy intensive and are typically used in controlled-environment agriculture to disinfect water (Runia and Boonstra, 2004). The photoreceptor for UVB photon is UVR8, which is reported to induce the opposite of shade avoidance responses, such as the inhibition of hypocotyl elongation, petiole elongation and rosette expansion (Casal, 2013; Fraser et al., 2016). More research on UVA is necessary. It shares its photoreceptors with B photons: cryptochrome and phototropin (Fraser et al., 2016). Jeong et al. (2020) treated cucumber seedlings with additional UVA and FR alongside PPFD. The UVA treatment partitioned more biomass into the stem but less into the leaf. The plant dry weight was lower than the FR treatment. In another lettuce experiment, Chen et al. (2019b) found that additional UVA enlarged the leaf area and the shoot dry weight, as well as the anthocyanin and ascorbic acid contents. Although UV may potentially improve biomass production and plant quality, its effects require further research.

6.2 PPFD, photoperiod and DLI

Three factors determine the cumulative amount of photosynthetic photons that reaches the plants: photosynthetic photon flux density (PPFD), photoperiod, and daily light integral (DLI). DLI is the product of instantaneous PPFD and photoperiod. Plants typically show an expo-linear growth pattern, i.e., initially the crop grows exponentially and gradually it will show linear growth (Goudriaan and Monteith, 1990). Since vertical farming provides a well-managed environment for crops, PPFD is typically the limiting factor and higher PPFD leads to a higher growth rate, which may possibly increase LUE.

Several studies have explored the effects of PPFD and tried to identify the optimal PPFD for lettuce growth. The plant dry weight doubled when PPFD was increased from 85 to 170 μmol m^{-2} s^{-1} (Yanagi et al., 1996). However Pennisi et al. (2020), who increased PPFD from 100 to 300 μmol m^{-2} s^{-1} with 50 μmol m^{-2} s^{-1} steps, found that the highest shoot dry weight was reached at the level of 250 μmol m^{-2} s^{-1}. Fu et al. (2012) grew lettuce under 100, 200, 400, 600 and 800 μmol m^{-2} s^{-1} levels. The fresh weight increased up to 600 μmol m^{-2} s^{-1}, but the 800 μmol m^{-2} s^{-1} treatment was surprisingly lower than the 200 μmol m^{-2} s^{-1} level. Carotti et al. (2021) carried out the experiment in a similar PPFD range. They set a targeted lettuce fresh weight (250 g $plant^{-1}$), and the growth duration was only shortened by 31% from 26 days to 18 days while the PPFD tripled from 200 to 750 μmol m^{-2} s^{-1}. Three different PPFDs were applied during this study, at 200, 400 and 750 μmol m^{-2} s^{-1}, together with different air and root temperatures. The 200 μmol m^{-2} s^{-1} PPFD treatment resulted in the lowest shoot fresh weight at harvest, while the 750 μmol m^{-2} s^{-1} PPFD treatment produced the highest shoot fresh weight in the same air and root temperature growth conditions. Cammarisano et al. (2020) did a comprehensive experiment with 12 PPFD levels ranging from 27 to 942 μmol m^{-2} s^{-1}. Their results showed that the crop dry weight increased with PPFD levels up to a plateau at about 330 to 518 μmol m^{-2} s^{-1}. This is evidence of a saturation effect: when the absorbed photosynthetic photons exceed the photosystem's capacity to utilize excitation energy, dissipation of the excess energy is necessary to avoid or to reduce the risk of photooxidative damage which causes efficiency to drop (Cammarisano et al., 2021).

While the studies previously mentioned all utilized a constant PPFD during the growth period, a gradually increasing PPFD could also increase light use efficiency. In the early stages of crop development, the incident PPFD may be wasted, falling on the floor. A mature crop can utilize more photosynthetic photons than a seedling. While keeping the total light integral the same, plants intercepted a higher fraction when under the influence of gradually increasing PPFD and thus produced the most biomass (Chapter 5). Similarly, Yamada et al. (2000) grew sweet potato seedlings for 15 days under a PPFD increasing stepwise (100, 200 and 300 µmol m^{-2} s^{-1}) or constant PPFD (200 µmol m^{-2} s^{-1}). The results showed that increasing PPFD produced 10% more biomass. Chapter 5 applied a PPFD range of 140-300 µmol m^{-2} s^{-1}, similar to Yamada et al. (2000) who applied PPFD from ~100 to 300 µmol m^{-2} s^{-1}. If the range were smaller, then a smaller difference in biomass production would be expected. Likewise, if the range of PPFD were much larger, the difference in biomass between increasing and decreasing PPFD would likely be larger. In that case, however, the constant light treatment might produce better results as high light intensities cause saturation, which makes growth plateau (e.g., Pennisi et al., 2020b). The results may be species- and cultivar-specific.

