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Article

Evaluation of Growth and Photosynthetic Rate of Cucumber Seedlings Affected by Far-Red Light Using a Semi-Open Chamber and Imaging System

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Abstract: Far-red light was excluded in photosynthetic photon flux; however, recent studies have shown that it increases photosynthetic capacity. In addition, there were few studies on the whole canopy photosynthetic rate and continuous changes of morphology on cucumber seedlings affected by far-red light. This study evaluated the effect of conventional white LEDs adding far-red light on cucumber seedlings using a semi-open chamber system for the measurement of the whole canopy gas exchange rate, and the Raspberry Pi-based imaging system for the analysis of a continuous image. In the image, through the imaging system, it was confirmed that far-red light promoted the germination rate of cucumber seedlings and enhanced early growth. However, the dry weight of the shoot and root did not increase. The measured net apparent CO_2 assimilation rate was improved by an increasing leaf area during the cultivation period. The conventional white LED light source with added far-red light increased the photosynthetic rate of cucumber seedlings' whole canopy. However, at the early seedling stage, plant height and leaf area of the whole canopy was increased by far-red light, and it was revealed that the image data saturated faster. It was considered that the photosynthetic efficiency decreased due to a shading effect of the limited planting density of the cell tray. The results found that using appropriate far-red light, considering planting density, could increase the photosynthetic rate of the whole canopy of crops, thereby promoting crop growth, but it was judged that the use of far-red light in the early growth stage of cucumber seedlings should be considered carefully.

Keywords: far-red light; whole canopy photosynthesis; cucumber seedling; Raspberry Pi

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1. Introduction

Crop yield is determined by the photosynthetic rate of the crop whole canopy. Photosynthetic capacity is related to photosynthetic characteristics (photosynthetic pigments, chlorophyll fluorescence, photosystem, and photosynthetic electron transport, etc.). These factors could be regulated by light quality. There have been many studies related to light quality and recently, many studies have been reported on far-red light. Kalaitzoglou et al. [1] investigated the effects of far-red light throughout the day or end-of-day on growth, morphology, light efficiency, and yield of tomatoes. Notably, this study showed that far-red light at the end-of-day on tomatoes could not replace the effects of far-red light throughout the day. Zhen et al. [2] reported the effects of far-red light on photosynthesis of a leaf and whole canopy in several crop species and proposed redefining the range of photosynthetic photon flux. Jin et al. [3] confirmed that adding far-red light to red-blue LEDs promoted the yield of lettuce at different planting densities. In addition, photosynthesis is carried out in two photosystems, which means that both photosystems work together actively at the same time when there is an appropriate proportion of far-red light, which accounts for a significant portion of the light above 680 nm. This indicated that far-red light is essential for the photosynthesis of plants. However, in the past, far-red light has been considered to be an inefficient light quality for the growth of crops and has not been considered for photosynthetic photon flux and artificial lighting for crop growth.

Therefore, many studies have shown that far-red light synergistically interacts with traditional photosynthetic light to take several advantages. Low red:far-red ratio in the canopy environment increases leaf length and leaf area and reduces leaf width, making leaves narrower than those under normal light [4,5]. As a result, far-red light increases photon use efficiency for plants [6–8]. Furthermore, far-red light optimizes light interception through the regulation of leaf angles to increase light reception [9,10]. Increasing far-red light reduces stomatal conductance and the numbers of stomata in some plants, but in other plants, leads to an increase in the numbers of stomata [11,12]. Furthermore, Li and Kubota [13] reported that conventional light with supplemental far-red light increased significantly the dry and fresh weight of lettuces and tomatoes. Far-red light affected leaf structure and chloroplasts. It expands the area of leaves and increases photosynthesis in some studies, but reduces leaf thickness [14,15]. In the case of photosynthetic characteristics, far-red light decreases the chlorophyll a/b ratio, increases the photosystem II/I ratio [16–18], and accelerates the cyclic electron transport around photosystem I [19,20]. In the case of photosynthate products, increasing far-red light compared to conventional light increased sucrose and starch contents in soybean [8] and reduced starch content in strawberry and peach [21].

In addition, far-red light regulates plant height and flowering. In the case of plant height, photoreceptors of plants are known as phytochrome A, a far-red light photoreceptor, and phytochrome B, a red-light photoreceptor [22,23]. When the ratio of red light decreases, phytochrome detects it and the following response mechanism occurs. A decrease in the ratio of red light may be caused by nearby plants, which may be perceived as competitors for light. Far-red light increases the photomorphogenesis suppressor phyA-105, which interacts with constitutive photomorphogenesis 1 to form E3 ubiquiton ligase that inhibits plant photomorphogenesis and promotes hypocotyl elongation by degrading phosphorylated phyA and the positive regulator long hypocotyl 5 [23–26]. Furthermore, the expression of phytochrome interacting factor 7 (PIF7) by far-red light leads to the increased binding of PIF7 to downstream target genes [27–29]. Then, indole-3-acetic acid is transported from leaves to stems, elongating the internodes and plant height [27]. Furthermore, phytochrome A promotes the induction of flowering. Far-red supplemented white light is much more effective than white light alone in promoting flowering, and this effect has been shown to be phytochrome A-dependent, and phytochrome A is also required for flowering induction when extending the day length with monochromatic FR or white light with supplemented far-red light [30].

Meanwhile, the response of crops to the environment could vary depending on the growing stage. Continuous phenotyping is feasible to confirm changes in the response of crops to the environment, and it is possible to know at what time the response is most affected. In other words, continuous phenotyping can make it easy to verify the effects of far-red light. Therefore, continuously phenotyping the effects of far-red light can be an interesting topic. However, there are few studies on phenotyping analysis for seedling morphology through continuously crop growth image during a cultivation period. In the meantime, it has been acquired through a method that relied on human analogue sensory observation. Recently, continuous monitoring of seedling emergence and early development via high-throughput phenotyping with an image system is a challenging topic of high interest in crop science [31]. The old, simple information is digitally converted into more diverse information by the development of convergence technology, which can be obtained automatically and efficiently. In the traditional way, in addition to the information that could not be obtained, the information can be visually confirmed using various sensors. A lot of data can be analyzed with high speed and precision in a non-destructive way for agricultural traits that have not been known before.

Therefore, this study evaluated the effect of far-red light on growth and morphology of cucumber seedlings using the semi-open chamber system for measurement of the whole canopy gas exchange rate and a camera system. We hypothesized that the growth and photosynthesis of cucumber seedlings could be promoted by setting more far-red light. The distinctive features of this study are as follows: the purpose of this study was to find out the optimal light quality for cucumber seedlings in a plant factory with artificial lighting. In addition, we set Raspberry Pi with a camera module for the continuous analysis of morphology during the cultivation period of cucumber seedlings. Through this, we identified how the morphology of cucumber seedlings affected the photosynthetic rate of whole canopy.

