

The Contribution of Different Spectral Sections to Increase Fresh Weight of Boston Lettuce

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Abstract. Six types of light sources [0G, 20G, 40G, cool-white light-emitting diode (LED CW), cool-white fluorescent lamp (FLCW), and plant light fluorescent lamp (TLRA)] were used as the sole light sources to cultivate boston lettuce (*Lactuca sativa* L. cv. *Ostinata*). The photosynthetically active radiation (PAR) range was separated into five sections and the contributions of each spectral section on fresh weight (FW) were quantified. The results indicate that the conventional method of separating PAR into red, green, and blue at 100 nm apart was not accurate enough to clarify the contribution of different spectral sections to FW of boston lettuce. Green light (525–575 nm) at less than 30% of PAR is even more important than red (625–700 nm) and blue (400–475 nm) to plant growth. Yellow light (575–625 nm) has very little effect on plant growth.

Light plays an important role in photosynthesis. Visible light is defined in the wavelength from 380 to 780 nm. Light within 400 to 700 nm, termed PAR, can be roughly separated into blue (B, 400–500 nm), green (G, 500–600 nm), and red (R, 600–700 nm). RGB light affects plant growth in different ways. For example, blue light induces biomass production but suppresses the hypocotyl elongation; red light boosts hypocotyl elongation and expands leaf (Johkan et al., 2010; McNellis and Deng, 1995). As for green light, researchers (Folta, 2004; Kim et al., 2004a, 2004b) found that it is related to leaf growth, stomatal conductance, and early stem extension. Green light is considered to be insignificant in driving photosynthesis due to the low absorptivity coefficient in the absorption spectra of purified chlorophylls (Sun et al., 1998).

Light-emitting diodes (LEDs) allow production of light that emit only the wavelengths of light corresponding to the absorption peaks of plant requirements. Other advantages such as energy efficiency, long lifetime, low heat generated at the side-facing plants, and dramatic decrease of cost make them popular light sources to enhance plant growth, increase

fruit yield, improve quality, control of plant morphology and hormone physiology in plant cultivation (Fan et al., 2013; Islam et al., 2014; Jao and Fang, 2004; Johkan et al., 2010; Kim et al., 2004a, 2004b, 2006; Xu et al., 2012). In this study, two types of light sources (LEDs and fluorescent lamps with various spectra) were used to cultivate boston lettuce (*L. sativa* L. cv. *Ostinata*). To obtain a more precise index about the effect caused by different wavebands, an analytical model involving five spectral sections was adopted. Understanding the correlation between light quality and the plant growth will be advantageous to plant cultivation and the development of optimum light spectrum in the future.

Materials and Methods

Experiment equipment. There were six light spectra used in this study. Three out of six were self-made with the capability of adjusting light intensity of R, G, and B LEDs (Everlight, Taiwan). They were red and blue LEDs (0G: relative peaks at 462, 640, and 660 nm), red and blue LEDs mixed with 20% green LEDs (20G: relative peaks at 462, 525, and 660 nm), and 40% green LEDs (40G: relative peaks at 462, 525, and 640 nm). Another three were commercially available. They were 6500 K LED CW (relative peaks at 447 and 569 nm, Wellypower, Taiwan), 6500 K FLCW (relative peaks at 436, 546,

and 611 nm, Wellypower), and TLRA (relative peaks at 436, 546, 611, and 660 nm, Wellypower). The ratio of blue light was between 20% and 26% in 0G, 20G, and 40G. Photosynthetic photon flux (PPF) of all treatments was adjusted to 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ measured with quantum meter (Quantum sensor Q46758 and LI-250A; LI-COR, USA) in a distance of 25 cm under light sources, and 96 points (16×6) were measured to calculate the mean and standard deviation values of PPF on the surface of a culture tray (120×60 cm). All Spectra were measured using Spectrometer (USB 4000; Ocean Optics, USA) in a distance of 25 cm under light sources (Fig. 1).