Extending the photoperiod to a 24-hour cycle is another way of increasing the DLI while maintaining a constant PPFD. Lee and Kim (2013) tested 12-, 16- and 20-hour photoperiods of white light and found that an increase in photoperiod from 12 (DLI = 9.9 mol m^{-2} d^{-1}) to 16 hours (DLI =13.2 mol m^{-2} d^{-1}) resulted in a sharp increase of dry mass, while there was only a limited effect when photoperiod was further increased from 16 to 20 hours (DLI =16.6 mol m^{-2} d^{-1}). Pennisi et al. (2020a) extended the photoperiod from 16 to 24 hours of 250 mol m^{-2} d^{-1} PPFD RB with R:B ratio at 3. The lettuce dry mass production increased, although the relative increase was lower than that of the total light increment. However, the fresh mass dropped as the photoperiod was extended. Kelly et al. (2020) tested different combinations of PPFD and photoperiod while maintaining the same DLI. They showed that the lettuce dry weight is determined by DLI rather than PPFD and photoperiod.

6.3 Planting density

In natural circumstances, under the influence of just solar radiation, a dense canopy with high planting density will mean that the plants within receive less PAR and a higher fraction of FR reflected from neighboring plants, and thus the plant beneath the canopy will perceive a lower R:FR ratio, inducing the shade avoidance responses mentioned before. Besides radiation cues, biogenic volatile organic compounds (BVOC) from neighboring plants can also trigger shade avoidance responses (Kegge and Pierik, 2010). Moreover, the touching of leaflet tips can induce one common sign of shade avoidance syndrome, hyponasty, which causes the leaflet to move upward (de Wit et al., 2012; Pierik and De Wit, 2014). Lastly, the increased mechanical stress of wind shielding in a high planting density induces stem elongation of tobacco (Anten et al., 2005), which may be less likely to happen in vertical farms.

The reported highest LUE from an lettuce cultivation experiment in vertical farming is 1.63 g mol^{-1} (Pennisi et al., 2019), which is close to the maximum theoretical LUE, 1.81 g mol^{-1} (Loomis and Williams, 1963). The former LUE is achieved through late transplantation when the lettuce is relatively large and by a short growing duration. Therefore, the calculation for the potential yield in vertical farms used the second highest experimental LUE, 1.23 g mol^{-1} (Carotti et al., 2021), derived from the transplantation of a smaller lettuce crop with a longer growing duration. Both LUEs suggest that vertical farming has great potential to achieve the maximum theoretical LUE, since environmental factors can be kept stable and the resources for crop growth can be supplied without limit. The underlying difference between the first and second highest experimental LUEs is the proportion of PPFD intercepted, which depends upon the crop size (i.e., the projected leaf area) and planting density. Within vertical farms, planting density has a profound effect on the proportion of light intercepted. A higher planting density results in fewer photons falling to the floor without being utilized by the crops. Since plants are relatively small in early developmental stages and grow larger, a gradually decreasing planting density is one way of maximizing utilization of light (Nicole et al., 2016). As a result, a gradually decreasing planting density applied alongside a gradually increasing PPFD may further promote LUE.

6.4 Nutritional quality

Lettuce generally has a high concentration (> 2500 mg kg^{-1} fresh weight) of nitrates when compared with other leafy vegetables (Santamaria, 2006). Although nitrates are not inherently hazardous, they can be metabolized into nitrites in the human body, which cause diseases (Gangolli et al., 1994; Chan, 2011). According to the Joint Expert Committee of Food and Agriculture and the European Commission's Scientific Committee on Food, the daily intake of nitrate should be between 0 and 3.7 mg kg^{-1} body weight (Santamaria, 2006). Some studies have observed that in lettuce the concentration of nitrate increases when the R:B ratio decreases, whereas the concentration of soluble sugars decreases (Chen et al., 2014b, 2021). Soluble sugars, a group which includes glucose, fructose, and sucrose, are important primary metabolites in lettuce. Since they are the substrates for respiratory metabolism, the retention of a certain level of soluble sugars may delay lettuce texture deterioration, i.e., preserve shape and crispness (Min et al., 2021).

