A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Growth, Yield, and Fruit Quality of Greenhouse Sweet peppers Grown under Far-red Supplemented Interlighting Conditions

원적색광이 추가된 군락 내 보광 하에서 재배한 온실 착색단고추의 생육, 수확량 및 과실 품질

BY

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AUGUST, 2022

MAJOR IN HORTICULTURAL SCIENCE AND BIOTECHNOLOGY

DEPARTMENT OF AGRICULTURE, FORESTRY, AND BIORESOURCES

THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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UNDER THE DIRECTION OF DR. JUNG EEK SON
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
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ABSTRACT

Far-red light (FR, 700-750 nm) has been used as a supplemental light source for horticultural crops due to its physiological activity related to phytochrome-mediated responses, called 'Shade avoidance response (SAR).' Interlighting is a method of supplemental lighting that has directly compensated for the lack of light in the middle and lower canopies. This study analyzed the effect of interlighting with an additional FR on photosynthesis, yield, and fruit qualities in sweet peppers. In the winter and summer period, three light treatments were treated: natural light (NL), red-blue interlighting (RB), and adding far-red with RB (RBFR). The RBFR was set to adding 55 μmol m^{-2} s^{-1} of FR to 71 μmol m^{-2} s^{-1} of RB (red: blue = 8:2). Daily light integral of NL in winter and summer was 16.6 and 32.2 mol m^{-2} d^{-1}, which was about twice as high in summer. The

shoot dry weight and fruit yields of RBFR were 20% and 17% higher than those in RB in winter, but no significant differences in growth, yield, and morphology were observed in summer. Therefore, the interlighting RB or RBFR did not show any yield increase in the summer of sufficient background light. To examine the adequate FR dose under the RB background, five FR light doses were treated (0, 20, 40, 60, and 80 μmol m^{-2} s^{-1}: FR 0, FR 20, FR 40, FR 60, and FR 80) in winter season. Adding FR to RB increased the total shoot dry mass and the highest under FR 60 of intensity. The three-dimensional simulation revealed that supplemented far-red increased total assimilation due to light-favored morphology. However, FR accelerated the leaf senescence with a faster decrease in photosynthetic capacity of V_{cmax}, and J_{max}, and reducing the total assimilation increment from morphological acclimation. FR 60 also showed a faster yield increase due to accelerated reproductive structure at the initial growth stage. Higher R: FR of solely RB interlighting may be less effective in increasing yield, leading to inefficient responses such as late reproductive development and higher biomass allocation to leaves. Adding FR increased yield from a small dose of FR 20 to FR 80, but saturated at 60 μmol m^{-2} s^{-1} of FR intensity. In addition to yield, fruit quality attributes such as soluble sugar, ascorbic acid, and carotenoid contents were analyzed in two sweet pepper varieties of red and yellow fruits in the winter season. Adding FR had increased yield and individual fruit size, but TSS showed no significant difference by FR. In carotenoids, RB and RBFR were higher than those under

NL, but the increment under RBFR was lessened in red and yellow fruits by 24% and 18% than under RB, respectively. Additional FR lighting may have a trade-off relationship between fruit yield and carotenoid content. This study is meaningful in that the overall effect of adding far-red to red-blue interlighting was evaluated in greenhouses, and the results will help derive an optimal growth of sweet pepper by precisely controlling the lighting spectrum.

Additional keywords: carotenoid, morphology, photosynthesis, ray-tracing simulation

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GENERAL INTRODUCTION

Greenhouse cultivation can provide year-round fruit production with efficient use of resources (Pardossi et al. 2004). However, in the high latitude regions of the Northern Hemisphere, the insufficient amount of light in winter is a limiting factor for the production. Supplemental lighting was introduced to solve the seasonal light deficiency (Heuvelink et al. 2005). The supplemental lighting is generally divided into top-lighting and interlighting depending on where the light is irradiated. The inter or intra-canopy lighting method selectively illuminates the light-limited area in the middle and lower canopies through LEDs to efficiently increase the yield (Hemming 2009). As horticultural light sources, red-blue LEDs have been most used due to their higher photosynthetic quantum yield (Jokinen et al. 2012; Tewolde et al. 2016), but recently, far-red wavelengths (700-750 nm) have been used due to their physiological activation related by plant photoreceptors called 'phytochrome.' Plants determine whether they are sunlit or shaded by the red and far-red light amount absorbed by the phytochromes. Under low red/far-red ratio (R: FR), the plant exhibits 'Shade avoidance response (SAR)' to enhance the fraction of unfiltered sunlight (Franklin 2008).

SAR shows morphological changes such as increased internode length and leaf expansion to trace sunlight and redistribution of plant assimilation products (Casal 2013). These changes in plant morphology could improve the light

interception efficiency of crops and thus increase the total assimilation amount (Sarlikioti et al. 2011). Furthermore, far-red light could increase the amount of photosynthetic assimilation by promoting electron transport by changing the protein balance in the photosystems of leaves (Hogewoning et al. 2012; Zhen and van Iersel 2017). Thus, the actual efficiency of far-red light can be similar to light in the photosynthetically active radiation (PAR, 400-700 nm) range in indoor cultivation (Zhen and Bugbee 2020).

However, in the long-term perspective, far-red light also acts as a signal that accelerates leaf aging (Rousseaux et al. 1996; Rousseaux et al. 2000). Low R: FR or low light intensity promotes a decrease in the chlorophyll and nitrogen content of leaves, which may reduce the assimilation efficiency of the PAR range when using interlighting due to accelerated leaf senescence in the middle or lower canopy (Wherley et al. 2005; Causin et al. 2006; Brouwer et al. 2012). Therefore, adding far-red light should be conducted to comprehensively judge these physiological responses, depending on the crop species or genotypes (Ji et al. 2021).

Several studies have applied far-red LEDs with background light sources such as HPS or red-blue LEDs. (Hao et al. 2016; Kalaitzoglou et al. 2019; Zhang et al. 2019). However, most studies on the far-red supplemental lighting have focused on tomatoes. Sweet pepper is also one of the most cultivated crops produced in greenhouses (Jovicich et al. 2004a), but studies on the far-red light in sweet pepper have been limited (Brown et al. 1995; Schuerger et al. 1997).

Far-red light is abundant in solar radiation and accounts for about 40-60% of photosynthetic photon flux of the red wavelength. The transmittance of far-red light from plant leaves is typically about 30-60%, and the stratified leaves of the higher plant play a role in bringing R: FR variance from the top to the bottom canopies (Smart 1986). The increase in plant leaf area deepens self- or mutual shading to create R: FR variation in the upper and lower parts. Therefore, supplemental far-red effects are changed by the plant growth or the natural light variation (Holmes and Smith 1977). Thus, the seasonal or domestic light environment also could affect interlighting efficiency.

This study analyzed the effect of the additional far-red light to red-blue interlighting on dry mass production, fruit yield, and qualities of sweet pepper. First, it was investigated whether the red-blue interlighting or adding far-red light is effective under the domestic light environment in winter and summer. Second, dry mass production was analyzed under different far-red doses and the appropriate intensity of far-red light on background red-blue light was determined. Finally, the effect of additional far-red on overall fruit qualities and carotenoids were evaluated for red and yellow sweet pepper cultivars.

LITERATURE REVIEW

Plant response to far-red radiation

Light is a source of photosynthesis in plants and a signal that recognizes their environment. Light quality changes in plants are achieved by several photoreceptors, including phytochromes, cryptochromes, phototropins, and the UVR8 family. Phytochrome is a photo-dimeric protein that exists in an active and inactive form (Sharrock 2008). Plant determines their light environment with the equilibrium of the two forms of phytochrome photoreceptor. The leaf surface absorbs the PAR range with a high absorption rate of over 90%, whereas the far-red range of 700-780 nm transmits relatively higher than red (Franklin and Whitelam 2005). Therefore, higher plants recognize the sunlit or shaded condition from the R: FR variance. Phytochrome regulates various developmental responses such as seed germination, flowering time, fruit quality throughout the plant life cycle, and morphological responses such as root elongation, internode elongation, and leaf expansion (Casal 2013). In the long-term aspect, the low R: FR promotes leaf senescence as proteins in old leaves are rapidly reduced for optimal resource distribution in the canopy (Rousseaux et al. 1996; Rousseaux et al. 2000).

Application of far-red light in horticulture

SAR caused under shading in conventional growing conditions may be undesirable for the horticultural crops. Plant development and growth in ornamental or vegetative crops could be controlled by changing the R: FR by artificial LED lighting or shading curtains. In ornamental flowers, the market value of flowers was increased by controlling plant architecture through internode elongation (Mata and Botto 2009), adjusting flowering dates in long-day plants, or inducing floral bud growth (Craig and Runkle 2012). In the greenhouse, simple red-blue light sources limited internode elongation (Islam et al. 2012) and curled leaf morphology (Trouwborst et al. 2010). When using an artificial light spectrum close to natural light, a desirable growth response of crops was shown, and a similar plant response can be induced by adding far-red light to red or blue (Hogewoning et al. 2010; Kang et al. 2020). Far-red light mainly promoted the morphological changes in plants (Meinen et al. 2012), and the changed plant architecture may also associate with an increase in total plant dry matter production in tomatoes (Kalaitzoglou et al. 2019; Zhang et al. 2019). In fruit quality, an increase in the assimilation product partitioning to fruit has been reported by far-red light as overexpression of genes related to carbon metabolism in tomatoes (Ji et al. 2019, 2020). In addition, the improvement of physiochemical properties of tomato fruits was reported under far-red supplementation (Kim et al. 2020).

Supplemental inter or intra-canopy lighting

Additional light is essential for high crop yields in greenhouse cultivation because natural sunlight is a regional and seasonal limitation factor (Hao and Papadopoulos 1999; Trouwborst et al. 2010). Supplemental lighting can be divided into two methods: top-lighting, which illuminates the upper part of the crop, and the inter- or intra-canopy method, which is illuminating light inside the canopy or between the canopy of the plant rows (Kumar et al. 2016). Due to the characteristics of greenhouses, where crops are grown at high planting density, the lower part of the crop is intensely shaded (Monsi and Saeki 2005). Therefore, the interlighting method was introduced to provide light in the middle part of the crop with a higher leaf area but a strongly shaded region (Hovi-Pekkanen et al. 2005). Interlighting mainly used wavelengths of red-blue LEDs with high photosynthetic quantum yield (McCree 1971) under the assumption that far-red light was already abundant in the lower canopy. Thus, the appropriate light red-blue ratio was explored without far-red (Kaiser et al. 2018). Recent studies have introduced far-red light into the intra-canopy lighting to enhance plant growth through the higher physiological activity of far-red light (Kim et al. 2019, 2020). However, the interlighting effect is dependent on natural irradiance level and plant growth stage during the interlighting period (Tewolde et al. 2016). Therefore, it is necessary to analyze the effect of red-blue interlighting with the additional far-red conditions according to the different cultivation conditions.

Growing sweet pepper in a greenhouse

Sweet pepper is one of the most-consumed vegetables worldwide due to its taste and higher nutrient values, such as vitamin C and carotenoid content (Kim et al. 2011). In addition to the sensory attributes, health properties, such as vitamin C and carotenoids, also emerge as factors determining quality of sweet pepper. Carotenoids determine the color of the fruit surface and function as antioxidants for scavenging free radicals that help protect cells from oxidation damage. In terms of the human diet, carotenoids are essential and broken down into provitamin A to improve eye health and decrease the risk of cardiovascular disease and cancers (Maoka et al. 2001; Abdel-Aal et al. 2013). Most sweet pepper production is concentrated in greenhouses, and it is crucial to maintain a year-round constant output (van Houten et al. 1995; Jovicich et al. 2004a). The sweet pepper plant is vertically trellised (Jovicich et al. 2004b), with one fruit on each node to control uniform fruit quality. For pruning, it is common to remain two leaves individually main shoot and lateral branch at each node. Therefore, supplemental lighting could assist in steadily producing high-quality fruits by supplementing the insufficient light amount in winter.

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CHAPTER 1

Growth and Yield of Sweet Peppers subjected to Far-red Supplemented Interlighting in Winter and Summer

ABSTRACT

Far-red light has been included in horticultural light sources due to its physiological activity related to phytochrome-mediated responses. Recent studies have shown that adding far-red light to red-blue interlighting LEDs improves the growth and production of greenhouse tomatoes. However, in long-term cultivation, the effects of interlighting with the additional far-red light may vary according to the seasonal light environment. This study aims to investigate the effect of adding far-red light to red-blue interlighting on the growth and yield of sweet pepper in winter and summer. Sweet pepper (*Capsicum annuum* L. 'Kori') plants were grown in greenhouses for five months under average daily light integrals of 16.6 and 32.2 mol m^{-2} day^{-1} in winter and summer, respectively. The interlighting with adding 55 μmol m^{-2} s^{-1} of far-red light to 71 μmol m^{-2} s^{-1} of red-blue light (red: blue = 8:2). The shoot dry weight and fruit yields were 20% and 17% higher, respectively, with additional far-red in the winter, but no significant differences in growth, yield, or morphology were observed in the summer. In summer, stem length and leaf

area of plants increased rapidly due to sufficient sun irradiance, resulting in a higher far-red fraction at the bottom canopy. Thus, the far-red fraction at the middle and bottom canopies would have been sufficient with sunlight alone. Moreover, additional far-red interlighting promotes a decrease in leaf photosynthetic capacity in the bottom canopies. Therefore, plant growth and morphology were not influenced by adding far-red to interlighting in the summer in the mid-latitude regions. This study concluded that the additional far-red could contribute to the growth and yield of sweet peppers in insufficient light conditions.

Additional keywords: far-red fraction, intra-canopy lighting, photosynthesis, supplemental lighting

INTRODUCTION

Over the past decade, light-emitting diodes (LEDs) have been used as supplemental light sources in greenhouses to compensate for low light intensity on crops grown with a high planting density. In particular, an interlighting method that places light sources inside the canopy effectively replenishes insufficient light conditions in the canopy for high-wired plants such as tomatoes, cucumbers, and sweet peppers (Trouwborst et al. 2010; Joshi et al. 2019; Paucek et al. 2020).

Red-blue LEDs are mainly used as interlighting sources because they can efficiently induce photosynthesis by matching the action spectrum of plant chlorophyll a and b (Pfündel and Baake 1990). Furthermore, red-blue light mediates a wide range of photosynthetic responses such as stomatal opening and chlorophyll synthesis in response to phytochromes and cryptochromes photoreceptors (Tennessen et al. 1994; Lin 2002; Shimazaki et al. 2007; Lin et al. 2013). For these reasons, red-blue wavelengths have been widely used for indoor cultivation (Wang et al. 2016; Pennisi et al. 2019) and greenhouse interlighting (Kaiser et al. 2018b). Far-red (FR) light is also a vital signal that induces various plant physiological responses recognized by the phytochrome (Sharrock 2008), which is a dimeric protein capable of interconverting between the inactive P_r (red light absorption) and physiologically active P_{fr} (far-red light absorption) forms (Klose et al. 2015). Far-red light activates phytochrome-

mediated downstream responses called shade avoidance responses, including stem and petiole elongation and leaf expansion (Casal 2013). The addition of far-red light to red-blue LEDs could increase net photosynthetic assimilation through efficient radiation capture by seedlings after morphological acclimation to low R: FR conditions (Park and Runkle 2017). In addition to morphological changes, the FR fraction enhances the photosynthetic rate by irradiating with red light (Emerson and Rabinowitch 1960). Far-red light changes the quantum yield of two types of photosystems, photosystem I (PS I) and photosystem II (PS II) (Hogewoning et al. 2012), promoting the positive effect of short and long-term light reactions by increasing PSII photochemistry and accelerating relaxation of non-photochemical quenching from solar irradiance (Kono et al. 2020; Zhen and van Iersel 2017). Adding FR light to red-blue light altered reproductive growth by mediating the overexpression of genes related to fruit assimilation metabolism, accompanying an increased dry matter fraction in fruits (Kim et al. 2019; Ji et al. 2020).

Due to FR light activating various positive physiological processes, several studies have tried adding FR to red-blue light for tomato production as top-lighting (Kalaitzoglou et al. 2019; Kim et al. 2019; Zhang et al. 2019; Ji et al. 2020). FR light has also been introduced as an interlighting source for the high-wired crop in greenhouses (Kim et al. 2020). For the interlighting spectrum, the use of red-blue LEDs alone led to impaired morphogenesis, such as leaf curling, which inhibits plant growth (Trouwborst et al. 2010). Plants therefore require

suitable ratios of blue, red, and FR light for desirable photoreceptor-mediated morphogenesis (Bantis et al. 2018; Kaiser et al. 2018b). However, the effect of interlighting could vary by region or season due to the natural light environment in greenhouses (Solbach et al. 2021). The intensity and ratio of red and FR light in natural irradiance changes seasonally due to solar altitude (Stanhill and Cohen 2001; Tewolde et al. 2016; Wald 2018). Difference in natural light has led to cases where interlighting with red-blue light was effective (Guo et al. 2016a; Kaiser et al. 2018b; Paponov et al. 2019; Paucek et al. 2020) as well as ineffective (Trouwborst et al. 2010; Tewolde et al. 2016). Palmitessa et al. (2020) reported supplemental interlighting with FR light did not affect growth and yield at Mediterranean latitudes. In order to re-analyze the conflicting results of previous studies, it is necessary to assess the effect of interlighting with or without additional FR light under a seasonally heterogeneous light environment.

Thus, interlighting with additional FR light might lead to differential growth, morphology, or photosynthetic acclimation patterns in summer compared to winter cultivation periods. This study aimed to investigate the effect of adding FR to interlighting on growth, yield, and leaf photosynthesis of greenhouse-grown sweet pepper both in summer and winter.

MATERIALS AND METHODS

Plant material and cultivation condition

Six-week-old sweet pepper seedlings (*Capsicum annuum* L. 'Kori') were transplanted on rockwool substrates (Grodan GT Master, Grodan, Roermond, The Netherlands) on August 21, 2019 (for winter cultivation) and on February 26, 2020 (for summer cultivation) in a Venlo-type glasshouse at Seoul National University, Suwon, Korea (37.2°N, 126.9°E). Nutrient solutions with 2.5 dS m^{-1} EC and 5.5 pH were applied with macronutrient concentrations of 10.28 mM NO_3^-, 0.91 mM NH_4^+, 0.84 mM PO_4^{3-}, 6.8 mM K^+, 2.20 mM Ca^{2+}, 1.25 mM Mg^{2+}, 1.25 mM SO_4^{2-}, and micronutrient concentrations of 12.9 μM Fe^{2+}, 2.24 μM Mn^{2+}, 1.42 μM Zn^{2+}, 3.6 μM BO_3^{3-}, 0.19 μM Cu^{2+}, and 0.2 μM MoO_4^{2-}. Each plant was drip-irrigated with 66 mL of nutrient solution whenever the accumulated solar radiation reached 0.5 MJ m^{-2}. At 20 days after transplanting (DAT), the nutrient solution concentration was adjusted to 2.8-4.0 dS m^{-1} according to vegetative or reproductive growth stages. The day/night temperatures in the greenhouse were generally maintained at 26°C/20°C and 28°C/22°C in winter and summer seasons, respectively. The greenhouse temperature was controlled by fans and sunscreens. The fans were operated by the set temperature with thermostats. Greenhouse heating was operated when the night temperature was below 20°C through the hot water pipes. The plants were grown at a planting density of 3.3 plant/m^2 before developing axillary

buds. After bud development, each plant was divided into two main stems, vertically trellised to a 'V' system (Jovicich et al. 2004). Sweet pepper fruits were harvested three times a week when the overall fruit color turned red.

Interlighting treatments

Three interlighting treatments were applied: natural light (NL, control), natural light with red (R, peak wavelength at 660 nm) and blue (B, peak wavelength at 450 nm) LEDs (RB), and natural light with RB and far-red (FR, peak wavelength at 730 nm) LEDs (RBFR). Two interlighting bars were installed between the V-shaped stems parallel to the cultivation bed. The irradiation angle of the light source was at 0 degrees, and irradiating light from both sides. The greenhouse spaces of 15 × 9 m were divided into three parts, and each sector was separated with an impermeable plastic film to prevent interference from light sources. The photosynthetic photon flux density (PPFD, 400-700 nm) of the single red-blue LED bar was adjusted to 71 μmol m^{-2} s^{-1} at a distance of 20 cm, which was measured by a portable spectrometer (LI-250A, LI-COR, Lincoln, NE, United States). The RB consisted of 80% red and 20% blue in PPFD, respectively, and the FR was supplemented to the RB in RBFR (Fig. 1-1A). The ratio of FR to RB was adjusted to 0.69 according to the phytochrome photostationary state (PSS) value by Sager et al. (1988), which showed a higher FR fraction than that in sunlight. In RBFR, the adjusted red/far-red ratio (R: FR) of light source was 1.01 (Table 1-1). The LED modules were installed at the

height of 0.9 and 1.1 m from the cultivation bed, and the interlighting treatments started when plant height reached about 1 m, when DATs were 58 and 45 in winter and summer, respectively. All interlighting treatments were carried out for 12 hours of daylength, from 6:00 to 18:00.

Light properties for winter and summer

The light intensity in the greenhouse was measured at the top of the canopy using a quantum sensor (SQ-110, Apogee Inc., North Logan, UT, United States) and collected with a data logger (CR1000, Campbell Scientific, North Logan, UT, United States). During the experimental period, the light intensity emitted from the interlighting contributed to 18.4% and 9.5% of the total daily light integral (DLI) in winter and summer, respectively (Table 1-1). Spectral properties of the canopy were measured with a portable spectroradiometer (C-7000, SEKONIC, Tokyo, Japan). Spectral measurements were carried out at the top, middle, and bottom parts of the canopy for each treatment on a clear day in the middle of the experimental period. Spectrum measurements were repeated at least five times for each canopy level.

Sampling for morphology, destructive sampling, and yield measurements

Plants sampling were conducted twice, at the middle and at the end of the growing period (80 and 120 DATs, respectively). Ten plants were sampled for each treatment. The vegetative organs were divided into leaf, stem, and petiole.

Morphological traits such as plant height and leaf area were measured. The leaf area was determined using a leaf area meter (LI-3000A, LI-COR). The dry masses of leaf, stem, petiole, and fruit tissues were measured after drying for 72 hours at 105°C in a forced-air drying oven (HB-503LF, Hanbaek CO. LTD, Bucheon, Korea). The specific leaf area (SLA) and specific stem length (SSL) were defined as ratio of leaf area and stem length to each organ's dry mass, respectively (Poorter et al. 2012). For the fruit yield, 36 plants per treatment were investigated during the whole growth period, excluding samples for destructive measurement. Individual fruit fresh weight was measured with a weighing scale 24 hours after harvesting.

Measurement of leaf photosynthetic capacity

Leaf photosynthetic rates were measured a week before the final harvest. Leaf positions were selected by numbering nodes from the fully expanded leaf closest to the meristem. The photosynthetic rates at the top, middle and bottom positions were measured on the 3^{rd}, 15^{th}, and 27^{th} node leaves, respectively. The light-response curves were measured using a gas exchange system (LI-6400XT, LI-COR) with a 2 × 3 cm leaf LED chamber (6400-02B, LI-COR). The light response curve of the leaves at the top, middle, and bottom positions was determined by reducing PPFD to 1,500, 1,200, 900, 600, 400, 200, 100, 50, and 0 μmol m^{-2} s^{-1} at an ambient CO_2 concentration of 400 μmol mol^{-1}, a leaf temperature of 25°C, and relative humidity of 60%.

Table 1-1. Interlighting treatments at different growth periods of sweet peppers

Treatment		PPFD[z] (unit)	Average DLI (unit)	Ratio of interlighting PPFD to natural light PPFD (%)	R/FR ratio	PSS
Natural light[y] (Control)	Winter	854.43	16.61	18.48	1.15 b	0.72 b
	Summer	1,256.52	32.21	9.53	1.25 a[x]	0.73 a
Interlighting	RB[w]	71.04	3.07	-	-	0.88 a
	RBFR	71.31	3.08	-	1.01	0.69 b

[z]PPFD, DLI, and PSS are photosynthetic photon flux density, daily light integral, and phytochrome photostationary state (Sager et al. 1988), respectively.

[y]Natural light was measured with a zenith direction from the greenhouse floor (n = 5), and PPFDs in winter and summer were measured at 12:00 on sunny days (Nov. 19 and May 20, respectively) in the middle of the experimental period.

[x]Different small letter denotes significance tested with Tukey's HSD test, $P < 0.05$ (n = 5)

[w]RB, RBFR, and natural light mean sunlight with red-blue, red-blue and far-red LEDs, and sunlight only, respectively.

