Rapid and semi-automated leaf net photosynthetic rate determination for numerous phosphor-converted white-LED lights of different spectral distributions

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Abstract

Phosphor-converted white LEDs (PCW-LEDs) of numerous types with different relative spectral photon-flux-density distributions (SPDs) are commercially available today. Some are regarded as promising light sources for use in plant factories with artificial lighting. The leaf net photosynthetic rate ($P_n$) measured under PCW-LED light is an important criterion for evaluating PCW-LEDs in terms of photosynthesis performance. To ascertain $P_n$ rapidly under dozens of PCW-LED lights having different SPDs, we have developed a rapid and semi-automated $P_n$-quantification method. The method uses a modified LED-artificial sunlight source system (LASS system) and a $P_n$-measurement system. The modified LASS system can produce light with an SPD, which can accurately approximate that of any PCW-LED light at a photosynthetic photon flux density (PPFD) of 150 μmol m$^{-2}$ s$^{-1}$. First, PCW-LED lights of 30 types at a PPFD of 150 μmol m$^{-2}$ s$^{-1}$ were produced using the modified LASS system within 2.5 h. We then measured the $P_n$ of cos lettuce, red-leaf lettuce, and green-leaf lettuce (Lactuca sativa L.) plants. In a 16-h $P_n$ measurement repetition, the modified LASS system supplied all the produced lights automatically and successively to an identical leaf of a lettuce plant. A $P_n$-measurement system simultaneously measured $P_n$ under the produced light. Results show that the mean $P_n$ values of the cos lettuce, red-leaf lettuce, and green-leaf lettuce under the 30 produced lights at 20 days after sowing were, respectively, 7.11–8.02, 5.76–7.11, and 4.83–6.17 μmol m$^{-2}$ s$^{-1}$. A rapid and semi-automated method was developed for successive measurement of $P_n$ under dozens of combined lights, of which each SPD approximated that of the selected PCW-LED lights, within days, which indicates that the method can determine the $P_n$ quickly under numerous PCW-LED lights. Results show that the system contributes to rapid selection of PCW-LED lights performing high $P_n$.

Key words: LED-artificial sunlight source system, Lettuce, Plant factories with artificial light, Spectral photon-flux-density distribution

Introduction

Because of their small size, long lifetime, and various relative spectral photon-flux-density distributions (SPDs), light-emitting diodes (LEDs) are particularly beneficial for cultivation in plant factories with artificial lighting (PFALs) (Bantis et al., 2018; Kozai, 2016; Massa et al., 2008). The related literature, of which Bantis et al. (2018) provides a review, includes many reports of investigations of LED-SPD effects on the growth and development of various plant species. Most such studies have been conducted using blue LEDs (460–470 nm typical peak wavelength) and red LEDs (660 nm typical peak wavelength), partially because red light reportedly drives photosynthesis efficiently and because adding small amounts of blue light to red light can increase photosynthesis and dry mass further for some species (Hernández and Kubota, 2016; Hogewoning et al., 2010; Kim et al., 2004; Lee et al., 2007; Ohashi-Kaneko et al., 2006; Wang et al., 2016). However, growers have difficulty diagnosing the health of plants grown under blue-LED and red-LED light (BR-light) (Massa et al., 2008). Adding green-LED light to BR-light (BGR-light) is one means of overcoming this difficulty because it produces white light (Kim et al., 2004). Several studies have revealed that the dry masses of some plants grown under BGR-light are equal to or greater than those grown under BR-light (Kim et al., 2004; Kong et al., 2015; Saengtharatip et al., 2018; Snowden et al., 2016). These reports have indicated indirectly that some white light can be more effective than BR-light for plant dry mass production.

