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Photosynthetic Physiology of Blue, Green, and Red Light: Light Intensity Effects and Underlying Mechanisms

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Red and blue light are traditionally believed to have a higher quantum yield of CO_2 assimilation (*QY,* moles of CO_2 assimilated per mole of photons) than green light, because green light is absorbed less efficiently. However, because of its lower absorptance, green light can penetrate deeper and excite chlorophyll deeper in leaves. We hypothesized that, at high photosynthetic photon flux density (*PPFD*), green light may achieve higher *QY* and net CO_2 assimilation rate (A_n) than red or blue light, because of its more uniform absorption throughtout leaves. To test the interactive effects of *PPFD* and light spectrum on photosynthesis, we measured leaf A_n of "Green Tower" lettuce (*Lactuca sativa*) under red, blue, and green light, and combinations of those at *PPFD*s from 30 to 1,300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The electron transport rates (*J*) and the maximum Rubisco carboxylation rate ($V_{c,max}$) at low (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and high *PPFD* (1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were estimated from photosynthetic CO_2 response curves. Both $QY_{m,inc}$ (maximum *QY* on incident *PPFD* basis) and *J* at low *PPFD* were higher under red light than under blue and green light. Factoring in light absorption, $QY_{m,abs}$ (the maximum *QY* on absorbed *PPFD* basis) under green and red light were both higher than under blue light, indicating that the low $QY_{m,inc}$ under green light was due to lower absorptance, while absorbed blue photons were used inherently least efficiently. At high *PPFD*, the QY_{inc} [gross CO_2 assimilation (A_g)/incident *PPFD*] and *J* under red and green light were similar, and higher than under blue light, confirming our hypothesis. $V_{c,max}$ may not limit photosynthesis at a *PPFD* of 200 $\mu mol\ m^{-2}\ s^{-1}$ and was largely unaffected by light spectrum at 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A_g and *J* under different spectra were positively correlated, suggesting that the interactive effect between light spectrum and *PPFD* on photosynthesis was due to effects on *J*. No interaction between the three colors of light was detected. In summary, at low *PPFD*, green light had the lowest photosynthetic efficiency because of its low absorptance. Contrary, at high *PPFD*, QY_{inc} under green light was among the highest, likely resulting from more uniform distribution of green light in leaves.

Keywords: photosynthesis, quantum yield of CO_2 assimilation, light spectrum, photosynthetic photon flux density, electron transport, $V_{c,max}$, light intensity, light quality

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Abbreviations: *PPFD*, photosynthetic photon flux density; RuBP, ribulose 1,5-bisphosphate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; VPD, vapor pressure deficit; FWHM, full width at half maximum; A_n, net CO_2 assimilation rate; R_d, dark respiration rate; $QY_{m,inc}$, maximum quantum yield of CO_2 assimilation; $A_{g,max}$, light-saturated gross assimilation rate; $QY_{m,abs}$, maximum quantum yield of CO_2 assimilation on absorbed light base; QY_{inc}, quantum yield of CO_2 assimilation based on incident *PPFD*; A_g, gross CO_2 assimilation rate; QY_{abs}, quantum yield of CO_2 assimilation on absorbed light base; *QY*, quantum yield of CO_2 assimilation; *A/Ci* curve, assimilation – internal leaf CO_2 response curve; RACiR, rapid A/C_i response technique; $V_{c,max}$, maximum rate of Rubisco carboxylation; *J*, rate of electron transport; CA1P, 2-carboxy-D-arabinitol-1-phosphate; NPQ, non-photochemical quenching.

INTRODUCTION

The photosynthetic activity of light is wavelength dependent. Based on McCree's work (McCree, 1971, 1972), photosynthetically active radiation is typically defined as light with a wavelength range from 400 to 700 nm. Light with a wavelength shorter than 400 nm or longer than 700 nm was considered as unimportant for photosynthesis, due to its low quantum yield of CO_2 assimilation, when applied as a single waveband (**Figure 1**). Within the 400–700 nm range, McCree (1971) showed that light in the red region (600–700 nm) resulted in the highest quantum yield of CO_2 assimilation of plants. Light in the green region (500–600 nm) generally resulted in a slightly higher quantum yield than light in the blue region (400–500 nm) (**Figure 1**; McCree, 1971). The low absorptance of green light is partly responsible for its low quantum yield of CO_2 assimilation. Within the visible spectrum, green leaves have the highest absorptance in the blue region, followed by red. Green light is least absorbed by green leaves, which gives leaves their green appearance (McCree, 1971; Zhen et al., 2019).

Since red and blue light are absorbed more strongly by photosynthetic pigments than green light, they are predominantly absorbed by the top few cell layers, while green light can penetrate deeper into leaf tissues (Nishio, 2000; Vogelmann and Evans, 2002; Terashima et al., 2009; Brodersen and Vogelmann, 2010), thus giving it the potential to excite photosystems in deeper cell layers. Leaf photosynthesis may benefit from the more uniform light distribution throughout a leaf under green light. Absorption of photons by chloroplasts near the adaxial surface may induce heat dissipation of excess excitation energy in those chloroplasts, while chloroplasts deeper into the leaf receive little excitation energy (Sun et al., 1998; Nishio, 2000). Blue and red photons, therefore, may be used less efficiently and are more likely to be dissipated as heat than green photons.

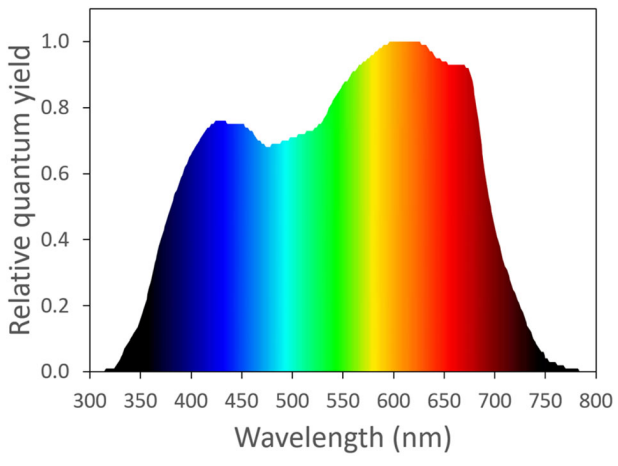

FIGURE 1 | The normalized action spectrum of the maximum quantum yield of CO_2 assimilation for narrow wavebands of light from ultra-violet to far-red wavelengths (McCree, 1971). Redrawn using data from Sager et al. (1988).

The misconception that red and blue light are used more efficiently by plants than green light still occasionally appears (Singh et al., 2015), often citing McCree's action spectrum or the poor absorption of green light by chlorophyll extracts. The limitations of McCree's action spectrum were explained in his original paper: the quantum yield was measured under low photosynthetic photon flux density (*PPFD*), using narrow waveband light, and expressed on an incident light basis (McCree, 1971), but these limitations are sometimes ignored. The importance of green light for photosynthesis has been well established in more recent studies (Sun et al., 1998; Nishio, 2000; Terashima et al., 2009; Hogewoning et al., 2012; Smith et al., 2017).

From those studies, one trend has emerged that has not received much attention: there is an interactive effect of light quality and intensity on photosynthesis (Sun et al., 1998; Evans and Vogelmann, 2003; Terashima et al., 2009). At low *PPFD*, green light has the lowest QY_{inc} (quantum yield of CO_2 assimilation on incident light basis) because of its low absorptance; at high *PPFD*, on the other hand, red and blue light have a lower QY_{inc} than green light, because of their high absorptance by photosynthetic pigments, which shifts much of the light absorption closer to the upper leaf surface. This reduces both the quantum yield of CO_2 assimilation in cells in the upper part of a leaf and light availability in the bottom part of a leaf.

The interactive effect between light quality and intensity was illustrated in an elegant study that quantified the differential quantum yield, or the increase in leaf CO_2 assimilation per unit of additional light (Terashima et al., 2009). The differential quantum yield was measured by adding red or green light to a background illumination of white light of different intensities. At low background white light levels, the differential quantum yield of red light was higher than that of green light, due to the low absorptance of green light. But as the background light level increased, the differential quantum yield of green light decreased more slowly than that of red light, and was eventually higher than that of red light (Terashima et al., 2009). The red light was absorbed efficiently by the chloroplasts in the upper part of leaves. With a high background level of white light, those chloroplasts already received a large amount of excitation energy from white light and up-regulated non-photochemical quenching (NPQ) to dissipate excess excitation energy as heat, causing the additional red light to be used inefficiently. Green light, on the other hand, was able to reach the chloroplasts deeper in the mesophyll and excited those chloroplasts that received relatively little excitation energy from white light. Therefore, with high background white light intensity, additional green light increased leaf photosynthesis more efficiently than red light (Terashima et al., 2009).