Ascorbic acid (AsA, Vitamin C) and dehydroascorbic acid (DHA) are major antioxidants in lettuce, preventing enzymatic pinking and browning after harvest (Min et al., 2021). Chen et al. (2011) observed that the AsA concentrations in lettuce were higher under monochromatic blue light and a combination of red and blue light than under monochromatic R. Likewise, under continuous LED light, the AsA concentration in lettuce increased with decreasing R:B ratios (Zha et al., 2020). The authors further concluded that a larger proportion of blue light increased AsA concentration due to the elevated AsA regeneration instead of biosynthesis. However, more recently another research group has found that monochromatic red light more effectively promotes AsA accumulation in lettuce than other treatments with blue light (Chen et al., 2021). Interestingly, in the same study, lettuce grown under increasing fractions of blue light (between 30 and 60%) exhibited increased AsA. These results suggest that AsA accumulation is sensitive to R:B ratio.

Secondary metabolites of low concentrations also play an essential role in plant quality. Light is one of the main factors affecting biosynthesis of these compounds (Thoma et al., 2020). Pennisi et al. (2019b) investigated the responses of phytochemical compound accumulation in lettuce to several R:B ratios. The authors found that an R:B ratio between 2 to 4 increased flavonoid concentrations when compared with a ratio of 0.5, as well as with fluorescent light. A further study found that a 3:1 ratio of R:B LED light applied during lettuce growth led to a greater accumulation of anthocyanins and flavonoids (and induced a corresponding gene expression) when compared with the lettuce grown under fluorescent light (Sng et al., 2021). Anthocyanin and flavonoids are stronger antioxidants than vitamin C in plants (Bagchi et al., 1998). In addition to the increment of anthocyanins and flavonoids, red and blue light also affect the carotenoid concentration in lettuce. Carotenoids are vital pigments which protect photosynthetic components from damage caused by the active triplet state of the chlorophyll molecule (Landrum and Bone, 2001; Chen et al., 2017). R combined with B increased the carotenoid content in lettuce compared to white LED light (Amoozgar et al., 2017). Sng et al. (2021) also observed that lettuce grown under RB showed a higher concentration of carotenoids than lettuce grown under fluorescent light. In this study, the concentration of secondary compounds related to flavor and taste, such as terpenoids and alkaloids, was also increased by the treatment with RB.

An increase in energy efficiency and crop yield should not come at the expense of quality. End-of-production practices which alter lighting conditions a few days before harvest can boost the quality of fresh products at harvest without having a huge impact on yield and plant morphology (Min et al., 2021). This is due to the biosynthesis of some secondary metabolites (e.g., anthocyanins, flavonols, isoflavonoid, stilbenoids) which are more active at the mature than the early stages of lettuce development (Sng et al., 2021), especially under the combined influence of R, B and high PPFD lighting.

6.5 Maximizing light use efficiency and energy use efficiency

Maximizing light use efficiency in vertical farming increases biomass production, particularly of the marketable organs. All environmental factors, such as temperature, CO_2 concentration, air humidity, air flow, as well as water and nutrient availability in the root zone, affect plant growth and thus will impact light use efficiency. These factors may also interact with the light conditions. Since vertical farms can provide crops with any climate, there will be an optimized growing technique, species- and cultivar-specific, which is the setpoints of all environmental factors.