In recent years, PCW-LEDs have become a common white light source for investigating white-light SPD effects on plants (Cope et al., 2014; Han et al., 2017; Metallo et al., 2018). A typical PCW-LED comprises a blue-LED chip and phosphors of one or more types incorporated in the encapsulation resin, of which the phosphors convert partial blue-LED light into green or red light (Cho et al., 2017). Some reports have described that PCW-LED light can enhance plant growth compared to BR-light (Han et al., 2017; Lu et al., 2019; Nozue et al., 2018), even to BGR-light (Saengtharatip et al., 2018). In addition, PCW-LEDs (blue-LED chip plus phosphors of one or more types) have been popularized rapidly for general lighting applications (Han et al., 2017), of which the cost can be less than 20% of that of common red LEDs (Kusuma et al., 2020). Because demand for PCW-LEDs is still increasing, their price can be expected to decrease further. Moreover, PCW-LED light sources generally consist of one type of LED, which can be easier to manufacture than those with two or more types of LEDs, such as BGR-light sources. Considering
these benefits of PCW-LEDs, they are expected to be promising plant cultivation light sources, especially for PFALs.

Currently, numerous types of PCW-LEDs with different relative SPDs are commercially available. In Japan, more than several dozen types of PCW-LED can be purchased from some LED manufacturers such as Nichia Corp. (Tokushima, Japan) and Stanley Electric Co. Ltd. (Tokyo, Japan). A PCW-LED SPD depends mainly on the phosphor type and their amounts contained in the encapsulation resin of the LED package. By adjusting these two factors, LED manufacturers can readily produce hundreds of thousands of PCW-LEDs with different relative SPDs. Several studies have compared the respective performances of PCW-LEDs of several types in growing plants. Their results have demonstrated that the relative SPD of PCW-LED light can strongly affect dry weight (Han et al., 2017; Lu et al., 2019; Nozue et al., 2018; Saengtharatip et al., 2018; Zou et al., 2020). The results also indicate that the relative SPD of PCW-LEDs should be selected carefully to maximize dry mass production.

Han et al. (2017) grew lettuce plants under PCW-LED lights with relative SPD of three types. Their results demonstrated that the PCW-LEDs that brought about higher \( P_n \) tended also to engender higher dry weights. This result indicates that the \( P_n \) might be used as an indicator to estimate the potential dry weights of the lettuce plants grown under PCW-LED lights with different relative SPDs. Indeed, the number of PCW-LED types used in their study is regarded as insufficient. Further research with PCW-LEDs of more types should be conducted to assess the validity of \( P_n \) as an indicator to estimate the potential dry weights of the lettuce plants. Such an investigation would require data of the \( P_n \) measured under more than dozen PCW-LED light regimes with different relative SPDs to find those among them to supply to cultivation experiments. This process might require more than a few months for preparation of all PCW-LED light sources. Moreover, measuring \( P_n \) with those PCW-LED light sources can be tedious because the operator taking measurements must repeat plant cultivation once for each light source to be tested. Therefore, a method that is able to provide \( P_n \) data rapidly and effortlessly will greatly improve the efficiency of the PCW-LED selection process.

This study was conducted to develop a rapid method for determining \( P_n \) under several dozen PCW-LED lights with different relative SPDs within days. For this purpose, we developed a rapid and semi-automated \( P_n \)-quantification method. The method uses two systems: a LASS system (Fujiiwara et al., 2013) and a photosynthesis measurement system. The LASS system, which includes 625 monochromatic LEDs with 32 peak wavelengths (385–910 nm), was developed to produce light with an SPD approximating that of arbitrarily selected light at wavelengths of 385–910 nm within a few minutes. It can also supply an arbitrary number of the produced lights automatically and successively. Furthermore, their irradiation periods can be set individually at any interval of more than 2 s. The LASS system can produce PCW-LED light with wavelengths mainly of 400–800 nm. Stated briefly, the developed method determines \( P_n \) under selected PCW-LED lights by measuring \( P_n \) successively under each of the corresponding produced lights that is incident on an identical leaf.

For this study, we first modified the original LASS system to improve the approximations accuracy and maximum PPFD of the produced lights. Using the original system, we produced SPDs that approximate those of some PCW-LED lights, but with inadequate approximation accuracy for a PPFD of 150 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) or greater. Subsequently, details of procedures used for the developed \( P_n \)-quantification method are described. Lastly, using the method, we ascertained the \( P_n \) of three lettuce cultivars under 30 PCW-LED lights with different relative SPDs at a PPFD of 150 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) within one day per cultivar per repetition. It is noteworthy that \( P_n \) was measured using the proposed method under incident light with different relative SPDs on a leaf surface at an identical PPFD. Therefore, the measured \( P_n \) is expected to be affected by the relative SPDs via light absorption and the photosynthetic quantum yield of the leaves. In addition, the performance of the lights produced with the modified LASS system (Fig. 1) and correlations between the determined \( P_n \) and the different wavelength-band light PFDs of the 30 PCW-LED lights were also discussed.