Plant material and growth conditions. Lettuce (*L. sativa* L. cv. *Ostinata*; Known-You Seed Co., Taiwan) was cultured hydroponically using the deep flow technique (DFT) in an environmental-controlled confined chamber (5 m²) of ILan University. Lettuce seeds were soaked for 5 h then sown into plug trays, using foam/sponge cube as growth media, and allowing germination and growth for 6 d (stage 1) under 6500 K LED CW with PPF at 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in a growth chamber. The temperature and humidity of the chamber were kept at 20 °C and 70% to 80% with a 24-h light period. In stage 2, the seedlings were transplanted to another room with temperature of 25 °C day/18 °C night and humidity of 70% to 80% and provided with various light sources (0G, 20G, 40G, LED CW, FLCW, and TLRA) with daily light integral (DLI) of 8.64 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ($16 \text{ h}\cdot\text{d}^{-1} \times 150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The CO₂ concentration was kept at 1200 ppm in light period. The soluble complex nutrient solution (Type 1, 7N–6P–19K; Hyponex) was used and the pH and electrical conductivity (EC) were maintained at 6 and 1.2 $\text{mS}\cdot\text{cm}^{-1}$ (1.03 $\text{g}\cdot\text{L}^{-1}$), respectively.

Plant densities were 180 plants/m² from days 7 to 17, and 40 plants/m² from days 18 to 39. All plants were harvested on the 39th day after seeding. The roots and shoots were separated and FW was measured. The shoots were dried for 3 d at 75 °C to determine dry weight (DW).

Model description. Albright et al. (1999) developed a vegetative growth model of boston lettuce based on environmental conditions, including air temperature, CO₂ concentration, light integral, and nitrate concentration of the nutrient solution. As shown below, coefficient “A,” in Eqs. [1] and [2], was the environmental coefficient related to the integrated PPF (*L*) during cultivation, and indoor CO₂ concentration (*C*). Parameter “*d*” represented the growth day from seeding, and “ $\exp(C_1d - C_2d^2)$ ” was normalized growth curve of DW. With the given “A” and “*d*,” the DW at day *d* (DW_{*d*}) can be derived.

$$\text{DW}_d = A \exp(C_1d - C_2d^2) \quad [1]$$

$$A = 1.14 \times 10^{-5} + 9.57 \times 10^{-6}L + 7.67 \times 10^{-8}C \quad [2]$$

Eqs. [1] and [2] can be combined to form Eq. [3] as shown below:

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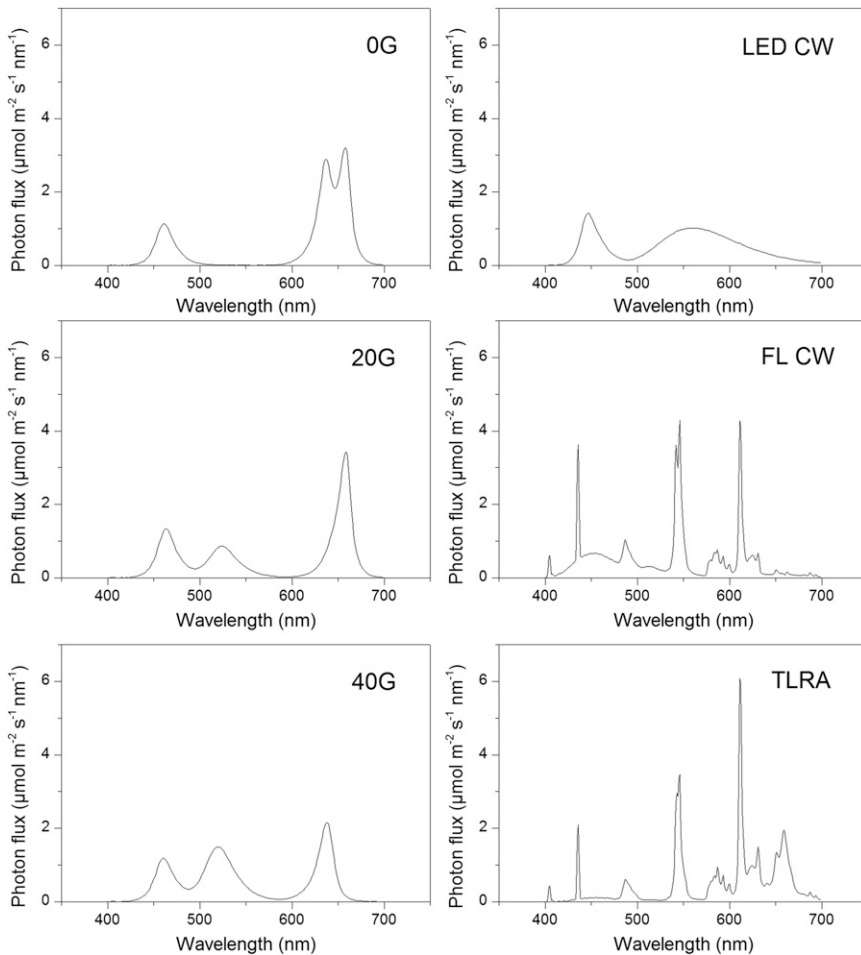