In this paper, we present a comprehensive study to explore potential interactive effect of light intensity and light quality on C_3 photosynthesis and underlying processes. We quantified the photosynthetic response of plants to blue, green, and red light over a wide *PPFD* range to better describe how light intensity and waveband interact. In addition, we examined potential interactions among blue, green, and red light, using light with different ratios and intensities of the three

narrow waveband lights. To get a better understanding of the biochemical reasons for the effects of light spectrum and intensity on CO_2 assimilation, we constructed assimilation – internal leaf CO_2 (C_i) response curves (A/C_i curves) under blue, green, and red light, as well as combinations of the three narrow waveband lights at both high and low *PPFD*. We hypothesized that effects of different light spectra would be reflected in the electron transport rate (J) required to regenerate consumed ribulose 1,5-bisphosphate (RuBP), rather than the maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) ($V_{c,max}$).

MATERIALS AND METHODS

Plant Material

Lettuce "Green Towers" plants were grown from seed in 1.7 L round pots filled with soilless substrate (Fafard 4P Mix, Sun Gro Horticulture, Agawam, MA, United States). The plants were grown in a growth chamber (E15, Conviron, Winnipeg, Manitoba, Canada) at 23.2 ± 0.8°C (mean ± SD), under white fluorescent light with a 14-hr photoperiod, vapor pressure deficit (VPD) of 1.20 ± 0.43 kPa and a *PPFD* of 200–230 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the floor level, and ambient CO_2 concentration. Plants were sub-irrigated when necessary with a nutrient solution containing 100 $mg \cdot L^{-1}$ N, made with a complete, water-soluble fertilizer (Peter's Excel 15-5-15 Cal-Mag fertilizer, Everris, Marysville, OH, United States).

Leaf Absorptance, Transmittance, and Reflectance

Leaf absorptance was determined using a method similar to that of Zhen et al. (2019). Three plants were randomly selected. A newly expanded leaf from each plant was illuminated with a broad-spectrum halogen bulb (70W; Sylvania, Wilmington, MA, United States) for leaf transmittance measurement. Transmittance was measured with a spectroradiometer (SS-110, Apogee, Logan, UT, United States). The halogen light spectrum was taken as reference measurement with the spectroradiometer placed directly under the halogen bulb in a dark room. Then, a lettuce leaf was placed between the halogen bulb and spectroradiometer, with its adaxial side facing the halogen bulb and transmitted light was measured. Leaf transmittance was then calculated on 1 nm resolution. Light reflectance of the leaves was measured using a spectrometer with a leaf clip (UniSpec, PP systems, Amesbury, MA, United States). Light absorptance was calculated as $1 - reflectance - transmittance$. We verified that this method results in similar absorptance spectra as the use of an integrating sphere. Absorptance of each of the nine light spectra used in this study were calculated from the overall leaf absorptance spectrum and the spectra of the red, green, and blue LEDs.

Leaf Photosynthesis Measurements

All gas exchange measurements were made with a leaf gas exchange system (CIRAS-3, PP Systems). Light was provided by the LEDs built into the chlorophyll fluorescence module (CFM-3, PP Systems). This module has dimmable LED arrays of different colors, with peaks at 653 nm [red, full width at half maximum (FWHM) of 17 nm], 523 nm (green, FWHM of 36 nm), and 446 nm (blue, FWHM of 16 nm). Nine different combinations of red, green, and blue light were used in this study (**Table 1**). Throughout the measurements, the environmental conditions inside the cuvette were controlled by the leaf gas exchange system. Leaf temperature was 23.0 ± 0.1°C, CO_2 concentration was 400.5 ± 4.1 $\mu mol \cdot mol^{-1}$, and the VPD of air in the leaf cuvette was 1.8 ± 0.3 kPa (mean ± SD).

Photosynthesis – Light Response Curves

To explore photosynthetic efficiency of light with different spectra, we constructed light response curves for lettuce plants using each light spectrum. Lettuce plants were exposed to 10 *PPFD* levels ranging from 30 to 1,300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (30, 60, 90, 120, 200, 350, 500, 700, 1,000, and 1,300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in ascending orders for light response curves. Photosynthetic measurements were taken on 40–66 days old lettuce plants. Lettuce plants were taken out of the growth chamber and dark-adapted for 30 min. Starting from the lowest *PPFD*, one newly expanded leaf was exposed to all nine spectra. Net CO_2 assimilation rate (A_n) of the leaf was measured using the leaf gas exchange system. Under each light spectrum, three A_n readings were recorded at 10 s intervals after readings were stable (about 4–20 min depending on *PPFD* after changing *PPFD* and spectrum). The three A_n readings were averaged for analysis. After A_n measurements under all nine light spectra were taken, the leaf was exposed to the next *PPFD* level and A_n measurements were taken with the light spectra in the same order, until measurements were completed at all *PPFD* levels. Throughout the light response curves, C_i decreased with increasing *PPFD*, from 396 ± 10 $\mu mol \cdot mol^{-1}$ at a *PPFD* of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 242 ± 44 $\mu mol \cdot mol^{-1}$ at a *PPFD* of 1,300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. To account for the potential effect of plants and the order of the spectra on assimilation rates, the order of the different spectra was re-randomized for each light response curve, using a Latin square design with plant and spectrum as the blocking factors. Data were collected on nine different plants.

TABLE 1 | List of light spectrum abbreviations and their spectral composition.

Light spectrum	Fraction of total photon flux (%)		
	Blue	Green	Red
100B	100	0	0
80B20G	80	20	0
20B80G	20	80	0
100G	0	100	0
80G20R	0	80	20
20G80R	0	20	80
100R	0	0	100
20B80R	20	0	80
16B20G64R	16	20	64

Regression curves (exponential rise to maximum) were fitted to the data for each light spectrum and replication (plant):

$$A_n = A_{g,max} \times (1 - e^{-QY_{m,inc} \frac{PPFD}{A_{g,max}}}) - R_d \quad (1)$$

where R_d is the dark respiration rate, $QY_{m,inc}$ is the maximum quantum yield of CO_2 assimilation (initial slope of light response curve, mol of CO_2 fixed per mol of incident photons) and $A_{g,max}$ is the light-saturated gross assimilation rate. The $A_{n,max}$ is the light-saturated net assimilation rate and was calculated as $A_{n,max} = A_{g,max} - R_d$. The maximum quantum yield of CO_2 assimilation was also calculated on absorbed light basis as $QY_{m,abs} = \frac{QY_{m,inc}}{light\ absorptance}$.

The instantaneous quantum yield of CO_2 assimilation based on incident *PPFD* (QY_{inc}) was calculated as $\frac{A_g}{PPFD}$ for each *PPFD* at which A_n was measured, where the gross CO_2 assimilation rate (A_g) was calculated as $A_g = A_n + R_d$. To account for differences in absorptance among the different light spectra, the quantum yield of CO_2 assimilation was also calculated based on absorbed light base, as $QY_{abs} = \frac{A_g}{PPFD \times light\ absorptance}$, where light absorptance is the absorptance of lettuce leaves for each specific light spectrum. The *differential QY*, the increase in assimilation rate per unit of additional incident *PPFD*, was calculated as the derivative of Eq. 1:

$$Differential\ QY = QY_{m,inc} \times e^{-QY_{m,inc} \frac{PPFD}{A_{g,max}}} \quad (2)$$

Photosynthesis – Internal CO_2 Response (A/C_i) Curves

To explore the underlying physiological mechanisms of assimilation responses to different light spectra, we constructed A/C_i curves. Typically, A/C_i curves are collected under saturating *PPFD*. We collected A/C_i curves at two *PPFD*s (200 and 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to explore interactive effects of light spectrum and *PPFD* on the assimilation rate. At a *PPFD* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, red light has the highest A_n and green light the lowest A_n, while at *PPFD* of 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, red and green light resulted in the highest A_n and blue light in the lowest A_n.

We used the rapid A/C_i response (RACiR) technique that greatly accelerates the process of constructing A/C_i curves (Stinziano et al., 2017). We used a Latin square design, similar to the light response curves. A/C_i curves were measured under the same nine spectra used for the light response curves. Nine lettuce plants were used as replicates. For each A/C_i curve, CO_2 concentration in the leaf cuvette started from 0 $\mu mol \cdot mol^{-1}$, steadily ramping to 1,200 $\mu mol \cdot mol^{-1}$ over 6 min. A reference measurement was also taken at the beginning of each replication with an empty cuvette to correct for the reaction time of the leaf gas exchange system. Post-ramp data processing was used to calculate the real A and C_i with the spreadsheet provided by PP systems, which yielded the actual A/C_i curves with C_i range of about 100–950 $\mu mol\ mol^{-1}$. Throughout the data collection, leaf temperature was 24.4 ± 1.3°C and VPD in the cuvette was 1.4 ± 0.2 kPa.