2. Materials and Methods

2.1. Target Crops and Environmental Condition

Cucumber seeds ('Joenbaekdadagi') were sown in six 20-cell plug trays filled with commercial medium (Numberone, Cham Grow, Inc., Hongseong, Republic of Korea) and cultivated for 14 days in the closed transplant production system with artificial lighting (Figure 1A), which were composed of the three-layered shelf. The seedling was grown at the daily average air temperature of 25 °C and relative humidity of 70% with a 16/8 h light photoperiod. The light intensity of the photosynthetic photon flux density (PPFD) was set at approximately 470 µmol m^{-2} s^{-1} for each treatment. The seedlings were sub-irrigated once every day during the cultivation period without a nutrient solution (Figure 1B). The irrigation was carried out until the top-soil water status was completely saturated.

Figure 1. (**A**) The closed transplant production system with artificial lighting, which were composed of the three-layered shelf and (**B**) cucumber seedlings cultivated in the closed transplant production system with artificial lighting.

2.2. Treatment of Far-Red Light

White LEDs (SG-BAR-28W, Future Green Co. Ltd., Yongin, Republic of Korea) at a distance of 250 mm in the vertical direction from the cultivation bed for plant cultivation were used as a light source, and far-red LED bars (FARREDLB, Forever Green Indoors, Seattle, WA, USA) were additionally installed to increase far-red photon flux. The photon flux density (PFD) in the wavelength range of 380–780 nm was set to the same in order to differ only the light quality. The light quality and quantity of each light environment (first and second bench of the three-layered shelf) were investigated using a spectrometer (LI-180, LI-COR Bioscience, Lincoln, NE, USA) in the wavelength range of 380 to 780 nm. In addition, the light source was configured by comparing the light quality and quantity at the time when photosynthesis can be most active in order to determine whether the conditions were appropriate for crop growth. The quality and quantity of sunlight was investigated under a Venlo-type greenhouse (35°53′ N 128°36′ E) at noon in springtime when the sunlight is the highest in Republic of Korea. The daily light intensity (DLI) of white LEDs (W) and white LEDs with added far-red light (WFR) treatment was calculated using the photon flux. The daily light intensity of sunlight was collected in a datalogger

(CR1000, Campbell Scientific Inc., Logan, UT, USA) using a thermopile sensor (CMP series, Campbell Scientific Inc., Logan, UT, USA).

The DLI was computed based on PFD rather than PPFD to confirm the effect of far-red light. Three samples were extracted for the measurement value and the significant difference was examined (p-value = 0.05) with Duncan's multiple range test. Different alphabets mean that they are significantly different (p-value = 0.01). The PFD was 487.3 μmol m^{-2} s^{-1} in white LEDs (W) treatment and 482.8 μmol m^{-2} s^{-1} in white LEDs with added far-red light (WFR) treatment (Table 1). This result showed that the PFD did not affect crop growth. Although the PFD of W and WFR treatments is lower than that of sunlight, considering a photo period was 16 h and characteristics of LEDs always have the same PFD, DLI of both treatments was higher than sunlight.

Table 1. The average photon flux (μmol m^{-2} s^{-1}) of the wavelength according to the light source.

Light Sources	Blue (400–500 nm)	Green (500–600 nm)	Red (600–700 nm)	Far-Red (700–780 nm)	PPFD (400–700 nm)	PFD (380–780 nm)	Red/Far-Red	DLI (mol)
W	57.3b	199.4b	172.0a	58.2b	428.7b	487.3a	3.0a	28.0a
(%) ^{z}	11.8	40.9	35.3	11.9	88.0	100		
WFR	47.1c	162.9c	145.5b	126.9a	355.5c	482.8a	1.15a	27.8a
(%)	9.8	33.7%	30.1	26.3	73.6	100		
Sunlight	160.9a	216.5a	179.8a	111a	557.2a	680.0b	1.6b	25.9b
(%)	23.7	31.8	26.4	16.3	81.9	100		

^{z} Mean percentage for PFD. Different letter indicated a significant difference within the column at the $p < 0.05$ according to Duncan's multiple range test.

2.3. Plant Growth Analysis

The plant height, stem diameter, number of leaves, leaf area, fresh weight, and dry weight were investigated. Plant height was measured as the distance from the soil surface to the growth point. The stem diameter was measured using a Vernier caliper (CD-20CPX, Mitutoyo Co., Kawasaki, Japan). The leaf area was measured by leaf area measurement (LI-3100c, LI-COR Bioscience, Lincoln, NE, USA). The measurement was performed by selecting six individuals for each treatment (six replicates). Compactness, leaf area index (LAI), leaf area ratio (LAR), and light use efficiency (LUE) of growth indicator to evaluate plant growth were computed according to the following equations:

$$\text{Compactness} = \frac{\text{shoot dry weight [mg]}}{\text{plant height [cm]}} \quad (1)$$

$$\text{LAI} = \frac{\text{leaf area } \left[\text{cm}^2\right]}{\text{plug tray area } \left[\text{cm}^2\right]} \quad (2)$$

$$\text{LAR} = \frac{\text{leaf area } \left[\text{cm}^2\right]}{\text{shoot dry weight [g]}} \quad (3)$$

$$\text{LUE} = \frac{\text{shoot dry weight [mg]}}{\text{daily light integral [mol]}} \quad (4)$$

2.4. Measurement of Whole Canopy Gas Exchange Rate

A semi-open chamber system for measuring the whole canopy gas exchange rate was installed in the closed transplant production system with artificial lighting (Figure 2). The infrared gas analyzer of the system was LI-850 (LI-COR Bioscience, Lincoln, NE, USA) for reference and LI-840 (LI-COR Bioscience, Lincoln, NE, USA) for sample. The whole canopy photosynthetic rate was calculated through the difference between the reference CO_2 concentration and sample CO_2 concentration. The measured value of changes in the whole canopy gas exchange rate was converted to the apparent net assimilation rate (A_n, μmol CO_2 m^{-2} s^{-1}) and transpiration (mmol H_2O m^{-2} s^{-1}). The tray of W treatment and WFR treatment was placed in each growth chamber of the semi-open chamber system for measuring the whole canopy gas exchange rate. For comparison of A_n for the whole canopy according to light quality treatment, the gas exchange rate was measured from 3 to 14 days after sowing. The A_n of the three trays for each treatment was calculated and the mean value was obtained. During the measurements, temperature and relative humidity inside the growth chamber were maintained at 25 °C and 70%, respectively. The light intensity was maintained at 471.1 and 469.0 µmol m^{-2} s^{-1} for each treatment, the same as the cultivation environment. Gas exchange measurements under a steady state of CO_2 concentrations in the growth chamber were maintained at 530 µmol CO_2 mol^{-1} of reference CO_2 concentrations. Changes of CO_2 and H_2O concentration in each growth chamber were recorded by a datalogger (CR1000, Campbell Scientific Inc., Logan, UT, USA).