Fig. 1. Spectral distribution of light from red and blue light-emitting diodes (LEDs) (0G), red and blue LEDs mixed with 20% green (20G), 40% green LEDs light (40G), 6500 K cool-white LEDs (LED CW), 6500 K cool-white fluorescent lamps (FLCW), and plant light fluorescent lamps (TLRA).

$$DW_d = (A_1 + A_2L + A_3C) \times \exp(C_1d - C_2d^2) \quad [3]$$

A_1 , A_2 , and A_3 are constant and weighing coefficient of integrated PPF (L) and CO_2 concentration (C). C_1 and C_2 were 0.5712 and 0.007742, respectively, according to Albright et al. (1999).

In this study, CO_2 concentration was fixed, the term A_3C becomes constant and can be merged with the term A_1 expressed in A'_1 . To analyze the effect of different spectral sections on plants growth, the PPF waveband was separated into various partitions, and each partition represents a specific range of wavelength. Eq. [3] could be modified as shown below:

$$DW_d = \left(A'_1 + \sum_{i=1}^n (B_i W_i) \right) \times \exp(C_1d - C_2d^2) \quad [4]$$

where W_i is the integrated PPF of separated spectral section, and B_i , the weighing factor of W_i , represents the contribution of related spectral range.

From the commercial point of view, the market price of lettuce was measured based on FW, Eq. [5] was derived.

$$FW_d = \left(A'_1 + \sum_{i=1}^n (B_i W_i) \right) \times \exp(C'_1d - C'_2d^2) \quad [5]$$

$$C'_1 : 0.1145$$

$$C'_2 : -0.001602$$

FW_d is the fresh weight at day d and “ $\exp(C'_1d - C'_2d^2)$ ” is the normalized growth curve of FW (R^2 : 0.9987) calculated from FW of boston lettuce from day 14 to day 35 (Wang, 2010).

A dramatic difference can be found in the coefficient of d^2 term. This term is highly related to the latter growth of lettuce due to value of days after seeding (d) was squared. The positive value of C_2 indicates that the growth rate of lettuce, expressed in terms of the value of DW and FW, was decreasing, that is approaching saturation, when d value gets bigger. However, the negative value of C'_2 indicates that the mass of lettuce will continue to increase without saturation. This is one of the major differences between plant growth under sunlight and under artificial light. When grown under sunlight, in the

same weather conditions, the amount of PPF will not vary too much whether the lettuce was young or matured. However, in a plant growth chamber using artificial light, the bigger the plant growth and the closer it gets to the light source, the more PPF it gets. It did not reach saturation within the experimental conditions of this study.

Number of spectral sections. Chlorophyll a and b were the primary phytochemical compounds. These compounds had highest absorption efficiency at 430, 453, 642, and 662 nm (Gross, 1991). There were other light-harvesting pigments, such as carotenoids, which absorbed light with the highest efficiency at 450 nm (Vierstra and Poff, 1981). To take account the peak wavelength of light sources and the highest absorption wavelength of photo pigments of plant, allowing all peak wavelengths within the middle of each sections as much as possible, the PAR range of 400 to 700 nm was unevenly divided into five sections: 400–475, 475–525, 525–575, 575–625, and 625–700 nm. To compare with previous studies, the data were also analyzed in a traditional way, which separated the PAR range into R (600–700 nm), G (500–600 nm), and B (400–500 nm) sections.

Statistical analysis. Each treatment (0G, 20G, 40G, LED CW, FLCW, and TLRA) was harvested in 15 plants for statistical analysis. Using 5% as the level of significance, statistical analysis was subjected to analysis of variance followed by Duncan's multiple range tests (SPSS, IBM). To obtain the indexes about the effect caused by different wavebands, B_i in Eq. [5] has been determined by Levenberg–Marquardt algorithm using commercially available software (1stOpt; 7D-Soft High Technology, China).