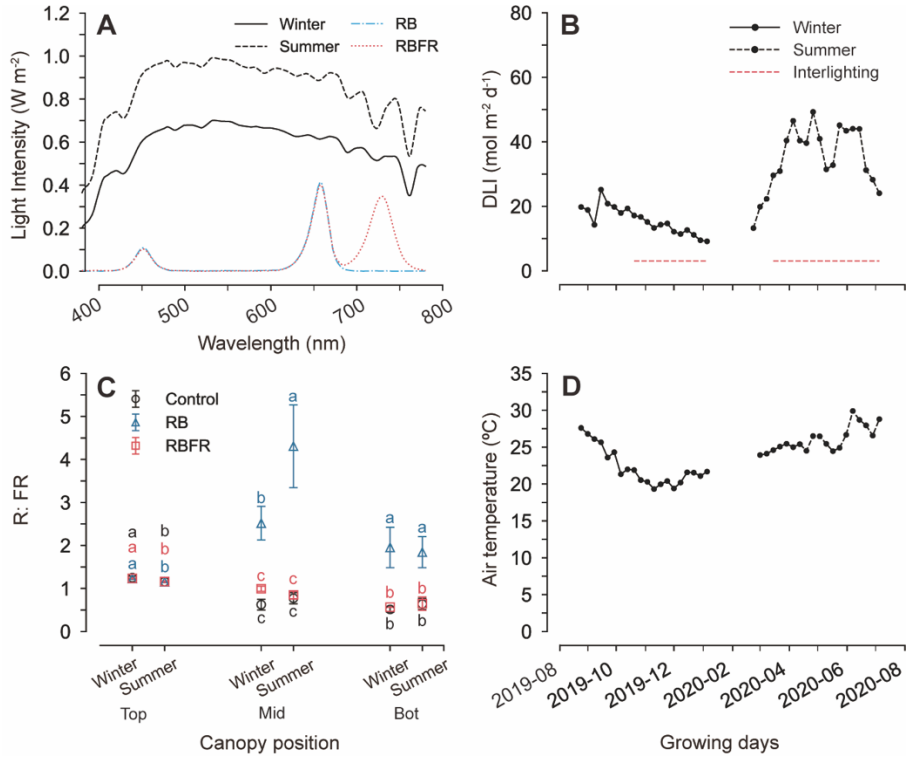

Fig. 1-1. Environmental conditions in the greenhouse during winter and summer periods: Spectral distributions of sunlight (at noon on Nov. 19 and May 21, respectively) and light-emitting diodes (LEDs) for interlighting (A), daily light integral (DLI, B), red/far-red ratio (R: FR) in the canopy (C), and air temperature (D). RB, RBFR, and control mean sunlight with red-blue, red-blue, and far-red LEDs, and sunlight only, respectively. Vertical bars indicate the mean ± SD (n = 5). Refer to Fig. 1-1 for the spectral distribution of the treatment.

The light response curves were measured by acclimating the leaves for 20 minutes at a PPFD of 1,500 μmol m^{-2} s^{-1} until the stomata were stabilized and gradually lowered light intensities to each PPFD measurement point. Each point proceeded to the next measurement point with an automated program of the LI-6400XT when the standard deviation of the photosynthesis and stomatal conductance values were less than 0.5 and 0.1, respectively, for 20 seconds.

Pigment analysis in leaves

The sampled leaves were dissected into 1 cm^2 and extracted with 3 mL N, N-dimethylformamide (Samchun pure chemical, Pyeongtaek, Korea). After extracting the leaf pigment for 72 hours in the dark at 25°C, the absorbance of the extracts was measured at 663.8, 646.8, and 480 nm using a UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States), and the concentration of chlorophyll a, b, and carotenoids were determined according to Wellburn (1994). The pigment samples at the top, middle, and bottom positions were picked on the 3rd, 15th, and 27th node leaves from the meristem.

$$Chl\ a = (12 * A_{664}) - (3.11 * A_{647}) \quad \text{Eq. 1-1}$$

$$Chl\ b = (20.78 * A_{647}) - (4.88 * A_{664}) \quad \text{Eq. 1-2}$$

$$\text{Total carotenoids} = (1000 * A_{480} - 1.12 * Chl\ a - 34.07 * Chl\ b)/245 \quad \text{Eq. 1-3}$$

Experimental set-up and statistical analysis

In order to minimize the interference of natural light, the treatment was arranged in the north-south direction and used all of the greenhouse sections from front to the end. The location of treatments was randomly determined. The experiment was conducted on a single plot to prevent environmental interference between neighbor plants. The mean and standard error of ten plants in one plot was calculated, and an analysis of variance (ANOVA) was performed. One-way ANOVA was conducted using R software version 3.6.1 and 'Agricolae' packages. For the post-hoc test, Tukey's test ($P < 0.05$) was used when the number of experimental groups was the same, and in other cases, the Bonferroni correction test ($P < 0.05$) was used. All graphics are plotted with python library 'Matplotlib,' version 3.4.3.

RESULTS

Plant morphology

In winter, the stem length per plant in the RBFR was 190.1 cm at the final harvest (140 DAT), which was longer than the 129.2 and 161.4 cm observed for the RB and control, respectively (Fig. 1-2). Leaf areas per plant were higher than the control in both RB and RBFR. At the intermediate harvest in winter, the RBFR showed a greater leaf area than the control (Fig. 1-2). Specific stem length showed no significant difference between the treatments. In winter, specific leaf area was significantly lower in the RBFR at the final harvest compared to control and RB. In summer, specific leaf area was lower in both RB and RBFR than the control at intermediate harvest. There was no significant difference in stem length and leaf area between the treatments in summer (Fig. 1-2), but specific leaf area was higher in control at the intermediate harvest.

Dry weight by plant organ

In winter, the dry weights of leaf, stem, and petiole were higher in RB and RBFR than in control, with stem dry weights in the RBFR being approximately 29% and 61% higher than in the RB and the control, respectively, at final harvest (Fig. 3). In summer, the dry weights of vegetative organs such as stem, leaf, and petiole were not significantly different between the treatments (Fig. 3). In winter, total shoot dry weight was 20% higher in RBFR compared to RB

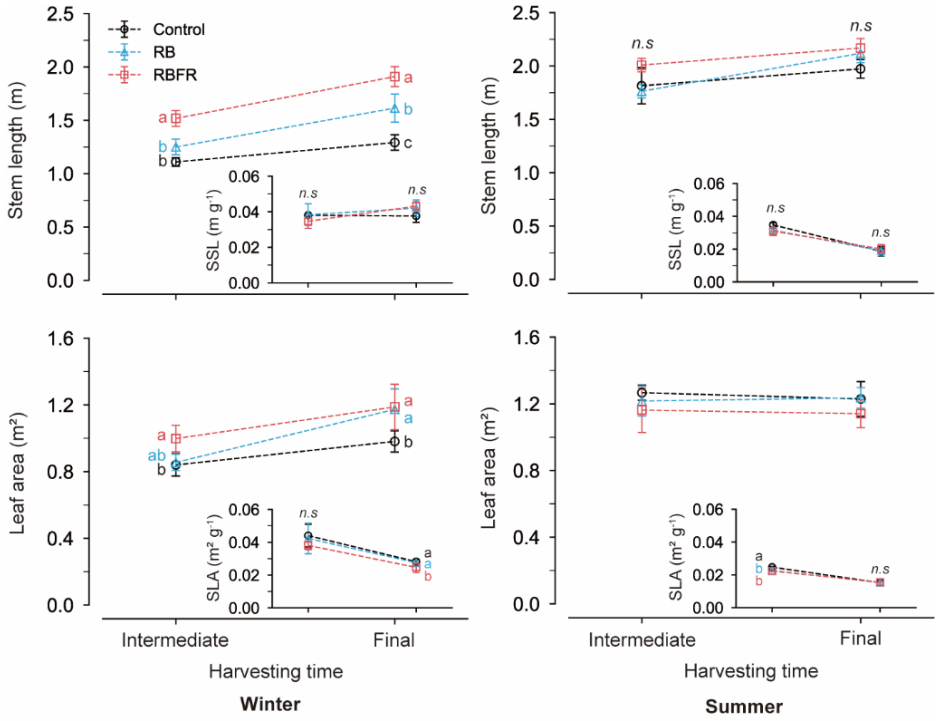

Fig. 1-2. Intermediate and final growth of sweet peppers in winter and summer periods at different interlighting treatments. SSL and SLA mean specific stem length and specific leaf area, respectively. Vertical bars indicate the mean ± SD (n = 10). The *n.s* indications are not significantly different for each parameter of figures according to harvesting time (Tukey's HSD test, $P < 0.05$). RB, RBFR, and control mean sunlight with red-blue, red-blue and far-red LEDs, and sunlight only, respectively. Refer to Fig. 1-1 for the spectral distribution of the treatment.

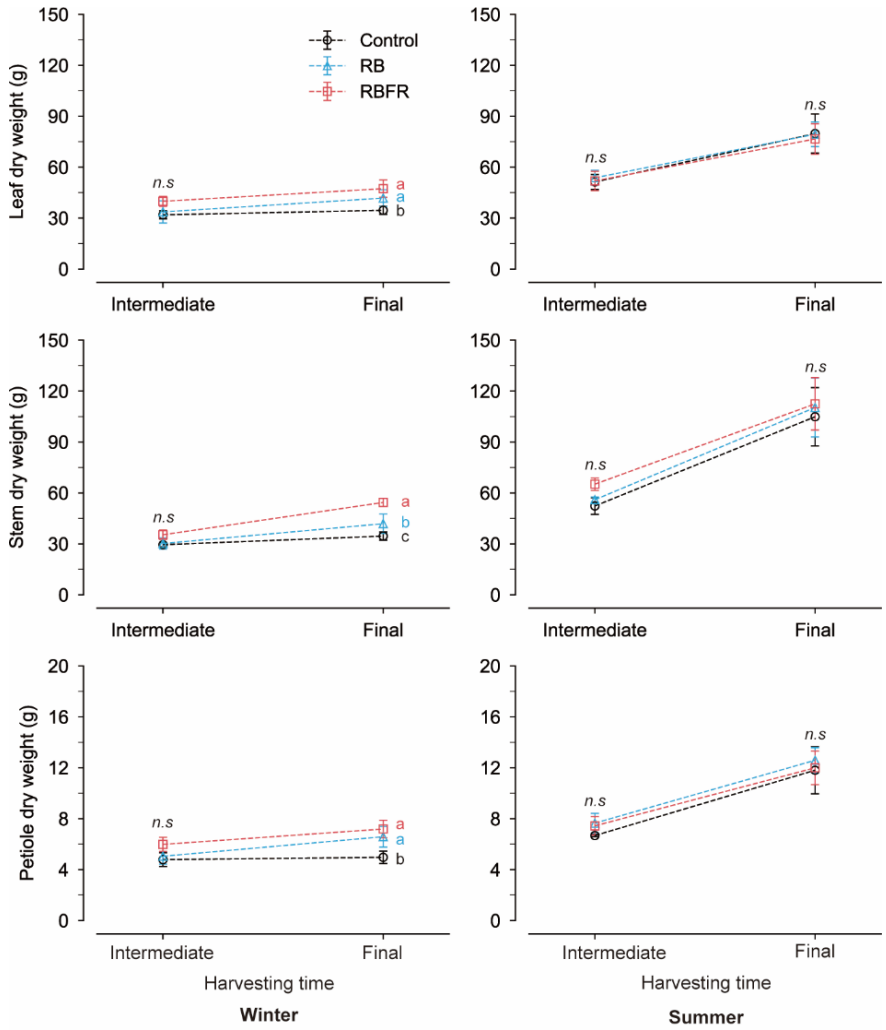

Fig. 1-3. Intermediate and final dry mass of sweet peppers in the winter and summer at different interlighting treatments. Vertical bars indicate the mean ± SD (n = 10). Different letters of the figure mean significantly different for each organ's dry weight according to interlighting treatment (Tukey's HSD test, $P < 0.05$). RB, RBFR, and control mean sunlight with red-blue, red-blue and far-red LEDs, and sunlight only, respectively. Refer to Fig. 1-1 for the spectral distribution of the treatment.

(Fig. 4). Total dry weight, including fruit, was 25% and 51% higher in RBFR than in control and RB, respectively (Fig. 4). In summer, fruit dry weight was significantly higher in RBFR than in control, but RB showed no significant difference despite the same PPFD intensity ($P = 0.12$) (Fig. 4).

Leaf photosynthetic capacity and pigment content

Leaf photosynthetic rates tended to decrease from the top to the bottom canopy in both winter and summer (Fig. 1-5). There were no significant differences in leaf photosynthetic rate for each treatment in winter (Fig. 1-5B). In summer, leaf photosynthetic rates at the bottom canopy were lower in RBFR than in RB, while no significant difference was observed in other canopy positions. RB showed higher leaf chlorophyll contents than in RBFR by approximately 14% and 19% at the bottom canopy in winter and the middle canopy in summer, respectively (Fig. 1-6). Carotenoid content showed no significant difference between the treatments. The chlorophyll a/b ratio in control was significantly higher than in RB and RBFR at the top canopy during winter.

Fruit production

In winter, fruit yield was highest in RBFR and lowest in control, with each treatment showing a significant difference (Table 1-2). RBFR displayed a 17% higher fruit yield than RB. Individual fruit fresh and dry weights were significantly higher in RBFR than those in RB.

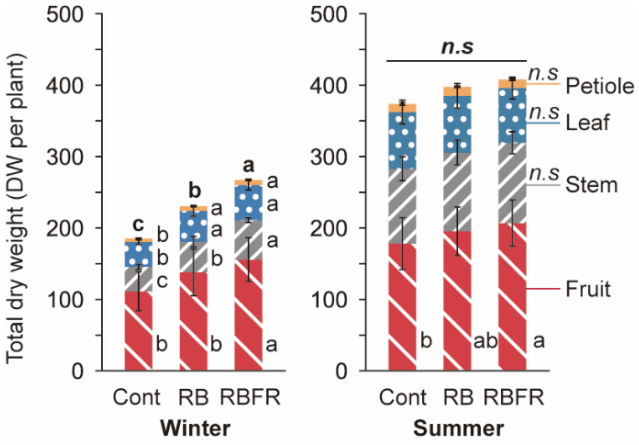

Fig. 1-4. The total and each organ dry mass of sweet peppers at the final harvest at different interlighting treatments. Different regular letters on the right side of the bar figure mean significantly different for each organ parameter according to interlighting treatment, and bold letters mean significance for the total dry weight of organs between the treatments (Tukey's HSD test, $P < 0.05$). RB, RBFR, and control (Cont) represent sunlight with red-blue, red-blue and far-red LEDs, and sunlight only, respectively. Refer to Fig. 1-1 for the spectral distribution of the treatment.

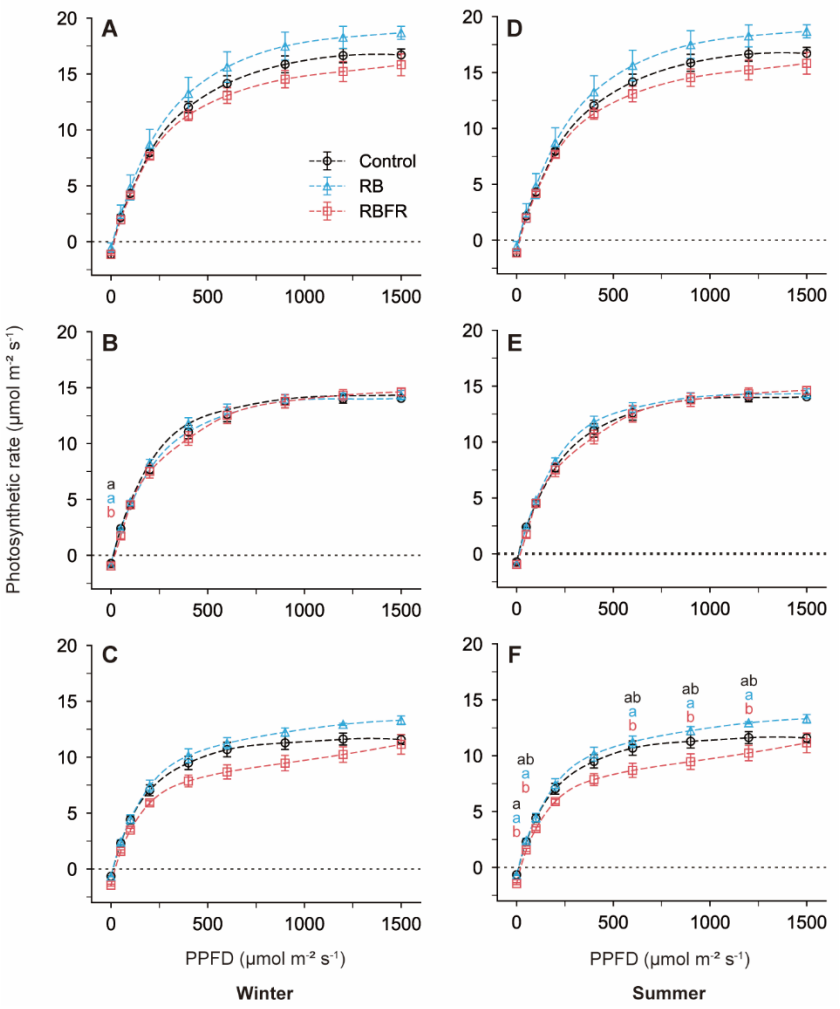

Fig. 1-5. Photosynthetic response curves of sweet pepper leaves to photosynthetic photon flux density (PPFD) at different interlighting treatments. A, B, and C are the measurement results at the top, middle, and bottom canopies of sweet peppers in winter, and D, E, and F in summer, respectively. Vertical bars indicate the mean ± SEM (n = 4). Small letters mean significance for the photosynthetic rate values between the treatments (Tukey's HSD test, $P < 0.05$). RB, RBFR, and control represent sunlight with red-blue, red-blue and far-red LEDs, and sunlight only, respectively. Refer to Fig. 1-1 for the spectral distribution of the treatment.

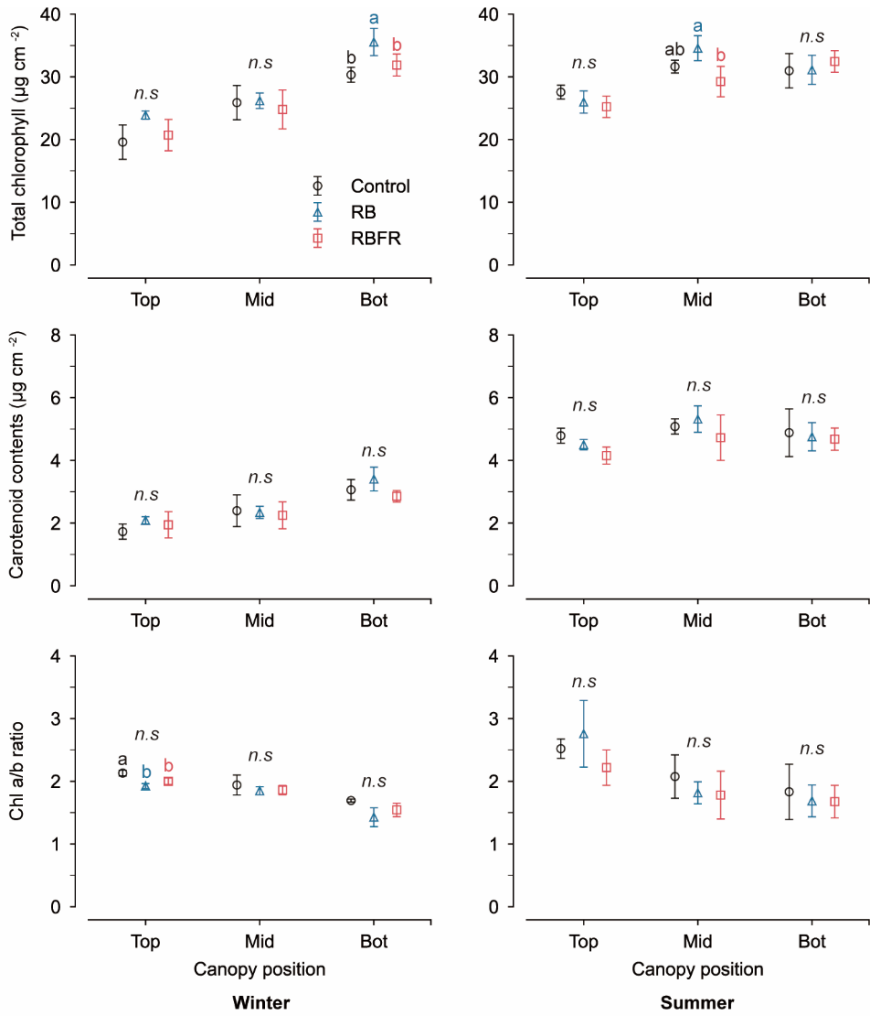

Fig. 1-6 Leaf pigment content to canopy positions (top-Top, middle-Mid, and bottom-Bot) in winter and summer periods at different interlighting treatments. RB, RBFR, and control represent sunlight with red-blue, red-blue and far-red LEDs, and sunlight only, respectively. Vertical bars indicate the mean ± SD (n = 9). Small letters mean significance for the photosynthetic rate values between the treatments (Tukey's HSD test, $P < 0.05$). Refer to Fig. 1-1 for the spectral distribution of the treatment.

Table 1-2. Fruit fresh and dry weights of sweet peppers at different interlighting treatments in winter and summer

Treatment	Winter			Summer		
	Fruit yield (kg m^{-2})	Fruit weight (g/fruit)	Dry weight (g/fruit)	Fruit yield (kg m^{-2})	Fruit weight (g/fruit)	Dry weight (g/fruit)
Natural light (Control)	5.4 c[z]	162.0 b[y]	16.8 b[x]	8.4 a	159.5 b	16.4 b
RB[w]	6.5 b	164.6 b	16.9 b	9.3 a	165.2 ab	17.1 ab
RBFR	7.5 a	172.8 a	18.2 a	9.4 a	169.5 a	17.9 a

[z]Upper letters of fruit yield values denote significance tested with Tukey's HSD test, $P < 0.05$ (n = 32).

[y]Upper letters of individual fruit fresh weight denote significance tested with the Bonferroni correction test, $\alpha = 0.05$ (n = 260, 315, 349, 424, 451, and 444 in the order of winter and summer in the control, RB, and RBFR, respectively).

[x]Upper letters of individual fruit dry weight denote significance tested with Tukey's HSD test, $P < 0.05$ (n = 30).

[w]RB, RBFR, and control mean sunlight with red-blue, red-blue and far-red LEDs, and sunlight only, respectively.

In summer, RB and RBFR showed no significant differences in fruit yield or individual fresh and dry weights. RB and RBFR showed slightly higher fruit yields by 10% and 11%, respectively than the control, but this was not a significant difference (P = 0.067 and 0.1, respectively). RBFR showed increased individual fruit fresh and dry weights compared to the control, regardless of season.

DISCUSSION

Supplemental lighting in greenhouses is not always necessary for regions with abundant sunlight. However, in high latitude regions, the external light environment drastically changes with the season, acting as a limiting factor for the yield of glasshouse cultivation (Cockshull et al. 1992). Recently, interlighting has been used to improve the uneven light distribution in the canopy by arranging LED light sources at the middle or bottom of the canopy (Tewolde et al. 2016). In addition to increasing the amount of light, optimization of the spectral composition of the light is also important, for instance by determining optimal red-blue ratios (Kaiser et al. 2018b) or adding green or FR light (Paradiso and Proietti 2021). Recently, several studies have demonstrated improvements in tomato growth and fruit yield by adding FR to RB light (Ji et al. 2019; Kim et al. 2019). FR light elicits various physiological responses by inducing the shade avoidance response in plants (Casal 2013). However, the plant response to FR light may vary depending on the ambient light environment outside greenhouses (Kalaitzoglou et al. 2019). This study analyzed how the growth and yield of sweet peppers change under different light environments during the winter and summer when FR supplemented with RB interlighting in greenhouses.

Microclimate in greenhouses

In the past, supplemental lighting frequently caused an increase in leaf temperature because of the traditional light sources, such as high-pressure sodium lamps, considered the peak wavelength around 815 nm as infra-red or heat-radiation (Guo et al. 2016b). In this study, the additional far-red LEDs was adopted with a peak wavelength of around 730 nm (Fig. 1-1A), and no heat was generated from the light spectrum. The LED upper board generated some heat that did not affect the leaf and air temperature of the canopy microclimate (Fig. A1-1).

Morphology and vegetative growth under adding FR to interlighting

RBFR could lead to different physiological and morphological responses compared to solely RB interlighting due to the R: FR (Huché-Thélier et al. 2016). The R/FR ratio increased in RB at the middle canopy where natural light was relatively insufficient, while similar or slightly higher R: FR was shown in RBFR (Fig. 1-1C). Despite the same interlighting treatments, relatively higher R: FR was observed in winter compared to summer for RB. In natural vegetation, preferential absorption of the red-blue light regions by selective transmittance of leaf pigments leads to the enrichment of less-absorbed spectra, such as green and FR regions (Franklin 2008). In summer, the fraction of the FR light transmitted to the lower part of the canopy was relatively high due to ample natural light (Kotilainen et al. 2020).

For this reason, the light responses of sweet pepper plants with interlighting differed depending on the season. First, differences in morphology and growth from FR lighting were found only in winter. The growth of vegetative organs was not different between the treatments in summer, which appears to be because interlighting DLI accounts for only 10% of the total light input during the entire growth period (Table 1-1). Similarly, interlighting would be ineffective when light is sufficient, such as in the northern hemisphere's summer (Tewolde et al. 2016) and Mediterranean greenhouses (Palmitessa et al. 2020). Regardless, adding FR light to RB did not affect vegetative growth due to sufficient FR in the natural light during summer. Only fruit production was approximately 10% and 11% higher in the RB and RBFR, respectively, than in control. On the other hand, in winter, RBFR showed higher shoot growth than RB due to a lower R: FR fraction (Fig. 1-3). Higher shoot growth under low R: FR accompanied morphological differences such as higher leaf area and longer internodes (Zhang et al. 2019). These morphological changes due to shade avoidance responses may have favored light interception and increased the gross assimilation by plants (Sarlikioti et al. 2011; Strauss et al. 2020). In this regard, plant growth and dry mass accumulation were facilitated with an additional FR light despite the same amount of photosynthetically active radiation (PAR, 400-700 nm) from the light sources (Park and Runkle 2017; Ji et al. 2019; Zhang et al. 2019).

