Morphological and Physiological Responses of Cucumber Seedlings to Different Combinations of Light Intensity and Photoperiod with the Same Daily Light Integral

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Additional index words. cellulose content, *CsCesA1*, light quantity, light use efficiency, specific leaf area

Abstract. Lighting strategies for morphological and physiological characteristics of horticultural crops often focus on the proper daily light integral (DLI); however, a suitable combination of photosynthetic photon flux density (PPFD) and photoperiod at the same DLI is conducive to optimize the light environment management in vegetable seedling production. In the present study, cucumber seedlings (*Cucumis sativus* L. cv. Tianjiao No. 5) were grown for 21 days under six different combinations of PPFD and photoperiod at a constant DLI of $11.5 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, corresponding to a photoperiod of 7, 10, 13, 16, 19, and 22 h·d⁻¹ provided by white light-emitting diodes (LEDs) under a controlled environment. Results showed that plant height, hypocotyl length, and specific leaf area of cucumber seedlings decreased quadratically with increasing photoperiod, and the opposite trend was observed in seedling quality index of cucumber seedlings. In general, pigment content and fresh and dry weight of cucumber seedlings increased as photoperiod increased from 7 to 16 h·d⁻¹, and no significant differences were found in fresh and dry weight of shoot and root as photoperiod increased from 16 to 22 h·d⁻¹. Sucrose and starch content of cucumber leaves increased by 50.6% and 32.3%, respectively, as photoperiod extended from 7 to 16 h·d⁻¹. A longer photoperiod also led to higher cellulose content of cucumber seedlings, thus improving the mechanical strength of cucumber seedlings for transplanting. *CsCesA1* relative expression level showed a trend similar to cellulose content. We propose that *CsCesA1* is the key gene in the response to cellulose biosynthesis in cucumber seedlings grown under different combinations of PPFD and photoperiod. In summary, prolonging the photoperiod and lowering PPFD at the same DLI increased the quality of cucumber seedlings. An adaptive lighting strategy could be applied to increase seedling quality associated with the reduction of capital cost in cucumber seedling production.

Vegetables play an important role in reducing cancer risk and preventing cardiovascular disease, which is largely due to the antioxidant effects of bioactive components, such as vitamins, flavonoids, and proanthocyanidins (Khan et al., 2018; Yi et al., 2020). In modern agriculture, the vegetable seedling stage and subsequent cultivation stage are often

separated due to environmental conditions, planting density, and management differences (He et al., 2020; Vatakait-Kairien et al., 2020). In China, the planting area of vegetables was more than 20 million hm² and the annual demand for vegetable seedlings was more than 680 billion plants in 2018 (Liu et al., 2018, 2019). Previous studies indicated that high-quality vegetable seedlings had higher compactness (Jeong et al., 2020), lower specific leaf area (Snowden et al., 2016), and higher shoot fresh weight (Hernández et al., 2016) compared with seedlings grown under undesirable conditions, thus affecting the subsequent crop production and corresponding economic benefits to growers. Thus, the effective production of high-quality seedlings is of great significance for researchers and farmers.

Morphological and physiological characteristics of vegetable seedlings are regulated by ambient light, including PPFD (Wei et al., 2019; Xu, 2015; Yan et al., 2019), photoperiod (Ji et al., 2020; Wei et al., 2020), light direction (Joshi et al., 2017), and light quality

(Hernández and Kubota, 2016; Wojciechowska et al., 2020). During the main cultivation season (winter and spring) of cucumber seedlings, the quality of cucumber seedlings is reduced because of the weak sunlight conditions, which affect the yield and nutritional quality of cucumbers (Ji et al., 2020). Lower PPFD or shorter photoperiod led to longer hypocotyl length (Ji et al., 2020), smaller leaf area (Yan et al., 2019), lower shoot fresh weight (Wei et al., 2019), less chlorophyll content (Wei et al., 2020), and lower seedling quality index (Sun et al., 2020) of vegetable seedlings. Most studies investigated the influence of PPFD or photoperiod on growth and quality of seedlings; however, PPFD and photoperiod independently cannot reflect the plant responses to light (Kelly et al., 2020; Kitaya et al., 1998). Lighting recommendations for plant growth in a greenhouse or plant factory with artificial lighting (PFAL) are not only based on the quantity of DLI, but are also related to lighting strategies or control algorithms. DLI is the product of PPFD and photoperiod and optimal DLIs were investigated in lettuce (Kelly et al., 2020; Zhang et al., 2018), sweet basil (Dou et al., 2018), petunia (Owen et al., 2018), cucumber seedlings (Ji et al., 2020), and sweetpotato seedlings (He et al., 2020). However, most studies focused on the suitable DLI for crop growth, without consideration to how the light energy was delivered over time (Palmer and van Iersel, 2020).