Results and Discussion

As shown in Table 1, a total of four LED and two fluorescent light (FL) sources were used. The LEDs performed significantly better than FLs in terms of FW and DW. The treatment 20G (23% green as shown in Table 2) has the greatest mean FW. However, it shows no significant difference with other LED light sources including less green (0G), more green (40G), and LED CW (51% green).

The DW of 20G was the lowest compared with 0G and 40G. The water content of 20G (97.72%) was significantly higher than 0G (96.86%) and 40G (97.11%). The reason might be the difference on the peak wavelength of red light. The red peak of 20G was 660 nm, but 0G was 640 and 660 nm, and 40G was 640 nm. The R_{660}/R_{640} ratios of 20G, 0G, and 40G were 3.29, 1.07, and 0.08 as shown in column 3 of Table 1. The same result can be derived for fluorescent lamps, the R_{660}/R_{640} ratios of FLCW (1.23) and TLRA (3.37) are close to 20G (3.29), thus leading to no significant difference on water content among these three treatments. The higher R_{660}/R_{640} ratio might reduce the DW and increase water content at the condition of

the same FW. This hypothesis requires further investigation.

FLCW lamps are often used for plant cultivation, but the results showed that the FW of lettuce under FLCW was significantly lower than other treatments.

The proposed model was used by separating *PAR* range into three and five sections as mentioned above. The R^2 values were 0.15 and 0.92, respectively (Tables 2 and 3). The dramatic difference on R^2 suggested that the conventional RGB model was not accurate enough to clarify the contribution of different spectral sections to FW of boston lettuce.

In plant physiology, blue light suppressed hypocotyl elongation, induced biomass production, and affected stomatal conductance (g_s). Red light induced hypocotyl elongation and expansion in leaf area (Johkan et al., 2010; McNellis and Deng, 1995). Green light also affected leaf growth, g_s , and early stem elongation (Folta, 2004; Kim et al., 2004a, 2004b).

As shown in Table 3, the data shown in the second row from the bottom entitled “contributions” indicated that almost all spectral sections had contributions to the increase of lettuce FW except for the section of yellow light (575–625 nm). The bottom row shows that relative contributions of blue (400–475 nm), blue-green (475–525 nm), green (525–575 nm), yellow (575–625 nm), and red (625–700 nm) sections were 23.62, 15.34, 100, 0, and 56.3, respectively. The range of the green section was the smallest (50 vs. 75 nm of red and blue), yet it has the most profound impact on growth under the given light source within its maximum and minimum range as shown in Fig. 2.

Further analysis focusing on lights with high green ratio was conducted based on the results derived from Table 3. Spectrum data of the yellow section and growth data using 0G and 20G LEDs were ignored. As shown in Table 4, there are four types of light sources: two LEDs and two FLs (40G, LED CW, FL CW, and TLRA) with the green ratio of 18.8% to 30.7%. The results shown in Table 4 indicate that the relative contributions of red (69.21) and blue (85.09) were higher than data shown in Table 3. This showed that the contribution of green light was decreased when the intensity of green light increased in the range of 0% to 30% of *PAR*. Column 3 of Table 4 also indicates that the range of blue-green (475–525 nm) has no impact on FW accumulation of lettuce under conditions of green light at 18% to 30% of *PAR*.

Green light was often assumed to be unimportant in driving photosynthesis mainly due to the low absorptivity in the absorption spectra of purified chlorophylls (Sun et al., 1998). In a spectrophotometer cuvette containing dissolved chlorophyll, the internal reflection did not occur, so the absorption of green light was considered to be very low (Salisbury and Ross, 1992). Although the chances of green wavelengths

Table 1. *PPF*, shoot FW, DW, and percentage of water of lettuce grown under six light sources.