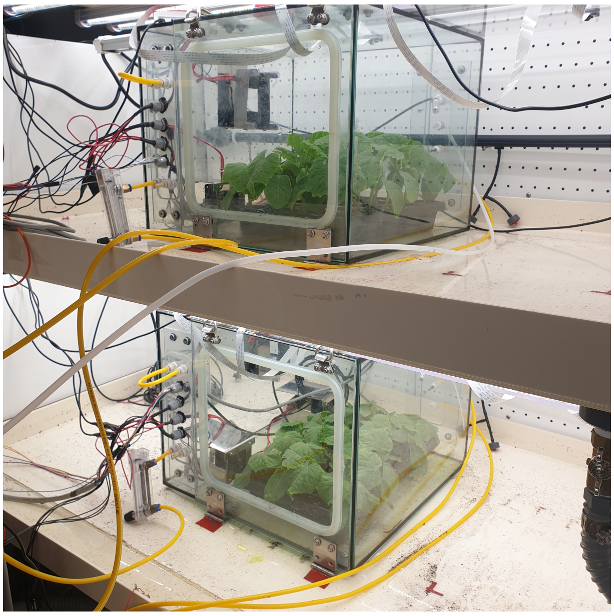

Figure 2. Measuring cucumber seedlings' whole canopy using a semi-open chamber system for the measurement of the crop whole canopy gas exchange rate during the cultivation period.

2.5. Imaging System Configuration

A microprocessor (Raspberry Pi model 3B+, Raspberry Pi foundation, Cambridge, UK) was used to acquire crop images. Since it has built-in Wi-Fi, it is advantageous for transmitting acquired data and it is also possible to set its own storage device. Raspberry Pi was connected to the Raspberry Pi camera module v2. The device was installed using a frame with acryl between the LEDs and cultivation shelves to obtain an accurate top-view image. The normalized difference vegetation index, called Excess Green (ExG), was used to separate the crops from the background. Filming was conducted at 20 min intervals from 8:00 to 23:00 when the LEDs were turned on. The crop region of interest (ROI) of the acquired image data were extracted. Image analysis was performed by extracting the pixel number and RGB color information from the obtained ROI.

2.6. Data Analysis

Statistical analysis of the growth factor was conducted using the SAS program (SAS 9.4, SAS Institute Inc., Cary, NC, USA). Significant differences were examined with Student's *t*-test (*p*-value = 0.05). Graphs were presented by the SigmaPlot program (SigmaPlot 12.5, Systat Software Inc., San Jose, CA, USA), and the Python program (Python 3.10.7, Python Software Foundation, Wilmington, DE, USA). Pearson correlation coefficients were obtained using analysis in the Python program for correlations among light quality and plant growth.

3. Results

3.1. Growth of Cucumber Seedlings in Different Light Quality Treatments

The growth of cucumber seedling in each treatment was compared 14 days after sowing. The mean plant height of the W and WFR treatment were 3.28 and 7.06 cm/plant, respectively, and W treatment was significantly shorter than WFR treatment (Table 2). There was no significant difference between the stem diameter of the two treatments. In addition, leaf length and leaf width were investigated to analyze leaf morphology. For both, the mean leaf length and leaf width of WFR were 7.3% and 14.5% longer than those of W treatment, and there was a significant difference. The results of plant length and leaf length all increased by far-red light were the same as previous studies, but through this study, it was confirmed that the increase in leaf length was weaker than other indicators. The mean leaf area of WFR treatment was greater than that of W treatment. Because the mean plant height and leaf area of the WFR treatment were significantly larger, the mean shoot fresh weight was also significantly higher than that of the W treatment. There was no significant difference in the mean root fresh weight of W and WFR treatments, which showed the same trend in root dry weight.

Table 2. Plant height, stem diameter, leaf length, leaf width, and leaf area of cucumber seedlings as affected by different light sources measured at 14 days after sowing.

Treatment	Plant Height (cm)	Stem Diameter (mm)	Leaf Length (cm)	Leaf Width (cm)	Leaf Area (cm^2)
W	3.28 ± 0.14	4.11 ± 0.28	5.93 ± 0.15	8.08 ± 0.28	72.15 ± 5.33
WFR	7.06 ± 0.39	4.17 ± 0.26	6.40 ± 0.14	9.25 ± 0.33	83.95 ± 6.95
Significance	***	NS	*	***	**

Significant differences were examined with Student's *t*-test (p-value = 0.05). NS: non-significant, ***: significant at $p < 0.001$, **: significant at $p < 0.01$, *: significant at $p < 0.05$.

The shoot fresh weight: root fresh weight (S/R) ratio was also not significantly different between treatments (Table 3). However, it was slightly higher in the WFR treatment, so it was considered that far-red light promoted the shoot growth. The compactness of the W treatment was significantly higher than that of the WFR treatment. It was judged that the plant growth was excessive compared to the dry weight, and it was judged that this was the result of inducing excessive growth before having complete photosynthetic ability (the state in which cotyledons appeared before true leaves appeared) in the early growth stage. In addition, there was no significant difference in LAR and LUE (Table 4). Although far-red light increased the dry matter weight of several crops, this study showed that far-red light did not increase dry matter weight. Nevertheless, adding far-red light to artificial lighting did not decrease the LUE. Therefore, it was judged that the addition of far-red light was suitable for further promoting the cucumber seedlings growth.

Table 3. Number of leaves, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight of cucumber seedlings as affected by different light sources measured at 14 days after sowing.

Treatment	No. of Leaves	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)	S/R Ratio
W	2.00 ± 0.00	3.05 ± 0.27	0.55 ± 0.08	1.83 ± 0.59	0.06 ± 0.02	1.8 ± 0.7
WFR	2.00 ± 0.00	3.55 ± 0.35	0.56 ± 0.10	1.87 ± 0.30	0.08 ± 0.00	2.0 ± 0.4
Significance	NS	**	NS	NS	NS	NS

Significant differences were examined with Student's *t*-test (p-value = 0.05). NS: non-significant, **: significant at $p < 0.01$.

Table 4. Compactness, LAI, LAR, and LUE of cucumber seedlings as affected by different light sources measured at 14 days after sowing.

Treatment	Compactness	LAI	LAR	LUE
W	0.17 ± 0.04	0.18 ± 0.01	132.2 ± 15.6	20.4 ± 3.0
WFR	0.08 ± 0.01	0.21 ± 0.02	152.7 ± 22.9	20.9 ± 3.7
Significance	***	**	NS	NS

Significant differences were examined with Student's *t*-test (*p*-value = 0.05). NS: non-significant, ***: significant at $p < 0.001$, **: significant at $p < 0.01$.