In addition to the morphological aspect of FR, recent studies reported that

FR photons have the same photosynthetic efficiency as shorter PAR wavelengths (Zhen and Bugbee 2020a). Thus, FR does not contribute to photosynthesis alone, but a combination of R and FR irradiance could synergistically enhance leaf-level photosynthesis (Emerson and Rabinowitch 1960). The photosystem of the light-harvesting complex consists of two reaction centers, PS I and PS II. The optimal spectra for PS I and PS II have asymmetries at 680 and 700 nm, respectively, due to their different pigment compositions (Evans 1986). Excitation experiments of the photosystems found that photochemical efficiency could be increased by adding FR photons with a longer wavelength to overexcite PSI compared to using only the 400-680 nm region of PAR (Hogewoning et al. 2012; Zhen and van Iersel 2017). Zhen and Bugbee (2020a) suggested that an additional FR could act as a photosynthetic source in higher plants, such as tomatoes and cucumbers. Considering this, direct enhancement from the additional FR is a factor in the higher total dry weight in RBFR compared to RB interlighting (Fig. 1-4). The increase in sweet pepper growth from the additional FR with solely RB or HPS light is the same as previously reported in cucumber and tomato (Kim et al. 2019; Meinen et al. 2012). Nevertheless, the enhancement of total assimilation under FR is difficult to analyze because morphological, photosynthetic acclimation and direct enhancement of photosynthesis from FR fractions are complexly intertwined. In summer, the RB and RBFR did not contribute to an increase in the vegetative growth due to abundant solar irradiance (Fig. 1-1B). Therefore, seasonal or

regional characteristics can affect the efficacy of interlighting.

Decreased photosynthetic capacity of the bottom canopy from RBFR interlighting in summer

This study analyzed whether the adding FR interlighting effect differs from conventional RB lighting on leaf photosynthesis and pigmentation in sweet pepper. In both seasons, photosynthetic capacity gradually decreased from the top to the bottom canopy leaves (Fig. 1-5). In greenhouse sweet pepper, as the leaf age naturally increases from the top to the bottom canopy, the photosynthetic capacity decreases from the top to bottom canopy during the ontogenetic process (Detto and Xu 2020). Numerous studies have revealed that light signaling from red or far-red is associated with ontogenetic leaf senescence (Sakuraba 2021), and thus the photosynthetic capacity of the canopy may vary under RB or RBFR interlighting. In summer, leaf photosynthetic capacity was lower in RBFR than RB at the bottom canopy (Fig. 5F). The rapid decline in the photosynthetic rates of RBFR at bottom canopies means that leaf senescence was accelerated under the higher far-red fractions (Rousseaux et al. 1996), as observed in other plants such as sunflower and tomato (Rousseaux et al. 2000; Ji et al. 2019). Chlorophyll contents also decreased in the middle and bottom canopies due to the addition of FR light (Fig. 1-6), suggesting FR contributed to faster leaf chlorophyll degradation (Hoffmann et al. 2015).

Contrary to FR, red light can delay leaf aging (Tucker 1981), and blue light plays a central role in maintaining leaf chlorophyll contents and photosynthetic proteins (Wang et al. 2015). Previous studies reported that RB interlighting led to higher photosynthetic capacity and chlorophyll contents in the bottom canopy of tomatoes and cucumbers (Trouwborst et al. 2010; Gómez and Mitchell 2016; Kumar et al. 2016; Kim et al. 2019). In this study, RB did not increase the leaf photosynthetic capacity compared to the control, which indicated leaf photosynthesis was mainly affected by ontogenic factors in sweet pepper (Kitta et al. 2014). The photosynthetic capacity in higher plants changes sub-optimally and cannot react quickly to sudden changes in light intensity (Retkute et al. 2015; Kaiser et al. 2018a). Natural light treatment may lead to light penetration in the middle and lower canopies in winter, even without supplemental lighting. Thus, intermittent sunlight into the lower canopies could be sufficient to delay leaf aging under natural light conditions.

However, a reduction in photosynthetic capacity was observed under RBFR in summer. Sufficient natural light in the summer resulted in faster growth in the leaf area (Fig. 1-2), which promoted intense shade in the lower part of the canopies. The R: FR in the lower part of the canopies may be much lower than that in the upper part. Thus, the additional FR from interlighting have contributed to a reduction in the photosynthetic capacity of the bottom canopies. In this study, photosynthetic rate and chlorophyll content were somewhat higher in RB than in RBFR at several canopy positions. The higher chlorophyll

content in RB was not observed in RBFR due to the increased FR fraction. However, favorable leaf photosynthetic properties, such as leaf photosynthetic capacity and chlorophyll concentration, were not always positively related to plant dry weight or fruit production (Hogewoning et al. 2010). Therefore, under additional FR lighting, crop morphology or source-sink modulation from the FR may be considered preferentially to the leaf photosynthetic properties (Park and Runkle 2017; Zhen and Bugbee 2020b).

Increased fruit production with an additional far-red interlighting in winter

During the winter experiment, the dry weight of fruits and stems increased in RBFR (Fig. 1-4), consistent with the altered biomass partitioning of plant organs due to additional FR lighting (Ji et al. 2019; Kalaitzoglou et al. 2019; Kim et al. 2019). Since previous interlighting studies targeted the RB spectrum in sweet pepper (Guo et al. 2016; Joshi et al. 2019), fruit production under the FR spectrum was less understood. Both RB and RBFR increased total fruit counts in winter (Table 1-1). Therefore, the additional amount of PAR induced improvement in the fruit set. RBFR contributed to additional yield improvement compared to RB with increases in individual fruit weight and the fruit number. In addition, RBFR showed an increased total fruit dry weight than RB in winter (Fig. 1-4). The increased dry weight of fruit in RBFR may be due to decreased dry matter partitioning to the leaves or roots (Kim et al. 2019). In

addition, FR radiation upregulated gene expression related to fruit sugar transport and metabolism in tomatoes, which increases the potential growth of individual fruit (Ji et al. 2020). In this study, increased fruit fresh and dry weight was consistent with a previous observation that in tomatoes (Kim et al. 2020), a relatively higher proportion of larger fruit occurs with the addition of FR light in winter.

In summer, no significant differences were observed in fruit yield and individual fruit weight within RB and RBFR (Table 1-2). Since sunlight already contains abundant FR light, additional FR did not contribute to higher fruit weight or yield during the summer due to sufficient FR supplied by sunlight (Palmitessa et al. 2020). RBFR light consumed 80% higher electricity than RB due to the additional FR fraction (Data not shown). Thus, using FR light in summer seems to be inefficient energy consumption. This study focused on leaf photosynthesis, morphology, growth, and yield responses to adding FR to RB interlighting. As well as fruit production, further studies are needed on the effect of FR on fruit quality, such as vitamin C and carotenoid contents, which are essential functional ingredients of sweet pepper.

CONCLUSIONS

This study analyzed the effect of adding far-red to interlighting on greenhouse-grown sweet pepper growth, yield, and leaf photosynthesis in both summer and winter. The additional far-red light improved plant growth, production, and individual fruit weight in winter under insufficient background light conditions. However, interlighting with an additional far-red did not increase fruit production in summer due to sufficient far-red light from sunlight. These results highlight that the effect of supplemental interlighting could change with seasonal variation in natural irradiance. In addition, higher natural irradiance in summer mainly contributed relatively faster growth, and the growth led to an accelerated decrease in leaf photosynthetic capacity or chlorophyll content in the middle and bottom canopies. The decreased leaf photosynthetic capacity of the lower canopy suggests that the height of interlighting lamps needs to be moved to the middle or upper canopy for long-term cultivation to optimize sweet pepper growth. Further studies are required to determine which physiological factors contributed to increased plant growth and dry weight under far-red light.

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CHAPTER 2

Morphological and Photosynthetic Acclimations to Dry Mass Increase of Sweet Peppers According to Far-red Dose Supplemented to Interlighting Using 3D Plant Model and Ray-tracing Simulation

ABSTRACT

Adding far-red (FR) to light capture may be a major factor affecting photosynthate production, but it is difficult to separate intertwined plant morphology and leaf acclimations, such as photosynthesis or leaf spectral properties. This study analyzes total dry mass production in sweet pepper (*Capsicum annuum*. L) according to the additional FR dose to red-blue interlighting and to evaluate the increment in dry mass assimilation attributable morphological and photosynthetic acclimation using 3D plant models and ray-tracing simulation. Additional FR doses of 0, 20, 40, 60, and 80 μmol m^{-2} s^{-1} were applied to red-blue background light (9:1) of 100 μmol m^{-2} s^{-1}. Morphologically, stem length increased linearly from FR 0 to FR 80, while leaf area was saturated at FR 40. Leaf photosynthetic capacity, P_{max}, V_{cmax}, and J_{max} decreased more quickly with increasing FR dose. The simulated light

distribution showed that morphological differences from FR resulted in increased total light interception. However, higher FR accelerated photosynthetic capacity reduction and attenuated photosynthate assimilation. The fruit yields from plants treated with FR 20, 40, 60, and 80 were higher by approximately 4%, 6%, 11%, and 10%, respectively, compared to FR 0. An accelerated reproductive structure was observed at FR 60 and 80, which contributed to the relative yield increase in the initial growth stage. In this study, morphological and photosynthetic acclimation under FR supplemented interlighting could be separately determined through 3D plant models and optical simulation. This study provides a physiological background for using far-red radiation for yield improvement.

Additional keywords: far-red, intra-canopy lighting, morphology, photosynthetic acclimation, 3D plant model

INTRODUCTION

Plants commonly exhibit light-driven reactions such as photomorphogenesis or photoperiodism by perceiving the light spectra, among which red to far-red wavelengths are recognized by a photoreceptor called phytochrome (Casal 2000). Plant photosynthetic and morphogenic responses are affected by spectral conditions such as red/far-red ratio or phytochrome steady-state (PSS) (Sager et al. 1988). In crop canopies, the wavelengths of light received by the leaf strata vary due to the leaf absorption spectra, so higher plants exhibit lower red:far-red and PSS values from top to bottom and show a shade avoidance response (SAR) to compensate for insufficient light (Casal 2013). SAR leads to morphological changes in plant structure, such as stem and petiole elongation, an intuitive response to 'forage' light in the complex canopy (Ballaré and Pierik 2017). SAR responses appear in field conditions in crop cultivation and excessively high planting density indicates a low yield response (Echarte et al. 2011; Yang et al. 2014). Simultaneously, shade conditions induce photophysiological consequences such as reduced leaf chlorophyll contents or accelerated leaf senescence in the bottom canopy to optimize acclimation to limited light on the leaf surface (Rousseaux et al. 1996). Therefore, SAR-induced morphological changes or leaf acclimation of crops accumulate over a long period during cultivation.

In greenhouses, intra-canopy lighting or interlighting can compensate for the plant light deficiency from mutual or self-shading at the bottom of the canopy and produce high-quality fruits annually (Kumar et al. 2016). Such interlighting supplements the lack of light at the bottom canopy from shading and aims to achieve functional advantages through spectral treatments. In general, red (600-700 nm) and blue (400-500 nm) light are the primary color supplemental LED lights because of their relatively higher photosynthetic efficiency over the range of photosynthetically active radiation (PAR) and the effect of blue light in suppressing the ‘red light syndrome' characterized by unresponsive stomata, a low photosynthetic capacity, leaf thickness and chlorophyll reduction (Hogewoning et al. 2010; Trouwborst et al. 2016). In several studies, improved growth, yield, and leaf photosynthesis have been reported with red-blue interlighting (Gómez and Mitchell 2016; Tewolde et al. 2016), and the optimum ratio of red-blue light also has been studied (Kaiser et al. 2018).

Far-red (700-750 nm) light has been employed as a horticultural light source outside the PAR. Generally, far-red light mediates physiological responses such as apical dominance or early flowering in *Arabidopsis thaliana* (Kim et al. 2008; Keuskamp et al. 2010) and tomatoes (Kim et al. 2019). In addition, far-red increases the relative allocation of photosynthetic products to tomato fruits (Kim et al. 2019; Ji et al. 2020). These studies conclude that the total photosynthetic products increase with far-red light and accompanying changes

in photosynthate allocation under additional far-red light (Ji et al. 2019; Kim et al. 2019). Two possible explanations for these increases in photosynthate can be considered: far-red is employed as a source of photosynthetic photons, or the observed effect is due to SAR-induced morphological changes. The physiological background of the enhancement of leaf photosynthesis by far-red light has been explored, and the quantum efficiency was similar to traditional red-blue light (Emerson and Rabinowitch 1960; Zhen and Bugbee 2020a; b).

Nevertheless, quantitative analysis is difficult due to the absence of a photosynthesis model reflecting these enhancements from spectral variance. In addition, morphological changes are also important aspects of plant light capture, allowing relative increases in photosynthetic potential by means of greater light interception (Brites and Valladares 2005; Sarlikioti et al. 2011). Thus, several simulation studies have attempted to analyze the changes in morphology due to light interception using the 3-dimensional (3D) plant model (Kalaitzoglou et al. 2019; Strauss et al. 2020). However, these previous studies have limitations in that they statistically analyzed light capture by fixed plant structures over a single growth stage.

Physiological acclimations, such as changes in morphology and photosynthesis induced by far-red light, occur throughout the entire cultivation period and ultimately determine the total assimilation of the plants. Therefore, quantitative evaluation of the combined effects on morphogenesis and photosynthesis under far-red light is essential for adequately applying

supplemental far-red to red-blue interlighting and understanding its physiological background throughout the period. However, the combined effects have not been quantified from morphogenesis and photosynthesis acclimation under the additional far-red light conditions. This study aims to understand the dose-response of adding far-red light to red-blue lighting on sweet peppers by comparing the simulated light distribution with the actual dry matter production and yield response.

MATERIALS AND METHODS

Plant growth, light treatment, and growth measurement

Plant material and cultivation condition

Sweet pepper seeds ('Mavera,' Enza Zaden, North Holland, the Netherlands) were sown into a soilless plug measuring, in cm, 2.0 W × 2.0 L × 2.7 H (Planttop Plug, Grodan, Roermond, The Netherlands). The plug was covered with vermiculite to retain moisture during germination. When two cotyledons were fully developed, the plug seedlings were transferred into stonewool blocks (Grodan Planttop, Grodan) and germinated in a commercial nursery greenhouse at Asan, Korea (36.8°N, 127.1°E). During the seedling period, a nutrient solution of EC 1.2 dS m^{-1} was supplied, including soluble NPK (3:1:1) fertilizer (Multifeed, Haifa Group, Haifa, Israel). After six weeks in the nursery (August 23, 2021) the seedlings were transplanted into stonewool substrates (Grodan GT Master, Grodan) in a multi-span greenhouse at Seoul National University, Suwon, Korea (37.2°N, 126.9°E). The greenhouse compartment was divided into five sub-compartments for conducting different far-red (FR) light treatments. Growing beds were placed in an east-to-west orientation. The distance between the rows was 1.05 m. One hundred milliliters of the nutrient solution was supplied by drip irrigation eight times a day, and the mean pH and EC (electrical conductivity) of the solution was controlled at 5.8 and 3.0 dS m^{-1}, respectively. Where main stems split into two nodes, each node was pruned

with a ‘V’ stem trellis system (Jovicich et al. 2004). Stem density was initially 3.0 stems/m^2 and increased to 6.0 stems/m^2 after pruning. Flowers were removed up to the first node after branching into two stems to maintain vegetative growth, and the fruit set started from the second node. Each fruit was harvested when more than 90% of the fruit surface turned red.

Interlighting treatments

Two layers of interlighting LED fixtures were installed at heights of 80 and 100 cm from the growing bed. The photosynthetic photon flux density (PPFD) of the LED fixtures was adjusted to 100 μmol m^{-2} s^{-1} through a measured value at a distance of 20 cm with a portable spectrometer (C-7000, SEKONIC, Tokyo, Japan). The red-blue ratio of the LED (peak wavelengths at 450 nm and 660 nm) was 9:1 in PPFD. Five FR doses: 0, 20, 40, 60, and 80 μmol m^{-2} s^{-1} were added to the red-blue (RB) interlighting (IL) of 100 μmol m^{-2} s^{-1} (Fig. 2-1A, Table S2-1). Thus, the percentage of far-red to the background RB was the same as the additional far-red intensity. The IL fixtures were located between the trained ‘V’ stems, and the light source irradiated both sides at 0 degrees of angle. IL started when the plant height reached approximately 60 cm when the meristems came close to the lighting lamp. The start date was October 2, 2021, 40 days after transplanting (DAT). All the IL treatments were carried out for 12 hours of daylength, from 6:00 to 18:00.

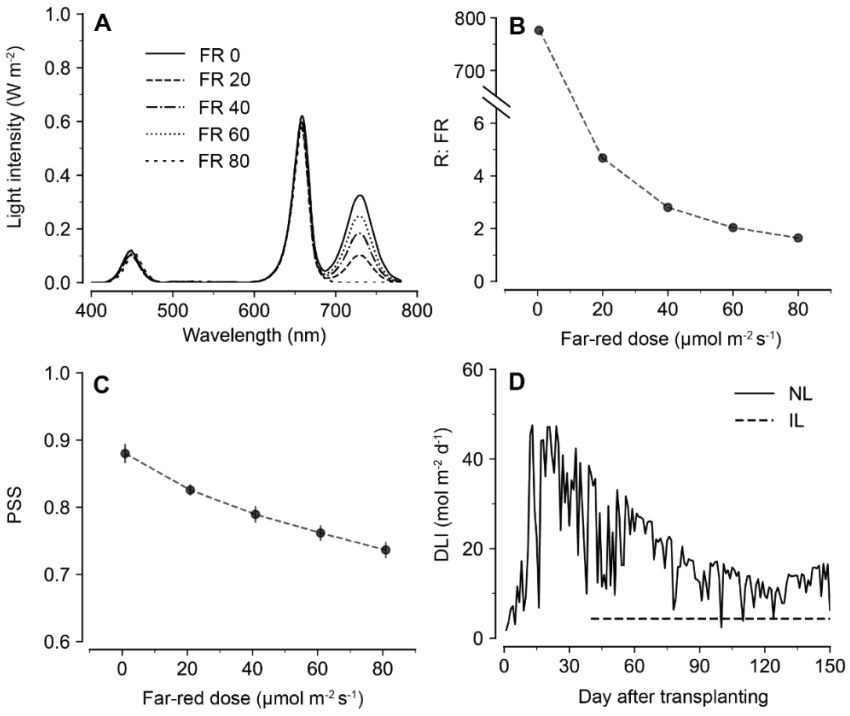

Fig. 2-1. Light environments under interlighting (IL) treatments in the greenhouse: the light intensities of interlighting treatments (A); red/far-red ratio (R: FR) according to FR dose (B); phytochrome steady state (PSS) (C); and daily light integrals (DLIs) of natural light (NL) and IL (D). FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interl ighting, respectively.

Each light treatment sector was blocked with an impermeable plastic film to prevent interference from other light treatments.

Morphology, growth, and fruit yield measurement

A non-destructive investigation was performed at 50 to 130 DAT. Stem length, node number, and fruit set number were measured every ten days. The stem length was measured as the length from the start of the stem branching point to the shoot apical meristem. The node number was counted from the branching point and counted only for each node more than 1 cm in length. Fruit count was measured as the number of harvested fruit sets per plant. After harvest, the fruit fresh weight, length, and width were measured. The measured fruits were dried in an oven at 80°C for 72 hours to measure dry weight. A destructive investigation was conducted for nine plants in each treatment at 150 DAT. First, the stem length, diameter, and leaf area were measured. Then, each plant organ was dissected into stem, leaf, and fruit to measure the fresh and dry weight. Specific leaf mass (SLM, g m^{-2}) was calculated by dividing leaf dry mass by leaf area as an indicator of the thickness of the leaf. Specific stem length (SSL, cm g^{-1}) was calculated by dividing stem length by stem dry mass to indicate the stem diameter. The dry matter fraction was calculated to analyze the partitioning of assimilation products by dividing the total shoot dry weight by the individual leaf, stem, and fruit dry weight.

Definition of leaf developmental age

Since the degree of growth could differ for each treatment group, the level of development was estimated using the non-destructive measured node numbers. For each light treatment, the leaf developmental age was calculated with the following function:

$$LDA = DAT_{meas} - \frac{1}{a}\ (node\ number_{meas}) \qquad (1)$$

where *LDA* is the leaf developmental age; DAT_{meas} is DAT at the measured date; $node\ number_{meas}$ is the node number counted from the branching point at the measured date; and *a* is the aging-dependent coefficient derived from the linear regression slope of the number of nodes measured every ten days.

Measurement of leaf photosynthetic acclimation

Leaf sampling and leaf optical properties

Five leaves were sampled individually at four canopy positions in five light treatments at 150 DAT (a total of 100 leaves). The leaves were picked from the stem branching point at the 6^{th}, 9^{th}, 12^{th}, and 15^{th} nodes (increasing from bottom to top). The light transmittance and reflectance of sweet pepper leaves were measured randomly at three locations on the sampled leaves. The optical properties were measured in the range of 300 to 900 nm with 1 nm intervals

using a spectroradiometer (BLUE-Wave Spectrometer, StellarNet Inc., Tampa, FL, United States), a halogen lamp (SL1, StellarNet Inc), and an integrating sphere (IC-2, StellarNet Inc.). Leaf transmittance and reflectance were calculated based on the adaxial surface of the leaf, and the transmittance and reflectance were determined by measuring the amount of light passing through the leaf and the amount of light reflecting the entrance of the integrating sphere, respectively. The leaf absorption spectrum was calculated as 1 – reflectance – transmittance.

Analysis of pigment and nitrogen content in leaves

The sampled leaves were dissected into 1 cm^2 piece and extracted with 3 mL N,N-dimethylformamide (Samchun Pure Chemical, Pyeongtaek, Korea). After extracting the leaf pigment for 72 hours in the dark at 25°C at room temperature, the absorbance of the extracts was measured at 663.8, 646.8, and 480 nm using a UV–vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States), and the concentrations of chlorophyll a, b, and carotenoids were determined according to Wellburn (1994):

$$Chl\ a = (12 * A_{664}) - (3.11 * A_{647}) \quad (1)$$

$$Chl\ b = (20.78 * A_{647}) - (4.88 * A_{664}) \quad (2)$$

$$Car = (1000 * A_{480} - 1.12 * Chl\ a - 34.07 * Chl\ b)/245 \quad (3)$$

Total nitrogen was quantified using Kjeldahl analysis. Dried leaf sample 0.1g was placed into a Kjeldahl flask. And then, a K_2SO_4 mixed catalyst and 10 mL of concentrated sulfuric acid (H_2SO_4) were added to the flask. In sequence, the mixed sample decomposed at 400°C for approximately 1 hour. After diluting the decomposed solution with distilled water, the solution was poured into a distillation flask, distilled, and then titrated with 0.01 or 0.1 N sulfuric acid solution in an automatic protein/nitrogen analyzer (Kjeltec auto 8400 System, Tecator AB, Sweden) to determine the nitrogen content. Nitrogen (%) was determined as follows:

$$\mathrm{N}(\%) = (T - B) \times N \times f \times \frac{14}{1000} \times \frac{1}{S} \times 100 \quad (4)$$

where T is the amount of sulfuric acid consumed for sample titration (mL); B is the amount of sulfuric acid consumed for blank titration (mL); S is the weight of the dried leaf sample (g); N is the normality of the standard sulfuric acid solution; and f is the correction factor of the sulfuric acid standard solution.

Gas exchange measurement and photosynthetic parameter estimation

Leaf gas exchange was measured using a gas exchange system (LI-6800, LI-COR, Lincoln, NE, United States) with a large light source chamber (6800-03, LI-COR). Leaf photosynthetic capacity was determined with leaf assimilation (A) to the intercellular CO_2 concentration (C_i) curve. A-C_i curve was derived using stepwise increasing ambient CO_2 concentrations of 400, 200, 50, 100,

150, 200, 300, 400, 600, 1,200, 1,400, and 1,800 μmol mol^{-1} using a flow rate of 300 mL min^{-1}. The chamber environment was set to 1,800 μmol m^{-2} s^{-1} of PPFD with a 9:1 ratio of RB LEDs (660 and 450 nm peak wavelengths, respectively), a leaf temperature of 25°C, and relative humidity of 60%. The stability time at each measurement point was set at 180 to 300 seconds to adapt stomata. For the A-C_i curve, measurements were conducted at 40, 70, 100, and 130 DAT. At 40 DAT. Only the 6th leaf was measured and the measurement position was increased to the upper leaves at three-node intervals. Finally, at 130 DAT, the 6th, 9th, 12th, and 15th leaves were measured. Each measurement point proceeded to the next point with an automated program of the LI-6800 when the standard deviation of the photosynthesis and stomatal conductance value was less than 0.5 and 0.1 for 20 seconds, respectively. The parameters of the Farquhar, von Caemmerer, and Berry (FvCB) model (Farquhar et al. 1980), V_{cmax}, J_{max}, and R_d, were estimated from the measured A-C_i curve data with an LI-6800 (LI-COR). Five variables, A, C_i, light intensity, sample CO_2 concentration, and leaf temperature, were used as inputs for the FvCB model parameter estimation. The model parameters were derived with nonlinear least-square fitting from the 'fitaci' function in the Plantecophys R package (Duursma 2015). The derived FvCB model parameters were used to calculate plant photosynthesis from the simulated light distribution.