Materials and Methods

After briefly introducing the original LASS system, this report describes some LASS system modifications. Finally, we describe procedures for a \( P_n \)-quantification method developed for successive evaluation of \( P_n \) for 30 PCW-LED lights with different relative SPDs.

Original LASS system

The original LASS system (Fujiiwara et al., 2013) comprises a light source unit, an LED temperature control system, and a spectral irradiance distribution (SID) control system. The light source unit consists of an LED module and a hollow conical reflection condenser. The LED module is equipped with 625 monochromatic LEDs with 32 peak wavelengths (385–910 nm). The condenser is used to mix lights from different LEDs and to gather them to the light outlet. The LED temperature control system is assembled to stabilize the LED module temperature. The SID control system can produce light with an SID (combined SID) that approximates a given SID (target SID) accurately within a 380–940 nm wavelength. Because an SID can convert to an SPD at the same wavelength range, as described in a review of the literature (Thimijan and Heins, 1983), the SID control system is therefore an SPD control system. This function can be achieved because it can control SIDs on the light outlet by adjusting the voltages applied to each type of monochromatic LED. When a desirable SID is obtained, users can save the voltage set (voltages of the 32 types of LED) in the SID control system. The LASS system can then readily re-irradiate a light with any combined SID by loading its voltage set and applying the voltages to the LEDs. Furthermore, the SID control system can automatically supply more than dozens of produced lights successively. Moreover, the irradiation period of each light can be set at an interval of more than 2 s.

Modified LASS system

Although the original LASS system can produce lights with different SPDs, accurately approximating those of the corresponding selected PCW-LED lights (a typical one is
presented in Supplementary Materials 1, Fig. S1A), the extent of the differences between some combined SPDs and the corresponding target SPDs in several wavelength ranges such as 440–460 nm, 510–550 nm, and 580–620 nm (a typical one is presented in Supplementary Materials 1, Fig. S1B) is not negligible. These differences might engender biases between $P_e$ measured under the produced lights and that measured under the corresponding PCW-LED lights. Another point to be improved was the low maximum-PPFDs of the produced lights with the original LASS system for PCW-LEDs, which were approximately 150 $\mu$mol m$^{-2}$ s$^{-1}$. Considering that the PPFD used in a PFAL can reach 300 $\mu$mol m$^{-2}$ s$^{-1}$ (Lu and Shimamura, 2018), the modified LASS system is expected to be capable of producing PCW-LED lights at a PPFD of more than 300 $\mu$mol m$^{-2}$ s$^{-1}$.

Therefore, we made the following modifications to the original LASS system to meet our requirements. First, we selected monochromatic LEDs of three types and PCW-LEDs of three types (Fig. 2; Supplementary Materials 2, Table S1) to be installed on the modified LED module. Their peak wavelengths differed from those of the original LED module. The three PCW-LEDs to be installed were selected because their relative SPDs included some wavelength ranges that are found only rarely in commercial monochromatic LEDs. In addition, their high photon fluxes per LED contribute to increased maximum PPFDs of the produced lights.

Then, we replaced monochromatic LEDs of eight types with selected LEDs of eight types with peak wavelengths that were similar to the original LED module; the LEDs of eight types have higher photon fluxes per LED. After those modifications, the modified LED module came to consist of monochromatic LEDs of 27 types (426–826 nm peak wavelengths) and PCW-LEDs of three types. Their relative SPDs, model codes, and peak wavelengths are presented in Fig. 2, Supplementary Materials 2, and Table S1.

Lastly, we coated the inner surface of the hollow conical reflection condenser with a water-based high-reflectance barium sulfate coating (Avian-B500; Avian Technologies LLC, New London, NH, USA) to increase the distribution uniformity of the combined PFDs at the light outlet.