3.2. Photosynthetic Rate of Cucumber Whole Canopy in Different Light Quality Treatments

Figure 3 shows the mean A_n for each treatment in this study. The mean CO_2 concentration in each growth chamber was maintained at 529.8 μmol mol^{-1}. The mean A_n was below 0 μmol m^{-2} s^{-1} until 6 days after sowing, and WFR treatment was significantly lower than W treatment. In other words, they did more respiration than photosynthesis. At 7 days after sowing, the mean A_n was greater than 0 μmol m^{-2} s^{-1}. The A_n from 7 to 10 days after sowing showed a higher tendency in the WFR treatment than W treatment (Figure 3). From 11 days after sowing, the W treatment showed a tendency in which the mean A of WFR treatment was reversed. However, the difference was only 0.65 μmol m^{-2} s^{-1}. This was thought to be due to the increase of LAI, and increasing the plant height and leaf area of WFR treatment. Nevertheless, the measured total net assimilation rate was high in the WFR treatment, and it was judged that this resulted in better growth in the WFR treatment. This state was maintained until 14 days after sowing. The total mean A_n during the cultivation period was 140.6 and 143.5 μmol m^{-2} s^{-1} for W and WFR, respectively. This result showed no significant difference of the shoot and root dry matter weight between treatments.

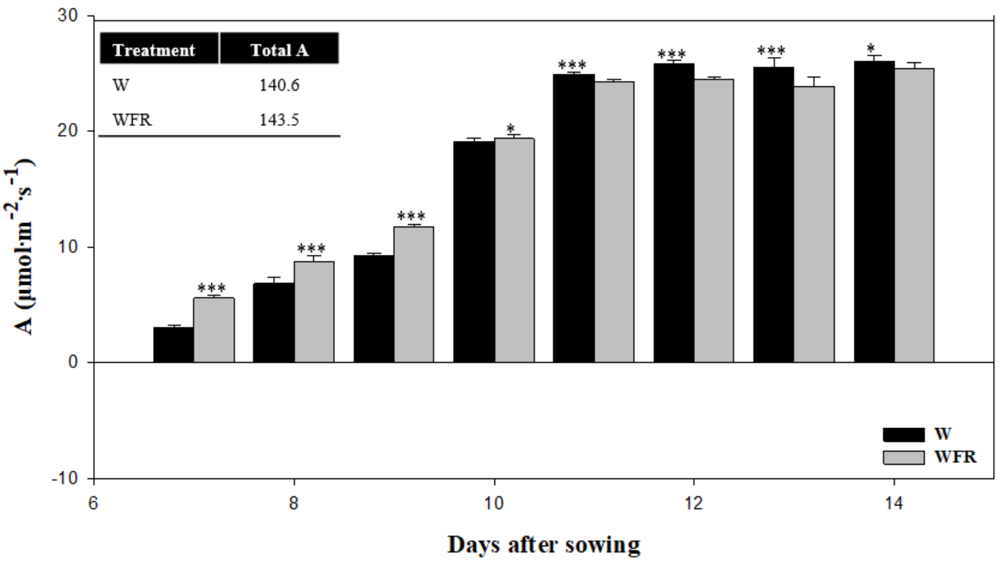

Figure 3. Changes of measured net CO_2 assimilation rate of cucumber seedlings' whole canopy affected by different light sources during the cultivation. Significant differences were examined with Student's *t*-test (*p*-value = 0.05). ***: significant at $p < 0.001$, *: significant at $p < 0.05$.

3.3. Growth of Cucumber Seedlings in Image

Through the imaging system, continuous image data could be acquired during the cultivation period (Figure 4). It could confirm the growth of cucumber seedlings through the top view and had sufficient resolution for analysis. Figure 5 is the result of separating the crop and the background using the ExG. The mean number of area pixels from the extracted images was used as area index. At 17:20 2 days after sowing, germination started first in the W treatment (Figure 5A). At 17:00 3 days after sowing, it was confirmed that germination of the WFR treatment progressed to the extent that it could be distinguished with the naked eye (Figure 5C). Likewise, the germination point of the WFR treatment was also confirmed. It was considered that far-red light accelerated the germination of cucumber seeds.

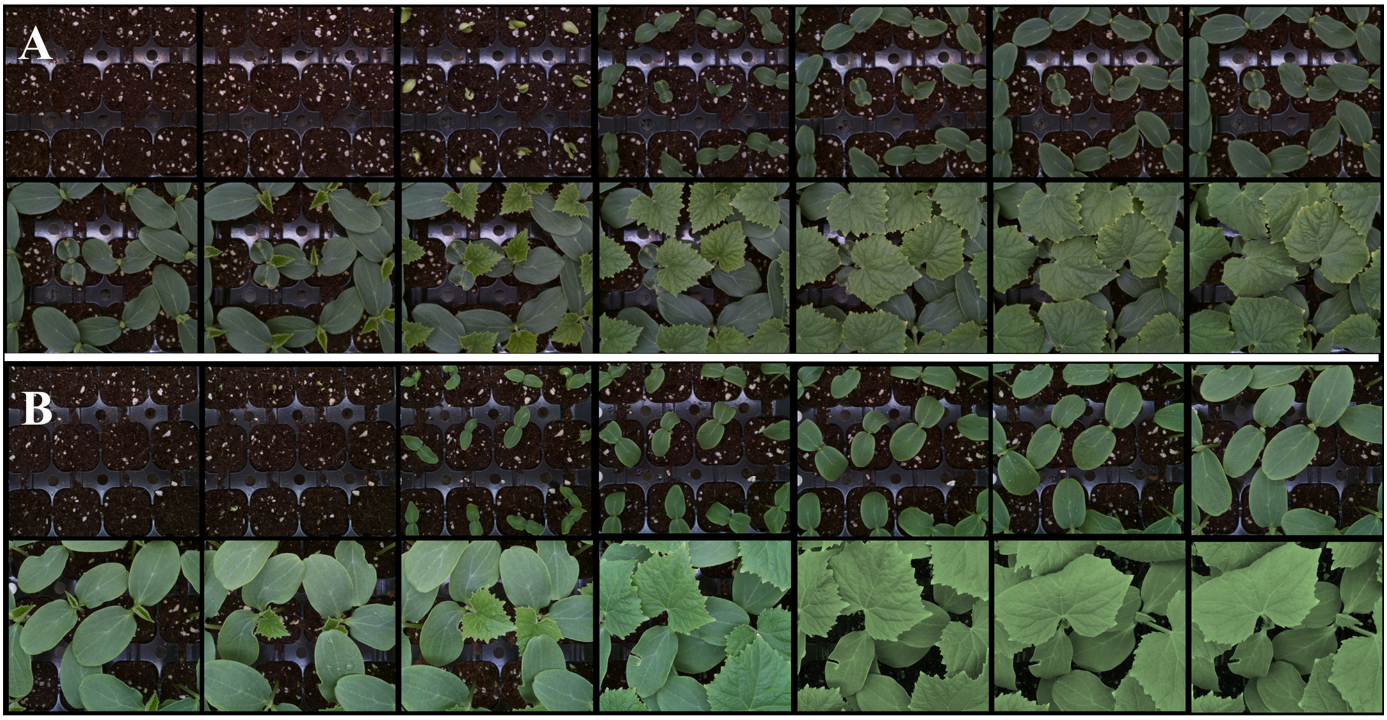

Figure 4. Image data for each treatment acquired through the imaging system during the cultivation period: (**A**) conventional white LEDs treatment; and (**B**) white LEDs with added far-red light treatment.