In-silico analysis of plant light distributions

Acquisition of the 3D scanned model of sweet pepper plants

The 3D scan models of sweet pepper plants were obtained at 40, 70, 100, and 130 DAT using a high-resolution portable 3D scanner (GO!SCAN50TM, CREAFORM, Quebec, Canada) and scan software (VXelement 7.0, CREAFORM). The resolution of the 3D scanner was set to 2 mm. Before 3D scanning, the scanner was calibrated to the ambient light environment in the greenhouse with a calibration plate, and circular retroreflective stickers were affixed to the plant to provide the scanner with the object 3D-reference positioning information. Fifteen plant meshes were acquired for five light treatments. Each scanned mesh was converted to a parametric surface using reverse engineering software (Geomagic Design X, 3D Systems, Rock Hill, SC, United States) for ray-tracing simulation (Kim et al. 2020a).

Greenhouse CAD modeling for ray-tracing simulation

All simulation environments were actualized as realistically as possible. The constructed 3D plant models were transferred to 3D CAD software (SOLIDWORKS, Dassault Systemes, Vélizy-Villacoublay, France), and the illumination on the plant surface was calculated using light modeling software (OPTISWORKS, Ansys Inc., Canonsburg, PA, United States). The greenhouse CAD model was constructed in the same way as the actual experimental greenhouse size of 12.5 × 15.0 × 4.5 m (L × W × H), and the optical properties

of the greenhouse glass, floor, and frames were applied to the simulation. The IL module, with a length of 2.6 m, was modeled and placed on the canopy for IL simulation. The 3D models of sweet pepper were placed at a 1.05 m row distance on the growing bed and 0.3 m planting space.

Natural light and interlighting simulation

Ray-tracing simulations were performed for 40, 70, 100, and 130 DAT, consistent with the date of collecting the 3D scanned data of plants. Simulations were performed independently with natural light (NL) and IL. NL was simulated with an analytical sky model system (CIE 2003) embedded in ray-tracing software (OPTISWORKS, Ansys Inc.). Sun location was determined by latitude, longitude, year, and time of day. Total daily light absorption from natural light was estimated by integrating hourly simulation data (Kaplanis 2006) from a simple cosine function. A ray-tracer (OPTISWORK, Ansys Inc.) adopted the Monte-Carlo algorithm with a forward ray-tracing method, which tracks trajectories of the shoot rays. The total number of shoot rays was two giga-rays. Each ray was eliminated when the light intensity of the ray was smaller than 10^{-6} Watt. To simulate the IL light distribution, the spectrum at each light treatment was applied to the modeled LED chips (Fig. 2-1A). Simulated NL and IL light distributions were used to calculate the amount of light interception, photosynthesis, and light use efficiency.

The simulated spectral range on the leaf surface

The measured leaf transmission and reflection of treatments were applied in 1 nm units for ray-tracing simulation. Spectral light distributions on the leaf surface were simulated for three spectral ranges: PAR (400-700 nm), R (655-665 nm), and FR (725-735 nm). The total light interception and photosynthesis were calculated for the PAR range. The peak wavelengths of the generally used red and FR LEDs were 660 and 730 nm, respectively. The R/FR ratio (R: FR) perceived by the plants was more precisely defined and revised by Franklin (2008) as follows:

$$\text{R: FR} = \frac{\text{photon irradiance between 665 and 675 nm}}{\text{photon irradiance between 725 and 735 nm}} \quad (6)$$

Calculation of light interception and photosynthesis

A light interception on the plant surface was calculated in the 3D point cloud coordinates from ray-tracing simulation. The ambient CO_2 concentration (C_a) of the greenhouse was converted to C_i using the Ball-Berry model with related humidity (RH) (Collatz et al. 1991; Katul et al. 2000) as follows:

$$\frac{C_i}{C_a} = 1 - \frac{1}{m}\frac{1}{RH} \quad (7)$$

where m is the fitted parameter from regression analysis from measured leaf gas exchange data, 5.3560 in this study; RH is the relative humidity in the greenhouse, fixed at 65% in simulated conditions.

Plant carbon uptake was estimated from the simulated light distribution using a modified FvCB C_3 photosynthesis model by Qian et al. (2012):

$$A_c = \left(\frac{V_c(C_i - \gamma^*)}{C_i + K_c\left(1 + \frac{O}{K_o}\right)}\right) - R_d \quad (8)$$

$$V_c = V_{cmax}\left(\frac{31 + \left(\frac{69}{1 + e^{-0.009(PAR-500)}}\right)}{C_i + K_c\left(1 + \frac{O}{K_o}\right)}\right) \quad (9)$$

$$A_j = \left(\frac{J(C_i - \gamma^*)}{4C_i + 8\gamma^*}\right) - R_d \quad (10)$$

$$J = \left(\frac{\alpha PAR + J_{max} - \sqrt{(\alpha PAR + J_{max})^2 - 4\theta J_{max}\alpha\, PAR}}{2\theta}\right) \quad (11)$$

where A_c is the ribulose 1,5-bisphosphate carboxylase/oxygenase carboxylation-limited photosynthetic rate; A_j is the ribulose 1,5-biphosphate regeneration-limited photosynthetic rate; Γ* is the CO_2 compensation point; K_c is the Michaelis–Menten constant of rubisco for CO_2; K_o is the Michaelis–Menten constant of rubisco for O_2; α is the efficiency of light energy conversion; and θ is the curvature of the light response of electron transport.

The photosynthetic rate at each point cloud was calculated by adopting the smaller values of A_c and A_j by substituting the simulated light distribution into the equation as follows:

$$\mathrm{A} = \sum_{i=0}^{n} \min\{A_c(i), A_j(i)\} - R_d \quad (12)$$

where *i* is the light intensity at each point cloud; *A* is the net photosynthetic rate;

Statistical analysis

The experiment was conducted by dividing the greenhouse section and arranging treatments in a generalized randomized block design. Data assessed on ten plants per plot were calculated averages and standard errors to give response per treatment. One-way analysis of variance (ANOVA) was conducted using R software version 3.6.1 and the 'Agricolae' package. For the post hoc test, Tukey's test ($P < 0.05$) was used when the number of experimental groups was the same, and in other cases, the Bonferroni correction test ($P < 0.05$) was used. All graphics were plotted using the Python library matplotlib, version 3.5.1.

The tendency under far-red light treatment was analyzed through polynomial fitting and linear fitting function. The model explains the effect of supplemental FR (x) on the tested variable (y):

$$y = ax^2 + bx + c \quad (13)$$

$$y = ax + b \quad (14)$$

The R^2 value was calculated as 1 – (sum squared regression error)/(sum squared total error). The fitting and score calculation was conducted with the Python NumPy package, version 1.22. All the definitions, units, and references of abbreviations used in the paper are summarized (Table 3-1).

Table 2-1. Abbreviations, variables, definitions, and units used in this paper

Abbreviation	Variable	Definition	Units
R: FR	Ratio of red to far-red light	Dividing spectral range of red (655-665 nm) to far-red (725-735 nm)	*a.u*
PSS	Phytochrome steady state	The ratio of the P_{fr} to the total P at equilibrium (Sager et al. 1988)	-
PPFD	Photosynthetic photon flux density	Photon flux density range of 400-700 nm	μ mol m^{-2} s^{-1}
DAT	Day after transplanting		
LDA	Leaf developmental age	Defined in function (1)	-
LMF	Leaf mass fraction	Leaf dry mass/total plant dry mass	g g^{-1}
SMF	Stem mass fraction	Stem dry mass/total plant dry mass	g g^{-1}
FMF	Fruit mass fraction	Fruit dry mass/total plant dry mass	g g^{-1}
P_{max}	The maximum value of the photosynthetic rate	The maximum potential photosynthetic rate derived from leaf gas exchange data	μ mol m^{-2} s^{-1}
J_{max}	The maximum rate of photosynthetic electron transport	Calculate from least square fitting with A-Ci curve data and FvCB model	μ mol m^{-2} s^{-1}
V_{cmax}	The maximum rate of rubisco carboxylase activity.	Calculate from least square fitting with A-Ci curve data and FvCB model	μ mol m^{-2} s^{-1}
NL	Natural light		
IL	Interlighting		
DPA	Daily photosynthetic assimilation	Integrated hourly simulated plant photosynthesis	mol m^{-2} d^{-1} $plant^{-1}$

RESULTS

Environments in the greenhouse during cultivation

The natural light in the greenhouse, the daily light integral (DLI), continued to decrease as winter progressed. At 40, 70, 100, and 130 DAT, the DLI measured at the top of the greenhouse was 35.5, 21.5, 16.6, and 14.3 mol m^{-2} d^{-1}, respectively (Fig. 2-1D). R: FR and PSS values decreased with increasing FR dose (Fig. 2-1, Table S2-1). The standard deviations of daily temperature and humidity were 0.26 and 0.85, respectively, which were controlled without significant differences between light treatments (Fig. A2-1).

Plant morphology and development

The leaf area per plant increased with increasing FR dose and then saturated under FR 60, showing a quadratic tendency (R^2 = 0.73) at the final harvest (Fig. 2-2A). Stem length increased linearly until FR 80 (R^2 = 0.97) (Fig. 2-2B). The pattern of SLA increased and bent similarly to that of the leaf area, and in the SSL, no particular trend was observed. The R^2 values in SLA and SSA were 0.36 and 0.51, respectively (Fig. 2-2). Plant development was affected by additional FR light. Under FR 0, the node number increased in the same pattern as other FR doses but was slightly lower, and the fruit number increased more slowly than with FR 20, 40, 60, and 80 up to 90 DAT (Fig. A2-2).

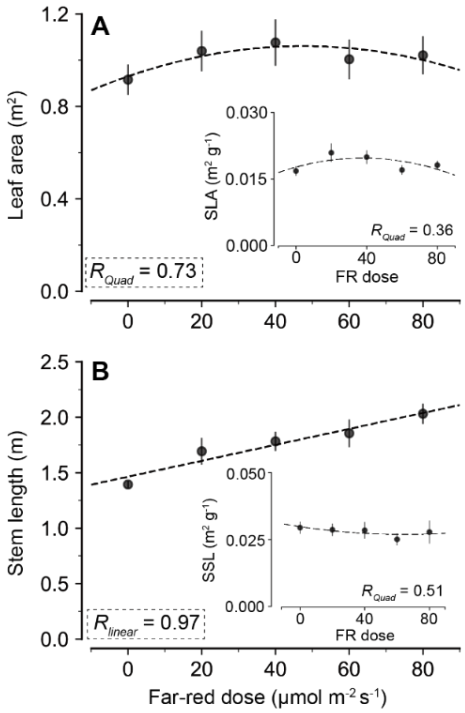

Fig. 2-2. Leaf area with specific leaf area (SLA) (A) and stem length with specific stem length (SSL) (B) under additional far-red (FR) doses to red-blue interlighting. A trendline with R^2 value depicts the higher fit values estimated with linear or quadratic regression. Different letters in the figure indicate statistical significance with Tukey's post-hoc test with mean ± SD (n = 9) values per treatment. FR 0 to 80 represent additional far-red doses of 0 to 80 µmol m^{-2} s^{-1} to red-blue interlighting, respectively.

Dry matter production and partitioning to plant organs

The total shoot dry weight increased until FR 60 and saturated, showing a quadratic tendency (R^2 = 0.92) but not showing a significant difference within FR 20, 40, 60, and 80 (Fig. 2-3A). FR 40, 60, and 80 showed approximately 20 to 30% higher shoot dry weight than FR 0. The leaf dry weight pattern was initially higher under FR 0, decreased under FR 20, and gradually increased (Fig. 2-3B). The stem dry weight and fruit dry weight increased but were saturated at FR 60 (Fig. 2-3C, D). There were significant differences in the leaf and stem mass fractions, but no significant difference was observed in the fruit mass fraction (Fig. 2-4). The leaf mass fraction was significantly higher under FR 0 than under the other FR doses (Fig. 2-4A). The stem mass fraction increased linearly with increasing far-red dose (R^2 = 0.98).

Change in photosynthetic capacity and leaf chemical components according to LDA

The photosynthetic parameters decreased as LDA increased in all far-red dose treatments. The photosynthetic parameters P_{max}, V_{cmax}, and J_{max} under FR 0 were maintained at relatively higher levels than under FR 20, 40, 60, and 80, even when LDA increased (Fig. 2-5). The slopes of P_{max}, V_{cmax}, and J_{max} were -0.113, -0.318, and -0.489 under FR 0, respectively, showing the lowest slope in photosynthetic parameters compared with the other FR doses (Table S2-2). There was no consistent tendency to decrease photosynthetic parameters within FRs 20, 40, 60, and 80 (Fig. 2-5, Table S2-2).

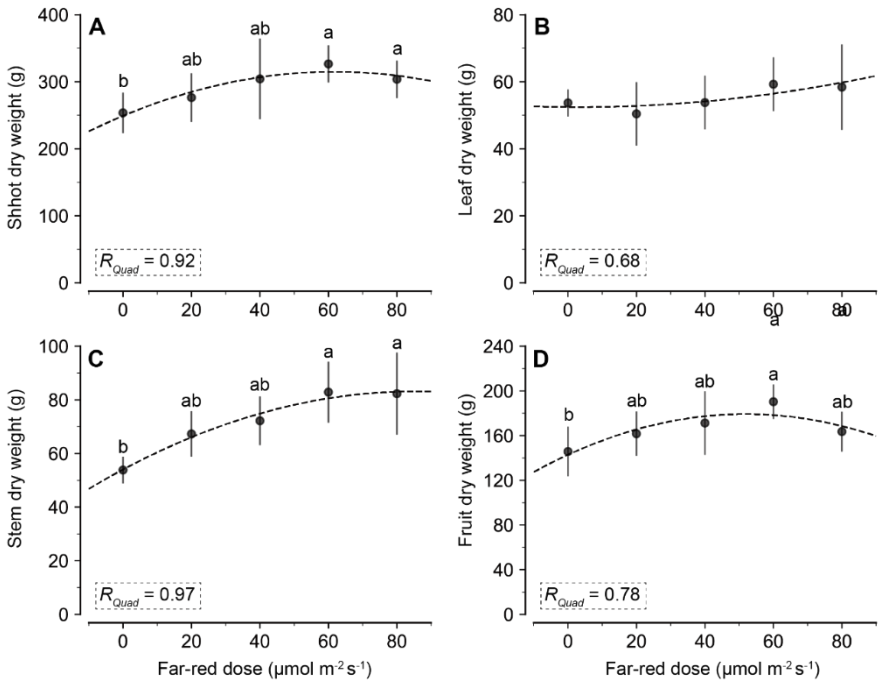

Fig. 2-3. Shoot dry weight (A), leaf dry weight (B), stem dry weight (C), and fruit dry weight (D) under additional far-red doses to red-blue interlighting at final harvest. A trendline with a R^2 value depicts the higher fit values estimated with linear or quadratic regression. Different letters indicate statistical significance with Tukey's post-hoc test with mean ± SD (n = 9) values per treatment. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interligh ting, respectively.

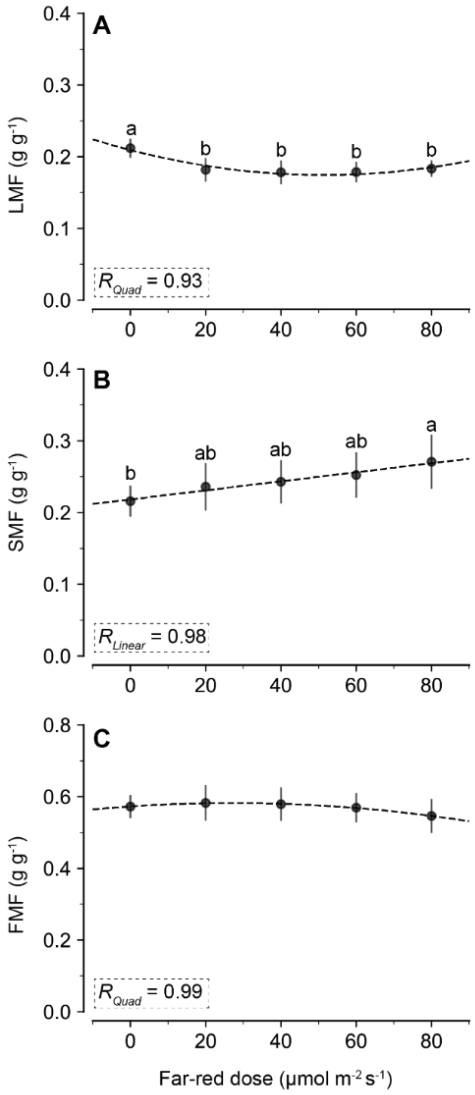

Fig. 2-4. Leaf mass fraction (LMF, A), stem mass fraction (SMF, B), and fruit mass fraction (FMF, C) under additional far-red doses to red-blue interlighting. A trendline with a R^2 values depicts the higher fit values estimated with linear or quadratic regression. Different letters indicate statistical significance with Tukey's post-hoc test with mean ± SD (n = 9) values per treatment. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlightin g, respectively.

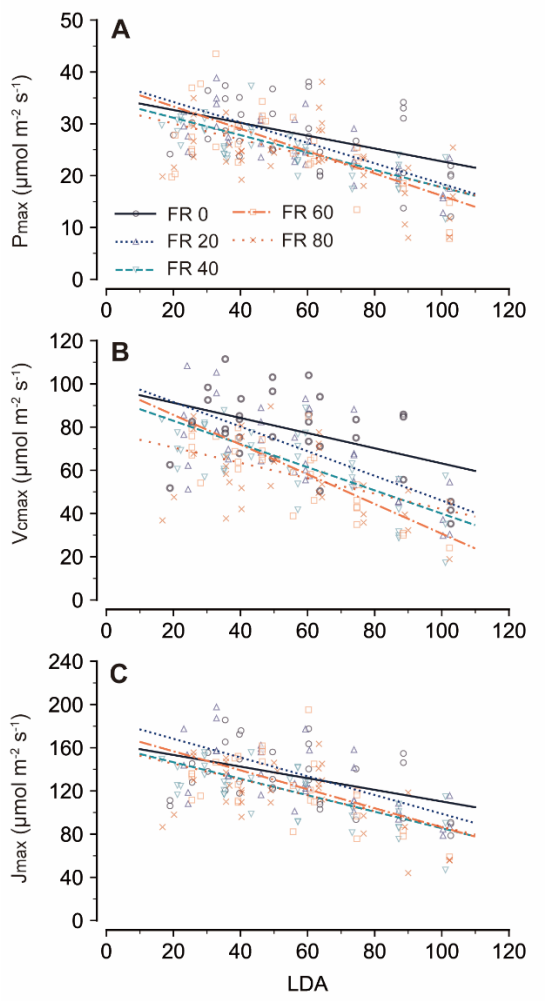

Fig. 2-5. Maximum potential photosynthetic rate (P_{max}, A), maximum rate of carboxylation (V_{cmax}, B), and maximum rate of electron transport (J_{max}, C) under additional far-red (FR) dose to red-blue interlighting according to leaf developmental age (LDA). FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively. A trendline depicts the fitted line estimated with linear regression.

The intercepts of P_{max}, V_{cmax}, and J_{max} were highest under FR 20, with values of 36.18, 97.35, and 176.8, and lowest under FR 80, with values of 31.58, 74.11, and 152.4, respectively (Table S2-2). The leaf nitrogen decreased with increasing LDA. The total chlorophyll and carotenoid contents were higher in the FR 0 treatment than in the other FR treatments, but the chlorophyll *a/b* ratios did not show significant tendencies (Fig. A2-3).

Simulated light distribution and photosynthesis of 3D plant models with growth stage

Ray-tracing was conducted with 3D scanned plant models at 40, 70, 100, and 130 DAT (Fig. A2-4). As the DAT increased, the leaf area and stem length of the scanned models increased, and the leaf area did not show a significant difference with FR dose, but the stem length was significantly higher under FR 80 (Fig. A2-5). The ray-tracing simulation in the greenhouse reflected the spatial light distribution of PAR, red, and FR light on the sweet pepper canopy under natural light (NL) and interlighting (IL) (Fig. 2-6B, C). NL light interception (mol d^{-1} per plant) did not significantly differ at 40 and 70 DAT (Fig. 2-7A). At 100 and 130 DAT, NL light interception was higher under FR 60 and 80 than under FR 0 and 20 (Fig. 2-7A). However, IL light interception was lower under FR 80 than under FR 40 (Fig. 2-7B). At 40 DAT, the area-based light interception showed no significance among treatments (Fig. A2-6). Area-based NL light interception was higher under FR 80 than other FR doses at 70 DAT, but IL light interception was significantly lower under FR 80 than other FR doses at 100 and 130 DAT (Fig. A2-6).

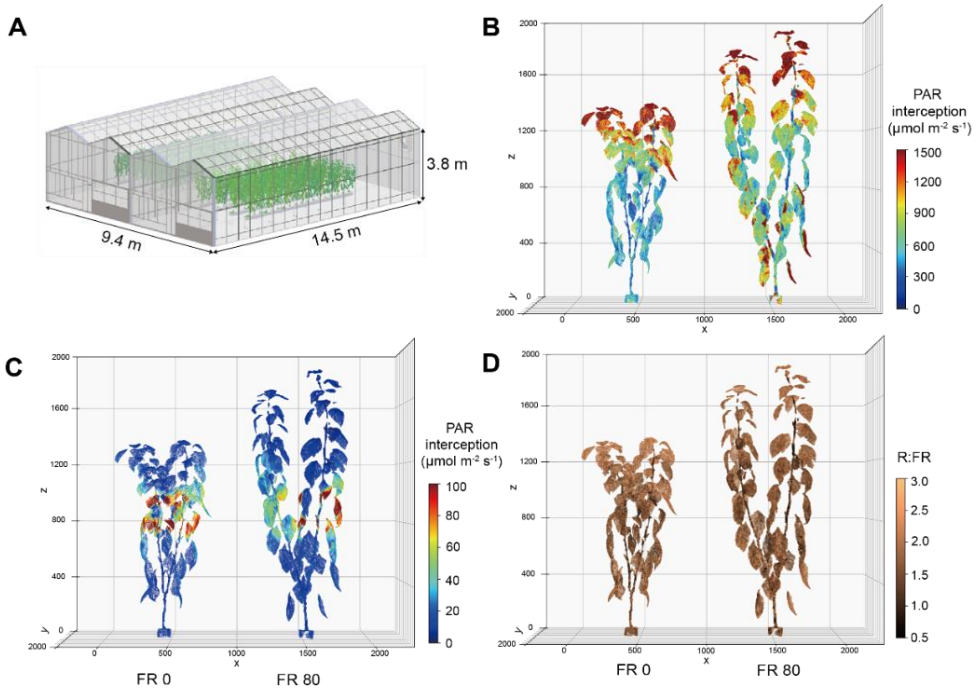

Fig. 2-6. Simulation setup and representative results of light distribution: view of greenhouse CAD model (A); light interceptions under FR 0 and 80 at 130 days after transplanting (DAT) under natural irradiance (B); under interlighting (C); red/far-red ratio (R: FR) under natural irradiance (D). Three-dimensional axes (x, y, z) represent the actual size of plants in mm. FR 0 and 80 represent additional far-red doses of 0 and 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

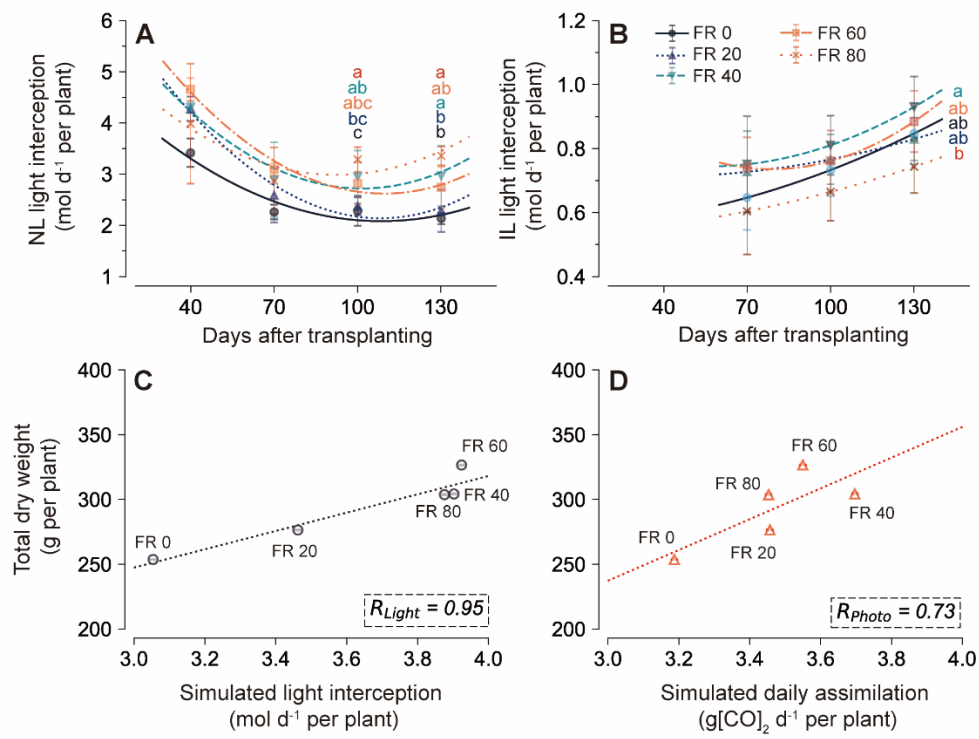

Fig. 2-7. Simulated daily light interception and its relationship to total dry weight at final harvest under different far-red light intensities: light interceptions for natural irradiance (A) and interlighting (B) at 40, 70, 100, and 130 days after transplanting (DAT), and total dry weights related to simulated light interception (C) and simulated daily assimilation (D), respectively. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively. Different letters indicate statistical significance with Tukey's post-hoc test with mean ± SD (n = 6) values per treatment. Unmarked letters in (A) and (B) are not statistically significant. A trendline depicts the fitted line estimated with quadratic regression in (A), (B) and linear regression in (C), (D).