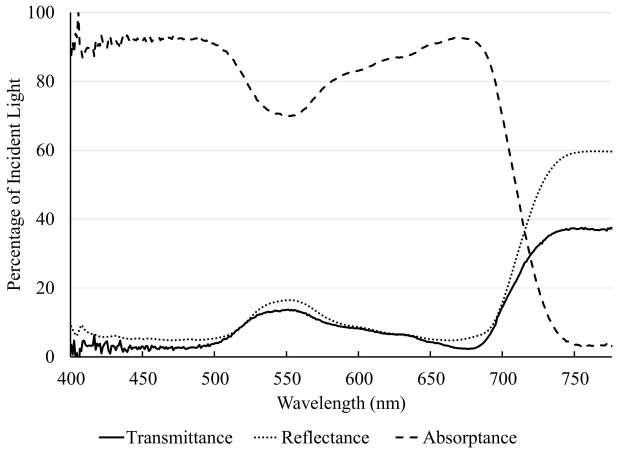

FIGURE 2 | Light absorptance, reflectance, and transmittance spectrum of a newly fully expanded "Green Towers" lettuce leaf.

Curve fitting for A/C_i curves was done by minimizing the residual sum of squares, following the protocol developed by Sharkey et al. (2007). Among our nine replicates, four plants did not show clear Rubisco limitations at low *PPFD* and for those plants Rubisco limitation ($V_{c,max}$) was not included in the model (Sharkey et al., 2007). We therefore report $V_{c,max}$ values for high *PPFD* only. The J was determined for all light spectra at both *PPFD*s. We therefore report $V_{c,max}$ was determined for all light spectra only at high *PPFD*. The quantum yield of electron transport [$QY(J)$] was calculated on both incident and absorbed *PPFD* basis as $QY(J)_{inc} = \frac{J}{PPFD}$ and $QY(J)_{abs} = \frac{QY(J)_{inc}}{light\ absorptance}$, respectively. We did not estimate triose phosphate utilization, because the A/C_i curves often did not show a clear plateau.

Data Analysis

The $QY_{m,inc}$, $QY_{m,abs}$, and $A_{g,max}$ were analyzed with ANOVA to determine the effects of light spectrum using SAS (SAS University Edition; SAS Institute, Cary, NC, United States). A_n, QY_{inc}, and QY_{abs} at each *PPFD* level and $V_{c,max}$ and J estimated from A/C_i curves were similarly analyzed with ANOVA using SAS. A_n at different *PPFD* levels were analyzed with regression analysis to detect interactive effect of blue, green, and red light on leaf assimilation rates using the fractions of red, blue, and green light as explanatory variables (JMP Pro 15, SAS Institute).

RESULTS

Leaf Absorptance

A representative spectrum of light absorptance, reflectance and transmittance of a newly fully expanded lettuce leaf is shown in **Figure 2**. In the blue region, 400–500 nm, the absorptance by "Green Towers" lettuce leaves was high and fairly constant, averaging 91.6%. The leaf absorptance decreased as the wavelength increased from 500 to 551 nm where the absorptance

minimum was 69.8%. Absorptance increased again at longer wavelengths, with a second peak at 666 nm (92.6%). Above 675 nm, the absorptance decreased steadily to <5% at 747 nm (**Figure 2**). The absorptance spectrum of our lettuce leaves is similar to what McCree (1971) obtained for growth chamber-grown lettuce, with the exception of slightly higher absorptance in the green part of the spectrum in our lettuce plants. Using this spectrum, the absorptance of the blue, green, and red LED lights were calculated to be 93.2 ± 1.0%, 81.1 ± 1.9% and 91.6 ± 1.1%, respectively. Absorptance of all nine spectra was calculated based on their ratios of red, green, and blue light (**Table 2**).

Light Quality and Intensity Effects on Photosynthetic Parameters

Light response curves of lettuce under all nine spectra are shown in **Figure 3**, with regression coefficients in **Supplementary Table 1**. It is worth noting that a few plants showed photoinhibition under 100B (decrease in A_n with *PPFD* > 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Those data were excluded in curve fitting for light response curves to better estimate asymptotes. Photoinhibition was not observed under other spectra.

The $QY_{m,inc}$ of lettuce plants was 22 and 27% higher under red light (74.3 $mmol \cdot mol^{-1}$) than under either 100G (60.8 $mmol \cdot mol^{-1}$) or 100B (58.4 $mmol \cdot mol^{-1}$), respectively (**Figure 4A** and **Supplementary Table 1**). Spectra with a high fraction of red light (64% or more) resulted in a high $QY_{m,inc}$ (**Figure 4A**), while 80G20R resulted in an intermediate $QY_{m,inc}$ (**Figure 4A**). To determine whether differences in $QY_{m,inc}$ were due to differences in absorptance or in the ability of plants to use the absorbed photons for CO_2 assimilation, we also calculated $QY_{m,abs}$. On an absorbed light basis, 100B light still resulted in the lowest $QY_{m,abs}$ (62.7 $mmol \cdot mol^{-1}$) and red light resulted in the highest $QY_{m,abs}$ (81.1 $mmol \cdot mol^{-1}$) among narrow waveband lights (**Figure 4B**). Green light resulted in a $QY_{m,abs}$ (74.9 $mmol \cdot mol^{-1}$) similar to that under red light, but significantly higher than that of blue light (**Figure 4B**). We did not find any interactions (synergism or antagonism) between lights of different colors, with all physiological responses under mixed spectra being similar to the weighted average of responses under single colors. Thus, for the rest of the results we focus on the three narrow waveband spectra.

Among the three narrow waveband lights, 100G resulted in the highest $A_{g,max}$ (20.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$), followed by red (18.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and blue light (17.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (**Figure 5** and **Supplementary Table 1**). As with $QY_{m,inc}$ and $QY_{m,abs}$, combining two or three colors of light resulted in an $A_{g,max}$ similar to the weighted averages of individual light colors.

QY_{inc} initially increased with increasing *PPFD* and peaked at 90–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, then decreased at higher *PPFDs* (**Figure 6A**). The QY_{inc} under 100R was higher than under either green or blue light at low *PPFD* (≤300 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Although 100G resulted in lower QY_{inc} than 100B at low *PPFD* (≤300 $\mu mol \cdot m^{-2} \cdot s^{-1}$), the decrease in QY_{inc} under 100G with increasing *PPFD* was slower than that with 100B or 100R. Above 500 $\mu mol\ m^{-2}\ s^{-1}$, the QY_{inc} with 100G was comparable to the QY_{inc} with 100R, and higher than with 100B (**Figure 6A**). The QY_{abs} with 100R was higher than that with either 100G or 100B at *PPFDs* from 60 to 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($p < 0.05$). The QY_{abs} with 100G was similar to 100B at low *PPFD*, but decreased slower than that with either 100R or 100B as *PPFD* increased. At *PPFD* ≥ 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, QY_{abs} was lowest under 100B among the three monochromatic lights ($p < 0.05$) (**Figure 6B**).

The differential *QY*, which quantifies the increase in CO_2 assimilation per unit of additional *PPFD*, decreased with increasing *PPFD*. The differential *QY* with 100R was higher than those with 100B and 100G at low *PPFD*. At a *PPFD* of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the differential *QY* was 70.5 $mmol \cdot mol^{-1}$ for 100R, 59.4 $mmol \cdot mol^{-1}$ for 100G, and 55.8 $mmol \cdot mol^{-1}$ for 100B (**Figure 7**). However, the differential *QY* with 100R decreased rapidly with increasing *PPFD* and was lower than the differential *QY* with 100G at high *PPFD* (**Figure 7**). At high *PPFD*, the differential *QY* with 100G was highest among three monochromatic light (**Figure 7**). For instance, at a *PPFD* of 1,300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the differential *QY* with 100G was 1.09 $mmol \cdot mol^{-1}$, while those with 100B and 100R were 0.64 $mmol \cdot mol^{-1}$ and 0.46 $mmol \cdot mol^{-1}$, respectively (**Figure 7**).

TABLE 2 | Light absorptance and transmittance of new fully expanded "Green towers" lettuce leaves under nine light spectra.

Light spectrum*	Light absorptance (%)	Light transmittance (%)
100B	93.2	2.2
80B20G	90.8	3.6
20B80G	83.6	7.8
100G	81.1	9.1
80G20R	83.2	8.1
20G80R	89.5	4.9
100R	91.6	3.9
20B80R	91.9	3.5
16B20G64R	89.8	4.7

*See **Figure 2** for the leaf absorptance spectrum.*
See spectral composition in **Table 1.*

Effect of Light Spectrum and Intensity on *J* and $V_{c,max}$

J of lettuce leaves at low *PPFD* was lowest under 100G (47.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$), followed by 100B (56.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and highest under 100R (64.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (**Figure 8A**). At high *PPFD*, on the other hand, *J* of leaves exposed to 100G (115.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 100R (112.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were among the highest, while *J* of leaves under 100B was the lowest (97.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (**Figure 8A**). At high *PPFD*, $V_{c,max}$ of leaves under blue light (59.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was lower than $V_{c,max}$ of leaves under 16B20G64R light (72.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$), but none of the other treatments differed significantly (**Figure 8**). When *PPFD* increased from 200 to 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, *J* under green light increased by 143%, while *J* under blue and red light increased by 73% and 75%, respectively (**Figure 8A**). *J* and $V_{c,max}$ at high *PPFD* were strongly correlated ($R^2 = 0.82$) (**Supplementary Figure 3**).