The RMSEs [mol m$^{-2}$ s$^{-1}$ nm$^{-1}$] of the combined SPDs and the target SPDs were used to evaluate the extent of their mutual difference. For lower RMSE, better combined SPD is obtained.

**Plant materials and growth conditions**

Cos lettuce (cv. Cos Lettuce; Takii Seed Co. Ltd., Kyoto, Japan), red-leaf lettuce (cv. Mother-red; Takii Seed Co. Ltd.), and green-leaf lettuce (cv. Mother-green; Takii Seed Co. Ltd.) plants were subjected to $P_e$ measurements at 20 and 25 DAS. They were cultivated in a growth chamber at 25 ± 1°C (mean ± SE) under a 16-h/8-h light/dark period cycle. A PCW-LED array (HMW120DC6 1N-40Y; Kyoritsu Densho Co., Ltd., Osaka, Japan), shown as PCW-LED light 16 in Fig. 3, was applied to provide a PPFD of 150 ± 10 $\mu$mol m$^{-2}$ s$^{-1}$ on the cultivation surface. The CO$_2$ concentration in the growth chamber was 1000 ± 50 $\mu$mol mol$^{-1}$ controlled by an infrared CO$_2$ controller (ZFP9; Fuji Electric Co. Ltd., Tokyo, Japan). Lettuce plants were cultivated in a nutrient solution (half-strength Otsuka-A nutrient solution; OAT Agro Co. Ltd., Tokyo, Japan) with electrical conductivity of 150 ± 0.1 mS m$^{-1}$.

**Procedures of the rapid and semi-automated $P_e$-quantification method**

**Step 1: Producing PCW-LED lights using the modified LASS system**

The general procedure to produce light with the modified LASS system (Fig. 4) is the following: 1) prepare the target-SID data, which include spectral irradiance at every 1 nm between 400 and 800 nm; then, 2) determine the voltage sets to gain the desired SID based on the target-SID data, and save it to the LASS system.

The target-SID data of 30 types of PCW-LEDs (Fig. 3; Supplementary Materials 3, Table S2) were converted from their SPDs, as calculated from the relative SIDs of the selected PCW-LEDs at a PPFD of 150 $\mu$mol m$^{-2}$ s$^{-1}$. The relative SIDs are usually shown as figures in their specification sheets.
Fig. 2. Relative spectral photon-flux-density distributions (SPDs) of the lights from LEDs of 30 types installed on the LED module of the modified LED-artificial sunlight source system: 27 types are monocolour LEDs (A); the remaining three types are phosphor-converted white LEDs (B). Black lines represent the relative SPDs of LEDs (original type) that were the same as or similar to those of the LEDs installed on the original LED module. Red lines represent the relative SPDs of the LEDs (exchanged type) that differed from those of any LEDs installed on the original LED module (LED model codes are presented in Supplementary Materials 2, Table S1).

Fig. 3. Spectral photon-flux-density distributions (SPDs) for 30 phosphor-converted white LEDs (PCW-LEDs, black lines) at a photosynthetic photon flux density (PPFD) of 150 μmol m⁻² s⁻¹. The SPDs (red lines) were measured at a leaf surface level using a spectroradiometer. They were obtained with the modified LED-artificial sunlight source system at a PPFD of 150 μmol m⁻² s⁻¹.
A free web-based program WebPlotDigitizer (Rohatgi, 2018) was used to obtain relative SIDs at every 1 nm between 400 and 800 nm, as shown in the figures of the specification sheets.

The target-SID data were then imported to the SID control system to determine the appropriate voltage set to obtain the desired SIDs, and therefore the desired SPDs. The SPDs were measured using a spectroradiometer (MS720; Eko Instruments Co. Ltd., Tokyo, Japan) at the same surface of a leaf enclosed in the leaf chamber of a portable photosynthesis system (LI-6400; Li-Cor Inc., Lincoln, NE, USA.; Fig. 1). It is noteworthy that, for \( P_n \) measurements, a steel plate was used as a connector to place the leaf chamber rigidly under the light outlet of the modified LASS system, just as it was placed in light production (Fig. 1). A steel plate was installed on the top of the leaf chamber and trepanned a hole, of which the shape and size were approximately the same as those of the light outlet.