Even though some optimists estimate that in the future the price of energy will drop sharply because of a massive and sufficient supply (Tsao et al., 2018), under the current circumstances energy consumption will remain the limiting factor in the development of vertical farming. Energy use efficiency (EUE) is a more comprehensive criterion when defined as the biomass production per unit of energy consumption for the overall vertical farms. Maximizing LUE or maximizing biomass production may not always be in line with maximizing EUE, since an optimal environment may not be the most efficient for energy consumption. Improving EUE is dependent upon the effective design and implementation of heating, ventilation, and air-conditioning systems (HVACs). For example, Zhang et al. (2016) proposed a perforated air tube with multiple rows applied to the growth area, providing a vertical air flow to improve air circulation and prevent tipburn. Whether the energy consumption per unit of harvestable biomass would be reduced in comparison with conventional ventilation (no air tubes in the growing area) remains uncertain. The effect of these air tubes on ventilation depends on the crop size and the structure of the growing area, like the distance between the canopy and LEDs. On the one hand, a larger space between the canopy and the LEDs may help to improve the air flow by reducing the resistance. On the other hand, photons attenuate in the air, so that fewer photons radiated from the LEDs would actually fall on the canopy. One solution might be to place the LEDs at different heights during different growing stages, decreasing photon attenuation and maintaining ventilation without increasing energy consumption. Additionally, vertical farms can cool down the interior air with heat exchangers when the outside air is at a lower temperature, avoiding the use of air-conditioning and reducing energy consumption. Though vertical farms can operate without interaction with the outside climate, specific designs can be made in accordance with the localized climate to lower operational energy consumption.

6.6 Suggestions for further research

The conclusions of the present research can be applied to vertical farming production in order to increase light use efficiency (LUE). There is still a gap between the experimental LUE and theoretical maximum LUE, which requires further exploration; present production systems have still quite some room for improvement. A dynamic light intensity, i.e., a gradually increasing PPFD throughout the period of growth, was shown to increase LUE (Chapter 5). On the basis of model simulations, dynamic planting density has been proposed as a means of improving LUE (Nicole et al., 2016). It may be postulated that dynamic control of the climate factors will improve LUE. Moreover, different combinations of climate factors, i.e., of light intensity and temperature of shoot and root environments, resulted in different LUE. Thus, a further premise can be proposed: dynamic combinations of climate factors during the day and throughout the cultivation cycle could further improve LUE; exploring this would require more research.

The energy price varies over 24 hours, typically being higher at times of high demand ("Actuele prijzen", 2022). These variations may be even stronger in the future when supply comes mainly from solar and wind energy. To mitigate the current developmental bottleneck of vertical farms, i.e., the high energy cost of lighting especially, energy consumption should primarily take place in periods with lower prices. For this reason, it is necessary to adapt dynamically to all climate factors. To give an example, air-conditioning consumes more energy at a higher light intensity because more heat is discharged from the lamps. A dynamic, adaptive program could contribute to the full utilization of low-cost energy by adjusting various environmental factors, for instance light intensity and temperature, under the assurance of supplying sufficient produce when required. Light intensity can be lowered when the price of electricity is high, which will also lower the cost of cooling. There are programs currently being tested in greenhouses with an aimed DLI (Albright et al., 2000; Hernandez et al., 2020; Hooks et al., 2021), the intermediate system of production between greenhouse and vertical farming. In the case of vertical farming, there is little knowledge of how dynamic climate management would adjust to the changing cost of energy, and its effects on crop biomass production and nutritional quality require further exploration.

One area in need of further research is the effect of extending or shortening the diel cycle (photoperiod + noctoperiod), which could possibly shorten the growth period and increase light use efficiency (Chen et al., 2022). A shortened diel cycle could utilize the lower price duration to lower the energy cost for vertical farming production.

Making efficient use of FR is another way of reducing energy consumption. To reach that efficiency, further research is needed into the minimum FR intensity and the maximum R:FR ratio. The minimum sufficiently effective FR intensity alongside the most effective waveband could reduce initial investment and daily energy consumption. Furthermore, additional FR should be applied in a dynamic manner. For example, a low fraction of additional FR can be applied to increase the efficiency of photosynthesis when the canopy is closed or almost closed as all PPFD is intercepted.

Finally, vertical farming requires special breeds of cultivar. Resistance to pests and diseases and the mechanism which allows acclimation to environmental stresses such as salinity or drought come at a cost: they limit crop growth potential. This resistance and this mechanism are unnecessary for vertical farming cultivation due to the fully controlled growth conditions. New cultivars without these adaptations can be expected to yield higher biomass in vertical farming.