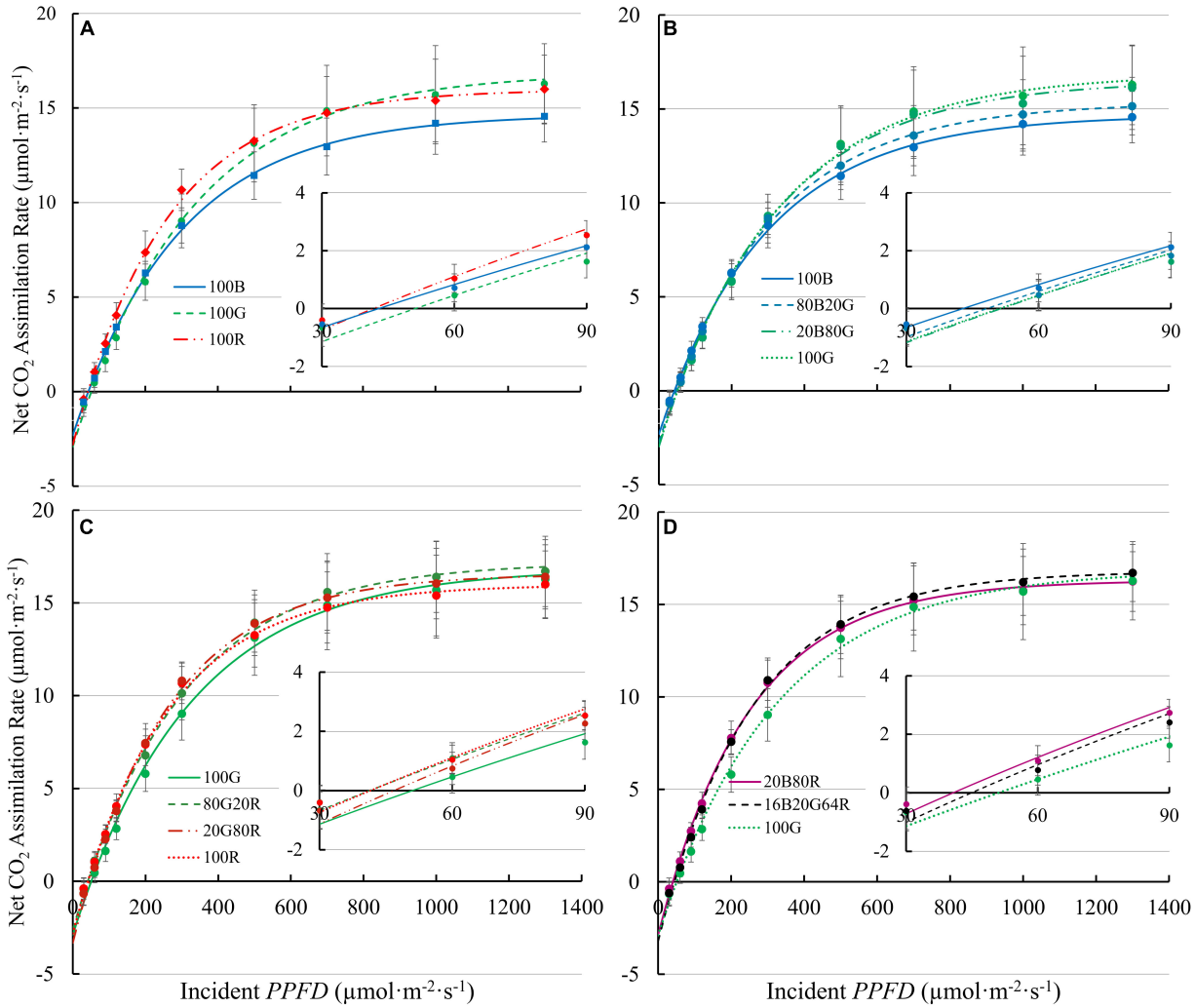

FIGURE 3 | Net assimilation (A_n) – light response curves of "Green Towers" lettuce under nine light spectra. Error bars represent the standard deviation (n = 9). Inserts show A_n against *PPFD* of 30-90 μmol·m^{-2}·s^{-1}s to better show the initial slopes of curves. The composition of the nine light spectra is shown in **Table 1**. The light spectra in the graphs are **(A)** 100B, 100G, and 100R; **(B)** 100B, 80B20G, 20B80G, and 100G; **(C)** 100G, 80G20R, 20G80R, and 100R; and **(D)** 20B80R, 16B20G64R, and 100G.

DISCUSSION

Interactive Effect of Light Spectrum and *PPFD* on Photosynthesis

There was an interactive effect of light spectrum and *PPFD* on photosynthetic properties of lettuce. Under low light conditions (≤200 μmol·m^{-2}·s^{-1}), the QY_{inc} of lettuce leaves under green light was lowest among blue, green, and red light (**Figure 6A**), due to its lower absorptance by lettuce leaves. After accounting for absorptance, green photons were used at similar efficiency as blue photons, while red photons were used most efficiently (**Figure 6B**). The $QY_{m,abs}$ under green and red light were higher than under blue light (**Figure 4B**). At high *PPFD*, green and red light had similar quantum yield, higher than that of blue light, both on an absorbed and incident light basis (**Figure 6A**). Multiple factors contributed to the interactive effect of light spectrum and *PPFD* on quantum yield and photosynthesis.

Light Absorptance and Non-Photosynthetic Pigments Determine Assimilation at Low *PPFD*

$QY_{m,inc}$ with blue and green light was lower than with red light (**Figure 4A**), consistent with McCree's action spectrum (McCree, 1971). But when taking leaf absorptance into account, $QY_{m,abs}$ was similar under green and red light and lower under blue light (**Figure 4B**). Similarly, at low *PPFD* (≤200 μmol·m^{-2}·s^{-1}), QY_{inc} of lettuce leaves was highest under red, intermediate under blue, and lowest under green light. When accounting for leaf absorptance, QY_{abs} under red light remained highest and QY_{abs} under both green and blue light were similar at low *PPFD* (**Figure 6A**). Consistent with our data, previous studies