**Step 2: Measurement of \( P_n \) under the produced lights**

Before \( P_n \) measurement started, the irradiation order of the 30 produced lights was determined randomly (Fig. 5). The irradiation period of each produced light was set as 0.5 h. During \( P_n \) measurement, the LASS system automatically supplied the produced light 16 (Fig. 3) for 1 h. Then the system supplied all 30 produced lights successively to an identical leaf according to the set order and period. Each \( P_n \) measurement was completed in 16 h. An example of \( P_n \) measurement with 30 produced lights is presented in Fig. 6. In a preliminary experiment, we measured the \( P_n \) of cos lettuce plants under four produced lights using the above irradiation method for approximately 28 h (Supplementary Materials 4: Fig. S2A). Results showed that the \( P_n \), measured under the produced lights in different periods was stable from

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**Fig. 4.** Procedure used for producing phosphor-converted white LED (PCW-LED) lights with the modified LED-artificial sunlight source system.

**Fig. 5.** Procedure of the automated leaf net photosynthetic rate \( (P_n) \) measurement. The modified LED-artificial sunlight source system (LASS system) automatically supplied the produced light 16 (Fig. 3) for 1 h. It then supplied all 30 produced lights successively to an identical leaf in a \( P_n \) measurement.
approximately four hours after the beginning.

A plant was used for the $P_n$ measurement approximately four hours after the beginning of the light period at 20 or 25 DAS. The timing was set based on our preliminary experiment results, which indicated that the $P_n$ measured under one produced light kept increasing for approximately six hours after the beginning of the light period (Supplementary Materials: Fig. S2B). A portable photosynthesis system (LI-6400) was used for $P_n$ measurements. In the leaf chamber of the LI-6400 system during $P_n$ measurements, the leaf temperature (mean ± SE), CO$_2$ concentration, relative humidity, and airflow rate were, respectively, 25.0 ± 0.1 °C, 1000 ± 1 µmol mol$^{-1}$, and 65 ± 8% and 500.3 ± 0.2 µmol s$^{-1}$. During $P_n$ measurement, the LI-6400 was set to record a dataset including $P_n$, CO$_2$ concentration, etc., automatically every 3 s and to match its two CO$_2$ and H$_2$O analyzers, respectively, for reference and sample lines after every ten datasets were recorded. On average, the LI-6400 recorded ten datasets (30 s) and matched its analyzers (approximately 30 s) in approximately one minute. Therefore, approximately 300 datasets were compiled for each produced light. The last 100 datasets were used to calculate the mean $P_n$ of that produced light. In all, five replications for each produced light with each lettuce cultivar were conducted at 20 and 25 DAS.

**Statistical analysis**

Differences in mean $P_n$ among the 30 produced lights were tested using a t-test with Holm–Bonferroni correction for multiple comparisons, with significance inferred for $p < 0.05$ using software R (version 3.6.1). Correlation analyses between the mean $P_n$, which was the mean value of the $P_n$ under the produced lights at 20 DAS and that at 25 DAS, and blue-light (400–500 nm) photon flux densities (PFDs), green-light PFDs (501–600 nm), red-light PFDs (601–700 nm), and far-red-light PFDs (701–800 nm) of the 30 PCW-LEDs were tested using Pearson correlation analysis, with significance inferred for $p < 0.05$, using the same software.