The R^2 values were 0.95 and 0.73 compared to simulated light interception and daily assimilation to total dry weight, respectively (Fig. 2-7C, D). Thus, simulated light interception better explained the total dry weight than simulated daily assimilation (Fig. 2-7D).

Relative contribution to total daily assimilation by varying morphology and photosynthetic acclimation from different far-red dose treatments

From 70 to 130 DAT, the contribution to daily photosynthetic assimilation (DPA) by changes in morphology and leaf photosynthetic acclimation was analyzed under the additional FR doses. At 70 DAT, the improvement of DPA by morphology was most remarkable under FR 60 (Fig. 2-8A). At 100 and 130 DATs, the improvement of DPA by morphology was the highest under FR 80. From the differential application of the photosynthetic parameters measured in the canopy, FR 80 had the greatest reduction in DPA at 70 and 100 DAT, 19% and 18% lower than those in photosynthetic parameters of FR 0 (Fig. 2-8B). The overall FR efficiency to total daily assimilation was the highest under FR 40 (Fig. 2-8C).

Fruit yield and individual fruit properties with additional FR doses

The total fruit yield was approximately 10 to 11% higher under FR 60 and 80 than under FR 0 (Fig. 2-9). The yield increment at the initial stage (107 DAT) was relatively higher than that at the intermediate (133 DAT) and final stages (159 DAT) (Fig. 2-9B). The individual fruit fresh weight, fruit length, and width were significantly lower under FR 0 than under the other FR doses (Table S2-3). There

were no significant differences in fruit fresh weight, length, or width between FR 20, 40, 60, and 80. The water content of the fruit did not show a significant difference within treatments.

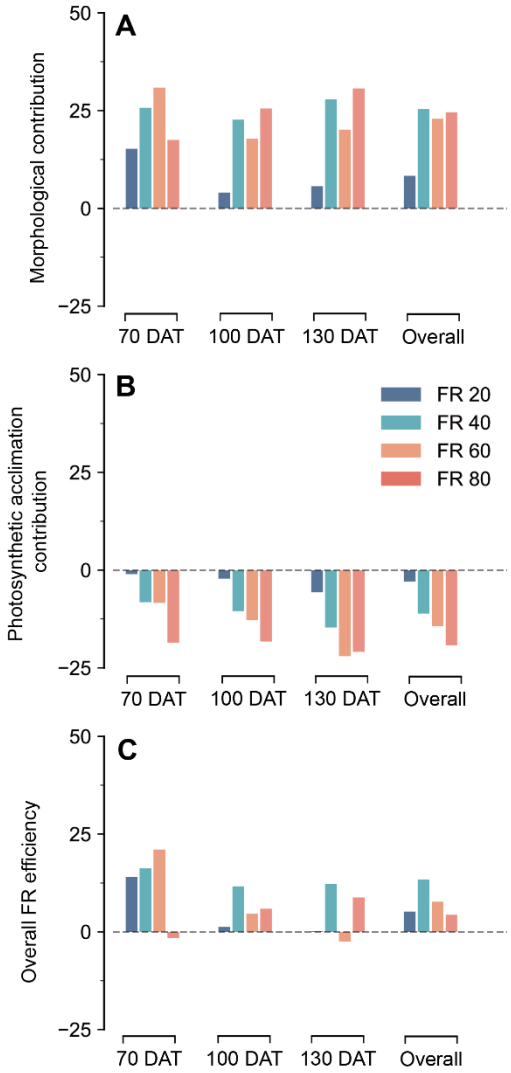

Fig. 2-8. Relative contributions of additional far-red doses to total daily assimilation by varying 3D structure and leaf photosynthetic capacity: morphological contribution (A), photosynthetic acclimation (B), and overall far-red efficiency (C) averaged with days after transplanting (DAT). FR 20 to 80 represent additional far-red doses of 20 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively. A gray line on zero indicates the FR 0 condition.

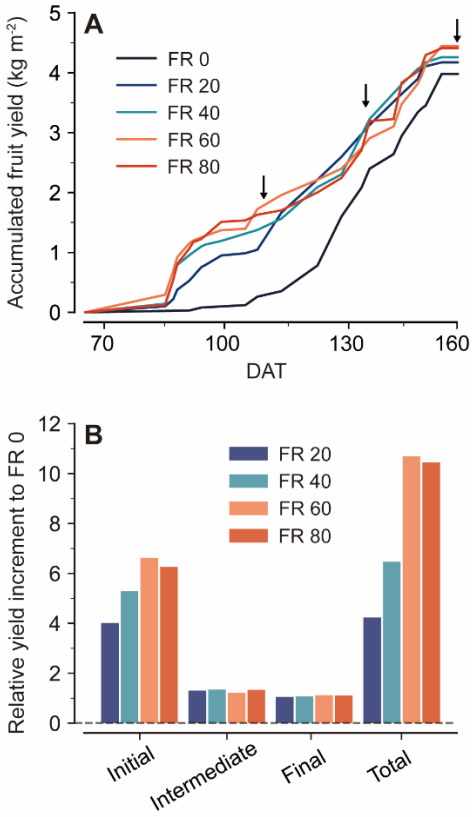

Fig. 2-9. Accumulated fruit yield (A) with days after transplanting (DAT) and relative yield increment to FR 0 under additional far-red (FR) doses to red-blue interlighting. Arrows in figure (A) indicate the initial, intermediate, and final stages (107, 133, and 159 DAT, respectively) for calculating the yield increment in figure (B). FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

DISCUSSIONS

Although RB LEDs were mainly used as light sources for IL to improve canopy photosynthesis in greenhouses, they do not emit FR light. Phytochrome-mediated physiological responses to FR light from artificial light sources were less pronounced due to their lower quantum yield (McCree 1971). However, several studies have reported that a relatively lower PSS from an artificial light source with an additional FR light can be advantageous for plant growth (Sager et al. 1988; Kalaitzoglou et al. 2019; Zou et al. 2019). In greenhouses, the necessity of FR light has not been emphasized because FR is thought to be abundant inside the canopy (Franklin 2008). This study investigated how the morphology, growth, and yield of plants were changed by different FR light doses ranging from 0 to 80 μmol m^{-2} s^{-1} under background RB IL. 3D structural model and leaf gas exchange data were collected from plants at the four growth stages of 40, 70, 100, and 130 DAT, and using these data the factors that significantly changed the growth of sweet pepper during the overall period were analyzed given different FR doses.

Plant morphology, growth, yield, and biomass allocation under different FR doses

First, crop growth under FR light was analyzed collectively after 160 days of cultivation. Leaf area and stem length affect plant light use efficiency (Sarlikioti et al. 2011). The leaf area continued to increase until FR 40 and then decreased, and the

stem length increased linearly with increasing FR dose (Fig. 2-2). Previous studies have reported that FR light induces a SAR reaction and promotes leaf expansion and internode elongation to seek further light in various horticultural crops, and a similar response was observed in greenhouse sweet pepper (Demotes-Mainard et al. 2016). The increase in stem length was not saturated even under FR 80 (Fig. 2-2B). However, the stem dry weight was saturated at FR 60 (Fig. 2-3C), so SSL was higher under FR 80 than under FR 60 (Fig. 2-2B). As the FR dose increased, the stem dry weight was saturated earlier than stem length. Thus, FR light intensity above 80 μmol m^{-2} s^{-1} could induce the stem overgrowth of sweet pepper under the 100 μmol m^{-2} s^{-1} of RB background IL used in this study. The total shoot dry weight showed a quadratic curve that was saturated at approximately FR 60 but increased with increasing FR light, and the fruit dry weight was also highest under FR 60. Only FR 60 showed a significantly higher fruit dry weight than FR 0. However, all the FR treatments showed 4 to 11% higher fruit yields than FR 0.

Similarly, several studies have reported that FR light can be beneficial in increasing yield in tomato plants (Ji et al. 2019; Kim et al. 2020b). These results imply that additional FR light is more effective in increasing assimilation product and yield than RB IL alone, even though a small amount of FR light was added to sweet peppers. In general, the increase in yield under FR light has been explained in three ways. The first is the improvement of light capture efficiency by SAR (Niinemets and Fleck 2002; Sarlikioti et al. 2011), and the second is the photosynthesis enhancement effect with additional FR light in the RB light background (Emerson and Rabinowitch 1960;

Zhen and Bugbee 2020b), and the third is an increase in assimilation product partitioning to fruits (Ji et al. 2020).

This study evaluated the assimilation product partitioning changes found under increasing FR light (Fig. 2-4). Under FR 0, the LMF was much higher using solely RB IL, but the higher LMF disappeared even with a small intensity of FR light of 20 μmol m^{-2} s^{-1}. Increases in leaf thickness and leaf morphological disorder were observed in higher blue light fractions (Hogewoning et al. 2010). Thus, a small amount of additional FR light can alleviate the inefficient distribution of assimilation products to leaves with RB. Excessive distribution of assimilation products to leaves mediates a thick leaf phenotype, which is a constraint to lowering the efficiency of CO_2 diffusion in mesophyll cells due to higher cell wall resistance (Syvertsen et al. 1995). In addition, an increased LMF under FR 0 was associated with a relatively decreased SMF (Fig. 2-4B). However, an increase in dry mass partitioning to fruit (Kim et al. 2019; Ji et al. 2020) was not observed in this study (Fig. 2-4C). Unlike tomatoes, sweet peppers maintain one fruit at each node, so the allocation signaling acting on sugar distribution may be weaker than in tomatoes, where 3 to 5 fruits are attached to the same node. Alternatively, the response to FR light may differ depending on the genetic variability of plant species (Ji et al. 2021). Thus, supplemental FR to IL was ineffective in increasing the dry mass fraction to fruits in the sweet pepper cultivar used in this study. Nevertheless, adding FR contributed to a higher shoot dry weight as the total photosynthetic assimilation increased.

Leaf photosynthetic acclimation under different FR doses

During the growing season, photosynthetic acclimation in the canopy can affect the total assimilation at the whole-plant level. Self- and mutual shading caused by stratified leaves alter the long-term potential of leaf photosynthesis from changes in the R: FR (Rousseaux et al. 2000). In this study, the addition of FR acted as a senescence signaling pathway for the photosynthetic capacity of leaves. The FvCB photosynthetic parameters and P_{max} were derived by measuring the A-C_i curve at four growth stages. The photosynthetic capacity slowly decreased under FR 0 with increasing LDA (Fig. 2-5). Additionally, as the FR dose increased, the photosynthetic parameters decreased steeply until FR 60. A decrease in leaf-level gas exchange was previously observed from additional FR light (Ji et al. 2019). Simultaneously, RB increased leaf photosynthesis compared to plants grown under NL (Tewolde et al. 2016; Jiang et al. 2017). However, this study showed that the improvement in photosynthesis was attenuated by adding FR to RB IL (Fig. 2-5). The leaf protein results showed that the nitrogen content constantly decreased according to the LDA in most FR treatments, whereas the total chlorophyll decreased rapidly at higher FR doses (Fig. A2-3C). In addition, the leaf absorption in the PAR range was also reduced by approximately 1% as the FR dose increased (Fig. A2-7). In general, far-red results in chlorophyll degradation with shade signaling in a wide range of plant species (Casal et al. 1987; Li and Kubota 2009; Brouwer et al. 2014). A decrease in chlorophyll is associated with a direct reduction in the measured leaf photosynthesis. Therefore, the decline in FvCB parameters derived from the measured leaf photosynthesis could be due to rapid chlorophyll degradation under FR radiation.

Additional FR light rebalanced source-sink responses, accelerating harvest time and increased initial fruit yield

The total fruit yield increased by 10% under FR 60 and 80, but the increase was concentrated at the initial stage (Fig. 2-9). Plant reproductive development was accelerated under supplemental lighting and reduced time to flowering and harvest. FR light promoted fruit flowering compared to other supplemental sources such as HPS light (Kim et al. 2019). In this study, the fruit set was relatively faster in the FR treatments than in the RB treatment alone, which resulted in a relatively fast yield increase under FR 60 (Fig. 2-9). Thus, adding FR can promote fruiting and early harvest of sweet pepper. However, the higher FR intensity did not guarantee an effective increase in yield, which may result from the excessive distribution of assimilation products to shoots (Fig. 2-4). During cultivation, the balance between vegetative and reproductive growth continuously changes with the source-sink balance (Gifford and Evans 1981; Brouwer 1983). The total number of fruits influences the distribution of assimilation products to fruits. Although there was no significant difference in the number of fruits in the second half of the planting period (Fig. A2-3), the final yield improved more under FR 60 and 80 because the increase in fruit number at early DAT induced the higher assimilation of products into fruits (Figs. 2-9A, S2-2).

Integrative analysis of natural light and interlighting by adding FR to RB interlighting via a 3D plant model and ray-tracing simulation

The simulation could interpret the effect of FR doses by dividing the simulated light sources into NL and IL by disposing of the actual greenhouse and scanned plants model in 3D space (de Visser et al. 2014). The simulation results clearly showed the local light distribution by NL (Fig. 2-6B) and IL (Fig. 2-7A). The DLI at 70 DAT was only approximately 40% at 40 DAT (Fig. 2-1D). As winter began, the intercepted light decreased due to the decreases in global hourly solar irradiation and sunshine duration (Fig. 2-7A). Despite the reduced light amount at 100 and 130 DAT, NL light interception was relatively higher under the FR supplemented treatments. The high efficiency of light interception from natural irradiance under the additional FR doses primarily contributed to the increase in leaf area. However, even when calculating NL interception per leaf area, a significantly higher light interception was observed (Fig. A2-6). This result means that high FR treatment alleviates self- or mutual shading from morphological traits under FR light (e.g., longer internode, petiole, or leaf angle), resulting in a more favorable architectural shape to capture NL (Falster and Westoby 2003; Sarlikioti et al. 2011). On the other hand, IL light interception steadily increased due to the increase in leaf area in the middle canopy during the cultivation period (Fig. 2-7B). IL light interception under FR 80 was lower than that under FR 40 at 130 DAT (Fig. 2-7B). The decrease in an IL light interception may be due to the wide distribution of leaves caused by increasing the internode length in the middle canopy where the IL was applied directly. Therefore, although high FR light intensity increases the light capture from NL, there was a decrease in the light use efficiency

of IL. In addition, IL light interception varies according to the DAT, suggesting that the position of the supplemental lighting bulb should be vertically adjusted according to the cultivation period.

Contributions of morphological and photosynthetic acclimation to total daily assimilation under additional FR doses

Although light interception under different FR doses was analyzed previously, whole-plant photosynthesis is entangled with leaf photosynthetic capacity according to vertical leaf positions. To find the appropriate FR dose, the contribution of daily assimilation by FR light was analyzed by dividing it into two factors: morphological and photosynthetic acclimation (Fig. 2-8). The efficiency of additional FR light was higher than FR 0 over most of the cultivation period (Fig. 2-8C). The enhancement of daily photosynthesis by the morphological change was the main factor rather than the decrease in photosynthetic capacity in the canopy by FR light. In this study, the overall FR efficiency observed in the simulations was high under FR 40, while the actual shoot dry weight and fruit yield peaked under FR 60 (Fig. 2-3A, Table S2-3). Additionally, simulated light interception reflected the final total dry weight better than daily assimilation with a higher R^2 value (Fig. 2-7C, D). There are two reasons why the simulated assimilation does not match the actual yield. First, the photosynthetic capacity under FR light may have been underestimated. In general, under high FR light, leaves acclimate with shade-avoidance traits such as thin leaf phenotype, lower chlorophyll concentrations, and rubisco contents (Hogewoning et al. 2012; Izzo et al. 2020). Leaf gas exchange was generally measured with RB light

sources (> 1500 μmol m^{-2} s^{-1} for sunlit leaves) for faster induction of stomata (Evans and Santiago 2014). Thus, leaves grown under FR light are disadvantageous in the measured leaf photosynthesis under RB chamber spectra. In previous studies, the measured photosynthesis under additional FR light with an open chamber at a moderate light intensity was not different (Ji et al. 2019; Zhang et al. 2019) or increased (Kalaitzoglou et al. 2019). Therefore, the photosynthetic capacity derived from the A-C_i curve may be underestimated under FR light. Another possibility is that the additional FR light to RB IL directly contributed to total photosynthesis. Zhen and Bugbee (2020a) reported that FR photons increased gross photosynthesis under background white light in several horticultural crops. Thus, gross assimilation may have improved because the PAR-background IL was conducted together with FR light. Since the current photosynthesis model cannot quantitatively evaluate leaf photosynthesis by FR light, there is a limitation because not all of these photophysiological characteristics are reflected in calculating whole-plant photosynthesis with simulation. Nevertheless, this study is meaningful to separately interpret long-term physiological acclimations according to the IL with additional FR doses.

R: FR absorbed by leaf surface and appropriate FR dose

Unlike top-lighting, IL is employed close to the leaves so that light distribution is spatially concentrated in the middle or lower part of the canopy (Fig. 2-6C). Since the light source is close to the leaves, physiological disturbance has been reported in light-sensitive crops such as cucumbers (Trouwborst et al. 2010). The intracellular

abundance of photoreceptors drives photophysiological responses in plants and is regulated by leaf absorption spectra (Li et al. 2012). R: FR or PSS, which describe the light source spectrum, attempt to quantify the light quality of plants (Sager et al. 1988), but the interpretation of plant responses is more complex (Rajapakse et al. 1992). This study analyzed how the R: FR absorbed by leaves changes during cultivation in the upper, middle, and lower canopies. The R: FR of natural irradiance decreased by self- or mutual shading as the growth stage increased (Fig. A2-8). Interestingly, in the greenhouse environment, the R: FR in the middle canopy was overall lower than that in the lower canopy by approximately 0.1 (Fig. A2-8C, D). Thus, mutual shading intensified in the middle canopy due to the growing conditions in greenhouses with higher planting density. Therefore, IL in the middle canopy may be more effective than in the lower canopy because of its relatively higher photosynthetic capacity and pursuit of vertical light uniformity. In addition, even with NL, the R: FR of FR 0 can be rather high with FR supplemented IL (Fig S2-8E), and the spectrum inside the canopy may not be completely suitable for plant growth.

CONCLUSIONS

This study confirmed that the total biomass accumulation of sweet peppers was curvilinearly improved with increasing far-red (FR) dose added to red-blue (RB) interlighting and then saturated at an FR dose of 60 μmol m^{-2} s^{-1} (FR 60). The light distribution was estimated using ray-tracing simulation combined with 3D-scanned plant models grown under five additional FR doses with RB background interlighting. A higher FR dose improved light capture under natural light through morphological adaptation such as longer internodes or higher leaf areas. Simultaneously, a higher FR dose accelerated photosynthetic senescence within the canopies, negatively affecting total canopy assimilation. Thus, a moderate FR dose (FR 40) showed higher simulated photosynthate assimilation than FR 60. Nevertheless, FR 60 had the most effective fruit yield. The simulated light interception yielded a better description of final dry weight than simulated photosynthate assimilation. Thus, the chamber-measured leaf photosynthesis is biased to underestimating the biomass production under FR. Furthermore, the higher FR improved the yield through acceleration to the reproductive stage in the initial cultivation period. Therefore, adding FR to RB could be one way to achieve higher fruit production by regulating fruit development or source-sink interactions. In addition, FR 20 and 40 at moderate doses helped reduce dry mass partitioning to the leaves and efficiently improved yield. This study may provide practical background knowledge to manipulate plant growth and development under FR supplemented lighting in cultivation.

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CHAPTER 3

Fruit yield, Quality, and Carotenoid Content of Sweet Peppers subjected to Far-red Supplemented Interlighting

ABSTRACT

Supplemental interlighting is commonly used in modern greenhouses improving lack of light, but the light spectrum affects fruit quality and color change. This study aimed to analyze the effect of interlighting with red-blue and additional far-red light on the fruit qualities and carotenoid contents of red ('Mavera') and yellow ('Florate') sweet pepper varieties (*Capsicum annuum* L.). Three light treatments were applied: natural light (NL), NL with red-blue LED interlighting (71 µmol m^{-2} s^{-1}) (RB), and RB with far-red light (55 µmol m^{-2} s^{-1}) (RBFR). Ascorbic acid, free sugars, and individual carotenoid content were quantified with HPLC analysis. Fruits were sampled on November 14, 2020 (Group 1) and January 03, 2021 (Group 2) from the plants grown under average light intensities of 335.9 and 105.6 µmol m^{-2} s^{-1}, respectively. Overall, total yields in RB and RBFR were 22% and 33% higher than those in NL in red fruits, 2% and 21% higher in yellow fruits, respectively. In both colored fruits,

ascorbic acid, total soluble sugar, and carotenoid content were more elevated in RB and RBFR than NL. In Group 1, ascorbic acid and total soluble sugar differed significantly from RB and RBFR only in red fruits. In Group 2, ascorbic acids in red and yellow fruits were 9% and 3% higher in RBFR than RB, but total soluble sugars were 4% and 2% lower, respectively. Carotenoid contents in red and yellow fruits were 3.0- and 2.1-fold higher in RB, and 2.0- and 1.4-fold higher in RBFR than in NL, respectively. In this study, interlighting significantly impacted fruit quality in Group 2, mainly due to seasonal changes increasing the interlighting fraction to the total light amount. In particular, red and yellow fruit yields were 9% and 19% higher in RBFR than RB, but carotenoid contents were 24% to 18% lower, respectively. Thus, providing the additional far-red to interlighting could achieved higher yield but decreased carotenoids in fruits then that with the red-blue interlighting only.

Additional keywords: ascorbic acid, fruit color, light environment, soluble sugar

INTRODUCTION

Light is a limiting factor for fruit yields and quality in the high latitude regions. Supplemental lighting was introduced to achieve year-round fruit production under insufficient sunlight in modern greenhouses. Among various light sources, light-emitting diodes (LEDs) can control the spectrum of light sources, thereby enable the photophysiological induction of greenhouse crops. Red-blue wavelengths are representative light spectra for higher quantum yields and photosynthetic efficiencies (McCree 1971). In addition to red-blue light, far-red (FR) light has been used as a light source that mediates the photoreceptor responses from plant phytochromes (Mitchell 2015). Thus, several studies have studied the effect of FR on tomatoes (Ji et al. 2019; Kalaitzoglou et al. 2019; Palmitessa et al. 2020).

In fruit quality attributes, adding FR to red light improved the chemical compositions, such as fruit sugar contents and mineral components, and increased the fruit yields of greenhouse tomatoes (Kim et al. 2020). Sweet pepper is one of the most-consumed vegetables worldwide due to its taste and higher nutrient values (Kim et al. 2011). In addition to the sensory attributes, vitamin C and carotenoids are essential compounds of the sweet pepper due to their role as an antioxidant in the human body and decrease the risk of cardiovascular disease and cancers (Maoka et al. 2001; Abdel-Aal et al. 2013). However, studies on fruit yields and quality in sweet peppers under red-blue or

FR supplemented light is not yet sufficient.

Phytochromes are homodimeric photoreceptors that shift between two equilibrium forms in plants: the biologically inactive form P_r has a maximum absorbance in red light (660 nm), and the biologically active P_{fr} form has a maximum absorbance in FR light (730 nm) and excited by absorbing red light (Sharrock 2008). Activated P_{fr} migrates from the cytosol to the nucleus and exerts various biological responses by binding to transcription factors, such as those in the phytochrome-interacting factor (PIF) family (Casal 2013). In tomatoes or sweet peppers, fruit ripening simultaneously proceeds during chlorophyll breakdown and accumulation of carotenoids (Seymour et al. 2013). The chlorophylls in immature fruit surfaces generate a self-shading effect due to preferential absorption of red light that maintains the phytochromes predominantly in the inactive P_r form and high PIF1a, levels of PIF homolog that suppress *PSY1* expression, which is the first enzyme responsible for carotenoid metabolism (Llorente et al. 2016). Thus, carotenoid production could be prevented under low R: FR by inhibiting the genes involved in the carotenoid biosynthesis pathway by activating PIFs. Against this background, a high proportion of FR light can be a trade-off for ripening progress or carotenoid production by maintaining a relatively higher equilibrium of inactive P_r.

In previous studies, the addition of photosynthetically active radiation (PAR) via LEDs resulted in similar responses at higher R: FR. Monochromatic red-

blue and white LED lighting was efficacious in increasing lycopene and lutein production in tomatoes (Dannehl et al. 2021). In addition, the lycopene and capsanthin syntheses of tomatoes and peppers have been enhanced under supplementary red or blue light LEDs with higher expression of carotenoid biosynthesis genes such as the *PSY*, *LCY*b, and *CCS* genes (Xie et al. 2019; Pola et al. 2019). These results supported that supplemental lighting with red-blue LEDs could result in higher carotenoid content in sweet peppers, but the effect of additional far-red light is unclear. Studies on fruit carotenoid accumulation by adding FR light to red-blue interlighting have rarely been conducted under greenhouse conditions. Therefore, it is necessary to analyze whether interlighting with additional FR light affects fruit quality in greenhouses with background sunlight.

This study aimed to analyze the effect of interlighting with red-blue and additional FR lighting on the fruit yields, quality, and carotenoid accumulation of sweet pepper fruits in greenhouses.

