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Asian Journal of Plant Science Research, 2022, 12 (6):1-9

Research Article

ISSN: 2249-7412
CODEN (USA): AJPSKY

Effect of Different Light Wavelengths on Growth and Quality of Lettuce

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Received date: February 18, 2022, Manuscript No. AJPSKY-20-3418; **Editor Assigned date:** February 21, 2022, PreQCNo. AJPSKY-20-3418(PQ); **Reviewed date:** March 07, 2022, QC No. AJPSKY-20-3418; **Revised date:** August 22, 2022, QI No. AJPSKY-20-3418; Manuscript No. AJPSKY-20-3418(R); **Published date:** September 19, 2022, DOI: 10.36648/2249-7412.12.6.224

Citation: Khan K (2022) Effect of Different Light Wavelengths on Growth and Quality of Lettuce. Asian J Plant Sci ResVol: 12 No: 6.

ABSTRACT

In order to investigate the effect of light wavelength on growth and physiology of lettuce plants, Romaine lettuce and sunny leaf lettuce were chosen as targets for the study. Using single color LED lights (white, red, green, blue), leaf absorption of light, photosynthetic rate, stomatal conductance and transpiration rate of romaine lettuce leaves were examined. The results showed low correlation of light absorption percentage and stomatal conductance to photosynthetic rate of romaine leaf. However, transpiration rate and photosynthetic rate have high correlation, suggesting a relationship between these two processes. Sunny lettuce plants were cultivated on rockwool using hydroponic method under different light source including fluorescent lamb and LED of different colors (white, red, green, blue and rainbow). Plants illuminated by fluorescent lamp gave the highest dry weight, while rainbow LED provided plants with the lowest fresh and dry weight. Plants under white, red, green or blue LED do not differ significantly about fresh and dry weight. These results suggest that combination between different types of LED is required to achieve the best yield of lettuce plants.

Keywords: *Lettuce plants; Romaine lettuce; Sunny leaf lettuce; Romaine lettuce*

Introduction

Light has been one of the most important factors which affects almost every physiological process of plants, not only photosynthesis, but also respiration, transpiration, metabolism and development. Despite being such an important factor, light's effects on plants have never been completely understood because plants perceive light from several different aspects, including intensity, photoperiod and light quality. While studies in previous decades mostly focus on light intensity and photoperiod effects, recent researches have paid more attention to the effects of light quality, or portions of different light wavelength, on the physiology of plants [1]. The availability of single color LEDs and significant improvements in light intensity, shelf life, energy efficiency as well as price reduction of these light sources recently have opened the possibility for the application of specific color LED in artificial light cultivation of plants, as well as paved a way for studies on the effect of light wavelength on plants. Several studies have confirm that light wavelength has profound effects on photosynthesis and growth, as well as stomatal conductance and transpiration. In regard to light quality, the impact of red and blue light is more pronounced, indoor cultivation system as compared to outdoor since these wavelengths are largely absorbed by photosynthetic pigments. Red light being crucial part of light spectrum, induce transformation in phytochrome, thereby effecting plant morphogenesis as well as regulating the synthesis of phytochemicals such as phenolics and oxalate. Likewise, blue light is vital for regulating biosynthesis of chlorophyll and anthocyanin along with, the chloroplast development, stomatal opening, and photo-morphogenesis.

This research focus on investigating the effects of different single color LED lights on the growth and physiology of lettuce. Due to the fast growth rate and ease of cultivation in hydroponics system, lettuce has been one of the most potential target plants for the application of artificial light cultivation in greenhouses and plant factories. In order to improve light usage efficiency and enhancing plant growth, many researches have been conducted about using LED lights with different colors, with many results have been published about the most effective ratio of colors for lettuce growth. However, less is known about the relationship among the changes in the plant physiological processes (photosynthesis, transpiration, stomata conductance, light absorption, etc.) under different light qualities. This research, through combining and comparing plant growth and the changes in plant physiology under different light colors, attempts to provide a better understanding of the mechanism by which light quality affects plant growth, which could help orienting the combination of different colors for better efficiency in artificial light cultivation of lettuce [2]