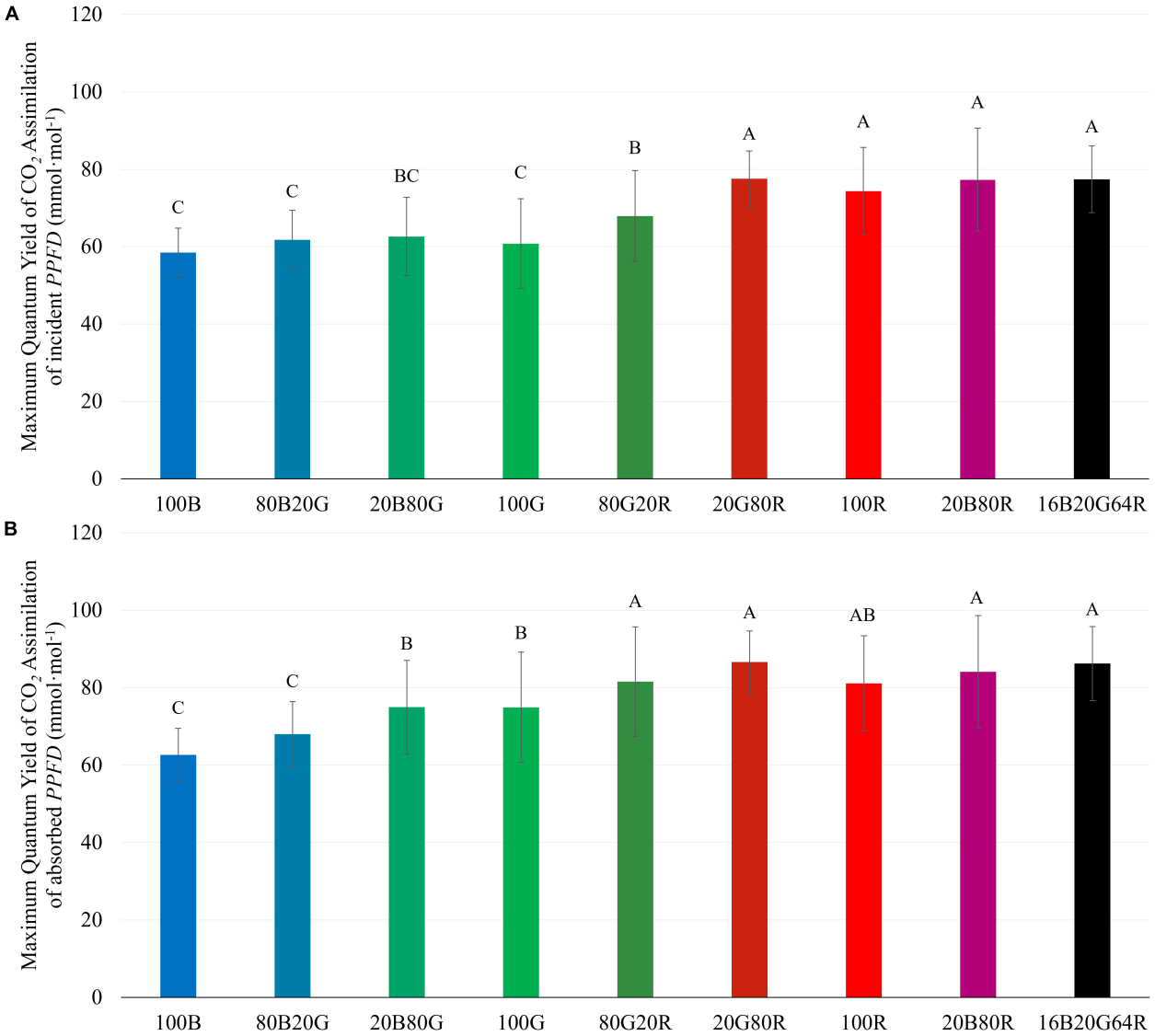

FIGURE 4 | Maximum quantum yield of CO_2 assimilation of "Green Towers" lettuce based on incident ($QY_{m,inc}$) **(A)** and absorbed light ($QY_{m,abs}$) **(B)** under nine different light spectra. Values are calculated as the initial slope of the light response curves of corresponding light spectra (see **Figure 3**). Bars with the same letter are not statistically different ($p \leq 0.05$). Error bars represent the standard deviation ($n = 9$). The composition of the nine light spectra is shown in **Table 1**.

also documented that, once absorbed, green light can drive photosynthesis efficiently at low *PPFD* (Balegh and Biddulph, 1970; McCree, 1971; Evans, 1987; Sun et al., 1998; Nishio, 2000; Terashima et al., 2009; Hogewoning et al., 2012; Vogelmann and Gorton, 2014). For example, the $QY_{m,abs}$ of spinach (*Spinacia oleracea*) and cabbage (*Brassica oleracea L.*) was highest under red light, followed by that under green light and lowest with blue light. But on incident light basis, $QY_{m,inc}$ of under green light was lower than under red or blue light (Sun et al., 1998).

Both our data (**Figure 4B**) and those of Sun et al. (1998) show that $QY_{m,abs}$ with blue light is lower than that with red and green light, indicating that blue light is used intrinsically less efficiently by lettuce. Blue light, and, to a lesser extent, green light is absorbed not just by chlorophyll, but also by flavonoids and carotenoids (Sun et al., 1998). Those pigments can divert energy away from photochemistry and thus reduce the QY_{abs} under blue light. Flavonoids (e.g., anthocyanins) are primarily located in the vacuole and cannot transfer absorbed light energy to photosynthetic pigments (Sun et al., 1998). Likewise, free carotenoids do not contribute to photochemistry (Hogewoning et al., 2012). Carotenoids in light-harvesting antennae and reaction centers channel light energy to photochemistry, but with lower transfer efficiency than chlorophylls (Croce et al., 2001; de Weerd et al., 2003a,b; Wientjes et al., 2011; Hogewoning et al., 2012). Therefore, absorption of blue light by flavonoids and carotenoids reduces the quantum yield of CO_2 assimilation. Thus, even with the high absorptance of blue light by green leaves, $QY_{m,abs}$ of leaves under blue light was the lowest among

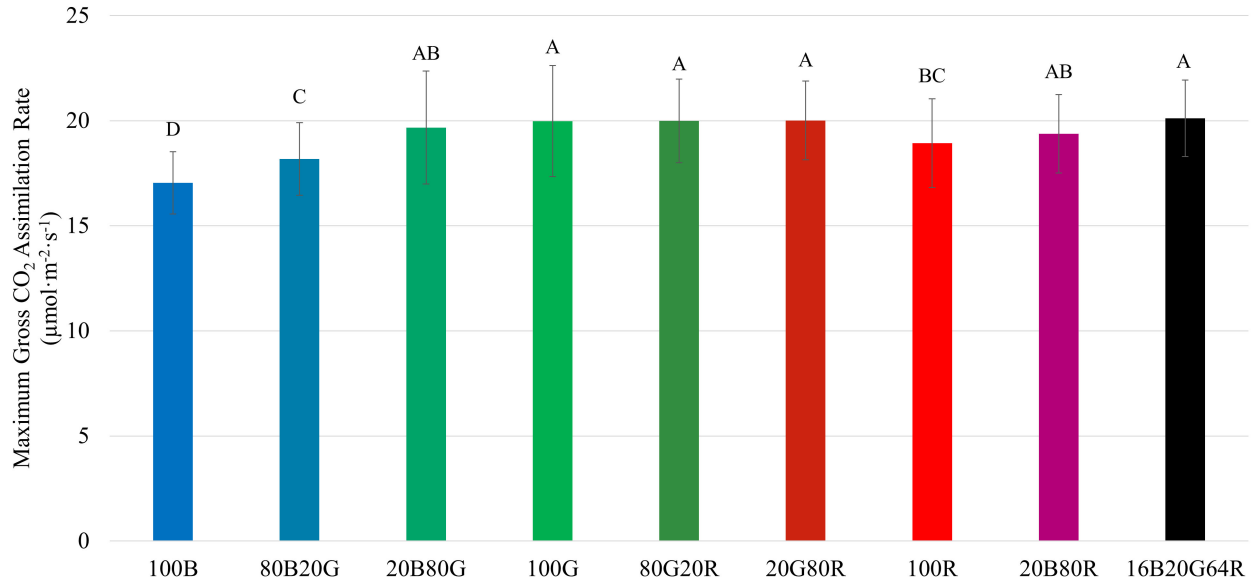

FIGURE 5 | Maximum gross assimilation rate ($A_{g,max}$) of "Green Towers" lettuce under different light spectra, calculated from the light response curves. Bars with the same letter are not statistically different ($p \leq 0.05$). Error bars represent standard deviation ($n = 9$). The composition of the nine light spectra is shown in **Table 1**.

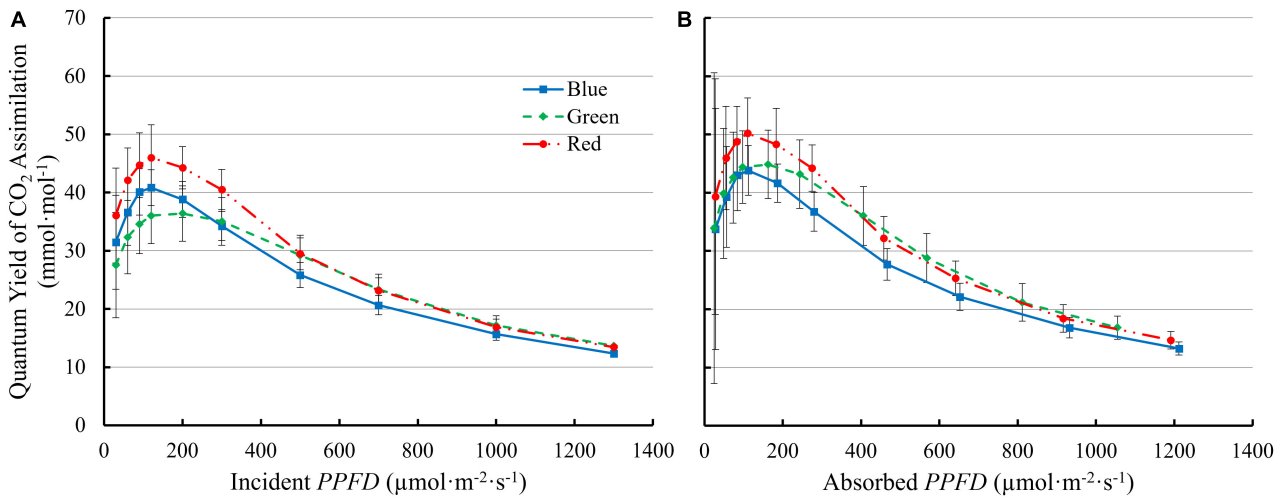

FIGURE 6 | The quantum yield of CO_2 assimilation of "Green Towers" lettuce as a function of incident (QY_{inc}) **(A)** and absorbed *PPFD* (QY_{abs}) **(B)** under blue, green, and red LED light. Error bars represent the standard deviation ($n = 9$).

the three monochromatic lights (**Figure 4B**). It is likely that the lower QY_{abs} under green light than that under red light was also due to absorption of green light by carotenoids and flavonoids (Hogewoning et al., 2012). At high *PPFD*, absorption of blue light by flavonoids and carotenoids still occurs, but this is less of a limiting factor for photosynthesis, since light availability is not limiting under high *PPFD*.