Light quality garnered great attention among researchers and in industry in recent years as a result of the recently built PFALs and rapid development of light-emitting diodes (LEDs); however, PPFD also had significant influences on plant shape of vegetable seedlings (Snowden et al., 2016; Yan et al., 2019). Currently available white LEDs showed higher energy efficiency, and He et al. (2020) suggested substituting fluorescent lamps in PFALs with white LEDs. Therefore, a suitable combination of PPFD and photoperiod provided by white LEDs is conducive to optimize the light environment management in vegetable seedling production.

Various combinations of PPFD and photoperiod had different impacts on growth of lettuce (Kelly et al., 2020; Mao et al., 2019; Palmer and van Iersel, 2020) and ornamental species (Elkins and van Iersel, 2020a). Previous studies indicated that shoot dry weight of *Rudbeckia* seedlings (Elkins and van Iersel, 2020a) and lettuce (Weaver and van Iersel, 2020) increased by more than 25% as photoperiod increased from 12 to 21 h·d⁻¹ with DLI at 12 and 17 mol·m⁻²·d⁻¹, respectively, which may be caused by the increased daily photochemical integral (the total electron transport through photosystem II integrated over a 24-h period). However, opposite results were reported by Kelly et al. (2020), who observed that no significant differences were found in dry weight of lettuce (cv. Rouxai) as photoperiod increased from 16 to 24 h·d⁻¹ with DLI at 10.4 mol·m⁻²·d⁻¹. A similar trend was found in lettuce seedlings grown under high CO₂ concentration (Kitaya et al., 1998). These results

Received for publication 21 July 2021. Accepted for publication 2 Sept. 2021.

Published online 21 October 2021.

This work was financially supported by National Key Research and Development Program of China (2019YFD1001901-13), Science and Technology of People-benefiting Project of Qingdao (21-1-4-ny-6-nsh), Modern Agricultural Industrial Technology System of Shandong Province (SDAITG05G06), and the Foundation for High-level Talents of QAU (663/1120098).

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demonstrated that morphological and physiological responses of plants to light environment vary between species and different combinations of PPFD and photoperiod.

As an important horticultural crop, cucumber (*Cucumis sativus* L.) is widely cultivated in the world and is sensitive to light treatment (García-Caparrós et al., 2020; Jeong et al., 2020). In China, planting area and production of cucumber reached 1.05 million hm² and 56.3 million tons, respectively, which represented 52.7% and 74.8% of the world cucumber planting area and production in 2018, respectively (FAO, 2020). It is still unclear how combinations of PPFD and photoperiod affected growth and quality of cucumber seedlings at the same DLI.

It is highly desirable to increase cellulose production in several plants, as it plays a key role in plant morphogenesis, mechanical strength, and biomass exploitation (Hu et al., 2018; Liu et al., 2016). Cellulose content is also a vital parameter for mechanical transplanting of vegetable seedlings in modern agriculture. Cellulose synthesis (*CesA*) is related to the organization, regulation, and other activities of the cellulose synthase complex (CSC) (McFarlane et al., 2014). *CesA* genes were investigated in past decades, however, the genetic manipulation of this family of genes associated with cellulose production is still unclear (Hu et al., 2018). Moreover, the expression of cellulose-related genes under different combinations of PPFD and photoperiod in cucumber seedlings also requires further study.

The objectives of this study were to quantify the suitable combination of PPFD and photoperiod at the same DLI by investigating leaf morphology, pigment content, biomass, and expression of cellulose-related genes of cucumber seedlings. The results could be used as guidelines for light management strategy for cucumber seedlings production under PFALs or greenhouse with supplementary lighting. It was important to understand how plants use the light effectively with the same light energy.

Plant materials and growth conditions. Seeds of cucumber (*C. sativus* L. cv. Tianjiao No. 5) were sown in 72-cell trays (53.5 cm × 27.5 cm × 4.0 cm) filled with a mixture of vermiculite, peat, and perlite at a ratio of 3:1:1 (v/v). Cucumber seedlings were grown in the growth chamber for 21 d with air temperature at 25 ± 1°C/20 ± 1°C during day/night. Relative humidity and CO₂ concentration were maintained at 60% to 70% and 400 ± 50 μmol·mol⁻¹ during the growth period. Hoagland's nutrient solution was used with an electrical conductivity of 1.8 to 2.0 mS·cm⁻¹ and pH of 6.0 to 6.5 and comprised the following components (mg·L⁻¹): Ca(NO₃)₂·4H₂O, 945; KNO₃, 607; MgSO₄·7H₂O, 493; NH₄H₂PO₄, 115; Na₂Fe-EDTA, 30; MnSO₄·4H₂O, 2.13; CuSO₄·5H₂O, 0.08; ZnSO₄·7H₂O, 0.22; H₃BO₃, 2.86; (NH₄)₆Mo₇O₂₄·4H₂O, 0.02, respectively. Tap water and one-half strength of the standard nutrient solution were used at 2 d after sowing and at cotyledon stage, respectively. A full-strength Hoagland's nutrient solution was used after the true leaf emerged.

Table 1. List of treatments applied to cucumber seedlings for 21 d. Six combinations of photosynthetic photon flux (PPF) density (P) and photoperiod (H) at the same daily light integral (DLI) in an indoor controlled environment.