MATERIALS AND METHODS

Plant material and cultivation condition

Sweet pepper seeds of red (‘Mavera,’ Enza Zaden, North Holland, the Netherlands) and yellow (‘Florate,’ Enza Zaden) fruit colors were sown into soilless plugs with sizes of 2.0 W × 2.0 L × 2.7 H cm each (Planttop Plug, Grodan, Roermond, The Netherlands), and the plug trays were covered with vermiculites to retain moisture during germination. When two cotyledons were fully developed, the plug seedlings were transferred into stonewool blocks (Grodan Planttop, Grodan) and germinated in a commercial nursery greenhouse at Asan, Korea (36.8°N, 127.1°E). During the seedling period, a nutrient solution of EC 1.2 dS m^{-1} was supplied with soluble NPK (3:1:1) fertilizer (Multifeed, Haifa Group, Haifa, Israel). After a six-week nursery period, the seedlings were transplanted into stonewool substrates (Grodan GT Master, Grodan) on August 26, 2020, in a Venlo-type glasshouse at Seoul National University, Suwon, Korea (37.2°N, 126.9°E). For the light treatments, a greenhouse was divided into three compartments, and the area of each sector was 2.3 W x 9.0 L m. The growing beds were placed in an east-to-west orientation, and the distance between rows was 1.05 m. The nutrient solution was supplied by drip irrigation based on the measured solar radiation inside the greenhouse, and the ± pH and electrical conductivity (EC) values of the solution were controlled at 5.8 and 3.0 dS m^{-1}, respectively. When the main stems split

into two nodes, each node was pruned with a 'V' stem trellis system (Jovicich et al. 2004). The stem density was initially 3.0 stems/m^2 and increased to 6.0 stems/m^2 after pruning.

Measurement of light environment

The DLI of natural light was monitored with three sensors on the top of the greenhouse using the quantum sensors (SQ-110, Apogee Instruments, Logan, UT, United States) equipped with a data logger (CR1000, Campbell Scientific, Logan, UT, United States). In order to analyze the spectral change under the supplementary interlighting, the spectral intensities at the upper, middle, and lower canopies were measured with a portable spectroradiometer (C-7000, SEKONIC, Tokyo, Japan) from November 16 to 20, 2020, four times a day between 10:00 to 16:00.

Interlighting treatments

Three light treatments were applied: natural light (NL, control), NL with red-blue LED interlighting (RB), and RB with FR light (RBFR). Two layers of customized interlighting LED fixtures (BISSOLLED, Seoul, Korea) were installed at 0.9 and 1.1 m above the growing beds and fixed during the cultivation period. The photosynthetic photon flux density (PPFD) of the single-layer LED fixture was adjusted to 71 μmol m^{-2} s^{-1} at a distance of 20 cm. The red: blue in the RB was 8: 2 in PPFD (Fig. 3-1A). Under the RBFR, the additional FR light intensity was set to 55 μmol m^{-2} s^{-1} in photon flux density.

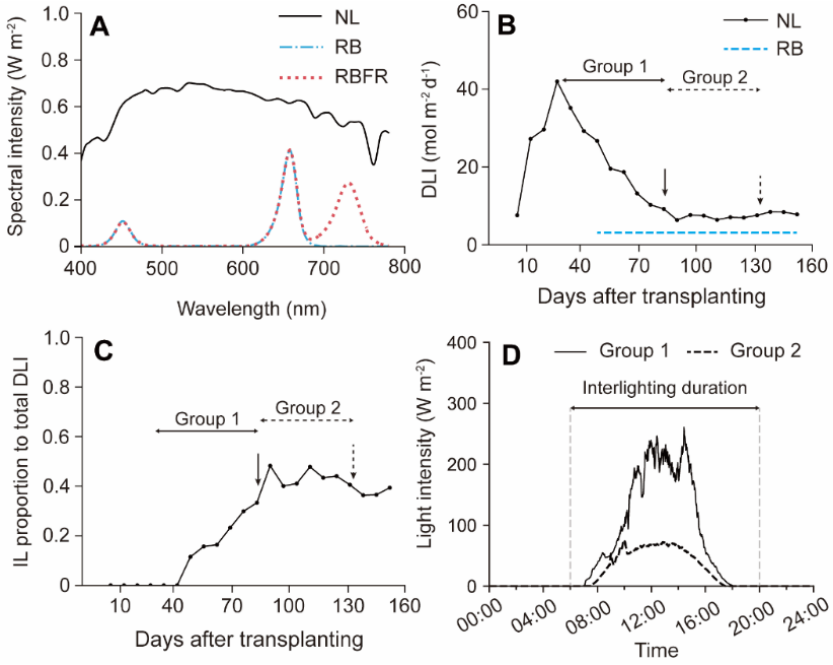

Fig. 3-1. Light environment in the greenhouse during cultivation: spectral composition (A), the weekly average of the daylight integral (DLI) (B), interlighting (IL) proportion to total DLI (C), and averaged daily light intensity (D). NL, RB, and RBFR mean natural light, NL with red-blue LED interlighting, and RB with far-red light, respectively.

At this time, the R: FR of the light source was 1.01. Interlighting began on September 15, 2020, 50 days after transplant (DAT), when the plant heights reached approximately 80 cm when the meristems reached the light sources. All interlighting treatments were carried out for 12 hours from 6:00 to 18:00 (Fig. 3-1D). Each treatment was blocked with an impermeable plastic film to prevent interference from light sources.

Fruit sampling and color measurement

Fruit sampling was divided into Groups 1 and 2 (Fig. 3-2A). Group 1 tracked the fruits that developed from 1-3 flowering nodes, and Group 2 was followed by tagging from the beginning of the Group 1 harvest. The fruits in Groups 1 and 2 were monitored with tag tape starting from 25 (September 20, 2020) and 80 DAT (November 14, 2019), respectively, and ten fruits in each group were monitored. At the tagged time, the fruits of Groups 1 and 2 were located about 50-75 and 75-100 cm above the growing bed, respectively (Fig. 3-2A). The L^*, a^*, and b^* color spaces were measured by averaging the measured values obtained with a portable spectrophotometer (CM-2500d, Konica Minolta, Tokyo, Japan) at four random locations on the tagged fruit surfaces. The time when the fruit widths reached 1 cm was regarded as fruit set and determined as one day after pollination (DAP). The coloration was measured at 10-day intervals until 30 DAP and at 3-day intervals thereafter in the tagged fruits. The tagged fruits were harvested at the mature stage when approximately 95% of

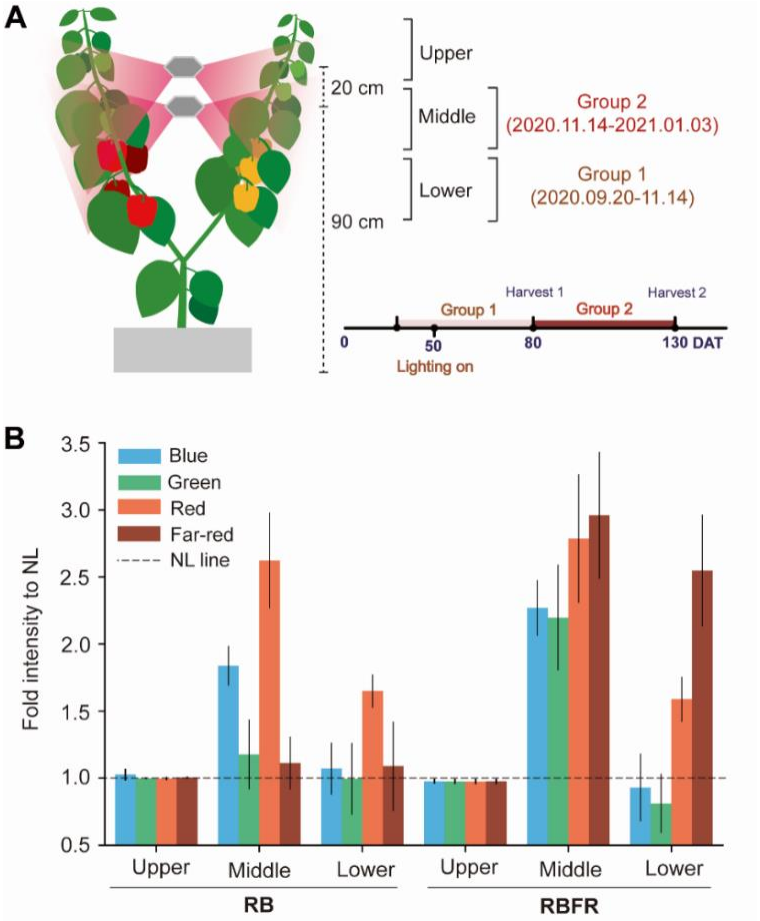

Fig. 3-2. Fruit sampling groups and spectral light distribution in sweet pepper canopy positions. The harvesting groups, sampling positions (A), and fold intensity of spectral distributions compared to NL from interlighting RB and RBFR were measured at the upper, middle, and lower canopy positions (B). NL, RB, and RBFR mean natural light, NL with red-blue LED interlighting, and RB with far-red light, respectively. DAT means days after transplanting, the spectral range of blue, green, red, and far-red were 400-500, 500-600, 600-700, and 700-750 nm, respectively. Values are the mean ± SD of 20 replicates.

the fruit surfaces were colored (Verlinden et al. 2005). The harvested fruits were stored in a deep freezer to analyze the fruit qualities and chemical compositions. Ten fruits for each treatment group were stored in a deep freezer (DF8520, Dongducheon, Korea). Immediately after harvest and when harvesting was completed, fruits were randomly mixed and crushed with a freezer mill (SPEX 6875D, SPEX, Metuchen, NJ, United States). All analyses were performed in the same manner for the red and yellow fruits.

Analysis of fruit quality

The quality of fully matured fruits was analyzed within 24 hours after harvesting. Individual fruit fresh weights were measured with a weighing scale. The fruit dry weights were measured after drying the fruits in a dry oven at 80°C for 72 hours. The firmness of fruits was measured with a texture analyzer (CT-3, Brookfield Co., Middleborough, MA, United States). The equatorial planes of the fruits were compressed by using a rounded tip probe of 3 mm diameter at a speed of 10 mm s^{-1} and strain of 5 mm. The total soluble sugar (TSS) contents of fruits were measured with a refractometer (PAL-1, Atago, Tokyo, Japan). Titratable acidity (TA) was measured using a fruit acidity meter (GMK-835N, Gwon-highteck, Seoul, Korea). Firmness, TSS, and TA were obtained by averaging the results from three repetitions of continuously harvested fruit in groups 1 and 2. The data obtain procedure was performed five times for each sampling group.

Quantification of total ascorbic acid

The total ascorbic acid contents were determined with some modifications based on the work of Lee et al. (2017). The reagents, L-ascorbic acid (AA), meta-phosphoric acid (MPA), and dithiothreitol (DTT) were supplied by Sigma–Aldrich (Darmstadt, Germany). To determine of the AA contents, sliced fruits were frozen with liquid nitrogen and stored at -80°C in a deep freezer (DF8520, IlShinBioBase, YangJu, Korea). Five grams of pulverized frozen sample were mixed with 15 mL of MPA solution. The mixed sample volume was adjusted to 50 mL (5 g/50 mL) and centrifuged for 10 minutes at 16,800 RCF. Five hundred microliters of supernatant were mixed with 500 μL of DTT solution and incubated for 30 minutes at 25°C. The aliquots were filtered with a 0.45-μm PVDF membrane filter and injected into high-performance liquid chromatography (HPLC) (YL 9100, Younglin, Anyang, Korea) with a UV detector set at 252 nm. The separation was carried out using a ZORBAX NH_2 column (4.6 x 250 mm, Agilent Technologies, Santa Clara, CA, United States) with 30 minutes of run time. Ten micromoles of ammonium dihydrogen phosphate (pH 2.7) were used as the mobile phase at a 1.0 mL min^{-1} flow rate.

Quantification of sucrose, glucose, and fructose

Three major free soluble sugars, sucrose, glucose, and fructose were measured using an HPLC (Dionex ultimate 3000, Thermo Fisher Scientific, Waltham, MA, United States) equipped with a Shodex RI-101 Detector (Showa Denko,

Tokyo, Japan). The glucose reagent was obtained from Junsei Chemical (Tokyo, Japan), and the sucrose and fructose reagents were obtained from Sigma–Aldrich. Five grams of pulverized frozen sample were mixed with triple distilled water, and the mixed volume was adjusted to 50 mL (5 g/50 mL). Each sample was filtered with a 0.45-μm PVDF membrane filter, and 10 μL of the filtrate was injected into the HPLC. The separation was conducted using a Sugar-Pak column (6.5 x 300 mm, Waters Corp., Perth, Australia) held at a 70°C oven temperature with 30 minutes of run time. Ten micromoles of HPLC-grade distilled water were used as the mobile phase at a 0.5 mL min^{-1} flow rate. The HPLC measurement results obtained the total soluble sugar contents by summing the individual sugar contents.

Quantification of individual carotenoid content

The separation of carotenoids was performed by HPLC (Dionex ultimate 3000, Thermo Fisher Scientific) with some modifications based on the work of Yoo et al. (2017). Nine carotenoids were purchased from Sigma–Aldrich and used for standard: capsanthin, capsorubin, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, violaxanthin, lutein, and zeaxanthin. All experiments were performed in the dark to prevent carotenoid degradation. First, 100 mg of pulverized frozen samples were homogenized for one minute with two 6 mm glass beads, 300 μL of tetrahydrofuran (THF), 300 μL of methanol (MeOH) containing 5% butylated hydroxyl-toluene (BHT), and 50 μL of Mg-carbonate

in a 2-mL tube using a TissueLyser II (QIAGEN, Hilden, Germany) and the solution was incubated at 4°C for 20 minutes. The extract was centrifuged at 2,700 RCF and 4°C for 5 minutes, and the supernatant was transferred to a new 2-mL tube. Next, the supernatant was mixed with 375 μL of petroleum ether and 150 μL of 25% NaCl, the mixed sample was spun down at 2,700 RCF and 4°C for 3 minutes, and the supernatant was dispensed into a new 2-mL tube. Then, the sample was dried with a high-speed vacuum centrifuge for 2 hours at 45°C. After dispensing 300 μL of 20% KOH+MeOH to the dried sample, shaking incubation was performed at 1 RCF for 10 minutes. Then, 600 μL of THF, 375 μL of petroleum ether, and 150 μL of 25% NaCl solution were added to the sample, and the supernatant was transferred into a new 2-mL tube by centrifugation for 3 minutes at 4°C. Supernatant separation was repeated with petroleum ether until the lower phase lost its carotenoid color. The extract was dried using a high-speed vacuum centrifuge for 2 hours at 45°C after dispensing 500 μL of acetone into the sample tube, vortexing, and the carotenoid particles were completely dissolved with a sonicator. HPLC was conducted after filtering the samples into amber vials.

Statistical analysis

One-way analysis of variance (ANOVA) was conducted using R software version 3.6.1 and the ‘Agricolae’ package. For the post hoc test, Tukey's test ($P < 0.05$) was used when the number of experimental groups was the same, and

in other cases, the Bonferroni correction test ($P < 0.05$) was used. All graphics were plotted using the Python library 'Matplotlib,' version 3.5.1.

RESULTS

Light environment under the interlighting

Sweet pepper plants received most of the total PPFD from natural light until the Group 1 fruit harvest (~80 DAT) (Table 3-1, Fig. 3-1B). After the plants nearly reached the interlighting LEDs, the operation of the LEDs began (50 DAT). During the Group 1 period, LED lighting was applied for one month. The proportion of light offered by interlighting was 13% of that provided by natural light in Group 1. As the amount of natural light decreased in winter (Fig. 3-1B), the proportion of light supplied by interlighting increased to 40% of the total PPFD during the Group 2 period (Fig. 3-1C). The average temperature and daytime humidity were maintained at 25°C and 56%, respectively (Fig. A3-1). For the spectrum, the red-blue or far-red light fractions increased by 2- to 3-fold in the middle canopy under the RB or RBFR, respectively (Fig. A3B). In the lower part of the canopy, the relative proportion of red light increased by 1.7-fold under the RB and RBFR compared to the NL, and the relative proportions of blue light exhibited no significant differences among the light treatments (Fig. A3B).

Fruit yield and individual fresh weight

The total fruit yields of the red and yellow sweet pepper cultivars were markedly increased under the RBFR compared to the NL and RB (Table 3-2). In particular, fruit yields under the RBFR were 9% and 19% higher than under

Table 3-1. Spectral light intensities (μmol m^{-2} s^{-1}) of the light treatments at different growth periods

Light treatment	Sampling period[z]	Photon flux density (μmol m^{-2} s^{-1})					Average YPFD[y]	R: FR[x]
		Blue	Green	Red	Far-red	Total		
NL[w]	Group 1 (2020.09.20-11.14)	64.6	90.5	101.5	47.8	335.9	216.7	2.12
RB		6.2	0.2	36.5	0.0	42.9	38.8	-
RBFR		6.2	0.2	36.7	40.4	43.1	46.4	0.91
NL	Group 2 (2020.11.14-01.03)	19.5	29.3	32.0	15.6	105.6	71.4	2.05
RB		10.3	0.3	60.8	0.0	71.4	64.7	-
RBFR		10.3	0.3	61.0	67.4	71.6	77.4	0.91

[z]Sampling period indicates the harvesting periods for Groups 1 and 2 refer to Fig. 3-1.

[y]YPFD = yield photon flux, normalized ranges of 360 to 760 nm (Sager et al. 1988; McCree 1971).

[x]R: FR = photon irradiance (666-775 nm) / photon irradiance (725-735 nm) (Frankin 2008).

[w]NL, RB, and RBFR mean natural light, NL with red-blue LED interlighting, and RB with far-red light, respectively.

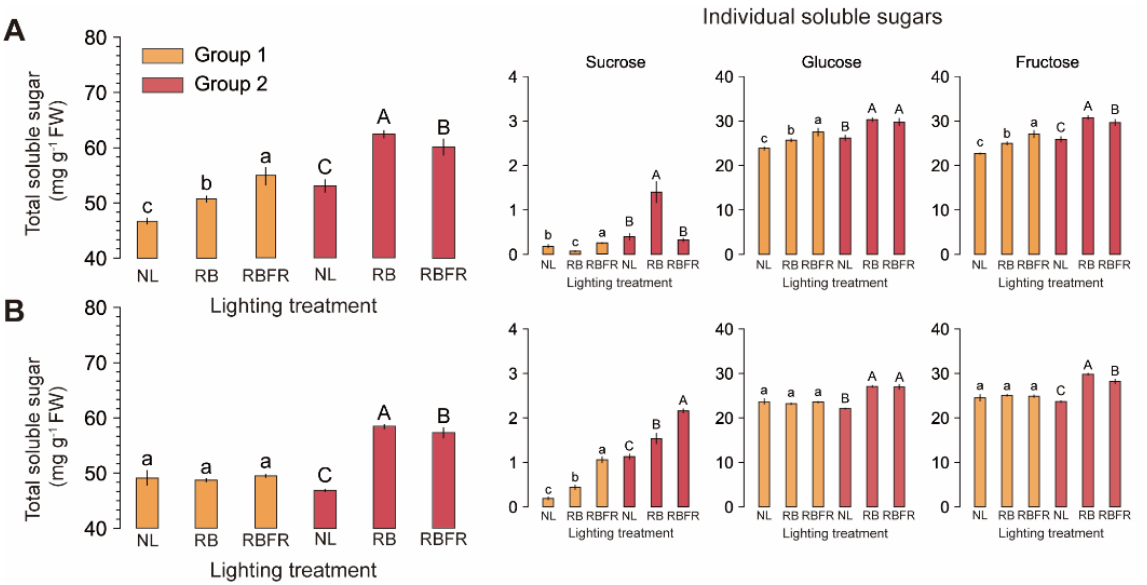

Fig. 3-3. Total and individual soluble sugar content of fully ripened red (A) and yellow (B) fruits of sweet peppers grown under natural light (NL), NL with red-blue LED interlighting (RB), and RB with far-red light (RBFR), respectively. Different letters indicate significance for light treatments within the same fruit color, as determined by Tukey's HSD test, $\alpha = 0.05$. Mean ± SD (n = 5). The total soluble sugar was calculated by adding the individual soluble sugars. Groups 1 and 2 indicate fruits sampled at different harvesting periods, as shown in Fig 3-1.

Table 3-2. Fruit yield, individual fruit weight, length and width of harvested red and yellow sweet peppers grown under natural light (NL), NL with red-blue LED interlighting (RB), and RB with additional far-red light (RBFR).

Treatment	Fruit color	Fruit yield (kg m^{-2})	Fruit weight (g/fruit)	Fruit length (cm/fruit)	Fruit width (cm/fruit)	DAP to harvest
NL	Red	1.207	190.9 a[z]	8.53 a	7.32 a	48.2
RB		1.477	172.6 b	7.84 b	6.95 b	47.6
RBFR		1.607	192.8 a	8.35 a	7.28 a	48.0
NL	Yellow	1.492	207.0 a	8.00 a	7.52 a	52.2
RB		1.516	194.0 b	7.72 a	7.16 b	51.3
RBFR		1.806	206.0 a	8.03 a	7.40 ab	50.5

[z]Different letters indicate significance among treatments within the same fruit color using the Bonferroni correction for multiple testing with a significance level of $\alpha = 0.05$ (n=91, 123, 124, 104, 113, and 126 for NL-Red, RB-Red, RBFR-Red, NL-Yellow, RB-Yellow, RBFR-yellow).

the NL for red and yellow fruits, respectively. Under the RB, the individual fruit weights per fruit were significantly lower than those for the NL and RBFR for both fruit colors. For the red fruits, the lengths and widths were shorter under the RB than under the NL and RBFR. Only the fruit widths were significantly smaller under the RB than the NL for the yellow fruits. There were no significant differences among the treatment groups in the period from the day after pollination (DAP) to harvest (Table 3-2).

TSS, titratable acidity, firmness, and ascorbic acid content

The TSSs of most fruit groups in the interlighting treatments were significantly higher than those under the NL. In Group 1, the red fruit showed approximately 13% and 11% higher TSS under the RB and RBFR than the NL, respectively. The yellow fruit showed approximately 24% higher TSS under the RB than the NL. In Group 2, only yellow fruit showed 24% and 16% higher TSS under the RB and RBFR than the NL, respectively. The titratable acidity (TA) showed no significant differences among the light treatments. The TSS: TA was higher under the RBFR than under the NL in the red fruits of Group 1 (Table 3-3). The ascorbic acid contents exhibited consistent differences among the light treatments and increased with additional FR light levels. Under RB and RBFR, the ascorbic acid contents were higher than those under NL, except for the yellow fruit of Group 1. The TSSs estimated from HPLC analysis were higher in the RB and RBFR than under the NL (Fig. 3-3). In the Group 1 samples, only the red fruits exhibited significant differences, which were in the order of RBFR,

Table 3-3. Total soluble sugar (TSS), titratable acidity (TA), TSS: TA, firmness, and ascorbic acid content of the sampled sweet pepper fruits under different light treatments

Treatment	Fruit color	Sampling period	TSS (Brix)	Titratable acidity (g/L)	TSS: TA	Firmness (kg LB/Newton)	Ascorbic acid content (μg/g FW)
NL	Red	Group 1 (2020.9.20-11.14)	6.13 b[z]	2.64 a	2.37 b	16.2 a	568.8 c
RB			6.90 a	2.79 a	2.50 ab	18.3 a	636.9 b
RBFR			6.83 a	1.92 a	3.63 a	19.3 a	711.0 a
NL	Yellow		6.10 b	2.24 a	2.74 a	14.6 b	722.5 b
RB			7.60 a	2.44 a	3.19 a	18.0 a	729.4 b
RBFR			6.77 ab	1.97 a	3.46 a	16.6 ab	846.4 a
NL	Red	Group 2 (2020.11.14-01.03)	7.90 A	2.93 A	2.70 A	20.6 B	704.9 C
RB			9.87 A	3.37 A	2.93 A	19.9 AB	818.0 B
RBFR			8.16 A	2.35 A	3.61 A	22.3 A	891.7 A
NL	Yellow		7.07 C	3.05 A	2.35 A	13.6 A	767.2 C
RB			8.80 A	3.18 A	2.83 A	15.5 A	1,773.3 B
RBFR			8.23 B	3.13 A	2.92 A	18.5 A	1,836.3 A

[z]Different letters indicate significance for light treatments within the same fruit color, as determined by Tukey's HSD test, $\alpha = 0.05$ ($n = 5$). Statistical tests in Groups 1 and 2 were indicated by lowercase and uppercase letters, respectively. The sampling period indicates the harvesting periods for Groups 1 and 2 refer to Fig. 3-1.

RB, and NL (Fig. 3-3A). In Group 2, both red and yellow fruits exhibited values in the order of RB, RBFR, and NL. The sucrose levels showed differences under several light treatments but accounted for 4% of the lower portion in all samples. All of the significant patterns of the glucose and fructose concentrations were consistent with the TSS concentrations except for the glucose concentrations in Group 2.