Materials and Methods

6 week-old romaine lettuce plants cultivated in greenhouse under natural light were used as materials. Randomly selected part of leaf area (5 × 5) cm without leaf veins were collected and used to measure leaf transmittance and reflectance with an integrating hemisphere (Figures 1 and 2). LED lights of different colors (white; red, 630 nm; green, 520 nm; blue, 465 nm) with the intensity of 20 μmol m^{-2} s^{-1} were used as light source [3]. The spectra of the LED lights were measured with Spectroradiometers SR9910-V7 (IRRADIAN Ltd.). The method of measuring leaf transmittance and reflectance is summarized in Table 1.

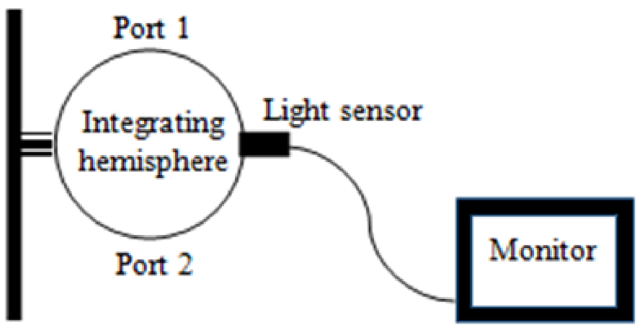

Figure 1: Integrating hemisphere for measuring leaf absorption of light.

Figure 2: SR9910-V7 spectroradiometer.

Table 1: Method of measuring leaf transmittance and reflectance.

Measurement	Symbol	Port 1	Port 2
100% transmittance/reflectance	(A)	LED light	Mirror
Leaf transmittance	(B)	LED light on top of a piece of leaf	Mirror
0% transmittance	(C)	LED light on top of a black rubber	Mirror
Leaf reflectance	(D)	LED light	A piece of leaf on top of the mirror
0% reflectance	(E)	LED light	A black rubber on top of the mirror

The measurement was repeated two times for each light color. Leaf absorption of light is calculated by the following equation:

$$\text{Leaf absorption } (\%) = 100 - \frac{(B-C)}{(A-C)} \times 100 - \frac{(D-E)}{(A-E)} \times 100$$

Net photosynthesis rate, intercellular CO_2 concentration, stomatal conductance and transpiration rate were measured by LI-COR 6400 (LI-COR, Inc., USA). The measuring chamber was mounted on an unseparated leaf of a 6 week-old romaine lettuce plant [4]. The leaf was illuminated with white, red (635 nm ± 5 nm), green (522 nm ± 5 nm) and blue (460 nm ± 5 nm) light from an integrated 6400-18 RGB light source. Light intensity was set at 600 µmol m^{-2} s^{-1}, CO_2 concentration 400 µmol mol^{-1}, temperature and relative humidity were not controlled. Each time light color was changed, the system was left for 30 minutes to stabilize, then measurement of net photosynthetic rate, intercellular CO_2 concentration, stomatal conductance and transpiration rate of the leaf were recorded. Plant growth, morphology and chlorophyll content of sunny lettuce grown under different light colors [5].

Sunny lettuce (Lactuca sativa var. crispa) were used as material plant for the experiment. The plants were germinated and grown on rockwool in greenhouse at Kashiwanoha Campus, Chiba University under natural light for 3 weeks. Every week Enshi nutrient solution with EC 1.2 and pH 7.0 was supplied to the plant [6]. After that, the plants were move to growth chambers illuminated with fluorescent lamps or LEDs of different colors; white, red (630 nm), green (520 nm), blue (470 nm) or rainbow (combination of 7 colors from red to purple) with the light intensity of 200 µmol m^{-2} s^{-1} and photoperiod of 14 hours [7]. The plants were allowed to grow for 3 more weeks with the same nutrient supply condition as previously. Plants which were kept in the greenhouse were used as control (Figures 3 and 4).