Treatment	PPF density (μmol·m ⁻² ·s ⁻¹)	Photoperiod (h·d ⁻¹)	DLI (mol·m ⁻² ·d ⁻¹)
P457-H7	457	7	11.5
P320-H10	320	10	11.5
P246-H13	246	13	11.5
P200-H16	200	16	11.5
P168-H19	168	19	11.5
P145-H22	145	22	11.5

$$\text{DLI (mol·m}^{-2}\text{·d}^{-1}\text{)} = \text{P (μmol·m}^{-2}\text{·s}^{-1}\text{)} \times \text{H (h·d}^{-1}\text{)} \times 3600 \text{ (s·h}^{-1}\text{)} \times 10^{-6}.$$

Treatment design. Cucumber seedlings were grown under various combinations of PPFD and photoperiods to maintain a constant DLI of 11.5 mol·m⁻²·d⁻¹ provided by white LEDs (W-18W; Weifang Hengxin Electric Appliance Co., Ltd, Shandong, China) for 21 d in a plant factory equipped with artificial lighting in the College of Horticulture, Qingdao Agricultural University, Qingdao, China. The combinations of PPFD (μmol·m⁻²·s⁻¹) and photoperiod (h·d⁻¹) are as follows: 457 × 7, 320 × 10, 246 × 13, 200 × 16, 168 × 19, and 145 × 22 (Table 1). Cultivation beds (L × W × H = 1.5 × 0.9 × 0.12 m) with the previously described LEDs were set in the plant factory. Opaque black-white plastic films were applied in each cultivation unit to avoid light contamination. To minimize border effects, cucumber seedlings adjacent to the plastic films were not sampled. There were 216 cucumber seedlings in each treatment, which were divided into 6 groups, and each group of 36 cucumber seedlings was considered as one replication. A spectrometer (PG100N; United Power Research Technology Corporation, Taiwan, China) was used to measure spectral distributions of the LED lamps. Fractions of blue (B, 400–499 nm), green (G, 500–599 nm), and red (R, 600–699 nm) lights of PPFD were 28.5%, 49.1%, and 22.4%, respectively (Fig. 1).

Plant morphology and growth characteristics. Plant height and stem diameter were measured from the media surface to the meristem tip and in the middle part between cotyledon and hypocotyl, respectively. Leaf area of cucumber seedlings were scanned (Yaxin-124 scanner; Beijing Yaxin Liyi Technology Co., Ltd., Beijing, China). The fresh weights of

leaves and roots were measured by an electronic analytical balance (FA1204B; BioonGroup, Shanghai, China), and then the leaves and roots were dried in an oven at 105 °C for 3 h and then set to 80 °C for 72 h for dry weight measurements. Seedling quality index and specific leaf area (SLA) were calculated according to Han et al. (2004) and Dou et al. (2018), respectively, where seedling quality index = (stem diameter/plant height + root dry weight/shoot dry weight) × total dry weight, and SLA = leaf area/leaf dry weight. Light use efficiency (g·mol⁻¹) based on fresh and dry weights of cucumber seedlings were calculated by dividing plant weight by the integrated light energy received by plants during cultivation period according to Pennisi et al. (2020).

Photosynthetic pigment contents of cucumber seedlings. Chlorophyll and carotenoid contents of seedling leaves were measured according to Lichtenthaler and Wellburn (1983). Briefly, leaf samples of 0.1 g were cut into small pieces and then extracted in 80% acetone (v/v) for 72 h in the dark. The absorbance of extracts was measured at 470, 645, and 663 nm by a spectrophotometer (1810; Shanghai Yoke Instrument Co., Ltd., Shanghai, China).

Sucrose, starch, and cellulose contents of cucumber seedlings. Sucrose content of cucumber leaves was determined by the resorcinol method (Zhang et al., 2009). Absorbance was measured using the spectrophotometer at wavelength of 480 nm. Starch content of cucumber leaves was measured using the colorimetric method (Takahashi et al., 1995). A factor of 0.9 was used to convert glucose to starch equivalents. The sulfuric acid anthrone method (Wang, 2006) was used to determine

Fig. 1. Relative photon flux density of white light-emitting diodes used in the experiment.

Fig. 2. Morphological characteristics of cucumber seedlings grown under different combinations of photosynthetic photon flux density (P) and photoperiod (H) with same daily light integral for 21 d in an indoor controlled environment.

cellulose content of cucumber seedlings. Absorbance at wavelength of 620 nm was used for measuring cellulose content.