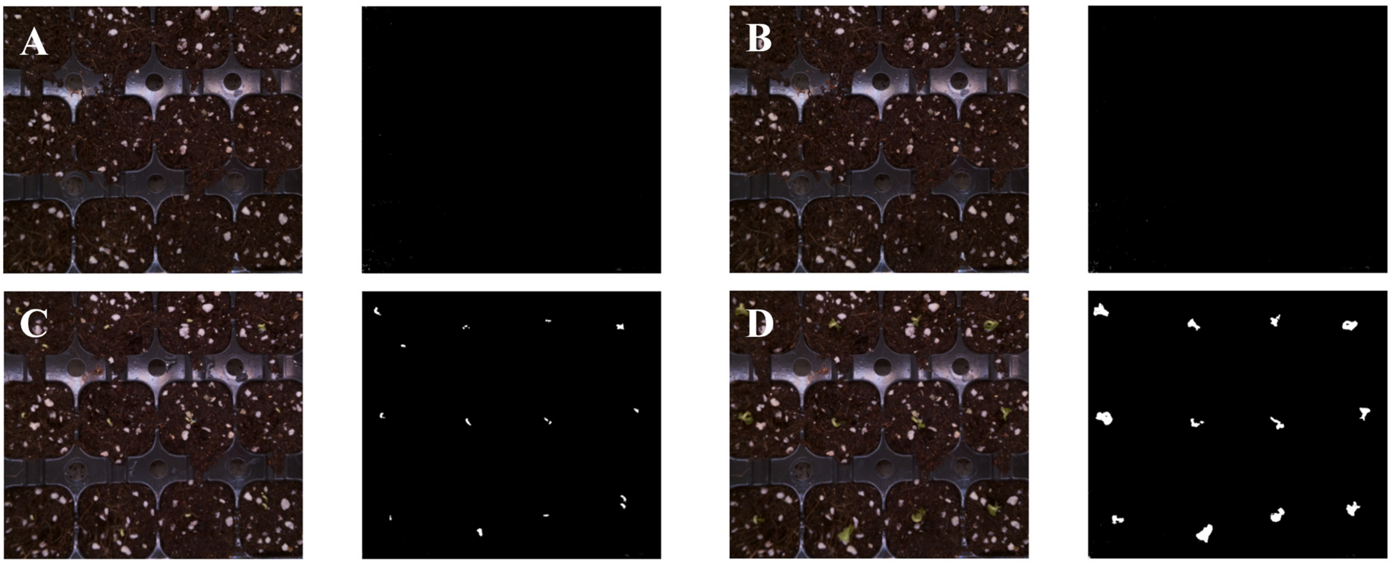

Figure 5. Germination point image of conventional white LEDs treatment acquired through the imaging system during the cultivation period: (**A**) 17:20 2 days after sowing; (**B**) 08:40 3 days after sowing; (**C**) 17:00 3 days after sowing; and (**D**) 08:20 4 days after sowing.

Figure 6 shows the change of area index based on the time of WFR treatment germination. Germination of the WFR treatment occurred first. Furthermore, from the time of 3 days after sowing, the difference in area index between the WFR and W treatment rapidly increased (Figure 6). During the cultivation period, the overall area index was maintained high in the WFR treatment (Figure 7A). However, after the crop image was saturated with leaves, the area index remained almost the same between treatments. Nonetheless, the WFR treatment showed an increase in leaf area in the saturated state. On the other hand, the W treatment showed a slow increase in leaf area in the saturated state. As a result of extracting the RGB color information, the WFR treatment was distributed in the red group as a whole and the W treatment was distributed in the blue group (Figure 7B).

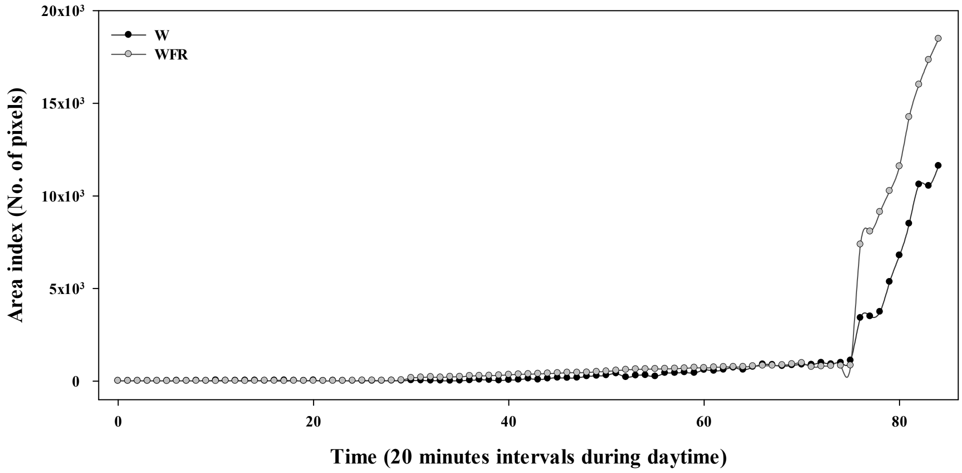

Figure 6. Changes of area index of conventional white LEDs treatment and white LEDs with added far-red light treatment at the germination point (time: from 13:20 2 days after sowing to 10:40 4 days after sowing).