Figure 3: LI-COR 6400 system.

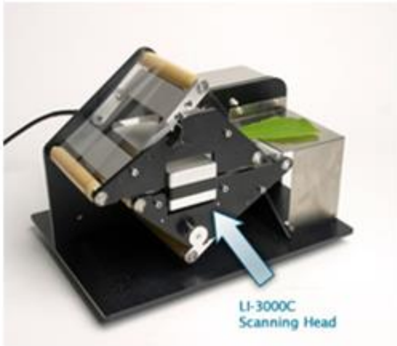

Figure 4: LI-3050C Transparent Belt Conveyor with mounted Li-3000C Leaf Area Meter for measuring leaf area.

At the end of experiment, leaf number, leaf length and fresh weight of plants in each treatment were recorded. Total leaf area was measured with Li-300°C Lea f Area Meter mounted on LI-305°C transparent belt conveyor (LI-COR, Inc., USA). For each plant one area of leaf without leaf veins was chosen to measure chlorophyll content with SPAD 502 plus chlorophyll meter (Spectrum Technologies, Inc., USA). Plant biomass were dried in 105°C for 3 hours, then stored in 80°C condition for 4 days and plant dry weight was recorded [8]. The experiment was replicated 3 times, with 6 plant for each replication. In order to test the accuracy of SPAD 502 plus chlorophyll meter, 18 measurements were performed on sunny lettuce leaves with both SPAD 502 plus chlorophyll meter and dualex scientific chlorophyll meter at the same location on the leaves. 0.56 cm^2 of leaf at the same location was also taken and chlorophyll was extracted with 1 ml N-N-dimetylformamide in dark for 4 days at 4°C.

The absorbance of the solution at 647, 664 and 750 nm was recorded by photospectrometer, and chlorophyll content was calculated by the following equation;

Chlorophyll (a+b)=7.12 × (A664 - A750)+17.67 × (A647-A750)

The data were calculated with Microsoft Excel 2013. Statistical analysis was performed with MSTATC 2.1 program from Michigan University, USA.

Results and Discussion

Light absorption, net photosynthetic rate, stomata conductance and transpiration rate of romaine leaf under different light colors. Light spectrum measurement of LED light with different colors showed that the single color LEDs have relatively sharp peak. Red LED has a peak dragging from 580 nm to 665 nm, maxing at 630 nm. Blue LED has a peak ranging from 425 nm to 530 nm, with highest point at 465 nm. The peak of green LED is relatively broad, covering a large range from 465 nm to 600 nm, with highest peak at 520 nm. Parts of the green LED peak and blue LED peak overlap each other. This may cause some indifference between the data from green LED and blue LED treatments [9].

As compared to single color LEDs, white LED has a unique spectrum, with 2 relatively distinctive peaks. The first peak is sharp, from 405 nm to 485 nm, with highest point at 450 nm, corresponding well to the blue LED peak [10]. The second peak is lower and broader, maxing at 545 nm and dragging a long tail up to 750 nm. With this spectrum, white LED may have some overlapped effects on plants with blue LED. However, high emission at the yellow region (560-600 nm) may give white LED light special effects on plants which are not observed even when combining all three types of single color LEDs [11].

It has been recognized early that the absorption spectrum of both chlorophyll a and b (two types of chlorophyll presenting in plants) have two peaks at the red (near 680 nm) and blue (near 460 nm) regions, while in the green region chlorophyll absorbs little light. Based on this, it was assumed that blue and red light are the most effective for photosynthesis and growth of plants, while green light was expected to have no contribution to photosynthesis and plant growth [12].

The measured absorption of different types of LED light by leaf indeed confirmed that romaine leaves absorb high percentage of red light and blue light (over 80%). Surprisingly, high portion of green light (69%) was also absorbed by romaine leaves. Light stimulates photoreceptors which act as morphogenetic signals in growth and development of plants and provides energy for their physiological activity. This might be the reason of improvement of shoot fresh weigh in the current investigation. The outcomes are in accordance who found light quality and nitrogen supply improved plant growth.