Fruit coloration and carotenoid content

The surface of sweet pepper fruit color is unevenly ripened (Fig. 3-4A). After the breaker stage (DAT 45), the surface color was not significant as it exhibited very large standard deviations across all treatments (Figs. 3-4B, C). There was no trend in fruit ripening rate in Group 1, but in Group 2, fruit ripening was partially promoted at DAT 40 to 50 under RB and RBFR in red and yellow fruits, respectively. Among the nine carotenoids analyzed in this study, eight were detected except for α-carotene, which were capsanthin, capsorubin, zeaxanthin, β-cryptoxanthin, and β-carotene in red fruits and violaxanthin, lutein, and zeaxanthin in yellow fruits (Fig. A3-2). In Group 1, only red fruits exhibited significant increases in carotenoid content from the RB and RBFR compared to the NL (Fig. 3-5A), and the total carotenoid contents were not significantly different between the RB and RBFR (Fig. 3-5). In Group 2, the total carotenoid content in red and yellow fruits was higher in the order of RB, RBFR, and NL (Fig. 3-5). The total carotenoid content in Group 2 was 3.0- and 2.1-fold higher than those for the NL in red and yellow fruits in the RB and were

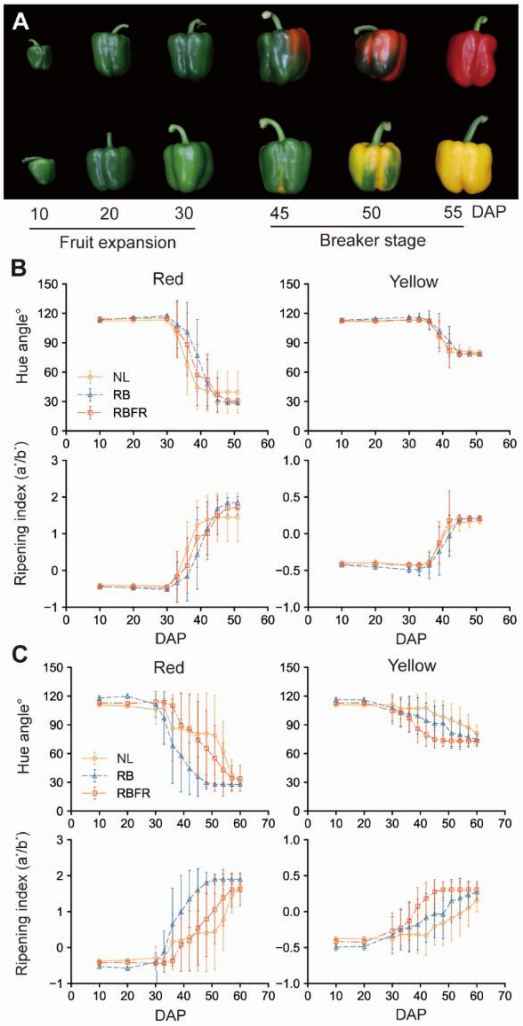

Fig. 3-4. Fruit coloration of red and yellow fruits of sweet peppers grown under natural light (NL), NL with red-blue LED interlighting (RB), and RB with far-red light (RBFR), respectively. The developmental process of sampled fruits (A), hue angles, and ripening index of red and yellow fruits according to DAP in Groups 1 (B) and 2 (C). The hue angles and ripening indices were calculated using the L^*, a^*, and b^* values. Hue angle was calculated with $180 + \tan^{-1}(b^*/a^*)$. Groups 1 and 2 indicate fruits sampled at different harvesting periods, as shown in Fig. 3-1. Values are the mean ± SD of 30 replicates.

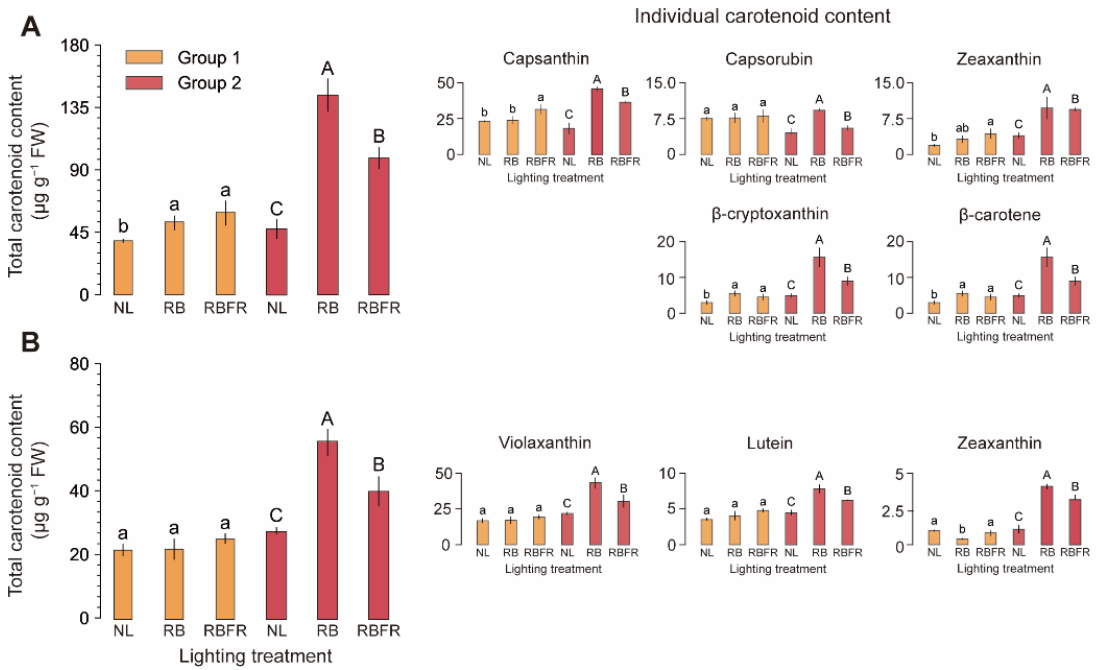

Fig. 3-5. Total and individual carotenoid content of red (A) and yellow (B) fruits of sweet pepper grown under natural light (NL), NL with red-blue LED interlighting (RB), and RB with far-red light (RBFR), respectively. Different letters indicate significance for light treatments within the same fruit color, as determined by Tukey's HSD test, $\alpha = 0.05$. Values are the mean ± SD of four replicates. Groups 1 and 2 indicate fruits sampled at different harvesting periods, as shown in Fig. 3-1.

were 2.0- and 1.4-fold higher under the RBFR, respectively (Fig. 3-5). The individual carotenoid content was significantly lower in red fruits under the RBFR than those under the RB, except for zeaxanthin, and in the yellow fruits, all individual carotenoid content was lower under the RBFR than under the RB. Total carotenoid content in red and yellow fruit was improved during the entire sampling period compared to NL, about 57% and 33% under the RB and 24% and 22% under the RBFR, respectively. Nevertheless, the RBFR showed approximately 18-24% lower total carotenoid content for both fruit colors than the RB during the overall period (Fig. 3-6).

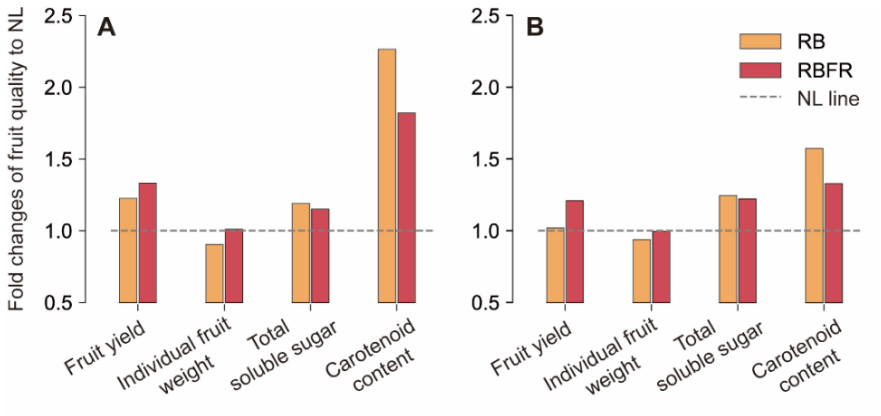

Fig. 3-6. Fold changes in fruit qualities of red (A) and yellow (B) fruits of sweet pepper to natural light (NL) during overall cultivation. RB and RBFR mean NL with red-blue LED interlighting and RB with far-red light, respectively. Total soluble sugar and carotenoids content were calculated from data overall sampling periods (Refer to Fig. 3-3, 4).

DISCUSSION

Interlighting effects according to the vertical position of fruits

Greenhouse crops such as tomato and cucumber have trellised training systems that continuously prune while winding the stems into the growing beds between two anchor posts (Hanafi and El-Fadl 2000). Therefore, the positions of the existing stems continue to move downward during cultivation. On the other hand, in sweet pepper, since it is vertically trellised upward (Jovicich et al. 2004), the shoot meristems of the plants move upward with increasing plant height with growth progress. In this study, since the interlighting modules were fixed, the effects of interlighting may vary from the vertical positions of the leaves or fruits. Thus, fruit sampling was performed twice during the growing period to equally analyze the fruit qualities in Groups 1 and 2, considering the heterogeneity of the vertical positions of the fruit sets in the canopy (Fig. 3-2). In Group 1, since the plants were grown when the amount of natural light was relatively high, the effect of the interlighting spectrum was relatively small (Fig. 3-1C, Table 3-1). In Group 2, the amount of natural light decreased as winter progressed, and interlighting was conducted in all periods after fruit setting (Table 3-1). In addition to the effect of natural light, the plants had not yet reached the position of the interlighting LEDs, so the lighting durations for Group 1 were relatively shorter for 30 days. Thus, fruit samplings were divided into two groups to evaluate the effects of interlighting on fruit qualities under

light variation during the total cultivation period.

Yield and individual fruit weight increase under RBFR interlighting

Several studies have reported increased tomato yields and individual fruit fresh weights under red-blue light with additional FR light (Kalaitzoglou et al. 2019; Kim et al. 2020). Similarly, this study observed greater individual fruit fresh weights and yields under the RBFR than under the RB for both red and yellow fruits (Table 3-2). The fruit lengths and widths were greater under the RBFR than under the RB, directly related to the higher individual fruit weights and yields. The increases in fruit yield have been explained by the increases in photosynthetic products (Zhen and Bugbee 2020) or by higher dry matter partitioning to fruits (Ji et al. 2019) induced under FR light. In this study, the RBFR did not change the individual fruit weights compared to the NL, but these decreased under the RB (Table 3-2). The RB showed much higher red-blue proportions than the NL in the middle canopies (Fig. 3-2B). Therefore, the individual weight losses under the RB may occur due to the spectral effect from higher R: FR or blue light. In the middle or lower canopy, the FR light generated by the natural shading from upper leaves may be insufficient because its intensity depends on entirely solar irradiance. This result suggests that adding FR light to the RB interlighting could benefit higher yields both in the top lighting and the inter or intra-canopy lighting method.

Physicochemical changes in fruits under the RB and RBFR interlighting

Fruit quality factors such as TSS, TA, and firmness are essential physicochemical factors that can determine the basic fruit taste or texture of sweet peppers (Ghasemnezhad et al. 2011). There have been studies on the fruit yields under red-blue light in sweet peppers (Joshi et al. 2019; Sobczak et al. 2020), but the effect of FR light on fruit quality under greenhouse conditions has rarely been investigated. In this study, the TSSs were higher under the RB or RBFR than under the NL but did not significantly increase with additional FR light (Table 3-3, Fig. 3-3). In tomatoes, when adding FR light to red light, higher TSS levels were reported than when using only red LEDs (Kim et al. 2020). It was also reported that FR light could upregulate sugar transportation and metabolism in tomato fruits by overexpressing those genes related to starch synthases in chamber conditions (Ji et al. 2020). In Group 2, the RB and RBFR increased the individual sugar contents of sucrose, glucose, and fructose compared to the NL (Fig. 3-3), but no increase in TSS (Brix°) with adding FR light (Table 3-3). This experiment was conducted without shading curtains, unlike previous studies conducted under limited solar irradiance (Ji et al. 2020), since interlighting provides a small portion of the total light. Thus, FR light intensity may not be sufficient to improve the fruit soluble sugar. Reasons other than natural light may be the fruiting properties of sweet peppers. Unlike tomatoes with 3 or 6 fruits hanging per truss (Abdalla and Verkerk, 1968), sweet peppers do not have more than one fruit on each node, so the total number of

fruits may vary depending on the light treatment. Since the total number of fruits in this study was lower under the RB, splitting photosynthetic products to each fruit may differ among treatments. Although higher TSSs were observed under both interlighting treatments than under the NL, additional PAR is thought to increase carbohydrate assimilation to the fruit due to the higher photosynthate production in leaves. However, additional FR light did not significantly affect the TA, TSS: TA, or firmness.

The ascorbic acid concentrations were higher under the RB and RBFR than under the NL in Group 2. Higher PAR levels resulted in increased ascorbate accumulations in tomato leaves and fruits (Massot et al. 2012; Zushi et al. 2020). In this study, ascorbate contents under the RBFR increased by 9% and 4% compared to RB in red and yellow fruits, respectively. In previous studies, a decrease in R: FR induced a decrease in ascorbate, but this decrease was accompanied with the low level of sun irradiance. Ntagkas et al. (2019) reported ascorbate decrease from exogenous FR lights on postharvest tomato fruits but have not analyzed the effect of FR during cultivation. In contrast with tomatoes, the ascorbate levels in sweet pepper fruits are highest during the immature green stage and gradually decrease during ripening. Therefore, further studies on sweet pepper fruit quality by light spectrum are needed.

Carotenoid accumulation in fruits affected by the RBFR interlighting

Eight carotenoid components were analyzed to investigate the effect of interlighting on the carotenoid content of red- and yellow-colored fruits. The major carotenoids are capsanthin and capsorubin in red fruits and violaxanthin and lutein in yellow fruits. In plants, the accumulations of carotenoids in fruit are closely related to light (Llorente et al. 2017). Previous research has revealed increased total carotenoid content under red or blue LED light in controlled environments (Naznin et al. 2019). There have been no reports on the effects of red-blue and FR interlighting on the carotenoid content of sweet peppers in greenhouse conditions. In this study, the RB showed a 3-fold higher total carotenoid content than the NL in Group 2. However, the total carotenoid content was relatively higher under the RBFR than under the NL by 2.1-fold but was approximately 40% lower than those under the RB (Fig. 3-5). This tendency was consistent for both red and yellow fruits. Despite the equal amounts of PAR light, the RBFR showed a lower total carotenoid content. Llorente et al. (2016) reported that several fruits, such as tomatoes, accumulate carotenoids after the breaker stage, and R: FR can be a signal to start carotenoid accumulation. This chapter speculated how the fruit carotenoids changed by adding FR to the RB lighting, even in a greenhouse with background sunlight. For the individual carotenoid components, the patterns of significance in the total carotenoid content were equally shown as RB > RBFR > NL in red and yellow fruits, which indicated that the two fruit colors responded similarly to

the interlighting. Unlike other studies on tomatoes in which the lighting changed the surface color, no significant differences in fruit color were found (Fig. 3-4B, C). This result seems to be because the color of the sweet pepper surface changes irregularly as fruits ripen (Fig. 3-4A). This study analyzed the qualities of fully ripened fruits, and there was no significant difference in the time it takes to harvest (Table 3-2). However, practically sweet pepper fruits were commonly harvested when about 80% of the color was colored for shelf-life. During Group 2, overall pigmentation was accelerated by RB and RBFR treatment for about 5-10 days (Fig. 3-4C). Thus, the light treatment can advance the harvest time like other fruits (Xie et al. 2019). In fruit carotenoids, additional FR light could result in the relatively lower carotenoid than those under RB interlighting alone. This trend was different depending on the harvesting group. In Group 1, no significant differences in total carotenoid content were found in red and yellow fruits, and the levels of only several individual carotenoids increased in red fruits. This result means that the effect of interlighting may also be different depending on the vertical position of the fruit hanging on the stem. Therefore, for the crops continuously attracted upward with harvesting, such as sweet pepper, it is necessary to move the lighting system upward to where the fruits or leaves are located to obtain the optimal lighting effects.

Fruit qualities under the RB and RBFR interlighting during the overall growth period

During the total growing period, the quality of sweet pepper fruits was improved under the RB or RBFR rather than the NL (Fig. 3-6). The fruit yield was higher under the RBFR than the RB, but the carotenoid was higher under the RB than the RBFR. Soluble sugars and ascorbic acid increased similarly in both treatments (Fig. 3-6, Table 3-3). When adding FR to RB interlighting, only the R: FR was changed with the same amount of PAR light. From these results, soluble sugar and ascorbic acid in sweet pepper might react more sensitively to light quantity, and carotenoids and individual fruit weight were affected by both light quantity and quality (e.g., R: FR, blue-far-red interaction) (Brown et al. 1995; Demotes-Mainard et al. 2016). In addition, carotenoid improvement may be attenuated by additional far-red light due to antagonism with red or blue light (Llorente et al. 2016; Park and Runkle 2019). Therefore, choosing whether to add far-red light to the interlighting wavelength is necessary, focusing on two different purposes: improving yield or fruit functional properties.

Overall fruit qualities relation to fruit cultivar

Of the two sweet pepper cultivars, red (‘Mavera’) and yellow (‘Florate’) cultivars, the yellow cultivar was more dominant to vegetative growth (Enza Zaden product page June 13, 2022). The effect of RB or RBFR may differ for each cultivar because FR is fundamentally involved in the reproductive structure (Kim et al. 2019). Therefore, fruit yield and quality changes may differ depending on the variety. Interestingly, there was no yield increase under RB in the yellow cultivar, which may be because RB supplementation may interfere with the transition to the reproductive stage. Under RB, relatively small fruit weights, length, and width were observed.

Fruit size potentials are difficult to analyze as they relate to factors such as total assimilation products or source-sink strengths (Schapendonk and Brouwer 1984). As one possibility, this study observed a compact fruit set in the plant grown under RB due to the narrow internode length (Fig. A3-3). Physical space can also be a factor in determining the size of fruits because the size or shape of a general fruit is the most important quality factor. Therefore, the basic characteristics of the cultivar, such as internode length and vegetative strength, may also have affected the lighting spectrum.

CONCLUSIONS

In this study, supplemental interlighting with red-blue light (RB) or additional far-red light (RBFR) could increase overall fruit yields and qualities such as fruit soluble sugar, ascorbic acid, and carotenoid content in sweet pepper compared to solely natural light conditions. Fruit yields and individual fresh weights were higher but carotenoid contents were 18-24% lower under the RBFR than the RB. This study showed that additional far-red lighting has a trade-off relationship between fruit yields and carotenoid content. Thus, it is necessary to provide an adequate light spectrum for cultivation purposes, such as improving yield or accumulating carotenoids in fruits. In addition, fruit quality differed according to the vertical position of fruit sets, which means the location of interlighting fixtures needs to be corrected to the upper canopy for maximizing light use efficiency.

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GENERAL DISCUSSION

This study analyzed the effect of adding far-red (FR) light to red-blue interlighting (RB) on the growth, yield, and fruit quality of sweet peppers. First, the effect of FR light on the increase in photosynthate production was described. Second, vegetative-reproductive balance by additional FR light were discussed. Finally, the effect of adding FR light on the fruit qualities was discussed.

Increased photosynthates production by adding FR light to RB

Several studies have reported enhancement of growth and photosynthesis by FR light in the leafy vegetables in indoor cultivation (Lee et al. 2015; Zou et al. 2019). In greenhouses, FR light was relatively less illuminated than light sources in photosynthetically active radiation ranges (PAR, 400-700 nm). FR supplementation has increased total dry weight or yield and morphological changes in greenhouse tomatoes (Park and Runkle 2017; Zhang et al. 2019). In Chapter 2, adding 40-100 μmol m^{-2} s^{-1} of FR light to 100 μmol m^{-2} s^{-1} of RB achieved an 8~12% increase in the total dry mass assimilation in sweet peppers.

The 3D simulation revealed that structural acclimation by adding FR light to RB contributed to the higher total plant assimilation. In the greenhouse, sunlight is the main source for plant photosynthesis. Interlighting as a supplemental light source, and its contribution to total PAR was less than 20%. Nevertheless, adding FR light to RB mediates the plant morphological

responses with low photon energy. Therefore, growth and yield could be improved by adding FR light to interlighting even with low energy input.

Adding FR light to vegetative-reproductive balance during cultivation

In fruit vegetables, the growth of reproductive organs is important in achieving a higher yield. Therefore, physiological stimulation is necessary to set the fruits in fruit vegetables. The reproductive transition in greenhouse crops has been mainly guided by mild stress induction by temperature, irrigation (EC, water deficit), and CO_2 control (Sato et al. 2006; Fanasca et al. 2007; Qian et al. 2012).

Generally, the photosynthetic source-sink balance is determined by the developmental level of vegetative and reproductive organs (Li et al. 2015). FR light also influences photosynthesis and plant developmental processes (Ji et al. 2020). FR light advanced harvest time and improved yield in sweet peppers (Fig. 2-9), but it is unclear whether these effects were due to the improved total photosynthate or promoted flowering. This study adopted pruning management with maintaining one fruit per node. Since the fruit load was the same within treatments, the improvement in yield may be due to sufficient plant vegetative growth or source capacity under the additional FR light. However, the assimilation product partitioning by FR light has not been studied when pruning or fruit management is not performed. Therefore, further research is needed without pruning to determine the source-sink balance by FR light for sweet pepper plants.

Conversely, with no FR fraction, RB interlighting increased leaf thickness and shorter internode length. RB interlighting also slows down the meristem growth and lessens total node development. A high blue fraction mediates that an inefficient morphogenetic reaction is induced when closely irradiated in cucumber plants (Trouwborst et al. 2010). In Chapter 2, the 20 μmol m^{-2} s^{-1} of additional FR light counteracted the blue-induced morphological responses such as thicken leaf. In another way, even a small level of about 6% of the blue fraction could offset the inadequate plant response to blue light (Kaiser et al. 2018). These studies have implied the proportion of red, blue, and far-red light could interact with each other. Therefore, introducing LED dimming for the red, blue, and far-red light is essential to control subsequent plant responses and derive optimal crop yields during cultivation. Moreover, the plant management of this study (e.g. temperature, pruning, EC) was constant, but the subjective judgment of the grower needs to be intervened the interlighting spectrum according to cultivation purpose or sweet pepper variety.

Effect of supplemental FR light on fruit quality

Adding FR light to RB increased the fruit qualities of sweet peppers, such as soluble sugar or ascorbic acid compared to natural light (Table 3-3). Soluble sugar or ascorbic acid increases were accompanied by enhancement of total photosynthates. In tomatoes, driving photosynthates to sink organs also was increased under FR light, which resulted in higher soluble sugar in fruits (Ji et

al. 2020). In sweet peppers, dry matter production was increased under FR light (Fig. 1-4, 2-3), but the dry mass partitioning to fruits did not show a significant difference. Therefore, this study did not find any relationships between far-red light and sink activity in the fruits. Thus, the increase in soluble sugars was presumed to be due to an increase in the total amount of photosynthesis. Thus, additional experiments are needed on a wider range of sweet pepper varieties for FR light and photosynthetic product partitioning to fruits.

Carotenoid contents were higher under solely RB than the adding FR light to RB. FR light changes phytochrome-interacting factors (PIF) abundance, which conduct central roles in fruit ripening and carotenoid biosynthesis linking to *the SIPSY1* gene in tomatoes (Llorente et al. 2016; Gramegna et al. 2019). This study showed a trade-off relationship between yield enhancement and carotenoid production by FR light (in Chapter 3). Although several studies have reported that the increased red light enhances carotenoids (Dannehl et al. 2021), the carotenoids change under the addition of FR light is the first to be studied in greenhouses.

In the future, it will help derive the optimal light quality by accumulating research on yield to fruit quality under red, blue, and far-red light in greenhouse sweet peppers. In addition, the optimal growth spectrum should be derived for each variety, considering vegetative-reproductive strength and morphological traits.

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GENERAL CONCLUSIONS

This study confirmed the overall effect of far-red light supplemented to red-blue interlighting on the growth, yield, and fruit qualities of greenhouse sweet peppers, and analyzed the contributing factor to the yield improvement. The results can be meaningful because the overall cultivation effect was evaluated under natural light. Far-red light effectively increased the fruit yield compared to solely red-blue interlighting under weak sunlight in winter. The dose-response to additional far-red confirmed the increase in photosynthate assimilation under higher far-red treatments. The simulation confirmed that cumulative morphology improvement under the additional far-red light contributed to the higher total assimilation than the decrease in long-term photosynthetic capacity. The higher yield was achieved at approximately 60% of the far-red dose to red-blue interlighting. In addition, Fruit quality was improved in soluble sugar and ascorbic acid when grown under red-blue or far-red light than grown under solely natural light. However, the carotenoid contents of red and yellow cultivars were decreased by approximately 24% and 18% with additional far-red light, respectively. In summary, adding far-red light to the interlighting increased plant growth from sun-favored morphologies. The increased photosynthates improved fruit quality. However, no significant improvement in fruit quality was observed between interlighting with and without far-red light. These results will be helpful in deriving an optimal growth condition of sweet peppers by precisely controlling the interlighting spectrum.