6.7 Conclusions

Based on the research in Chapters 2-5 and my survey of the relevant literature, I come to the following conclusions:

- Lettuce grown in vertical farming has the highest light use efficiency based on cumulative incident photosynthetic photon flux (PPFD) when compared to greenhouse and open-field farming.
- The highest reported light use efficiencies of lettuce cultivation in vertical farms are close to the theoretical maximum but vertical farming has the potential to

reach the theoretical maximum light use efficiency due to its ability to keep all environmental factors at an optimal level.

- Adding far-red (FR) to red and blue (RB) promoted leaf expansion and increased light interception, resulting in a higher biomass production and LUE. This effect was smaller at higher planting densities.
- Additional FR increases the instantaneous net photosynthesis rate of lettuce grown either in FR-enriched or in FR-absent environments. However, this effect of additional FR on photosynthesis was smaller than that of additional RB at the same photon flux density.
- Gradually increasing PPFD during the growth period resulted in higher biomass production than gradually decreasing or constant PPFD, without affecting shelf life.

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Summary

Vertical farming is a production system where crops are grown in a closed system, typically in stacks, and all light is provided by lamps, making a substantially greater production efficiency per land area feasible. It allows for strict hygiene and in principle zero pesticide usage. All growth factors are under full control in vertical farms. Vertical farms have, therefore, a great potential to address the ever-increasing demand for fresh vegetable and fruit production in an efficient and sustainable way. However, the major challenge for vertical farming is its high energy consumption, especially for lighting. To counteract this, the light use efficiency (LUE: shoot dry weight at harvest divided by cumulative incident photosynthetic photon flux density (PPFD: 400-700nm); g mol^{-1}) of vertical farms needs urgently to be increased. In **Chapter 1** the concept of vertical farming is introduced, and improved light use efficiency is discussed as a possible solution to its high energy requirements. Far-red light (FR: 700-800 nm) provides one example of how changing the light quality in vertical farming might benefit lettuce production, although when applied independently it barely contributes to photosynthesis. When combined with photosynthetically active radiation (PAR: 400-700 nm), FR promotes leaf photosynthesis and leaf expansion. Furthermore, light use efficiency also depends on the PPFD applied during growth and the light strategy, i.e., dynamic light intensity instead of a constant intensity across the whole crop cycle. This requires further exploration. The overall aim of this research is to improve the LUE of lettuce in vertical farms by investigating and explaining the effects of additional far-red on lettuce morphology and photosynthesis and exploring the effect of dynamic light intensities. This chapter provides the detailed research objectives and hypotheses.

In **Chapter 2** a literature study was conducted to compare the lettuce cultivation LUE of greenhouse, open-field, and vertical farming. There are 53 studies of vertical farming, 13 of greenhouse, 8 of open-field. These studies collected data on other relevant aspects of cultivation besides LUE, such as lettuce weight at harvest, cultivation period (plant age at harvest), daily light integral, cumulative daily light integral for the whole cultivation period, planting density and CO_2 concentration. The average LUE for lettuce grown in a vertical farm was 0.55 g mol^{-1} which was higher than the 0.39 g mol^{-1} average of greenhouse-grown lettuce. Both were substantially higher than the field-grown lettuce average, 0.23 g mol^{-1}. The maximum measured LUE for lettuce grown in a vertical farm, 1.63 g mol^{-1}, is close to the published maximum theoretical value, which ranges between 1.26 and 1.81 g mol^{-1}. Since all environmental factors can be fully controlled, vertical

farming could potentially achieve its theoretical maximum LUE. Using the highest reported LUE based on shoot fresh weight (44 g mol^{-1} at 200 μmol m^{-2} s^{-1} PPFD and 16-hour photoperiod), it is estimated that each layer of a vertical farm can potentially produce annually up to 700 kg of lettuce per m^2 at 500 μmol m^{-2} s^{-1} of continuous light.