This result showed that romaine leaves contain many other pigments beside chlorophylls which can absorb green light [13]. On the other hand, white LED has very low absorption percentage by romaine leaves (only 55%). This could be due to high portion of light energy in the yellow region, which is poorly absorbed by plant pigments (Figure 5).

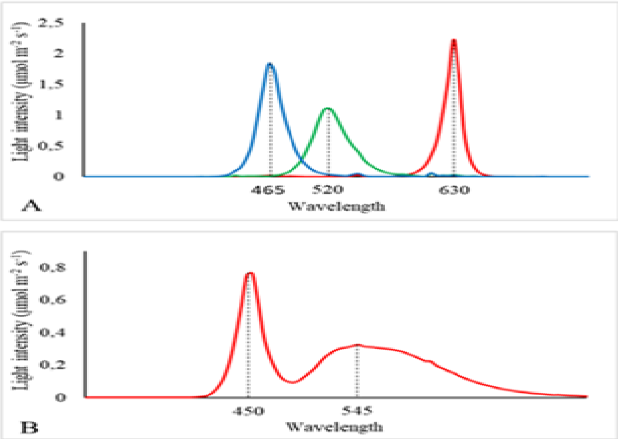

Figure 5: Emission spectrum of red, green, blue (A) and white (B) LED lights.

Contradictory to light absorption profile, photosynthetic rate of romaine lettuce leaf was lowest when illuminated by blue light, while that of red light was highest. Surprisingly, both green and white LED lights provide high net photosynthetic rate (89% and 93% as compared to red LED, respectively), even higher than that caused by blue LED light, despite the low light absorption percentage of these two LED lights [14].

Red light seemed to reduce stomata opening and transpiration, while blue light increased the stomatal conductance as well as transpiration rate of romaine leaf (Figure 6 and Table 2).

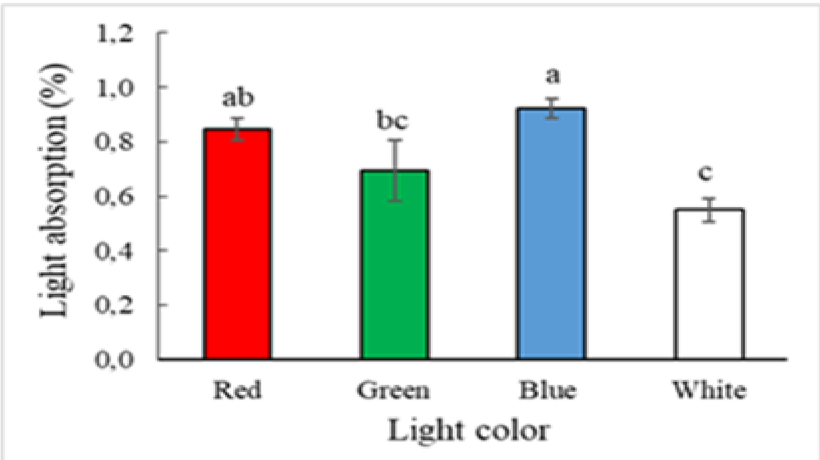

Figure 6: Absorptionof LED light with different colors by romaine lettuce leaf.

Table 3: Net photosynthetic rate, intercellular CO_2 concentration, stomatal conductance to H_2O and transpiration rate of romaine lettuce leaf.