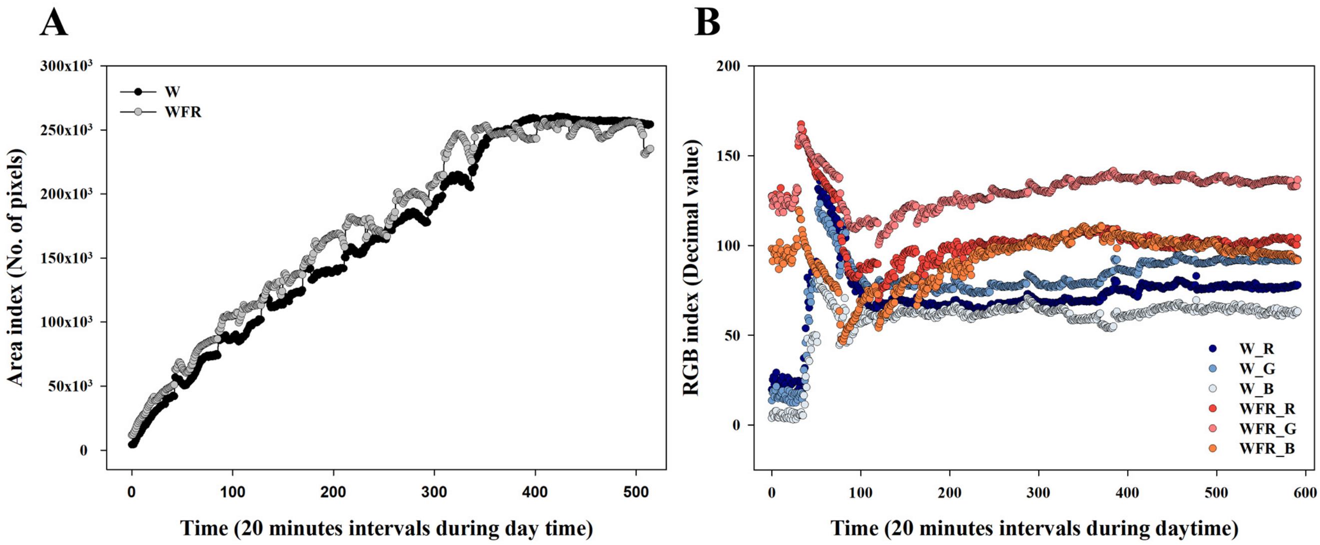

Figure 7. Changes of (**A**) the mean area index and (**B**) RGB color information of conventional white LEDs treatment and white LEDs with added far-red light treatment during the cultivation (time: from 08:00 4 days after sowing to 10:00 14 days after sowing).

In the last stage of cultivation, the increase of area index of images showed a similar tendency for both treatments, but this was because the entire image was maintained in a screen saturated with leaves. The time point of saturation was faster in the WFR treatment, and even after saturation, it was confirmed that the leaf expansion was greater than that of the W treatment. On the other hand, the W treatment showed little leaf expansion. It was judged that the shading effect appeared prominently in the WFR treatment because the artificial light installed in the closed transplant production system formed a vertical direction. Accordingly, it was confirmed that the photosynthetic rate of the WFR treatment was also slightly lowered 11 days after sowing. Despite the shading effect, the total net apparent assimilation rate of the whole canopy during the cultivation period was higher in WFR. Therefore, it was judged that far-red light increased the photosynthetic rate of the whole canopy.

3.4. Pearson Correlation between Light Quality and Plant Growth

To analyze a correlation between plant growth and light quality treatment, a linear correlation was quantified using a Pearson correlation coefficient (Table 5). The highest Pearson correlation coefficient with light quality treatment was plant height and its value was 0.92. In addition, the high correlation coefficient with light quality treatment was in the order of leaf width, leaf area, and shoot fresh weight, which were 0.9, 0.71, and 0.64, respectively. Root fresh weight, root dry weight, and stem diameter had little correlation with light quality treatment. In the case of leaf length, the correlation coefficient was 0.53, indicating an intermediate level. Similarly, plant height, leaf width, leaf area, and shoot fresh weight, which were highly correlated with light quality treatment, showed a high correlation with each other. It was revealed that the WFR treatment affected a significant effect on plant height, leaf width, leaf area, and shoot fresh weight. However, since the compactness was the only negative correlation with far-red light, it was judged that it was necessary to consider the excessive growth rate of plant height in far-red light use.

Table 5. Pearson correlation between light quality and cucumber seedlings growth 15 days after sowing.

Pearson Correlation with Far-Red Light							
	Plant Height	**Stem Diameter**	**Leaf Length**	**Leaf Width**	**Leaf Area**	**Shoot Fresh Weight**	**Shoot Dry Weight**
coefficient	0.92 ***	0.12 *	0.53 **	0.90 ***	0.71 **	0.64 **	0.05 NS
	Root fresh weight	Root dry weight	S/R ratio	Compactness	LAI	LAR	LUE
coefficient	0.04 NS	0.45 **	0.10 *	−0.21 *	0.71 ***	0.49 **	0.07 NS

Significant differences were examined with Student's *t*-test (*p*-value = 0.05). NS: non-significant, ***: significant at $p < 0.001$, **: significant at $p < 0.01$, *: significant at $p < 0.05$.

4. Discussion

Far-red light increased photomorphogenesis inhibitors, increasing hypocotyl elongation, plant height, leaf length, and decreasing leaf width, while increasing overall leaf area [32,33]. The increase in leaf area was induced because far-red light increased the extensibility of the leaf cell wall. In this experiment, unlike previous studies, far-red light increased leaf width and the leaf area was increased by 1.16 times. The leaf morphology of cucumber differs from tobacco and tomato leaf morphology used in the previous study. The leaf of the cucumber is unifoliate, not a compound leaf. Therefore, it was judged that the reason for the increase in leaf width was the difference in cultivar. In addition, Ai et al. [34] reported that far-red light increased the fresh and dry matter weight of tomatoes by 28% and 33%, respectively. Similarly, fresh and dry matter weight of cucumber seedlings increased by 16.3% and 2.2%, respectively. However, there was no significant difference among dry matter weight. This was due to the early stage with two true leaves of cucumber seedlings or the state in which cotyledons appeared before true leaves appeared, so it was judged that photosynthesis of sufficient dry matter production capacity was not secured. It could also be confirmed through the photosynthetic rates measured. The photosynthetic rate is directly involved in the production of dry matter. The total photosynthetic rate slightly increased during the cultivation period, and the same tendency of significance as dry matter weight was confirmed. In order to see a significant difference in dry matter weight, it was judged that an additional experiment through a longer cultivation period was needed or it was necessary to additionally confirm the results of far-red light treatment after the stage that the true leaf appeared. In addition, since there was a study result that far-red light stimulated the dry mass partitioning to fruits and production of fruits in tomato [35], it was judged that there was a need to see the effect on far-red light after transplanting in a greenhouse.

The volume of a cell plug tray was filled with 280 mL of a fully composted medium, which was small compared to the semi-open chamber (54,000 mL). In addition, it was possible that the effect of CO_2 release was smaller than the cultural medium by microbial decomposition due to a short-term experiment (14 days). Therefore, it was possible to compare the net photosynthetic rate in the treatments. It was confirmed that far-red light increased the photosynthetic rate during the early growth stage. Far-red light increased stomatal conductance and the number of stomata in some crops, which was also shown to be effective in cucumbers [36]. It was thought that the increasing of the leaf area increased the number of stomata and stomatal conductance. Accordingly, it was considered that this showed a higher photosynthetic rate because they were grown in the same environment as the W treatment. This could be seen in the Emerson effect [37]. When crops are exposed to light with a wavelength greater than 680 nm, then only PSII is activated, resulting in the formation of ATP only. However, when crops are exposed to light with a wavelength less than 680 nm, the photosynthetic rate decrease. Therefore, on giving appropriate far-red light, both PSI and PSII were working together at the same time, resulting in a higher yield.

However, the photosynthetic rate decreased in the WFR treatment from 11 days after sowing. While the leaf area increased, it was judged that the decrease was due to the shading effect caused by the limited area of the cell tray. There was a result of minimizing the shading effect by inducing the angle adjustment of the leaf to reduce the overlapping part of the leaf by far-red light [38], but this could not be confirmed in this experiment. Therefore, it was determined that it was necessary to compare the photosynthesis by securing sufficient planting density through an experiment after transplanting, not at the early seedling stage. In addition, it was judged that far-red light increased the photosynthetic rate of the whole canopy, and that LUE could be enhanced even in the early growth stage of cucumber seedlings if appropriate planting density was considered.