Light Dependence of Respiration and Rubisco Activity May Reduce the Quantum Yield at Low *PPFD*

At *PPFD*s below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the QY_{inc} and QY_{abs} of lettuce showed an unexpected pattern in response to *PPFD* (**Figure 6**). Unlike the quantum yield of PSII, which decreases exponentially with increasing *PPFD* (Weaver and van Iersel, 2019), QY_{inc} and QY_{abs} increased initially with increasing *PPFD* (**Figure 6**). A similar pattern was previously observed by Craver et al. (2020) in petunia (*Petunia* × *hybrida*) seedlings. This pattern could result from light-dependent regulation of respiration (Croce et al., 2001), alternative electron sinks such as nitrate reduction (Skillman, 2008; Nunes-Nesi et al., 2010), or Rubisco activity (Campbell and Ogren, 1992; Zhang and Portis, 1999). In our calculations, we assumed that the leaf respiration in the light was the same as R_d. However, leaf respiration in the light is lower than in the dark, in a *PPFD*-dependent manner

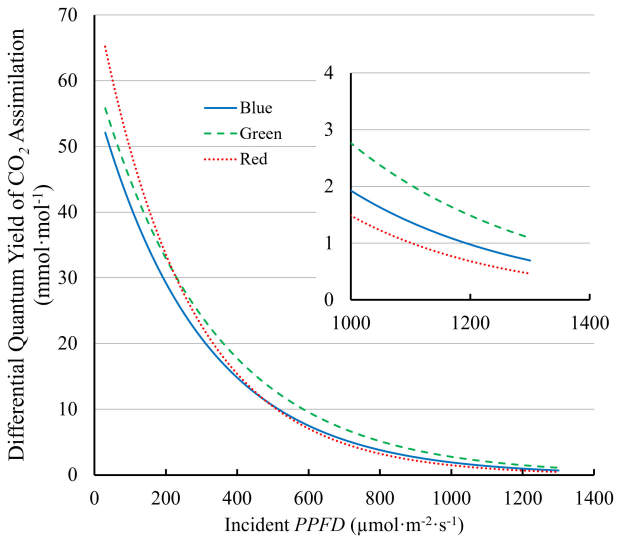

FIGURE 7 | The differential quantum yield of CO_2 assimilation (*differential QY*) of "Green Towers" lettuce under blue, green, and red LED light as a function of the *PPFD*. The *differential QY* is the increase in net assimilation per unit additional *PPFD* and was calculated as the first derivate of the light response curves (**Figure 3**). The insert shows the differential quantum yield plotted at *PPFDs* of 1,000–1,300 μmol m^{-2} s^{-1}s to better show differences at high *PPFD* (note the different *y*-axis scale).

(Brooks and Farquhar, 1985; Atkin et al., 1997), which can lead to overestimation of A_g with increasing *PPFD*. When we accounted for this down-regulation of respiration, using the model by Müller et al. (2005) to correct A_g, QY_{inc}, and QY_{abs}, we found that depression of respiration by light did not explain the initial increase in QY_{inc} and QY_{abs} we observed (**Supplementary Figure 4**). Alternative electron sinks in the chloroplasts that are upregulated in response to light can explain the low QY_{inc}, and QY_{abs} at low *PPFD*, because they compete with the Calvin cycle for reducing power (ferredoxin/NADPH). Such processes include photorespiration (Krall and Edwards, 1992), nitrate assimilation (Nunes-Nesi et al., 2010), sulfate assimilation (Takahashi et al., 2011) and the Mehler reaction (Badger et al., 2000) and their effect on QY_{inc}, and QY_{abs} would be especially notable under low *PPFD* (**Supplementary Figure 5**).

Upregulation of Rubisco activity by Rubisco activase in the light may also have contributed to the increase in QY_{inc} and QY_{abs} at low *PPFD* (Campbell and Ogren, 1992; Zhang and Portis, 1999). In the dark, 2-carboxy-D-arabinitol-1-phosphate (CA1P) or RuBP binds strongly to the active sites of Rubisco, preventing carboxylation activity. In the light, Rubisco activase releases the inhibitory CA1P or RuBP from the catalytic site of Rubisco, in a light-dependent manner (Campbell and Ogren, 1992; Zhang and Portis, 1999; Parry et al., 2008). At *PPFD* < 120 μmol·m^{-2}·s^{-1}, low Rubisco activity may have limited photosynthesis.

Light Distribution Within Leaves Affects *QY* at High *PPFD*

Except for the initial increase at low *PPFD*, both QY_{inc} and QY_{abs} decreased with increasing *PPFD*. QY_{inc} decreased slower under green than under red or blue light (**Figure 6A**). At a *PPFD* ≥ 500 μmol·m^{-2}·s^{-1}, QY_{inc} under green light was higher than that under blue light (**Figure 6A**). Accordingly, A_n under blue light was lower than under green and red light at *PPFD*s above 500 μmol·m^{-2}·s^{-1} (**Figure 3A**). The lower QY_{inc} under blue light than under green and red light at high *PPFD* can be explained by disparities in the light distribution within leaves.

Blue and red light were strongly absorbed by lettuce leaves (93.2 and 91.6%, respectively), while green light was absorbed less (81.1%) (**Table 2**). Similar low green absorptance was found in sunflower (*Helianthus annuus* L.), snapdragon (*Antirrhínum majus* L.) (Brodersen and Vogelmann, 2010), and spinach (Vogelmann and Han, 2000). In leaves of those species, absorption of red and blue light peaked in the upper 20% of leaves, and declined sharply further into the leaf. Absorption of red light decreased slower with increasing depth than that of blue light (Vogelmann and Han, 2000; Brodersen and Vogelmann, 2010). Green light absorption peaked deeper into leaves, and was more evenly distributed throughout leaves, because of low absorption of green light by chlorophyll (Vogelmann and Han, 2000; Brodersen and Vogelmann, 2010). The more even distribution of green light within leaves, as compared to red and blue light, can explain the interactive effects between *PPFD* and light spectrum on leaf photosynthesis. It was estimated that less than 10% of blue light traveled through the palisade mesophyll and reached the spongy mesophyll in spinach, while about 35% of green light and 25% of red light did so (Vogelmann and Evans, 2002). It was also estimated that chlorophyll in the lowermost chloroplasts of spinach leaves absorbed about 10% of green and <2% of blue light, compared to chlorophyll in the uppermost chloroplasts (Vogelmann and Evans, 2002; Terashima et al., 2009).

The more uniform green light distribution within leaves may be a key contributor to higher leaf level QY_{inc} under high *PPFD* because less heat dissipation of excess light energy is needed (Nishio, 2000; Terashima et al., 2009). Reaction centers near the adaxial leaf surface receive more excitation energy under blue, and to a lesser extent under red light, than under green light, because of the differences in absorptance. Consequently, under high intensity blue light, NPQ is up-regulated in the chloroplasts near the adaxial leaf surface to dissipate some of the excitation energy (Sun et al., 1998; Nishio, 2000), lowering the QY_{inc} under blue light. Since less green light is absorbed near the adaxial surface, less heat dissipation is required. When incident light increased from 150 to 600 μmol·m^{-2}·s^{-1}, the fraction of whole leaf CO_2 assimilation that occurred in the top half of spinach leaves remained the same under green light (58%), but decreased from 87 to 73% under blue light. This indicates more upregulation of heat dissipation in the top of the leaves under blue, than under green light (Evans and Vogelmann, 2003). On the other hand, the bottom half of the leaves can still utilize the available light with relatively high QY_{inc}, since the amount of light reaching the bottom half is relatively low, even under high *PPFD* (Nishio, 2000). By channeling more light to the under-utilized bottom part of leaves, leaves could achieve higher QY_{inc} even under high intensity green light. In our study, high QY_{inc} under green light and low QY_{inc} under blue light at high