Light color	Net photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)	Intercellular CO_2 concentration (µmol CO_2 mol^{-1})	Conductance to H_2O (mol H_2O m^{-2} s^{-1})	Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)
White	18.8	307	0.535	2.89
Red	20.1	295	0.49	2.77
Green	17.8	309	0.5	2.99
Blue	17.3	313	0.52	3.09

Regression analysis showed that the correlation between net photosynthetic rate and light absorption, as well as between net photosynthetic rate and stomatal conductance, sharply decline. These results clearly showed that the effects of light colors on photosynthesis cannot be explained simply by leaf absorption spectrum, nor can it be explained through indirect effect of light colors on stomata opening. It could be due to the differences between light absorption spectrum and photosynthetic action spectrum of plant leaf [15]. McCree measured the absorption spectrum and quantum yield spectrum of 22 different crops including lettuce. The data showed that although lettuce plant absorbed blue light and red light equally, the quantum yield of photosynthesis was much lower in the region of 350 nm-475 nm than in the region of 550 nm-675 nm of the spectrum. It is possible that the high energy state of blue light photon is not at suitable level for driving photosynthesis reaction, and a large portion of the energy was converted to heat or undesired chemical reactions (photorespiration, oxidative reaction, etc.) and wasted [16].

Transpiration rate also didn't have a high correlation with stomatal conductance. On the other hand, photosynthetic rate seemed to correlated well with transpiration rate and internal CO_2 concentration. These results suggested that there may be a relation between net photosynthetic rate and transpiration rate of romaine under the effect of different light wavelengths (Figure 7).

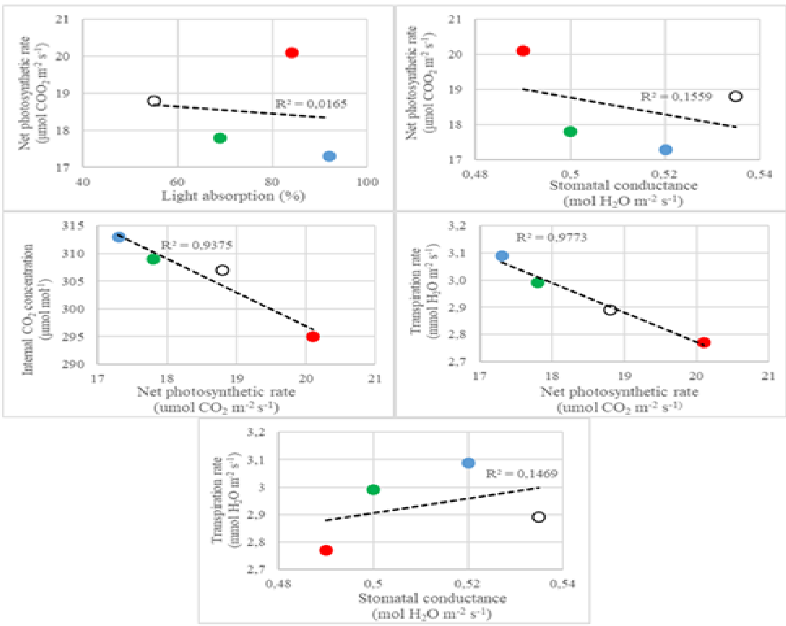

Figure 7: Relationship of light absorption, A) stomatal conductance; B) internal CO_2 concentration; C) transpiration rate; D) to net photosynthetic rate, as well as between transpiration rate and stomatal conductance; E) of romaine lettuce leaf illuminated by LED with different colors.

Plant growth, morphology and chlorophyll content of sunny lettuce grown under different light colors. Regression analysis between chlorophyll contents measured by chlorophyll meters and extraction method showed low correlation. This could be due to high amount of anthocyanin presenting in sunny lettuce leaves, which could interfered with chlorophyll content measurement by the chlorophyll meters. Among the two meters, SPAD 502 Plus gave a higher R2 value as well as higher slope than Dualex Scientific [17]. Therefore, the first chlorophyll meter was chosen as equipment for measuring chlorophyll content in next experiment (Figure 8).