In this study, it was possible to confirm the effect of far-red light during the cultivation period through continuous image analysis. This made it possible to confirm that at some point during the difference of growth was caused by far-red light. However, in addition to the image feature analyzed in this experiment, additional extractable features may include the center, contour, length, central axis, color space distribution, growth distortion, and so on. Analysis of additional image features requires a separate imaging system, such as a depth camera, or no overlap of the crops. Therefore, it was expected that a more detailed image analysis would be possible if the image analysis of each individual crop was performed as well as the image analysis of the whole canopy.

5. Conclusions

In this study, the effect of far-red light on the growth of cucumber seedlings was evaluated using a semi-open chamber system for the measurement of the whole canopy gas exchange rate and Raspberry Pi-based imaging system. The net apparent assimilation rate was calculated by measuring the gas exchange rate through the semi-open chamber system. The image data through the imaging system was separated from the background by using ExG. The mean area index and RGB color information was obtained from the extracted images. Through the area index extracted over time during the cultivation period, it was confirmed that far-red light induced germination of cucumber seedlings and promoted early growth. In addition, a conventional white LED light source adding far-red light increased the total photosynthetic rate of the cucumber seedlings' whole canopy. Furthermore, far-red light increased plant height, leaf length, leaf width, shoot fresh weight, and leaf area. However, crop saturation in the image was achieved faster by far-red light, and then showed that the shading effect due to rapid growth and increased leaf area appeared. As a result, due to the limited planting density, far-red light reduced the net assimilation rate of the whole canopy in the cultivation late period. Furthermore, while the plant height of cucumber seedlings increased, the dry weight of the shoot and root did not increase. Notably, it was judged that far-red light could cause unsuitable growth in the early growth stage of cucumber seedlings in a plant factory with limited planting density. These results showed that using appropriate far-red light considering planting density and growth stage could increase the photosynthetic rate of the whole canopy of crops, thereby promoting crop growth. Furthermore, it was considered necessary to properly add far-red light to commercially artificial lighting.

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References

1. Kalaitzoglou, P.; van Ieperen, W.; Harbinson, J.; van der Meer, M.; Martinakos, S.; Weerheim, K.; Nicole, C.C.S.; Marcelis, L.F.M. Effects of Continuous or End-of-Day Far-Red Light on Tomato Plant Growth, Morphology, Light Absorption, and Fruit Production. *Front. Plant Sci.* **2019**, *10*, 322. [CrossRef] [PubMed]
2. Zhen, S.; Bugbee, B. Far-Red Photons Have Equivalent Efficiency to Traditional Photosynthetic Photons: Implications for Redefining Photosynthetically Active Radiation. *Plant Cell Environ.* **2020**, *43*, 1259–1272. [CrossRef] [PubMed]
3. Jin, W.; Urbina, J.L.; Heuvelink, E.; Marcelis, L.F.M. Adding Far-Red to Red-Blue Light-Emitting Diode Light Promotes Yield of Lettuce at Different Planting Densities. *Front. Plant Sci.* **2021**, *11*, 2219. [CrossRef] [PubMed]
4. Kasperbauer, M.J.; Peaslee, D.E. Morphology and Photosynthetic Efficiency of Tobacco Leaves That Received End-of-Day Red and Far Red Light during Development. *Plant Physiol.* **1973**, *52*, 440–442. [CrossRef] [PubMed]
5. Zhang, Y.T.; Zhang, Y.Q.; Yang, Q.C.; Li, T. Overhead Supplemental Far-Red Light Stimulates Tomato Growth under Intra-Canopy Lighting with LEDs. *J. Integr. Agric.* **2019**, *18*, 62–69. [CrossRef]
6. Shibuya, T.; Endo, R.; Kitaya, Y.; Hayashi, S. Growth Analysis and Photosynthesis Measurements of Cucumber Seedlings Grown under Light with Different Red to Far-Red Ratios. *HortScience* **2016**, *51*, 843–846. [CrossRef]
7. Bae, J.H.; Park, S.Y.; Oh, M.M. Supplemental Irradiation with Far-Red Light-Emitting Diodes Improves Growth and Phenolic Contents in *Crepidiastrum denticulatum* in a Plant Factory with Artificial Lighting. *Hortic. Environ. Biotechnol.* **2017**, *58*, 357–366. [CrossRef]
8. Yang, F.; Liu, Q.; Cheng, Y.; Feng, L.; Wu, X.; Fan, Y.; Raza, M.A.; Wang, X.; Yong, T.; Liu, W.; et al. Low Red/Far-Red Ratio as a Signal Promotes Carbon Assimilation of Soybean Seedlings by Increasing the Photosynthetic Capacity. *BMC Plant Biol.* **2020**, *20*, 148. [CrossRef]
9. Girardin, P.; Tollenaar, M. Effects of Intraspecific Interference on Maize Leaf Azimuth. *Crop Sci.* **1994**, *34*, 151–155. [CrossRef]
10. Maddonni, G.A.; Otegui, M.E.; Andrieu, B.; Chelle, M.; Casal, J.J. Maize Leaves Turn Away from Neighbors. *Plant Physiol.* **2002**, *130*, 1181–1189. [CrossRef]
11. Momokawa, N.; Kadono, Y.; Kudoh, H. Effects of Light Quality on Leaf Morphogenesis of a Heterophyllous Amphibious Plant, *Rotala hippuris*. *Ann. Bot.* **2011**, *108*, 1299–1306. [CrossRef] [PubMed]
12. Kim, S.J.; Hahn, E.J.; Heo, J.W.; Paek, K.Y. Effects of LEDs on Net Photosynthetic Rate, Growth and Leaf Stomata of Chrysanthemum Plantlets In Vitro. *Sci. Hortic.* **2004**, *101*, 143–151. [CrossRef]
13. Li, Q.; Kubota, C. Effects of Supplemental Light Quality on Growth and Phytochemicals of Baby Leaf Lettuce. *Environ. Exp. Bot.* **2009**, *67*, 59–64. [CrossRef]
14. Lee, M.J.; Park, S.Y.; Oh, M.M. Growth and Cell Division of Lettuce Plants under Various Ratios of Red to Far-Red Light-Emitting Diodes. *Hortic. Environ. Biotechnol.* **2015**, *56*, 186–194. [CrossRef]
15. Zhen, S.; van Iersel, M.W. Far-Red Light Is Needed for Efficient Photochemistry and Photosynthesis. *J. Plant Physiol.* **2017**, *209*, 115–122. [CrossRef]
16. Fan, D.Y.; Hope, A.B.; Smith, P.J.; Jia, H.; Pace, R.J.; Anderson, J.M.; Chow, W.S. The Stoichiometry of the Two Photosystems in Higher Plants Revisited. *Biochim. Biophys. Acta BBA Bioenerg.* **2007**, *1767*, 1064–1072. [CrossRef] [PubMed]