Expression analysis of several cellulose-related genes of cucumber seedlings. Samples extracted from stems of cucumber seedlings in each treatment were used for total RNA extraction by using total RNA extraction kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), following the manufacturer's instructions. Subsequently, we reversely transcribed RNA samples into complementary DNAs (cDNAs) by using the RevertAid First Strand cDNA Synthesis Kit (Accurate Biotechnology (Hunan) Co., Ltd., Hunan, China) according to the manufacturer's protocols. Using as a reference the genes related to cellulose synthesis in *Arabidopsis thaliana* (Chundawat et al., 2011; Gong et al., 2015), we blasted the genes in CuGenDB (<http://cucurbitgenomics.org/>), after which, seven genes related to cellulose synthesis were obtained. The ACT3 gene (Accession number: DQ115883) was used as internal reference gene of cucumber (Li, 2015). Real-time polymerase chain reaction (PCR) analysis was performed using ChamQ SYBR Color qPCR Master MIX (Vazyme Biotech, Nanjing, China). Each pair of primers was designed using Primer Express 5.0 (Applied Biosystems, Waltham, MA). The primer sequences used in this study are listed in Table 2. The relative level of gene expression was determined by using the $2^{-\Delta\Delta C_t}$ method (Pfaffl, 2001).

Statistical analysis. One-way analysis of variance was performed to test effects of combinations of PPFD and photoperiod with

the same daily light integral on morphologic and physiologic characteristics of cucumber seedlings using SPSS 18.0 software (IBM, Inc., Chicago, IL), followed by the least significant difference test to compare the means between treatments ($P < 0.05$). The results were reported as the mean $\pm$ SD values. Regressions between treatments and morphological characteristics of cucumber seedlings were performed using Microsoft Excel 2016 software (Redmond, WA).

Results

Influence of PPFD and photoperiod on pigment content, morphology, and growth of cucumber seedlings with same DLI. Longer photoperiod led to higher chlorophyll and carotenoid contents compared with those grown under shorter photoperiod with higher PPFD at the same DLI (Table 3); however, no significant differences were found in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content in cucumber seedlings as photoperiod was extended from 16 to 19 h·d⁻¹. Total chlorophyll content of cucumber seedlings grown under photoperiod of 22 h·d⁻¹ decreased by 12.8% compared with those grown under 16 h·d⁻¹.

Plant height, hypocotyl length, and SLA of cucumber seedlings decreased in a quadratic way as photoperiod increased; however, seedling quality index increased quadratically with increasing photoperiod (Fig. 3). Plant height and hypocotyl length decreased by 37.0% and 43.6%, respectively, as photoperiod increased from 7 to 22 h·d⁻¹. Seedling quality index increased by 137.7% with increasing

photoperiod from 7 to 22 h·d⁻¹. No significant differences were observed as photoperiod increased from 16 to 22 h·d⁻¹ in hypocotyl length, SLA, and seedling quality index of cucumber seedlings (Figs. 2 and 3). Similar trends were observed in leaf area, and fresh and dry weights of shoot and root of cucumber seedlings. Fresh and dry weight of cucumber shoots increased by more than 20% as photoperiod increased from 7 to 22 h·d⁻¹. No significant differences were observed in cucumber seedlings in leaf fresh weight and root fresh weight as photoperiod increased from 13 to 22 h·d⁻¹ (Table 4).

Light use efficiency of cucumber seedlings grown under different combinations of PPFD and photoperiod with same DLI. Generally, light use efficiency based on fresh and dry weights of cucumber seedlings increased with increasing photoperiod under shorter photoperiod at the same DLI; however, no significant differences were observed in light use efficiency as photoperiod increased from 16 to 22 h·d⁻¹. Light use efficiency based on fresh and dry weights of cucumber seedlings grown with photoperiod at 19 h·d⁻¹ increased by 43.7% and 36.0%, respectively, compared with seedlings grown with photoperiod at 7 h·d⁻¹ (Fig. 4).

Impact of combinations of PPFD and photoperiod on physiological characteristics of cucumber seedlings. In general, starch and cellulose content of cucumber seedlings increased first, and then no significant differences were observed as photoperiod increased from 7 to 22 h·d⁻¹; however, sucrose content of cucumber seedlings decreased as photoperiod increased from 19 to 22 h·d⁻¹ (Fig. 5). Sucrose, starch, and cellulose content of cucumber seedlings grown at PPFD at 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ combined with photoperiod at 16 h·d⁻¹ increased by 50.6%, 32.3%, and 69.6% compared with those grown at 457 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ combined with photoperiod at 7 h·d⁻¹.

Effects of combinations of PPFD and photoperiod on cellulose gene expression. Relative expression level of *CsCesA1* in cucumber seedlings first increased and then decreased with increasing photoperiod from 7 to 22 h·d⁻¹. The relative expression level increased significantly in seedlings grown with photoperiod of 19 h·d⁻¹, and the highest *CsCesA3* relative expression level was obtained with photoperiod of 22 h·d⁻¹, which increased by 17- and 18-fold compared with photoperiod of 7 h·d⁻¹. Higher *CsCesA6* relative expression level was observed in cucumber seedlings grown with shorter photoperiods and the highest *CsCesA6* relative expression level was obtained in those grown with photoperiod at 10 h·d⁻¹. In general, *CsCesA4* relative expression level was lower. *CsCesA5* and *CsCesA7* relative expression levels were highest in treatments with photoperiod of 13 and 16 h·d⁻¹, respectively, which was upregulated by 3.5- and 15-fold compared with those grown with photoperiod of 7 h·d⁻¹ (Fig. 6).