**Results**

**Improved production of PCW-LED lights using the modified LASS system**

The SPDs of the 30 produced lights obtained with the modified LASS system approximated, with adequate accuracy, those of the corresponding target PCW-LED lights (Fig. 3). In several produced lights, such as produced lights 27, 28, and 30 in Fig. 3, the differences between their SPDs and the corresponding target SPDs were slightly greater at wavelengths of 440–460 nm than those in other wavelength ranges. When the PPFDs of the target PCW-LED lights were set to 150 µmol m$^{-2}$ s$^{-1}$, the differences of the PPFDs among the 30 produced lights obtained with the original LASS system and those obtained with the modified one were within 5 and 2 µmol m$^{-2}$ s$^{-1}$, respectively (Supplementary Materials 5, Table S3). The root-mean-square errors (RMSEs) at every 1 nm between 400 and 800 nm of the 30 target and combined SPDs in the modified LASS system were all smaller than those in the original one at a PPFD of 150 µmol m$^{-2}$ s$^{-1}$ (Supplementary Materials 6, Table S4). The mean RMSE in the modified one was 0.08 µmol m$^{-2}$ s$^{-1}$ nm$^{-1}$, which was approximately 50% of that in the original one. These results demonstrate quantitatively that the differences between the target and the produced SPDs in the modified LASS system at a PPFD of 150 µmol m$^{-2}$ s$^{-1}$ were markedly smaller than those in the original system at the same PPFD.

When the PPFD of the target PCW-LED lights were set at 300 µmol m$^{-2}$ s$^{-1}$, the mean PPFD of the 30 produced lights in the modified LASS system was 301 µmol m$^{-2}$ s$^{-1}$, whereas that in the original one was 260 µmol m$^{-2}$ s$^{-1}$ (Supplementary Materials 5, Table S3). It is noteworthy that the maximum PPFD of produced light 1 obtained with the modified LASS system was 292 µmol m$^{-2}$ s$^{-1}$ (Supplementary Materials 5, Table S3). The mean RMSE at a PPFD of 300 µmol m$^{-2}$ s$^{-1}$ in the modified LASS system was 0.22 µmol m$^{-2}$ s$^{-1}$ nm$^{-1}$, which was greater than that at a PPFD of 150 µmol m$^{-2}$ s$^{-1}$ in the modified LASS system (Supplementary Materials 6, Table S4).
**Pₙ measurement**

The mean \( P_\text{n} \) under the 30 produced lights at 20 days after sowing (DAS) and 25 DAS were 7.11–8.02 and 7.27–8.17 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) for the cos lettuce plants (Fig. 7), 5.76–7.11 and 5.89–7.18 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) for the red-leaf lettuce plants (Fig. 8), and 4.83–6.17 and 5.18–6.50 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) for the green-leaf lettuce plants (Fig. 9). For the three lettuce cultivars, the mean \( P_\text{n} \) under produced light 1 was significantly greater than that under the produced light 7–30 at 20 and 25 DAS. Moreover, the mean \( P_\text{n} \) under the produced light at 20 DAS and that at 25 DAS were comparable in the cos lettuce and green-leaf lettuce but not in the green-leaf lettuce, for which the value at 25 DAS tended to be greater than that at 20 DAS.

**Correlations between \( P_\text{n} \) and different wavelength-band light PFDs**

In the three lettuce cultivars, the mean \( P_\text{n} \), which was the mean values of the \( P_\text{n} \) under the produced lights at 20 DAS and that at 25 DAS, was correlated significantly and negatively to the blue-light (400–500 nm) and green-light (501–600 nm) PFDs of the PCW-LED lights, and was significantly and positively correlated to the red-light (601–700 nm) and far-red-light (701–800 nm) PFDs (Fig. 10).

**Discussion**

To ascertain \( P_\text{n} \) rapidly under dozens of PCW-LED lights with different relative SPDs, we developed a rapid and greatly automated \( P_\text{n} \)-quantification method. For this study, we demonstrated that the developed method can provide the \( P_\text{n} \) of 30 PCW-LED lights in one measurement automatically within one day.

**Evaluating reliability of the determined \( P_\text{n} \) in terms of RMSEs of the target and combined SPDs**

The reliability of the \( P_\text{n} \) determination made using the proposed \( P_\text{n} \)-quantification method depends primarily on the extent of spectral-photon-flux-density differences between the target and combined SPDs. The RMSE calculated from the spectral photon-flux-densities [\( \mu \text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1} \)] of a target and the corresponding combined SPD at wavelengths of 400–800 nm was then used as an index to evaluate the average extent of the spectral-photon-flux-density differences between the two SPDs. Low RMSE, as calculated from the two SPDs, was associated with small spectral photon-flux-density differences between them. The RMSEs of the 30 target and corresponding combined SPDs were less than 0.2 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Supplementary Materials 6, Table S4). In fact, differences of the PPFDs among the 30 combined SPDs were within 2 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Supplementary Materials 5, Table S3) at the same PPFD level. In several produced lights (produced lights 27, 28, and 30 in Fig. 3), the differences between the target and combined SPDs were slightly greater at wavelengths of 440–460 nm than those in the other wavelength ranges, but these small differences might not engender significant differences between the \( P_\text{n} \) under the target and the corresponding produced lights. Therefore, we inferred that the \( P_\text{n} \) measured under the