17. Glick, R.E.; McCauley, S.W.; Melis, A. Effect of Light Quality on Chloroplast-Membrane Organization and Function in Pea. *Planta* **1985**, *164*, 487–494. [CrossRef] [PubMed]
18. Wientjes, E.; Philippi, J.; Borst, J.W.; van Amerongen, H. Imaging the Photosystem I/Photosystem II Chlorophyll Ratio inside the Leaf. *Biochim. Biophys. Acta BBA Bioenerg.* **2017**, *1858*, 259–265. [CrossRef]
19. Laisk, A.; Talts, E.; Oja, V.; Eichelmann, H.; Peterson, R.B. Fast Cyclic Electron Transport around Photosystem I in Leaves under Far-Red Light: A Proton-Uncoupled Pathway? *Photosynth. Res.* **2010**, *103*, 79–95. [CrossRef]
20. Chow, W.S.; Hope, A.B. Electron Fluxes through Photosystem I in Cucumber Leaf Discs Probed by Far-Red Light. *Photosynth. Res.* **2004**, *81*, 77–89. [CrossRef]
21. Zahedi, S.M.; Sarikhani, H. The Effect of End of Day Far-Red Light on Regulating Flowering of Short-Day Strawberry (*Fragaria* × *ananassa* Duch. cv. Paros) in a Long-Day Situation. *Russ. J. Plant Physiol.* **2017**, *64*, 83–90. [CrossRef]
22. Jorissen, H.J.M.M.; Quest, B.; Lindner, I.; Marsac, N.T.D.; Gärtner, W. Phytochromes with Noncovalently Bound Chromophores: The Ability of Apophytochromes to Direct Tetrapyrrole Photoisomerization. *Photochem. Photobiol.* **2002**, *75*, 554–559. [CrossRef] [PubMed]
23. Wang, H.; Deng, X.W. Dissecting the Phytochrome A-Dependent Signaling Network in Higher Plants. *Trends Plant Sci.* **2003**, *8*, 172–178. [CrossRef] [PubMed]
24. Zheng, X.; Wu, S.; Zhai, H.; Zhou, P.; Song, M.; Su, L.; Xi, Y.; Li, Z.; Cai, Y.; Meng, F.; et al. Arabidopsis Phytochrome B Promotes SPA1 Nuclear Accumulation to Repress Photomorphogenesis under Far-Red Light. *Plant Cell* **2013**, *25*, 115–133. [CrossRef] [PubMed]
25. Sheerin, D.J.; Menon, C.; Oven-Krockhaus, S.Z.; Enderle, B.; Zhu, L.; Johnen, P.; Schleifenbaum, F.; Stierhof, Y.D.; Huq, E.; Hiltbrunner, A. Light-Activated Phytochrome A and B Interact with Members of the SPA Family to Promote Photomorphogenesis in Arabidopsis by Reorganizing the COP1/SPA Complex. *Plant Cell* **2015**, *27*, 189–201. [CrossRef]
26. Seo, H.S.; Watanabe, E.; Tokutomi, S.; Nagatani, A.; Chua, N.H. Photoreceptor Ubiquitination by COP1 E3 Ligase Desensitizes Phytochrome A Signaling. *Genes Dev.* **2004**, *18*, 617–622. [CrossRef]
27. Xie, Y.; Liu, Y.; Ma, M.; Zhou, Q.; Zhao, Y.; Zhao, B.; Wang, B.; Wei, H.; Wang, H. Arabidopsis FHY3 and FAR1 Integrate Light and Strigolactone Signaling to Regulate Branching. *Nat. Commun.* **2020**, *11*, 1995. [CrossRef]
28. Keuskamp, D.H.; Pollmann, S.; Voesenek, L.A.C.J.; Peeters, A.J.M.; Pierik, R. Auxin Transport through PIN-FORMED 3 (PIN3) Controls Shade Avoidance and Fitness during Competition. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 22740–22744. [CrossRef]
29. Ma, L.; Li, G. Auxin-Dependent Cell Elongation during the Shade Avoidance Response. *Front. Plant Sci.* **2019**, *10*, 914. [CrossRef]
30. Sheerin, D.J.; Hiltbrunner, A. Molecular Mechanisms and Ecological Function of Far-Red Light Signalling. *Plant Cell Environ.* **2017**, *40*, 2509–2529. [CrossRef]
31. Samiei, S.; Rasti, P.; Ly Vu, J.; Buitink, J.; Rousseau, D. Deep Learning-Based Detection of Seedling Development. *Plant Methods* **2020**, *16*, 103. [CrossRef] [PubMed]
32. Hoecker, U.; Xu, Y.; Quail, P.H. SPA1: A New Genetic Locus Involved in Phytochrome A–Specific Signal Transduction. *Plant Cell* **1998**, *10*, 19–33. [CrossRef] [PubMed]
33. Saijo, Y.; Sullivan, J.A.; Wang, H.; Yang, J.; Shen, Y.; Rubio, V.; Ma, L.; Hoecker, U.; Deng, X.W. The COP1–SPA1 Interaction Defines a Critical Step in Phytochrome A-Mediated Regulation of HY5 Activity. *Genes Dev.* **2003**, *17*, 2642–2647. [CrossRef] [PubMed]
34. Ai, K.; Su, H.; Zhou, H.; Cao, K.; Zou, Z. Effects of Different R:Fr Ratio on Chlorophyll Biosynthesis in Tomato Leaves under Salt Stress. *North Hortic.* **2019**, *424*, 14–22.
35. Ji, Y.; Nuñez Ocaña, D.; Choe, D.; Larsen, D.H.; Marcelis, L.F.M.; Heuvelink, E. Far-Red Radiation Stimulates Dry Mass Partitioning to Fruits by Increasing Fruit Sink Strength in Tomato. *New Phytol.* **2020**, *228*, 1914–1925. [CrossRef]
36. Shibuya, T.; Endo, R.; Yuba, T.; Kitaya, Y. The Photosynthetic Parameters of Cucumber as Affected by Irradiances with Different Red:Far-Red Ratios. *Biol. Plant.* **2014**, *59*, 198–200. [CrossRef]
37. Woolley, D.W.; Shaw, E.; Med, B.J.; Pharmacol Exptl Therap, J.; Robert Emerson, B.; Chalmers, R.; Cederstrand, C. Some factors influencing the long-wave limit of photosynthesis. *Proc. Natl. Acad. Sci. USA* **1957**, *43*, 133–143. [CrossRef]
38. Tan, T.; Li, S.; Fan, Y.; Wang, Z.; Ali Raza, M.; Shafiq, I.; Wang, B.; Wu, X.; Yong, T.; Wang, X.; et al. Far-Red Light: A Regulator of Plant Morphology and Photosynthetic Capacity. *Crop J.* **2022**, *10*, 300–309. [CrossRef]