ABSTRACT IN KOREAN

원적색광(Far-red, FR, 700-750 nm)은 '음지 회피 반응(Shade avoidance response)' 이라는 피토크롬이 매개하는 다양한 생리적 활성에 관여하고 있기 때문에 원예작물 보광에 추가되고 있다. 수관 내부 보광(Interlighting)은 중단 및 하단부 작물 수관에의 광 부족을 보완하기 위한 조명 방법이다. 본 연구에서는 착색단고추의 광합성, 수확량 및 과일 품질에 대해 FR이 추가된 수관 내부 보광의 효과를 분석했다. 먼저, 겨울과 여름 기간에 자연광, 적색-청색 수관 내부 보광(RB) 및 RB에 FR을 추가한(RBFR)의 3가지 광 처리를 매일 12시간의 광 주기로 처리했다. 겨울과 여름의 일적산광(DLI)은 16.6과 32.2 mol m^{-2} s^{-1}로 여름에 약 2배 높았다. RBFR에서는 겨울의 RB보다 작물의 건물중과 과실수량이 20%, 17% 높았으나 여름에는 생육, 수확량 및 형태형성에 유의한 차이가 관찰되지 않았다. RB보광 하에서 FR의 적절한 선량을 조절하기 위해 100 μmol m^{-2} s^{-1}의 RB 하에서 5가지 FR 조건 (0, 20, 40, 60, 80 μmol m^{-2} s^{-1}: FR 0, FR 20, FR 40, FR 60, FR 80)에 대하여 그 효과를 분석하였다. 겨울 작기에 RB에 FR을 추가하면 FR 60 조건에서 가장 큰 총 건물중의 증가를 보였다. 시뮬레이션 결과는 건물증 증가가 개선된 광형태형성으로 인한 것임을 보여주었다. 그러나 FR은 잎 노화를 가속화하고 V_{cmax}와 J_{max}의 광합성 능력의 더 빠른 감소를 가져와 형태적 순응으로 인한 총 동화 증가를 감소시켰다. 그 외에도 높은 FR 광도는 초기 생육 단계에서 가속화된 생식생장 구조로 인해 더 빠른 수확량 증가에 기여하였다. 단독 RB 수관 내부 보광의 더 높은 R: FR 비율은 수확량 증가에 덜 효과적일 수 있으며, 이는 늦은 생식생장 진행 및 잎에 바이오매스 할당을 높게 분배하는 등의 수확량에 비효율적인 생리 반응과 연계될 수 있다. FR을 추가하면 FR 20의 소량부터 FR 80까지 모두 수확량 증가에 기여했지만 이 효과는 60 μmol m^{-2} s^{-1} 세기에서 포화되었다. 겨울 작기에 대한

결과를 중심으로, 적색과 노란색 두 가지 착색단고추 품종에서 과실 수확량, 유리당, 아스코르브산, 카로테노이드 함량 등의 과실 품질을 분석하였다. FR을 추가하면 전체 수확량과 개별 과일 크기가 개선되었으나, TSS는 FR에 의해 유의한 차이를 나타내지 않았다. RB와 RBFR의 카로테노이드 함량은 자연광에서 보다 전반적으로 개선되었지만 RBFR에서의 카로테노이드 증분은 RB보다 적색 및 노란색 품종에서 각각 24%와 18% 감소하였다. 따라서 추가 FR 보광은 과일 수확량과 카로테노이드 함량 사이에 상충 관계가 있을 수 있다. 본 연구는 원적색광을 적색-청색 보광에 추가했을 때의 전체적인 효과를 온실에서 평가하였다는 점에서 의의가 있으며, 본 연구결과는 군락 내 보광 스펙트럼을 정밀하게 제어함으로써 착색단고추의 최적 생육을 위한 광 환경을 도출하는데 도움이 될 것이다.

추가 주요어: 광합성, 광 추적 시뮬레이션, 카로테노이드, 형태형성

학번: 2019-36049

APPENDIX

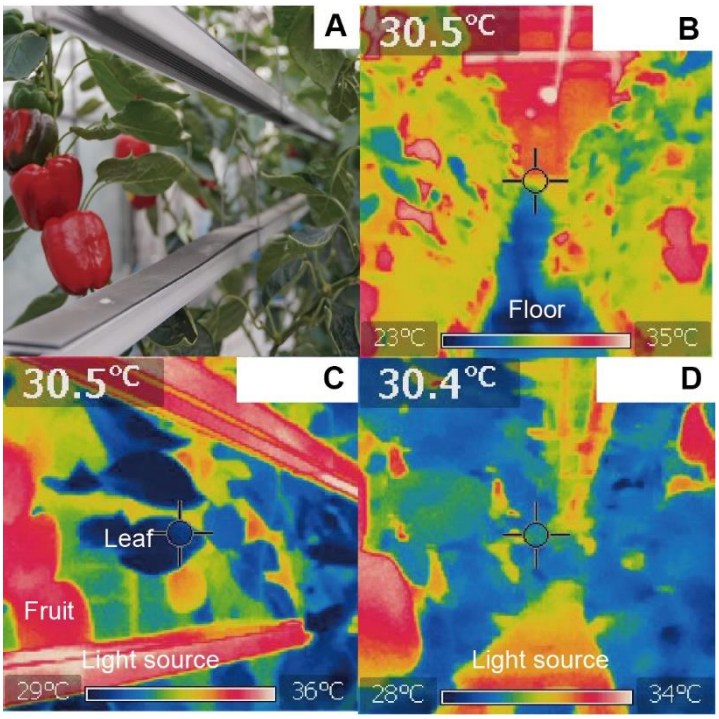

Fig. A1-1. Photo and thermal images in the greenhouse. Photo of interlighting bars of red-blue (A), thermal images of control (B), red-blue (C), and red-blue-far-red (D) treatments. Refer to Fig. 1-1 for the spectral distribution of the interlighting treatment.

Table A2-1. Spectral light properties (µmol m^{-2} s^{-1}) of light treatments

Photon flux density (µmol m^{-2} s^{-1})								
Treatment	Blue (400-500 nm)	Green (500-600 nm)	Red (600-700 nm)	Far-red (700-800 nm)	PPFD[z] (400-700 nm)	Total PFD (400-800 nm)	R: FR[y]	PSS[x]
NL[w]	154.5	233.0	258.4	195.8	646.0	842.0	1.03	0.72
FR 0[v]	11.1	1.5	86.7	0.3	99.3	99.6	783	0.88
FR 20	10.7	1.1	87.4	20.0	99.2	119.2	4.67	0.83
FR 40	10.6	0.9	90.3	40.0	101.8	141.8	2.81	0.79
FR 60	11.1	1.0	89.9	59.6	102.0	161.6	2.04	0.76
FR 80	11.2	0.7	90.6	79.7	102.5	182.2	1.66	0.74

[z]PPFD = photosynthetic photon flux density (µmol m^{-2} s^{-1}) (McCree, 1971)

[y]R: FR = red/far-red spectral ratio (655-665 nm / 725-735 nm)

[x]PSS = phytochrome photostationary state (Sager et al. 1988)

[w]Interlighting spectrum were measured at a distance of 20 cm and the light spectrum of natural light (NL) was measured 130 days after transplanting with ten repetitions from 10:00 to 14:00.

[v]FR 0 to 80 represent additional far-red doses of 0 to 80 µmol m^{-2} s^{-1} to red-blue interlighting, respectively.

Table A2-2. Slope and intercept of P_{max} and J_{max} on V_{cmax} derived from linear regression from leaf photosynthesis data collected at 40, 70, 100, and 130 days after transplanting under additional far-red doses to red-blue interlighting

	Slope			Intercept		
Treatment	P_{max}	V_{cmax}	J_{max}	P_{max}	V_{cmax}	J_{max}
FR 0[z]	-0.113	-0.318	-0.489	33.89	94.75	158.6
FR 20	-0.179	-0.518	-0.789	36.18	97.35	176.8
FR 40	-0.152	-0.488	-0.694	32.84	88.30	154.2
FR 60	-0.196	-0.625	-0.798	35.49	92.56	165.4
FR 80	-0.140	-0.324	-0.663	31.58	74.11	152.4

[z]FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

Table A2-3. Fruit yield and basic properties under additional far-red doses to red-blue interlighting

Treatment	Fruit yield ($kg\ m^{-2}$)	Fruit number	Fruit weight (g/fruit)	Fruit length (cm/fruit)	Fruit width (cm/fruit)	Water content ($g\ g^{-1}$)
FR 0[z]	4.02	153	197.4 b[y]	87.4 b	82.1 b	8.38 a
FR 20	4.19	142	223.3 a	92.2 a	85.4 a	8.04 a
FR 40	4.28	147	218.7 a	92.9 a	86.0 a	7.73 a
FR 60	4.45	146	228.0 a	93.6 a	87.3 a	8.19 a
FR 80	4.44	152	219.1 a	93.3 a	87.0 a	8.59 a

[z]FR 0 to 80 indicate additional far-red doses of 0 to 80 μmol $m^{-2}\ s^{-1}$ to red-blue interlighting, respectively.
[y]Different letters indicate statistically significant with Tukey's post hoc test with values per treatment (n = 9).

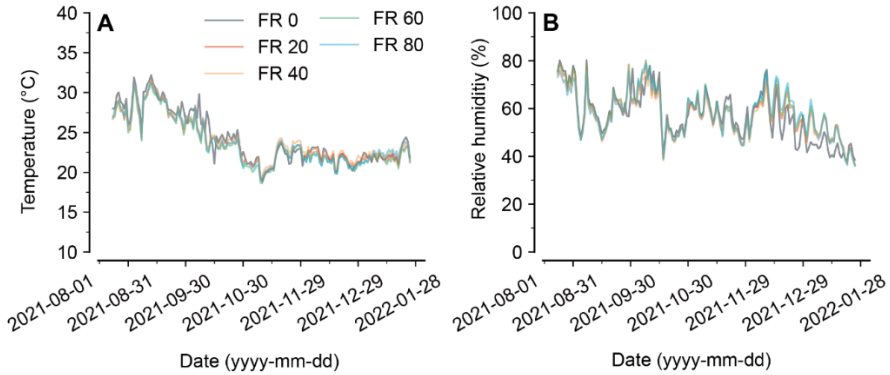

Fig. A2-1. Greenhouse environment under different light treatments under additional far-red doses to red-blue interlighting. (A) Temperature; (B) relative humidity. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

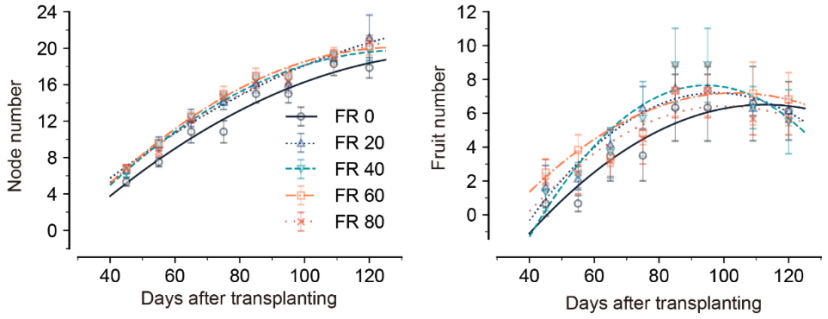

Fig. A2-2. Non-destructive measured node and fruit development under additional far-red doses to red-blue interlighting every ten days. (A) Node number; (B) Fruit number. A trendline depicts the fitted line estimated with quadratic regression. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

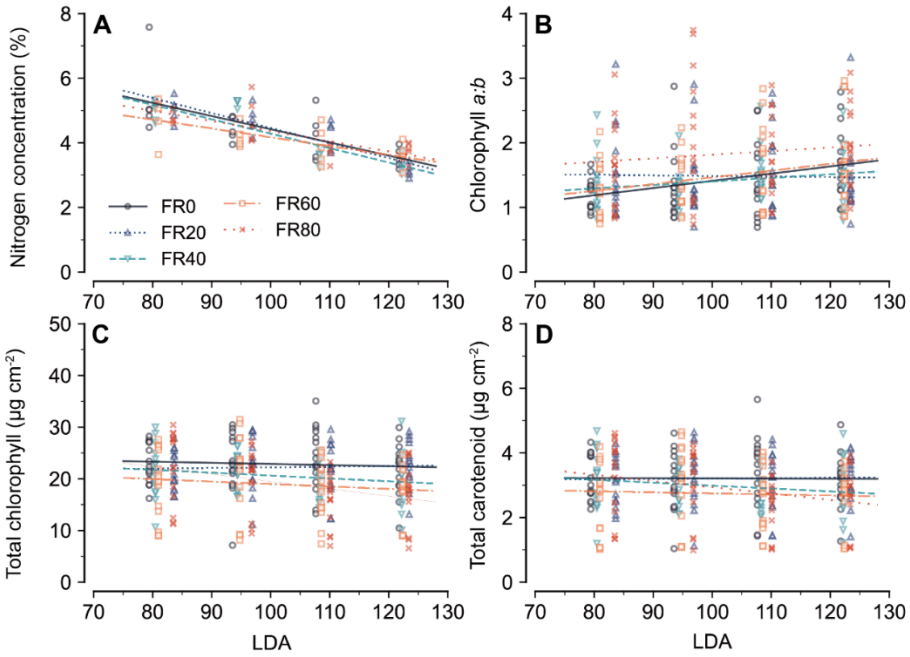

Fig. A2-3. Leaf nitrogen and chlorophyll concentrations according to leaf developmental age (LDA) under additional far-red doses to red-blue interlighting. (A) Nitrogen concentration; (B) chlorophyll a, b ratio; (C) total chlorophyll concentration; (D) total carotenoid. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

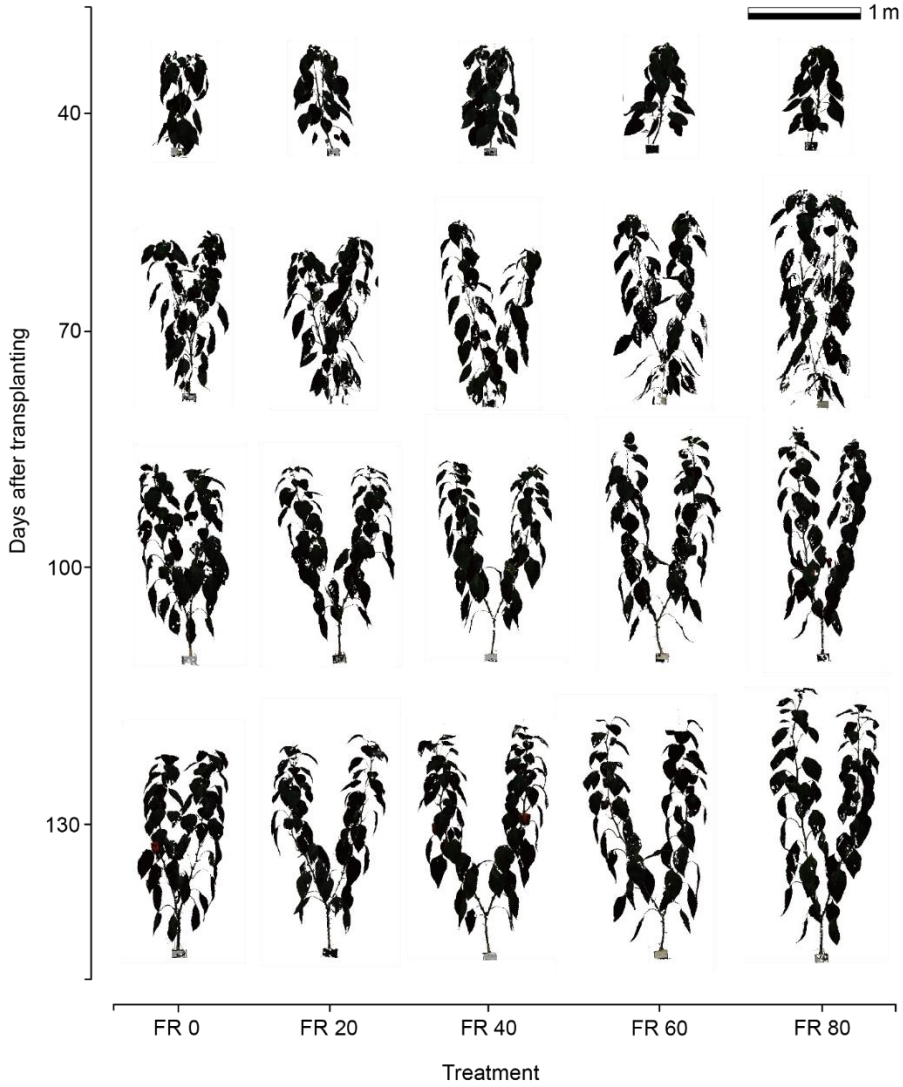

Fig. A2-4. Representative 3D scanned models used in ray-tracing simulations at 40, 70, 100, and 130 days after transplanting under additional far-red doses to red-blue interlighting. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

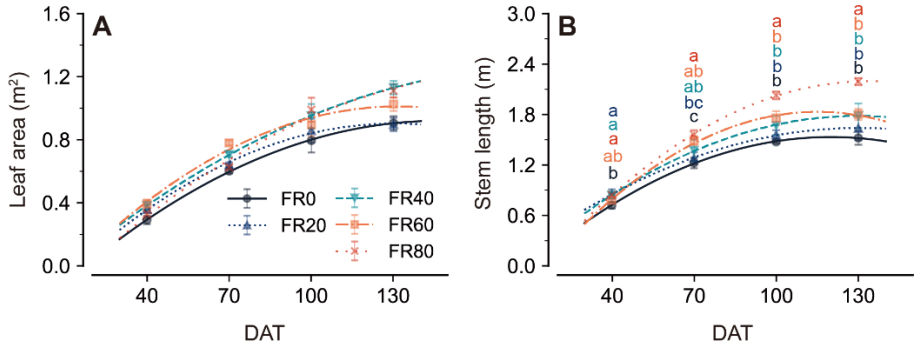

Fig. A2-5. Morphological trait extracted from 3D scanned data under additional far-red doses to red-blue interlighting. (A) Leaf area; (B) stem length. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

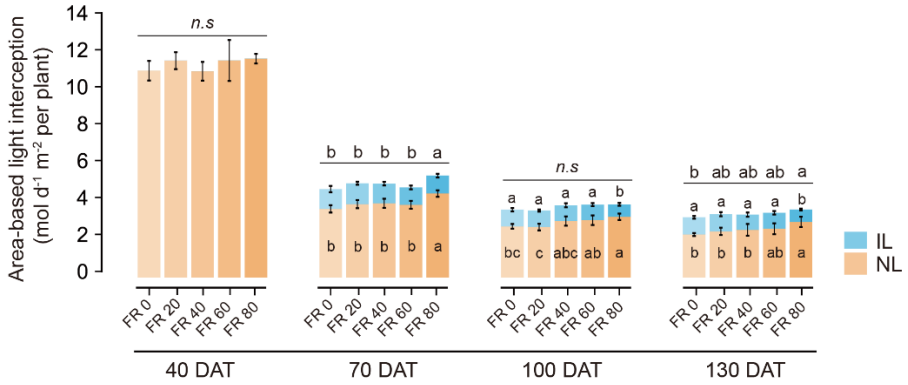

Fig. A2-6. Area-based light interceptions (mol d^{-1} m^{-2} per plant) at 40, 70, 100, 130 days after transplanting (DAT) under additional far-red doses to red-blue interlighting. FR 0 to 80 represent additional far-red doses of 0 to 80 µmol m^{-2} s^{-1} to red-blue interlighting, respectively.

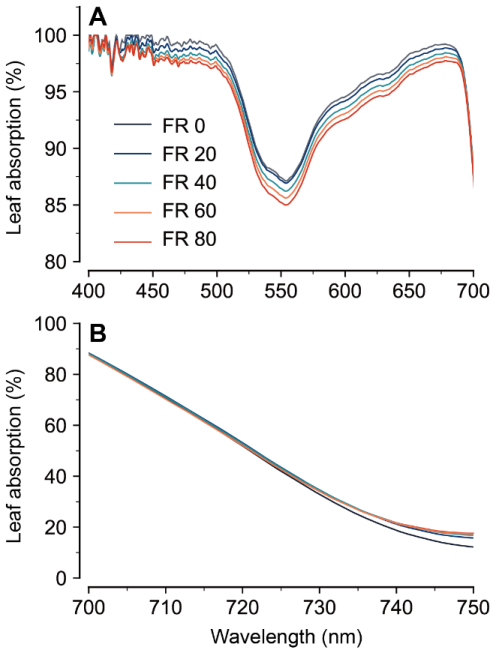

Fig. A2-7. Leaf absorption spectra under additional far-red doses to red-blue interlighting applied to the optical simulations. (A) Leaf absorption in the photosynthetically active radiation (PAR) (400-700 nm) range; (B) absorption in the far-red range (700-750 nm). FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

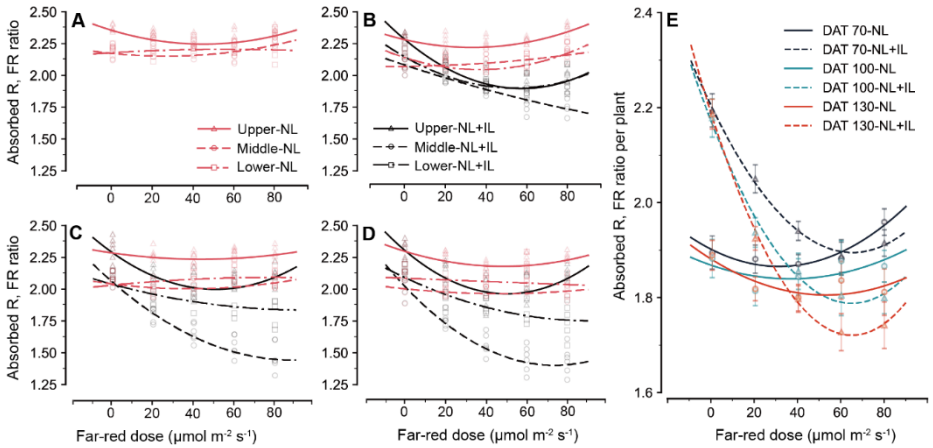

Fig. A2-8. Absorbed red (R)/far-red (FR) ratios under natural light only (NL) and NL+interlighting (IL) with additional FR doses at upper, middle, and lower positions and the whole plant level. A, B, C, and D indicate 40, 70, 100 and 130 days after transplanting. The upper, middle, and lower positions were divided into three parts based on the height axis of the plant. A trendline depicts the fitted line estimated with quadratic regression. FR 0 to 80 represent additional far-red doses of 0 to 80 μmol m^{-2} s^{-1} to red-blue interlighting, respectively.

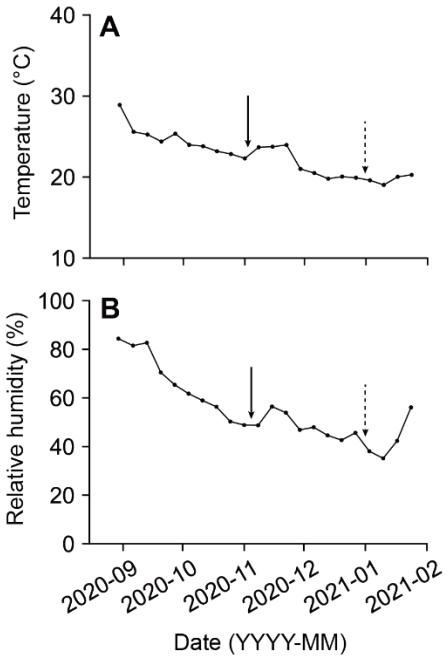

Fig. A3-1. Temperature (A) and relative humidity (B) in the greenhouse during cultivation.

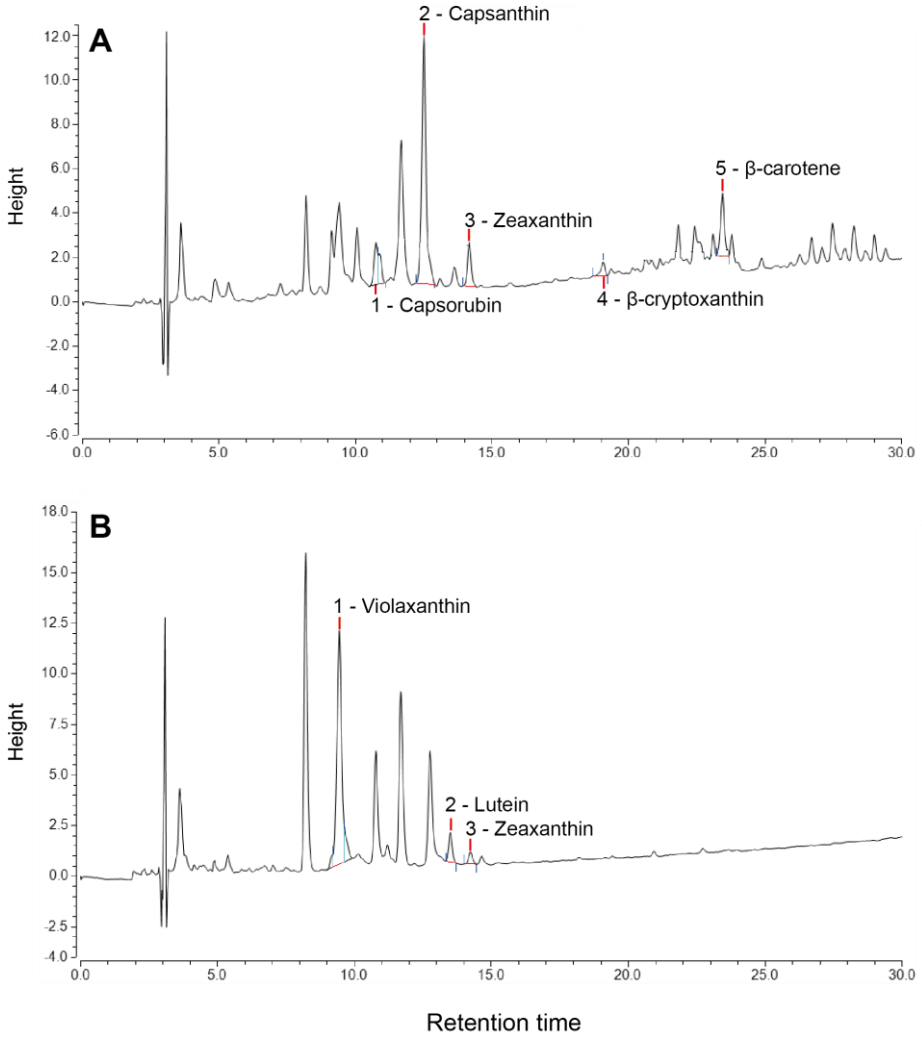

Fig. A3-2. Chromatograms of the carotenoid profiles in harvested red (‘Mavera,’ A) and yellow (‘Florate,’ B) sweet peppers.

Fig. A3-3. Sweet pepper fruit developmental disorder due to lack of physical space under red-blue interlighting.