![Fig. 7. Mean leaf net photosynthetic rate (\( P_\text{n} \)) of cos lettuce plants with the 30 produced lights at 20 (A) and 25 (B) days after sowing. Error bars represent standard errors of the mean \( P_\text{n} \). Different letters denote significant differences (\( p < 0.05 \)) by \( t \)-test with Holm–Bonferroni \( p \)-value adjustment (\( n = 5 \)).](image-url)
Fig. 8. Mean leaf net photosynthetic rates \( (P_n) \) of red-leaf lettuce plants with the 30 produced lights at 20 (A) and 25 (B) days after sowing. Error bars represent standard errors of the mean \( P_n \). Different letters denote significant differences \( (p < 0.05) \) by \( t \)-test with Holm–Bonferroni \( p \)-value adjustment \( (n = 5) \).

Fig. 9. Mean leaf net photosynthetic rates \( (P_n) \) of green-leaf lettuce plants with the 30 produced lights at 20 (A) and 25 (B) days after sowing. Error bars represent standard errors of the mean \( P_n \). Different letters denote significant differences \( (p < 0.05) \) by \( t \)-test with Holm–Bonferroni \( p \)-value adjustment \( (n = 5) \).
30 produced lights is sufficiently reliable to represent the $P_n$ measured under the corresponding PCW-LED lights at a PPFD of 150 μmol m$^{-2}$ s$^{-1}$.

The mean PPFD of the 30 SPDs obtained with the modified LASS system has achieved 300 μmol m$^{-2}$ s$^{-1}$, which is expected to be sufficient for a PFAL to grow leafy plants. It is noteworthy that the maximum PPFD of produced light 1 with the modified LASS system was 292 μmol m$^{-2}$ s$^{-1}$. In addition, the mean RMSE of the obtained SPDs at a PPFD of 300 μmol m$^{-2}$ s$^{-1}$ in the modified LASS system was 0.22 μmol m$^{-2}$ s$^{-1}$ nm$^{-1}$, which was approximately three times greater than that at a PPFD of 150 μmol m$^{-2}$ s$^{-1}$. These results indicate that the present LASS system requires further modifications to obtain produced lights with low RMSEs at a PPFD of 300 μmol m$^{-2}$ s$^{-1}$, similarly to those at a PPFD of 150 μmol m$^{-2}$ s$^{-1}$.

Limitations of the modified LASS system and possible solutions

For this study, the selected PCW-LEDs include a blue LED chip (approximately 460 nm peak wavelength) and one or more phosphors. White LEDs of two other types (Ye et al., 2010) are commercially available. One is the near-ultraviolet LED type, which comprises a near-ultraviolet LED chip (approximately 410 nm peak wavelength) and some phosphors. White LEDs of this type have also been used to investigate the effects of SPD on $P_n$ (Zou et al., 2020). Another is the BGR type, which comprises blue, green, and red LED chips. The modified LASS system is applicable to light production, of which each SPD accurately approximates that of BGR-type white LED lights. However, it is not applicable to near-ultraviolet-type white LED lights because monocolor LEDs with peak wavelengths shorter than 426 nm.
were removed from the present LED module. This limitation can be resolved simply by installing near-ultraviolet-type monochromatic LEDs of several types to the LED module.

Another limitation of the present LASS system is that it irradiates an area of only 7.1 cm². This limited irradiated area can be sufficient for a small leaf chamber such as the LI-6400 or a small whole-plant chamber (Kölling et al., 2015), but it is insufficient for a plant-organ chamber (Fortineau and Bancal, 2018) or a whole-plant chamber (Jauregui et al., 2018). This limitation, however, cannot be resolved easily because it requires a scaling up of the LED module by more than several times and a corresponding scaling up of the power supply for the LED module. Moreover, such a light source is expected to provide a uniform distribution on an irradiated area in terms of the PFD and relative SPD (Yano and Fujiwara, 2012), which indicates that the arrangement of the LEDs installed on the LED module might need to be re-assessed.