In **Chapter 3** lettuce was grown at three planting densities (23, 37 and 51 plant m^{-2}) with and without additional FR to explore the effect of FR on leaf expansion and biomass production. It was hypothesized that the addition of FR increases partitioning to the shoot, resulting in increased biomass production via enlarged leaf area and hence light interception. Plants were grown at 218 μmol m^{-2} s^{-1} red and blue light (RB) with 89% of red (R) and 11% blue (B). The additional FR intensity was 0 or 52 μmol m^{-2} s^{-1}. Adding FR increased the plant dry weight after 4 weeks by 46–77% (largest effect at lowest planting density) and leaf area by 58–75% (largest effect at middle planting density). Radiation use efficiency (RUE: plant dry weight per unit of incident radiation, 400–800 nm) increased by 17–42% and incident LUE increased by 46–77% when FR was added; the largest FR effects were observed at the lowest planting density. Intercepted light use efficiency (plant dry weight per unit of intercepted PPFD) was increased by adding FR (8–23%). Neither specific leaf area nor net leaf photosynthetic rate were influenced by FR. This led to the conclusion that additional FR increased plant biomass production mainly through faster leaf area expansion, resulting in increased light interception. The increased intercepted light use efficiency may also contribute to the increased biomass production.

In **Chapter 4**, if adding FR (700-750nm) to PAR light (400-700nm) is equally efficient in promoting photosynthesis as adding PAR light was explored. Besides quantifying the instantaneous effects of FR on photosynthesis the research was aiming to study if plants acclimate to FR and whether this results in a different photosynthetic response to FR. Finally, research was also aiming to quantify if the radiation use efficiency of plants is higher for plants grown under a mixture of PAR and FR or only PAR. Lettuce plants were grown in a climate chamber at two levels of PPFD (200 and 400 μmol m^{-2} s^{-1}), and at each PPFD level 25% of PAR or FR was added. In all treatments response curves of leaf photosynthesis to additional FR and PAR were determined. Furthermore, based on growth analyses, light and radiation use efficiencies of the plants were determined. Results showed that adding FR on top of red and blue light (RB) increased net photosynthesis rate distinctly, but this increase was to a smaller extent than the increase due to adding RB with the same intensity. At 200 μmol m^{-2} s^{-1} PPFD, the increase in P_n due to adding 50% FR was 39% of the increase due to adding 50% RB, while at 400 μmol m^{-2} s^{-1} PPFD, this increase in P_n by adding 50% FR was 45% of the increase due to adding

50% RB. Hence, when FR is combined with PAR light it is photosynthetically active but not with similar efficiency. Plants grown under FR also had lowered chlorophyll a and b content as well as lower light-saturated photosynthesis rate at all levels of background PPFD – indicating acclimation. Both substituting PAR by FR or adding FR to PAR increased light use efficiency based on incident and intercepted PPFD. Substituting PAR by FR also increased radiation use efficiency.

In **Chapter 5** an experiment was conducted to investigate the effects of gradually increasing or decreasing photosynthetic photon flux density (PPFD) during cultivation compared to a constant PPFD on biomass production. Lettuces were grown in climate rooms, and every three days the PPFD was either increased by 18 μmol m^{-2} s^{-1} (from 140 to 300 μmol m^{-2} s^{-1} from day 0 to 30), decreased (from 300 to 140 μmol m^{-2} s^{-1}), or kept constant (221 μmol m^{-2} s^{-1}), while the total light integral at the end of the cultivation period (30 d) remained the same for all three treatments. Gradually increasing PPFD resulted in a 16 or 12% increase in total plant dry weight compared to treatments with decreasing or constant PPFD, respectively. This increase was explained by a higher light interception, since most of the light was provided at a period of high leaf area index, and because of a higher specific leaf area in the early crop phase. Although LUE based on incident PPFD was highest when PPFD gradually increased, the LUE based on intercepted PPFD was highest when PPFD gradually decreased during cultivation. Despite the higher shoot dry weight during the gradual increase of PPFD, shoot fresh weight was not significantly affected by the light treatments. This difference in response between fresh and dry weight resulted from a higher shoot dry matter content when PPFD gradually increased. Gradually increasing PPFD had a positive effect on dry weight accumulation and increased dry matter content but did not affect shelf life.

In **Chapter 6** the findings of Chapters 2 to 5 were summarized and discussed through the lens of increasing light use efficiency in vertical farms through better understanding the effects of FR (on plant morphology and photosynthesis), PPFD (including daily light integral and photoperiod), and planting density and dynamic lighting effects on biomass production and nutritional quality. Other possible ways of maximizing light use efficiency were proposed and discussed. Suggestions for further research were put forward.