Discussion

Representing two classes of photosynthetic pigments in higher plants, chlorophylls

Table 2. Primer sequences for real-time polymerase chain reaction described in this study.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
<i>CsCesA1</i>	CAACACGATACGGGGTCAAG	CACTGGGTCAAATGGAGAAGAA
<i>CsCesA2</i>	CAGTGGTGAGCAAAGGAATGTG	GTGAGGCAGTAGAAGATGGTGATG
<i>CsCesA3</i>	GGATTCGTGATTCCGGTAGTGC	CATCTGAAAGGGGAGGTGGA
<i>CsCesA4</i>	CGGCTTTTGCTGTAACTGTGAA	TCTGGGTGATTGGTGGTGTG
<i>CsCesA5</i>	CGTTTTCCGAATGATTAGGCT	CGTCACAAAGGTTACCCAG
<i>CsCesA6</i>	CAACGGCAAGGTGAGGATAGA	GATACAAGCGTCTCAAAGGTAGTCC
<i>CsCesA7</i>	TGAGAATGACTGAGATGAGATGCC	GATGAGAACTTGCTGATGGATGC
Actin	GGCAGTGGTGGTGAACATG	TTCTGGTGATGGTGTGAGTC

Table 3. Pigment content of cucumber seedlings as affected by photosynthetic photon flux density (P) and photoperiod (H) with same daily light integral (DLI) in an indoor controlled environment.

Treatment	Chlorophyll a content (mg·g ⁻¹)		Chlorophyll b content (mg·g ⁻¹)		Total chlorophyll content (mg·g ⁻¹)		Carotenoid content (mg·g ⁻¹)	
P457-H7	0.53 ± 0.06	c	0.25 ± 0.04	d	0.78 ± 0.07	d	0.13 ± 0.03	b
P320-H10	0.88 ± 0.04	b	0.37 ± 0.03	c	1.25 ± 0.03	c	0.17 ± 0.01	a
P246-H13	0.96 ± 0.09	ab	0.41 ± 0.05	b	1.38 ± 0.14	b	0.17 ± 0.01	a
P200-H16	1.09 ± 0.09	a	0.47 ± 0.03	a	1.56 ± 0.08	a	0.18 ± 0.02	a
P168-H19	1.07 ± 0.14	a	0.43 ± 0.03	ab	1.50 ± 0.12	ab	0.18 ± 0.03	a
P145-H22	0.96 ± 0.06	ab	0.40 ± 0.02	b	1.36 ± 0.08	b	0.17 ± 0.01	a

Means (± SD) followed by different letters within one column differ significantly ($P < 0.05$) as established by the least significant difference test.

and carotenoids absorb light energy and drive photochemistry (Yamori, 2016; Yan et al., 2020). Leaf chlorophyll content of cucumber seedlings was significantly affected by different combinations of PPFD and photoperiod (Table 3). Cucumber seedlings grown at higher PPFD showed lower leaf pigmentation than at lower PPFD, indicating that excess PPFD ($>320 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) had negative influence on photosynthetic pigment accumulation. Similar trends were reported by Fu et al. (2017) and He et al. (2020). Kelly et al. (2020) observed that SPAD value of lettuce (cv. Rex) grown with PPFD of $216 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was higher than those grown with PPFD of $270 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with the same DLI at $15.6 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, but these trends were not found in lettuce grown under DLI at 8.4 and $10.4 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. In addition, the effects of different combinations of PPFD and photoperiod on leaf pigmentation under the same DLI can also be cultivar dependent (Kelly et al., 2020). In general, lengthening photoperiod under lower PPFD could compensate for the low PPFD, which was beneficial for pigment accumulation, thus affecting the plant's ability to absorb light energy more effectively with the same DLI (Mao et al., 2019). Longer

photoperiod resulted in increased chlorophyll content, which could increase photochemistry and light absorption of cucumber seedlings. Previous study hypothesized that longer photoperiod, which led to higher chlorophyll content, may be because chlorophyll synthesis in plants is dependent on light (Langton et al., 2003). Moreover, higher chlorophyll content based on weight also observed in strawberry runner plants (Zheng et al., 2019), lettuce (Fu et al., 2017; Song et al., 2020), and sweet basil (Dou et al., 2018) grown under lower PPFD. This may be the result of plants building larger light-harvesting antennae and synthesizing more chlorophyll to capture more light energy to adapt to the lighting environment. However, chlorophyll content based on leaf area increased with the increasing PPFD (Feng et al., 2019; Yu et al., 2016), which was associated with higher concentration of the RuBisCo enzyme, thus affecting photosynthesis. A long photoperiod with lower PPFD led to a decreasing trend in chlorophyll contents. A decreasing trend was also found in lettuce as photoperiod increased from 16 to 18 h·d⁻¹ (Virsilie et al., 2019) and continuous light duration increased from 12 h to 24 h (Bian et al., 2018), suggesting that an extended photoperiod may lead to