**Correlation between \( P_a \) and different wavelength-band light PFDs**

In the three lettuce cultivars, the mean \( P_a \) which represents the mean value of the \( P_a \) under the produced lights at 20 DAS and that at 25 DAS, was highly positively correlated with red-light (601–700 nm) PFDs for PCW-LED light, although it was strongly negatively correlated with the green-light (501–600 nm) PFDs (Figs. 10B and 10C). Many studies have demonstrated that red light can bring about a greater \( P_a \) than green light in various plants at a low PPDF level (Hogewoning et al., 2012; Inada, 1976; McCree, 1971; Sun et al., 1998). Results from those studies indicate that the red light in the PCW-LED lights might be more efficient than green light for driving the photosynthesis of an identical lettuce leaf at a PPDF of 150 \( \mu m\ s^{-1} \).

Mean \( P_a \) was found to be negatively correlated with the blue-light (400–500 nm) PFDs (Fig. 10A). However, the absolute values of the \( R \) are lower than that of correlation between mean \( P_a \) and the other monochromatic light PFDs (Figs. 10B, 10C, and 10D). This result might be attributable mainly to variation of \( P_a \) at blue-light PFDs of 20–30 \( \mu m\ s^{-1} \), which is much larger than that at the other blue light PFDs (Fig. 10A). The large variation of \( P_a \) at the blue-light PFD range might be partly attributable to the large variation of red-light PFDs in the same range of the blue-light PFD (Supplementary Materials 7, Fig. S3A), which can engender greater variation of \( P_a \) than for other monochromatic light because the red light is expected to be more efficient than other monochromatic light for driving photosynthesis.

Mean \( P_a \) was found to be highly positively correlated with far-red-light (701–800 nm) PFD for all lettuce cultivars (Fig. 10D). It is generally acknowledged that far-red light alone is much less efficient than green or red light for driving photosynthesis. Therefore, this result is expected to be attributable mainly to the fact that far-red light PFDs are positively correlated with red-light PFDs and negatively correlated with green-light PFDs (Supplementary Materials 7, Fig. S3B and 3C): a PCW-LED providing a high far-red light PFD tends to include a high red-light PFD along with a low green-light PFD, which might yield a high \( P_a \) (Fig. 10B and 10C).

In summary, it can be expected that increasing the red-light proportion in a PCW-LED light might increase \( P_a \) of lettuce plants at a low PPDF level. It can be readily verified whether increasing the red-light proportion in a PCW-LED light might yield a higher \( P_a \) using the modified LASS system. We have demonstrated that it can produce an arbitrary relative SPD of PCW-LED lights at 400–800 nm wavelengths. For instance, we can increase the red-light proportion of the produced light No. 1 to test whether we can propose a relative SPD of PCW-LED lights able to perform higher \( P_a \) than that measured under produced light No. 1, which is the largest value we measured for any of the 30 produced lights.

**Conclusions**

To ascertain the value of \( P_a \) under dozens of PCW-LED lights with different relative SPDs within days, we developed a rapid and automated \( P_a \)-quantification method that uses a modified LASS system and a photosynthesis measurement system. The mean RMSEs of the 30 produced lights, of which each SPD obtained with the modified LASS system approximated that of the 30 selected PCW-LED lights, were 0.08 \( \mu m\ s^{-1} \) at a PPDF of 150 \( \mu m\ s^{-1} \) and 0.22 \( \mu m\ s^{-1} \) at a PPDF of 300 \( \mu m\ s^{-1} \). Using the developed method, we ascertained the individual \( P_a \) of three lettuce cultivars under 30 PCW-LED lights at a PPDF of 150 \( \mu m\ s^{-1} \) within one day per cultivar per repetition. These results indicate that the developed method can contribute to the rapid selection of PCW-LEDs offering superior photosynthesis performance from numerous commercially available PCW-LEDs.

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**References**