Type of lights	<i>PPF</i> ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	R_{660}/R_{640}^z	Parameter ^y		
			FW (g)	DW (g)	Water (%)
0G	149.56 ± 24.89	1.07	85.40 ab	2.58 ab	96.86 bc
20G	149.11 ± 24.37	3.29	90.94 a	2.08 cd	97.72 a
40G	150.87 ± 23.75	0.08	83.26 ab	2.42 abc	97.11 bc
LED CW	147.76 ± 18.66	0.64	87.01 ab	2.77 a	96.80 c
FLCW	147.46 ± 12.49	1.23	72.21 c	1.96 d	97.33 ab
TLRA	150.15 ± 21.70	3.37	78.33 bc	2.20 bcd	97.29 ab

^zThe ranges for R_{640} and R_{660} were 635–645 nm and 655–665 nm.

^yMeans followed by different letters in three columns were significantly different at 5% level by Duncan's multiple range test.

DW = dry weight; FW = fresh weight; LED CW = cool-white light-emitting diode; FLCW = cool-white fluorescent lamp; *PPF* = photosynthetic photon flux; TLRA = plant light fluorescent lamp.

Table 2. Contribution of equally distributed three spectral sections within photosynthetically active radiation (400–700 nm) range to the harvested shoot FW of boston lettuce at day 39 after seeding under six light sources.

Types of light	Range			FW (g)
	400–500	500–600	600–700	
	100 nm	100 nm	100 nm	
0G	21 (%)	0	79	85.40
20G	26	23	51	90.94
40G	26	40	34	83.26
LED CW	28	51	21	87.01
FLCW	36	40	23	72.21
TLRA	12	30	58	78.33
Calculated results	B_1	B_2	B_3	R^2
Contributions	9.549E-03	9.204E-03	1.014E-02	0.15
Relative contributions	103.75	100.00	110.21	

FW = fresh weight; LED CW = cool-white light-emitting diode; FLCW = cool-white fluorescent lamp; TLRA = plant light fluorescent lamp.

Table 3. Contribution of five unequally distributed spectral sections within photosynthetically active radiation (400–700 nm) range to the harvested shoot FW of boston lettuce at day 39 after seeding under six light sources.

Types of light	Range					FW (g)
	400–475	475–525	525–575	575–625	625–700	
	75 nm	50 nm	50 nm	50 nm	75 nm	
0G	16.0 (%)	3.95	0.26	7.70	72.1	85.40
20G	18.6	15.8	12.3	2.16	51.1	90.94
40G	18.1	26.3	18.8	7.57	29.2	83.26
LED CW	24.2	9.41	30.7	24.3	11.4	87.01
FLCW	25.4	14.8	27.5	24.6	7.71	72.21
TLRA	6.73	5.72	20.1	32.9	34.6	78.33
Calculated results	B_1	B_2	B_3	B_4	B_5	R^2
Contributions	5.22E-03	3.39E-03	2.21E-02	1.52E-16	1.24E-02	0.92
Relative contributions	23.62	15.34	100.00	0.00	56.30	

FW = fresh weight; LED CW = cool-white light-emitting diode; FLCW = cool-white fluorescent lamp; TLRA = plant light fluorescent lamp.

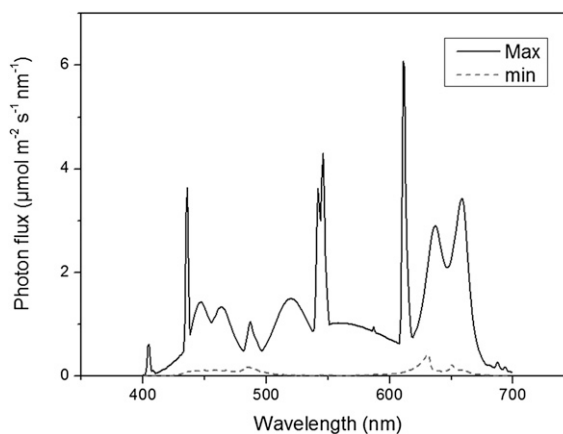


Fig. 2. Maximum and minimum intensity of spectral distribution of six light sources used in this study (0G, 20G, 40G, cool-white light-emitting diode, cool-white fluorescent lamp, and plant light fluorescent lamp).

Table 4. Contribution of five unequally distributed spectral sections within photosynthetically active radiation (400–700 nm) range to the harvested shoot FW of boston lettuce at day 39 after seeding under four light sources.