physiologic disorders, such as leaf chlorosis and chlorophyll degradation. Lower PPFD with longer photoperiod was beneficial for seedling quality than those grown under higher PPFD with shorter photoperiod at the same DLI. Similar trends were reported by Mao et al. (2019), who found that photoperiod at 16 h·d⁻¹ led to higher shoot and root fresh weights of lettuce than those grown with photoperiod at 12 h·d⁻¹ at the same DLI, which was due to the increased chlorophyll contents. Previous studies indicated that leaf chlorophyll content increased with the increase of photoperiod in kale (Lefsrud et al., 2006) and lettuce (Weaver and van Iersel, 2020). Similar results were found in our study (Table 3).

High-quality seedlings should exhibit morphological characteristics, such as firm stem, dark green leaves, and large white roots (Wei et al., 2018; Yan et al., 2019). Previous studies have indicated that seedlings grown under undesirable lighting environments had less dry weight (Johkan et al., 2010), fewer node numbers (Pramuk and Runkle, 2005), lower yield (Lee et al., 2012), and lower nutritional quality (Yan et al., 2019) compared with robust seedlings after transplanting. Cucumber seedlings grown under higher

Fig. 3. Relationships between photoperiod with same daily light integral and morphological characteristics of cucumber seedlings grown for 21 d after sowing in an indoor controlled environment.

Table 4. Growth and morphological characteristics of cucumber seedlings grown under six combinations of photosynthetic photon flux density (P) and photoperiod (H) with same daily light integral in an indoor controlled environment.

Treatment	Stem diam (cm)	Leaf area (cm ²)	Shoot fresh wt (g/plant)	Root fresh wt (g/plant)	Shoot dry wt (g/plant)	Root dry wt (g/plant)
P457-H7	0.307 ± 0.007 b	92 ± 7 b	3.41 ± 0.55 b	0.77 ± 0.20 c	0.268 ± 0.039 b	0.038 ± 0.009 c
P320-H10	0.321 ± 0.009 b	97 ± 7 b	3.83 ± 0.43 ab	1.10 ± 0.28 b	0.290 ± 0.046 ab	0.045 ± 0.013 b
P246-H13	0.333 ± 0.012 ab	104 ± 5 ab	4.29 ± 0.77 ab	1.25 ± 0.33 ab	0.322 ± 0.060 ab	0.057 ± 0.012 b
P200-H16	0.361 ± 0.030 a	111 ± 7 a	4.46 ± 0.91 a	1.35 ± 0.21 ab	0.337 ± 0.035 a	0.070 ± 0.013 ab
P168-H19	0.343 ± 0.035 ab	100 ± 10 ab	4.47 ± 1.07 a	1.50 ± 0.17 a	0.329 ± 0.040 a	0.074 ± 0.008 a
P145-H22	0.342 ± 0.002 ab	103 ± 8 ab	4.62 ± 0.76 a	1.51 ± 0.09 a	0.349 ± 0.036 a	0.076 ± 0.008 a

Means (± sd) followed by different letters within one column differ significantly ($P < 0.05$) as established by the least significant difference test.

PPFD with shorter photoperiod developed thin stem, higher height, and longer hypocotyl, which were easier to suffer from diseases and damage. Plants grown under low PPFD may have shade acclimation characteristics, including higher plant height (Ji et al., 2020), longer leaf length (Yan et al., 2019), and lower carbohydrate accumulation (Lu et al., 2017; Pennisi et al., 2020). Plants grown under lower PPFD tended to have longer leaves to increase light interception (Palmer and van Iersel, 2020). Yan et al. (2019) observed that leaf length of lettuce seedlings decreased with increasing DLI, indicating that leaf shape changed to acclimate to the lower PPFD or DLI environment. A relatively large canopy was beneficial for intercepting light efficiently in plant growth (Elkins and van Iersel, 2020a). Lower PPFD with longer photoperiod did not lead to a shade-avoidance response, based on the leaf morphological characteristics of cucumber seedlings in the present study. Lower PPFD with longer photoperiod led to larger area than those grown with higher PPFD (Table 4), which was used for light absorption. Plant height and hypocotyl length of cucumber seedlings decreased in a quadratic function with increasing photoperiod (Fig. 3), suggesting that plant height and hypocotyl length of cucumber seedlings was due to the effects of photoperiod, rather than PPFD for plants grown at the same DLI. Photoperiod regulates different processes of plants, such as photomorphogenesis, growth, and circadian rhythm. Important components in detection of the day length are the circadian clock and photoreceptors, which were used to perceive time and distinguish between dark and light, respectively (García-