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About the author

Wenqing Jin (金文卿) was born in Changchun, China, on 11th August 1987, from a Korean minority family. He thought he will be a teacher as most of the relatives from his grown-up family are teachers and he never thought to be an agriculture practitioner as he grew up in the city. In 2007, he left Changchun to Beijing for bachelor at college of water resources and civil engineering, China Agricultural University, majored in agricultural facility and environmental engineering and a secondary major of law. In 2011, he started his master program in the same college with specialization of environmental engineering of greenhouse. He did the experiment in a plant factory with artificial lighting and participated a 3-month training program in Chiba University, Japan, before graduation. The training program triggered his passion on greenhouse horticulture and encouraged him got the job in Priva. He started as a sales assistant in Priva Beijing office and grew into an account manager afterwards. He intended to try a different path of career and decided to pursuit further development out of his comfort zone. Fortunately, he got an opportunity of being a PhD in Horticulture and Product Physiology group, Wageningen University. The present thesis is the outcome of his work from the past 5 years (2017 to 2022). Now, he is starting in GreenV indoor, a newly started vertical farming company, as an agronomy manager with all his experience, knowledge and passionate on vertical farming. He will be happy to reach out via jinwenqing@outlook.com.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Title of review / title project proposal (7 ECTS)

- Light use efficiency in vertical farm

Post-graduate courses (3.9 ECTS)

- Fundamentals of crop physiology in a changing world; PE&RC (2018)
- 20th Annual CEAC greenhouse crop production & engineering design short course; Controlled Environment Agriculture Center, Arizona (2021)
- Statistical uncertainty analysis of dynamic models; PE&RC (2017)

Deficiency, refresh, brush-up courses (3 ECTS)

- Crop, physiology and environment; Horticulture and Product Physiology Group, Wageningen university (2017)
- Greenhouse technology; Farm Technology Group, Wageningen University (2018)

Competence strengthening / skills courses (2.85 ECTS)

- Research data management; Wageningen University & Research Library (2017)
- Scientific writing; Wageningen Into Language (2021)
- Adobe InDesign; Wageningen University & Research Library (2021)

Scientific integrity / ethics in science activities (0.45 ECTS)

- Research integrity; Wageningen Graduate School (2017)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC First year weekend (2017)
- PE&RC Day (2019, 2021)

Discussion groups / local seminars or scientific meetings (9.3 ECTS)

- Frontier literature in plant physiology (2017-2019)
- Functional-structure plant modelling discussion group (2017-2020)
- International symposium on environment control technology for value-added plant production (2019)
- The sixth international forum on protected horticulture; Shouguang, China (2021)

International symposia, workshops and conferences (3.8 ECTS)

- Greensys; Angers, France (2019)
- VertiFarm; Wageningen, the Netherlands (2019)

Societally relevant exposure (1.5 ECTS)

- Urban greenhouse challenge (2019-2020)

Lecturing / supervision of practicals / tutorials (0.6 ECTS)

- Advanced methods for plant-climate research in controlled environments (2019)

BSc/MSc thesis supervision (33 ECTS)

- Clara Wedmore Von Baintner: Effect of far-red supplemented fluorescent lighting on the growth of lettuce Lactuca sativa expertise in a closed plant production system (2018)
- Jorge Leigh Urbina: Disentangling the contribution of R:FR to the plant density effect on crop biomass (2018)

- Emilio Villar Alegria: FSPM of shade avoidance response of lettuce under different light treatments and plant densities (2018)
- Michele Butturini: Functional-structural plant modelling of the effect of plant density and far-red light on the transpiration of lettuce grown under indoor farming conditions (2018)
- Julia Winkeler: Functional-structural plant modelling of lettuce in a vertical farm as a function of light interception and photosynthesis (2018)
- Alex van Klink: Developing a dynamic functional-structural plant model of lettuce growing in vertical farming (2019)
- Yang Huang: Effects of dynamic light intensity on the growth of lettuce (2020)
- Yingyu Zhang: Introducing light intensity as a variable into expolinear growth model for lettuce (*Lactuca sativa*) (2021)
- Yawen Gu: Comparison between the effect of supplementary FR and RB under two levels of PAR on lettuce growth, morphology, and postharvest quality (2021)
- Yingyue Peng: FR enhancement and FR acclimation effect on lettuce photosynthesis on top of two levels of PAR light (2021)
- David Formiga Lopez: Meta-analysis of light use efficiency of lettuce and strawberry in vertical farm, greenhouse, and field (2021)

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