Types of light	Range					FW (g)
	400–475	475–525	525–575	575–625	625–700	
	75 nm	50 nm	50 nm	50 nm	75 nm	
40G	18.1 (%)	26.3	18.8	7.57	29.2	83.26
LED CW	24.2	9.41	30.7	24.3	11.4	87.01
FLCW	25.4	14.8	27.5	24.6	7.71	72.21
TLRA	6.73	5.72	20.1	32.9	34.6	78.33
Calculated results	B ₁	B ₂	B ₃		B ₅	R ²
Contributions	1.19E-02	2.15E-16	1.71E-02		1.46E-02	0.99
Relative contributions	69.21	0.00	100.00		85.09	

FW = fresh weight; LED CW = cool-white light-emitting diode; FLCW = cool-white fluorescent lamp; TLRA = plant light fluorescent lamp.

being absorbed by chlorophylls were small, the wavelengths that were not absorbed would be repeatedly reflected from chloroplast to chloroplast between the photosynthetic cells. With each reflection, a small percentage of the energy was absorbed until half or more was absorbed and used in photosynthesis (Kim et al., 2006). In addition, the active spectra for photosynthesis showed that green light was in an effective spectral region to power up photosynthesis in higher plants (McCree, 1972). In thylakoids, most of the carotenoids could efficiently transfer their excitation energy to the same reaction centers like chlorophylls, so they also contributed to photosynthesis (Siefertmann-Harms, 1987; Taiz and Zeiger, 1991).

Kim et al. (2004a, 2004b) reported that lettuce showed better growth and higher photosynthetic rates with the addition of 5% supplemental green light (500–600 nm) compared with only using the red (600–700 nm) and blue (400–500 nm) LEDs, and the addition of 24% green light (500–600 nm) could positively enhance plant growth. Leaves in the lower canopy would be able to use the transmitted green light in photosynthesis and perhaps reduce levels of leaf senescence and/or shedding within the canopy (Preece and Read, 1993). Green light was effectively absorbed by green leaves, and efficiently drove electron transport (Sun et al., 1998). Terashima et al. (2009) indicated that green light mixed with strong white light drove photosynthesis more effectively than red light in sunflower leaves.

The contribution indexes of different spectral sections to the growth of lettuce (B_i) are shown in Table 3. It was a combination of effects related to plant photosynthesis, leaf transmittance, g_s , and carbon fixation. The index of spectral section from 575 to 625 nm was 1.52E-16, which indicated that this portion of wavelength made no contribution to FW of lettuce.

Researchers categorized wavelengths from 500 to 600 nm as “green” light and concluded that additional green light promotes plant growth. This study further clarified that green light only within the range of 525 to 575 nm promotes plant growth.

Dougher and Bugbee (2001) suggested that “yellow” light from 580 to 600 nm suppressed chlorophyll or chloroplast formation. In *Euglena*, Schwartzbach (1990) determined that “green” light (peak at 597 nm) was less effective than blue in the synthesis of chlorophyll when light was saturating. Al-Wakeel and Hamed (1996) showed more growth suppression under “yellow” (peak at 595 nm) than “green” (peak at 520 nm) light in cucumbers. This study had consistent results and agreed with these studies.

The proposed model only considered the effect of single spectral section on lettuce and it did not consider the effect of interaction between spectra sections. For example, adjusting the ratio of blue and green light could stimulate the stomatal opening (Frechilla et al., 2000; Kim et al., 2004a; Talbott et al., 2002) and Emerson enhancement effect involved far red light (Govindjee, 1964). Therefore, this model can only be used to analyze spectrum of light sources within the minimum and maximum ranges as shown in Fig. 2. Nevertheless, optimum light spectral design can be derived from such models with further investigation.

Conclusions

A model was proposed to investigate the effects of various spectral ranges on the growth of plants in terms of FW and/or DW. Boston lettuce was used as the example. The low R^2 value suggested that the traditional analytical method which separated *PAR* range into R, G, and B sections was somehow misleading. This study separated the same range into five sections and revealed that the green light (525–575 nm) has much higher contribution than red (625–700 nm) and blue (400–475 nm). The researchers categorized wavelengths from 500 to 600 nm as “green” light and concluded that additional green light promotes plant growth. This study further clarified that “green” only within the range of 525 to 575 nm promotes plant growth. “Green-yellow” range from 575 to 625 nm has relatively very little effect on the growth of boston lettuce.

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