Caparrós et al., 2020; Hoffman et al., 2010). Miyazaki et al. (2015) indicated that hypocotyl elongation and the period of circadian oscillation were associated with the Zeittupe family, which was one of the five different photoreceptors in plants. SLA is a measurement of the amount of leaf area available for light capture per unit dry mass, which is an important trait explaining growth variations of plant grown under different light conditions. Previous studies showed that high PPFD with the same photoperiod led to lower SLA in sweet basil (Dou et al., 2018), lettuce (Ghorbanzadeh et al., 2020), and cucumber plants (Yu et al., 2016), indicating that thickness of plant leaves increased with increasing PPFD. This may be because of the growth of palisade tissue or the deep development of the spongy layer (Fan et al., 2013). However, a decreased trend in SLA was observed in cucumber seedlings with the increase of photoperiod (the decrease of PPFD) at the same DLI (Fig. 3), which was consistent with the study reported by Elkins and van Iersel (2020a), who observed that SLA of *Rudbeckia* seedlings decreased linearly as photoperiod increased from 12 to 21 h·d⁻¹ with the same DLI at 12 mol·m⁻²·d⁻¹. This may be because the increased chlorophyll content had a direct consequence of the decreased SLA with longer photoperiods.

Generally, higher PPFD or longer photoperiod often led to higher carbohydrate accumulation in cucumber seedlings (Ji et al., 2020), lettuce seedlings (Kitaya et al., 1998; Yan et al., 2019), and sweet basil (Dou et al., 2018), indicating that plant growth was promoted by increasing DLI. However, different results were observed in previous studies at

the same DLI. No differences were found in shoot fresh and dry weights in cucumber seedlings as photoperiod increased from 10 to 22 h·d⁻¹ with DLI at 11.5 mol·m⁻²·d⁻¹ in this study (Table 4). Similar trends were reported by Zhang et al. (2018), who indicated that no significant differences were found in fresh and dry weights in lettuce leaves grown under different combinations of PPFD and photoperiod with DLI at 8.64 mol·m⁻²·d⁻¹ provided by fluorescent lamps and LEDs. Kitaya et al. (1998) also found similar results in lettuce seedlings grown with DLI at 8.6 and 17.3 mol·m⁻²·d⁻¹ with CO₂ at 800 μmol·mol⁻¹. However, Palmer and van Iersel (2020) observed that shoot fresh weight of lettuce and mizuna increased by 16.0% and 18.7% as photoperiod increased from 10 to 20 h·d⁻¹ with DLI at 16 mol·m⁻²·d⁻¹. Elkins and van Iersel (2020a) found that shoot dry weight and root dry weight of *Rudbeckia* seedlings grown in a greenhouse increased by 30% and 24% as photoperiod increased from 12 to 21 h·d⁻¹ with DLI at 12 mol·m⁻²·d⁻¹. Similar trends were also found in lettuce reported by Weaver and van Iersel (2020) and Mao et al. (2019). These differences may be because of the range of DLI, a DLI threshold, species, and other variables. Kelly et al. (2020) reported that different influences were observed in lettuce between different combinations of PPFD and photoperiod at the same DLI and different cultivars.

Longer photoperiod increased plant growth with lower PPFD at the same DLI; this was because of decreased photosynthetic efficiency with increasing PPFD. Photoperiod exceeding 19 h·d⁻¹ did not induce any additional benefits but it did reduce the pigmentation and sucrose

Fig. 4. Light use efficiency based on fresh and dry weight of cucumber seedlings grown with the same daily light integral for 21 d after sowing in an indoor controlled environment. Means followed by different letters within each parameter are significantly different based on least significant difference test ($P < 0.05$). Error bars show ± sd.

Fig. 5. Sucrose, starch, and cellulose contents of cucumber seedlings as influenced by photosynthetic photon flux density (P) and photoperiod (H) with same daily light integral for 21 d after sowing in an indoor controlled environment. DW = dry weight. Means followed by different letters within each parameter are significantly different based on least significant difference test ($P < 0.05$). Error bars show $\pm$ SD.

accumulation of cucumber seedlings. Short photoperiod with high PPFD resulted in lower carbohydrate accumulation, which may be because of excess light energy causing photo-oxidative damage (Fan et al., 2013). Elkins and van Iersel (2020b) indicated that longer photoperiod with lower PPFD was considered to increase plant growth and photosynthetic efficiency, as daily photochemical integral increased linearly with increased photoperiod, which was because plants exhibited an increase in the quantum yield of photosystem II (Φ PSII) with decreasing PPFD. Previous research indicated that these simulated increases in daily photochemical integral did indeed correspond to improved growth in lettuce (Elkins and van Iersel, 2020b) and spinach beet (Soffe et al., 1977). In addition, previous studies had indicated that the Φ PSII of lettuce (Elkins and van Iersel, 2020b), sweetpotato seedling (He et al., 2020), and bayberry (Gao et al., 2019) decreased with increasing PPFD, indicating that light drives photochemistry efficiently under lower PPFD (Elkins and van Iersel, 2020b; Weaver and van Iersel, 2020). Similar trends were reported by Zhang et al. (2018) and Yan et al. (2019), who observed that efficiency of light energy use in lettuce decreased with increasing PPFD. This may be because a higher proportion of light energy converted to dissipated heat as PPFD increased. Influences of different combinations of PPFD and photoperiod at the same DLI on leaf morphology, growth, and seedling quality needed to be

considered; moreover, capital cost and operating expenses are also of great importance in commercial production for growers.