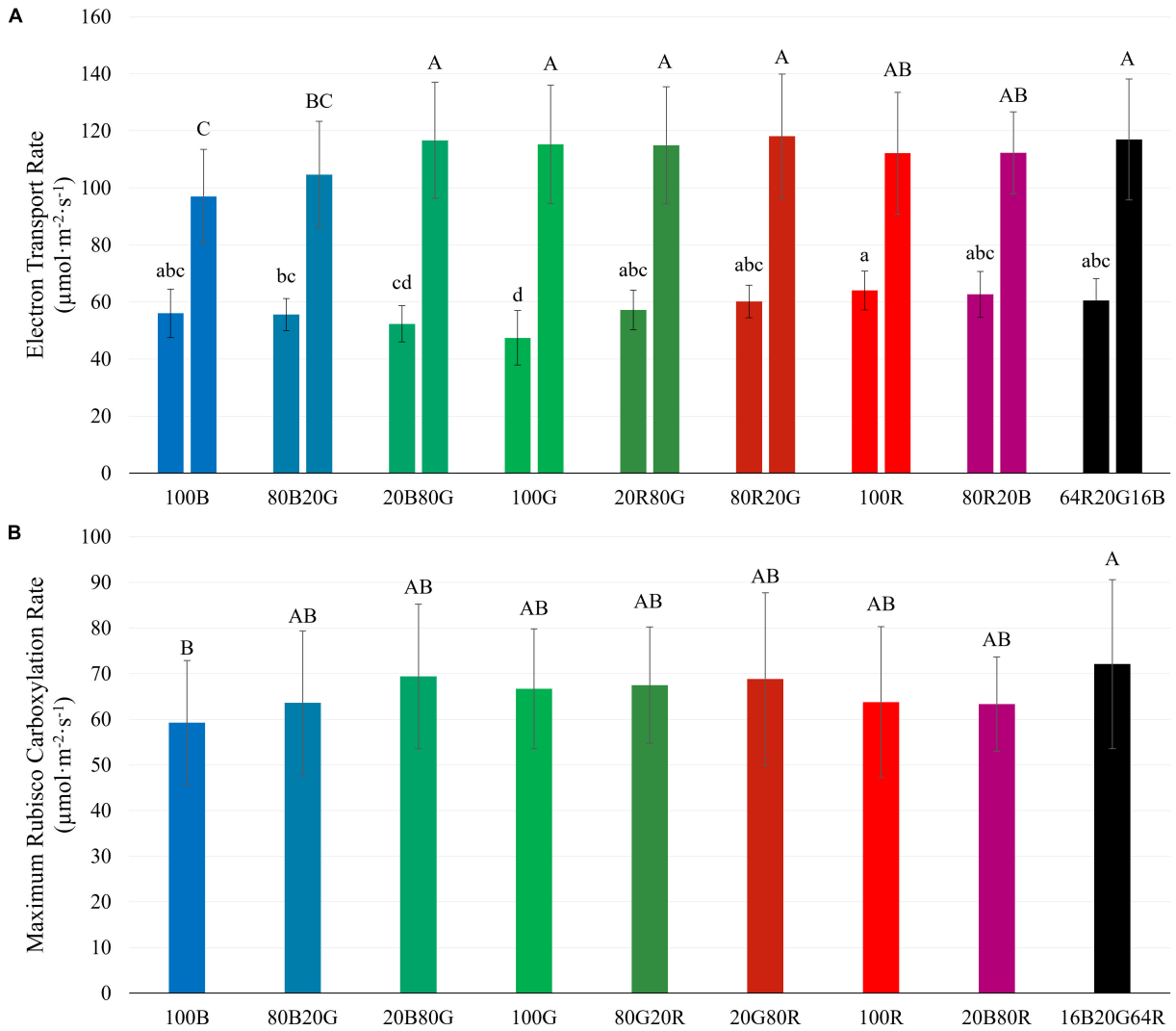

FIGURE 8 | Electron transport rate (*J*) at *PPFD*s of 200 (left bars) and 1,000 μmol m^{-2} s^{-1} (right bars) **(A)** and maximum Rubisco carboxylation rate ($V_{c,max}$) at a *PPFD* of 1,000 μmol m^{-2} s^{-1} **(B)** of "Green Towers" lettuce, as estimated from A/C_i curves under different light spectra. Bars with the same letter are not statistically different ($p \leq 0.05$). Error bars represent the standard deviation ($n = 9$). The light composition of the nine light spectra is shown in **Table 1**.

PPFD (**Figure 6**) can be thus explained by the large disparities in the light environment in chloroplasts from the adaxial to the abaxial side of leaves due to differences in leaf absorptance. Similarly, differential *QY* of lettuce leaves was highest under green light and lower under blue and red light at high *PPFD* (>300 μmol·m^{-2}·s^{-1}) (**Figure 7**), also potentially because of the more uniform distribution of green light and the uneven distribution of blue and red light in leaves.

Along the same line, A_n of lettuce leaves was the lowest under blue light at *PPFD* > 500 μmol·m^{-2}·s^{-1} (**Figure 3**). Also, A_n of lettuce leaves approached light saturation at lower *PPFD*s under blue and red light, than under green light (**Figure 3A**). Under blue, green, and red light, lettuce leaves reached 95% of $A_{n,max}$ at *PPFD*s of 954, 1,110 and 856 μmol·m^{-2}·s^{-1}, respectively. This can be seen more clearly in the differential *QY* at high *PPFD* (**Figure 7**). At a *PPFD* of 1,300 μmol·m^{-2}·s^{-1}, green light had a differential *QY* of 1.09 mmol·mol^{-1}, while that of red and blue light was only 0.46 and 0.69 mmol·mol^{-1}, respectively (**Figure 7**). Green light also resulted in a higher $A_{g,max}$ (22.9 μmol·m^{-2}·s^{-1}) than red and blue light (21.8 and 19.3 μmol·m^{-2}·s^{-1}, respectively) (**Figure 5**). As discussed before, the high $A_{g,max}$ under green light resulted from the more uniform light distribution under green light, allowing deeper cell layers to photosynthesize more. Previous research similarly found that at high *PPFD* (>500 μmol·m^{-2}·s^{-1}), A_n of both spinach and cabbage were lower under blue light than under white, red and green light (Sun et al., 1998). Overall, under high *PPFD*, the differences in light distribution throughout a leaf are important to quantum yield and assimilation rate, since it affects NPQ up-regulation (Sun et al., 1998; Nishio, 2000). However, light distribution within a leaf is less important at low than at high *PPFD*,

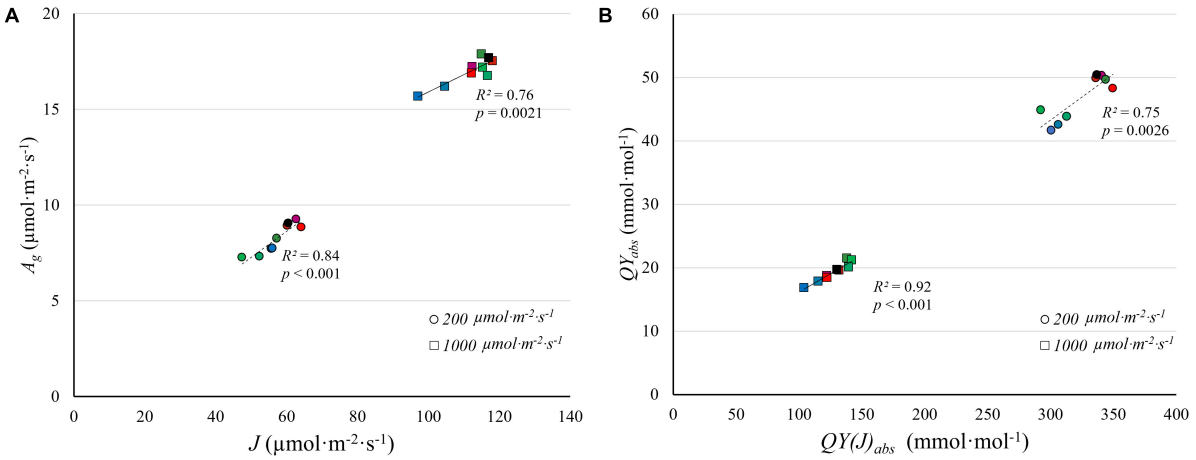

FIGURE 9 | The correlation between gross CO_2 assimilation rate (A_g) estimated from light response curves and electron transport rate (J) estimated from A/C_i curves **(A)**, and between the quantum yield of CO_2 assimilation (QY_{abs}) and the quantum yield of electron transport on an absorbed light basis [$QY(J)_{abs}$] **(B)**, under low *PPFD* (200 μmol m^{-2} s^{-1}) and high *PPFD* (1,000 μmol m^{-2} s^{-1}) under nine light spectra averaged over nine "Green Towers" lettuce plants. The color scheme representing the nine spectra is the same as **Figure 8**.

because upregulation of NPQ increases with increasing *PPFD* (Zhen and van Iersel, 2017).

Light Spectrum Affects *J*, but Not $V_{c,max}$

We examined the effect of light quality and intensity on J and $V_{c,max}$ (**Figure 8**). For the light-dependent reactions, the interactive effect between light spectra and *PPFD* found for CO_2 assimilation and quantum yield was also observed for J (**Figure 8A**). At low *PPFD* (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$), green light resulted in the lowest J and red light in the highest J among single waveband spectra. But at a *PPFD* of 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, red and green light resulted in the highest J and blue light in the lowest J (**Figure 8A**), similar to the differences in A_g.

There was no clear evidence of Rubisco limitations to photosynthesis at a *PPFD* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, so the rate of the light-dependent reactions likely limited photosynthesis. This is corroborated by the strong correlation between A_g and J at a *PPFD* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Although Rubisco limitations to photosynthesis were observed at a *PPFD* of 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, there were no meaningful differences in $V_{c,max}$ in response to light spectrum, in contrast to J (**Figure 8**).