Optimal lighting strategy should be applied to cultivate high-quality seedlings and in consideration of energy use efficiency in the controlled environment. Previous studies had investigated light use efficiency of tomato (Goto, 2011), perilla (Lu et al., 2017), basil (Janssen et al., 2019), and lettuce (Pennisi et al., 2020) grown in a controlled environment. Lu et al. (2017) demonstrated that light use efficiency decreased with increased DLI (PPFD) with the same electrical conductivity in both green and red perilla production. A similar trend was found in strawberry runner plants grown in the LED plant factory (Zheng et al., 2019). However, quadratic functions were observed between DLI and light use efficiency in indoor cultivation of lettuce and basil; moreover, the light use efficiencies of lettuce and basil were the highest with DLI at $14.4 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ (Pennisi et al., 2020). Light use efficiency based on fresh and dry weights of cucumber seedlings increased with increasing photoperiod from 7 to $13 \text{ h} \cdot \text{d}^{-1}$, and no significant differences were observed as photoperiod increased from 16 to $22 \text{ h} \cdot \text{d}^{-1}$ with the same DLI (Fig. 4). These results were associated with the trends of fresh and dry weights of cucumber seedlings, as the same DLI was applied among treatments. Using the light energy effectively has large impacts on the economics of a plant factory: not only as a

large impact in production, but also on the energy use (Janssen et al., 2019). The result was important for commercial production of cucumber seedlings grown in a plant factory or greenhouse supplemented with artificial lighting. Under same DLI, the higher light energy efficiency in the plant factory can be attributed to the proper lighting environment with longer photoperiod.

Carbohydrate metabolism (starch and sucrose) of cucumber seedlings was influenced by various combinations of PPFD and photoperiod, indicating that longer photoperiod led to accumulation of starch and sucrose (Fig. 5). This was because longer photoperiod restricted the transportation of photosynthetic products out of cucumber leaves; similar results were found in garlic (Atif et al., 2019, 2020). Cucumber synthesizes stachyose in mature leaves and transports this oligosaccharide in the phloem. The stachyose is converted to sucrose after long-distance transport into peduncles, and then catabolized to fructose and glucose after entering the fruit (Miao et al., 2007; Pharr et al., 1985). Sucrose and starch are the main storage carbohydrates in many plants and the major form in which carbon is transported through the plants. Starch was synthesized during the day and consumed at night, which was used to sustain sucrose synthesis and energy supply for plant growth (Shen et al., 2019). The capacity to alter the carbohydrate components may be dependent on the light requirements, species and, other environmental variables.

Fig. 6. Effects of combinations of photosynthetic photon flux density and photoperiod with same daily light integral on expression of cellulose-related genes of cucumber seedlings grown for 21 d after sowing in an indoor controlled environment. Means followed by different letters within each parameter are significantly different based on least significant difference test ($P < 0.05$). Error bars show $\pm$ SD.

As one of the main classes of polysaccharides in plant cell walls, cellulose contributes to plant morphogenesis (Liu et al., 2016). It is valuable to determine the regulatory aspects that control cellulose synthase complex behaviors for understanding the plant growth and directed cell (McFarlane et al., 2014). Previous study had indicated that overexpression of the *AtCesA6* gene enhanced secondary cell wall deposition, which improves the mechanical strength and leads to higher biomass of transgenic mature plants in *Arabidopsis thaliana* (Hu et al., 2018). However, the number of *CesA* genes differs between plant species. This study indicated that the *CsCesA1* relative expression level showed a similar trend with cellulose contents; we suggested that *CsCesA1* was the key gene in response to cellulose biosynthesis in cucumber seedlings grown under

different combinations of PPFD and photoperiod. *CsCesA3* and *CsCesA6* relative expression levels showed significant response in cucumber seedlings grown under longer and shorter photoperiod, respectively. However, the gene functions also require further study.

Conclusions

Longer photoperiod combined with lower PPFD at the same DLI led to higher photosynthetic pigment content, thicker leaves, and compact cucumber seedlings with lower plant height and shorter hypocotyl as photoperiod increased from 7 to 16 h·d⁻¹. The accumulation of cellulose in cucumber seedlings was due to the regulation of *CsCesA1* relative expression level. *CsCesA3* and *CsCesA6* are the key genes of cellulose biosynthesis in

cucumber seedlings grown under longer and shorter photoperiod at the same DLI, respectively. Under same DLI, the prolonged photoperiod could promote seedling quality of cucumbers by compensating for lower PPFD. Extending photoperiod to 16 to 19 h·d⁻¹ was beneficial for accumulation of sucrose, starch, and cellulose, which could improve the mechanical strength of cucumber seedlings for transplanting. Moreover, growers can extend the photoperiod associated with fewer LED lamps in commercial production, reducing the capital cost without affecting seedling quality.

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