When *PPFD* increased 5×, from 200 to 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, there was only a 1.7 to 2.4× increase in J, indicating a lower $QY(J)_{inc}$ at higher *PPFD*. This matches the lower QY_{inc} and the asymptotic increase in A_n in response to increasing *PPFD* (**Figure 3**). The relative increase of J under green light (143%) was greater than that under both blue and red light (73 and 75%, respectively) as *PPFD* increased. This similarly can be attributed to a more uniform energy distribution of green light among reaction centers throughout a leaf and weaker upregulation of non-photochemical quenching with increasing green light intensity (Sun et al., 1998; Nishio, 2000; Evans and Vogelmann, 2003), as discussed before.

There was a strong correlation between J and A_g under the nine light spectra at both *PPFD* levels (**Figure 9A**). QY_{abs} and $QY(J)_{abs}$ are similarly strongly correlated (**Figure 9B**). Unlike J, $V_{c,max}$ was largely unaffected by light spectra (**Figure 8B**) and was not correlated with A_g (data not shown). There was, however, a strong correlation between J and $V_{c,max}$ at a *PPFD* of 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($R^2 = 0.82$, **Supplementary Figure 3**), suggesting that J and $V_{c,max}$ are co-regulated. Similarly, Wullschleger (1993) noted a strong linear relationship between J and $V_{c,max}$ across 109 C_3 species. The ratio between J and $V_{c,max}$ in our study (1.5–2.0) similar to the ratio found by Wullschleger (1993). These results suggest that the interactive effect of light spectra and *PPFD* resulted from effects on J, which is associated with light energy harvesting by reaction centers, rather than from $V_{c,max}$.

No Interactive Effects Among Blue, Green, and Red Light

The Emerson enhancement effect describes a synergistic effect between lights of different wavebands (red and far-red) on photosynthesis (Emerson, 1957). McCree (1971) attempted to account for interactions between light with different spectra when developing photosynthetic action spectra and applied low intensity monochromatic lights from 350 to 725 nm with white background light to plants. His results showed no interactive effect between those monochromatic lights and white light (McCree, 1971). We tested different ratios of blue, green, and red light and different *PPFD*s, and similarly did not find any synergistic or antagonistic effect of different wavebands on any physiological parameters measured or calculated.

Importance of Interactions Between *PPFD* and Light Quality and Its Applications

The interactive effect between *PPFD* and light quality demonstrates a remarkable adaptation of plants to different light intensities. By not absorbing green light strongly, plants open up a "green window," as Terashima et al. (2009) called it, to excite chloroplasts deeper into leaves, and thus facilitating

CO_2 assimilation throughout the leaf. While red light resulted in relatively high QY_{inc}, QY_{abs} and A_n at both high and low *PPFD* (**Figures 3**, **6**), it is still mainly absorbed in the upper part of leaves (Sun et al., 1998; Brodersen and Vogelmann, 2010). Green light can penetrate deeper into leaves (Brodersen and Vogelmann, 2010) and help plants drive efficient CO_2 assimilation at high *PPFD* (**Figures 3**, **5**).

Many early photosynthesis studies investigated the absorptance and action spectrum of photosynthesis of green algae, e.g., Haxo and Blinks (1950) or chlorophyll or chloroplasts extracts, e.g., Chen (1952). Extrapolating light absorptance of green algae and suspension of chlorophyll or chloroplast to whole leaves from can lead to an underestimation of absorptance of green light by whole leaves and the belief that green light has little photosynthetic activity (Moss and Loomis, 1952; Smith et al., 2017). Photosynthetic action spectra developed on whole leaves of higher plants, however, have long shown that green light effectively contributes to CO_2 assimilation, although with lower QY_{inc} than red light (Hoover, 1937; McCree, 1971; Inada, 1976; Evans, 1987). The importance of green light for photosynthesis was clearly established in more recent studies, emphasizing its role in more uniformly exciting all chloroplasts, which especially important under high *PPFD* (Sun et al., 1998; Nishio, 2000; Terashima et al., 2009; Hogewoning et al., 2012; Smith et al., 2017). The idea that red and blue light are more efficient at driving photosynthesis, unfortunately, still lingers, e.g., Singh et al. (2015).

Light-emitting diodes (LEDs) have received wide attention in recent years for use in controlled environment agriculture, as they now have superior efficacy over traditional lighting technologies (Pattison et al., 2018). LEDs can have a narrow spectrum and great controllability. This provides unprecedented opportunities to fine tune light spectra and *PPFD* to manipulate crop growth and development. Blue and red LEDs have higher efficacy than white and green LEDs (Kusuma et al., 2020). By coincidence, McCree's action spectrum (**Figure 1**; McCree, 1971) also has peaks in the red and blue region, although the peak in the blue region is substantially lower than the one in the red region. Therefore, red and blue LEDs are sometimes considered optimal for driving photosynthesis. This claim holds true only under low *PPFD*. Green light plays an important role in photosynthesis, as it helps plants to adapt to different light intensities. The wavelength-dependent absorptance of chlorophylls channels green light deeper into leaves, resulting in more uniform light absorption throughout leaves and providing excitation energy to cells further from the adaxial surface. Under high *PPFD*, this can increase leaf photosynthesis. Plant evolved under sunlight for hundreds of millions of years, and it seems likely that the relatively low absorptance of green light contributes to the overall photosynthetic efficiency of plants (Nishio, 2000).

CONCLUSION

There was an interactive effect of light spectrum and *PPFD* on leaf photosynthesis. Under low *PPFD*, QY_{inc} was lowest under green and highest under red light. The low QY_{inc} under green light at low *PPFD* was due to low absorptance. In contrast, at high *PPFD*, green and red light achieved similar QY_{inc}, higher than that of blue light. The strong absorption of blue light by chlorophyll creates a large light gradient from the top to the bottom of leaves. The large amount of excitation energy near the adaxial side of a leaf results in upregulation of nonphotochemical quenching, while chloroplasts near the bottom of a leaf receive little excitation energy under blue light. The more uniform distribution of green light absorption within leaves reduces the need for nonphotochemical quenching near the top of the leaf, while providing more excitation energy to cells near the bottom of the leaf. We also found that the interactive effect of light spectrum and *PPFD* on photosynthesis was a result of the light-dependent reactions; gross assimilation and *J* were strongly correlated. We detected no synergistic or antagonistic interactions between blue, green, and red light.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

JL and MI designed the experiment, discussed the data, and revised the manuscript. JL performed the experiment, analyzed data, and prepared the first draft. Both authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.619987/full#supplementary-material

Supplementary Figure 1 | (Related to **Figure 6**) Quantum yield of CO_2 assimilation of "Green Towers" lettuce as a function of incident (QY_{inc}) **(A,C,E,G)** and absorbed *PPFD* (QY_{abs}) **(B,D,F,H)** under nine light spectra (see **Table 1**). Error bars represent standard deviation ($n = 9$).

Supplementary Figure 2 | (Related to **Figure 7**) Differential quantum yield of CO_2 assimilation (*differential QY*) of "Green Towers" lettuce under nine light spectra as a function of the *PPFD*. Inserts show *differential QY* at *PPFD*s of 1,000–1,300 $\mu mol \cdot m^{-2}\ s^{-1}$s to better show differences at high *PPFD* (note the different *y*-axis scale). The composition of the nine light spectra is shown in **Table 1**. The light spectra in the graphs are **(A)** 100B, 100G and 100R; **(B)** 100B, 80B20G, 20B80G and 100G; **(C)** 100G, 80G20R, 20G80R and 100R; and **(D)** 20B80R, 16B20G64R and 100G.

Supplementary Figure 3 | (Related to **Figure 6**) The correlation between electron transport (*J*) and maximum Rubisco carboxylation rate ($V_{c,max}$) of "Green Towers" lettuce estimated from A/C_i curves under *PPFD* (1000 $\mu mol\ m^{-2}\ s^{-1}$) under nine light spectra ($p < 0.001$).

Supplementary Figure 4 | (Related to **Figure 6**) The comparison between QY_{inc} before **(A)** and after **(B)** correcting for light-suppression of respiration under blue, green, and red LED light. Note that the initial increase in QY_{inc} became more pronounced after correction of light suppressed respiration.

Supplementary Figure 5 | The comparison between QY_{abs} before **(A)** and after **(B)** correcting for alternative electron sinks under blue, green, and red LED light. Assuming a simplified electron sink that diverts energy of 15 μmol m^{-2} s^{-1} of absorbed photons (an arbitrary value used for illustrative purposes only) away from the Calvin cycle under all *PPFD*s, the corrected QY_{abs} was calculated based on remaining photons available to support Calvin cycle processes **(B)**. Note that the pattern of QY_{inc} after correcting of alternative electron sink **(B)** is similar to quantum yield of PSII measured by chlorophyll fluorescence by Weaver and van Iersel (2019).

Supplementary Table 1 | Dark respiration rate (R_d), maximum quantum yield of CO_2 assimilation ($QY_{m,inc}$) and maximum gross assimilation rate ($A_{g,max}$) of "Green towers" lettuce derived from the light response curves for nine different spectra using Eq. 1. The light response curves are shown in **Figure 3**. *See light composition of nine lights presented here in **Table 1**.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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