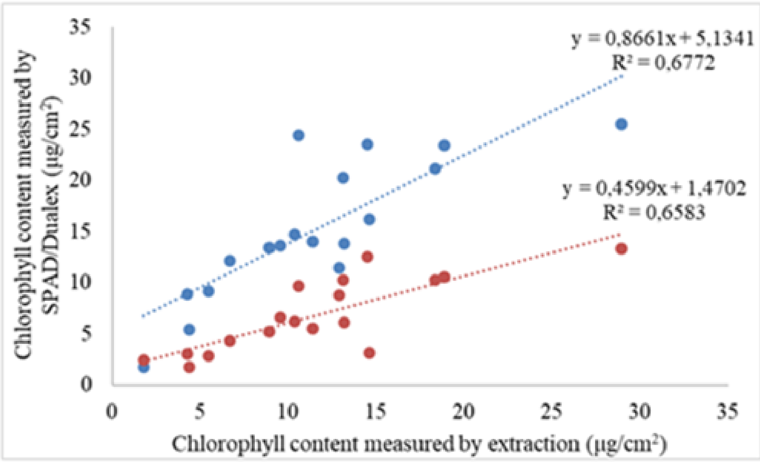

Figure 8: Correlation of chlorophyll content measured by SPAD 502 Plus and Dualex Scientific chlorophyll meter against measured by extraction method with N-N-dimetylformamide.

Table 3: Leaf length, leaf number, leaf area and chlorophyll content of sunny lettuce grown under different light colors.

Treatment	Leaf length (cm)			Leaf number		Leaf area (cm^2)			Chlorophyll content ($\mu g/cm^2$)		
Control	19.5			7.8		442.4			15.7		
Fluorescent	15.8	c[y]	**z	7.2	ns	433.7	a	ns	15.3	a	ns
White	18.4	bc	ns	7.5	ns	482.7	a	ns	12.2	a	ns
Red	21.8	a	ns	9.5	ns	508.3	a	ns	7.3	b	**
Green	21.4	ab	ns	7.5	ns	409.1	ab	ns	5.7	b	**
Blue	20.1	ab	ns	7.3	ns	479.7	a	ns	13.8	a	ns
Rainbow	19.4	ab	ns	7.2	ns	306.7	b	ns	8.3	b	**
ANOVA[x]	**			ns		*			**		
CV (%)	6.6			11.6		14.3			13.2		

Note: x: ns, *, **; non-significant or significant at $p \leq 0.05$ or 0.01, respectively.
Y; Means in the same column followed by the same letters are not significantly different at the 5% or 1% level by LSD Test.
z: ns, **; non-significantly or significantly different to control at $p \leq 0.01$.

As showed, different types of light caused significant changes in leaf length and leaf area of sunny lettuce plants. Red LED light gave the biggest leaves both in length and in area, while fluorescent light provided the shortest leaves, significantly shorter than control and most of the other treatments except white LED. Rainbow LED gave the smallest leaves in term of leaf area. Red, green and rainbow LED lights also significantly reduced chlorophyll content in leaves as compared to control and other treatments (Table 3).

Among all treatments, fluorescent light gave the highest dry weight, equaling with control plants in greenhouse, although the fresh weight of plants in this treatment is low. Among the LED light treatments, blue light gave the highest dry weight, although not significantly different from white, red and green treatments. Both fresh weight and dry weight of plants under green and rainbow LEDs are low, which could be due to low chlorophyll content as well as low efficiency in absorbing and using light. Red light, on the other hand, gave moderate fresh weight and dry weight despite the low chlorophyll content. This could be the result of high quantum yield of plant in the red region of light spectrum.

Based on these results, it is observed that florescent light produced plants with high percentage of dry matter (9.9%), which are somewhat similar to control plants grown in greenhouse. This might be due to the emission spectrum of fluorescent light is quite similar that of natural light, with the spectrum spreading somewhat evenly from 380 to 780 nm. White LED light, on the other hand, has low emission at around 480 nm and 600 nm-780 nm region (red), which could lead to significant reduction in plant growth and dry weight accumulation. Therefore, it may be required that white LED is combined with monochromatic LED lights to provide the most suitable spectrum for plant growth. Similary results were found. The results of this study were agreed with the findings (Figures 9 and 10).

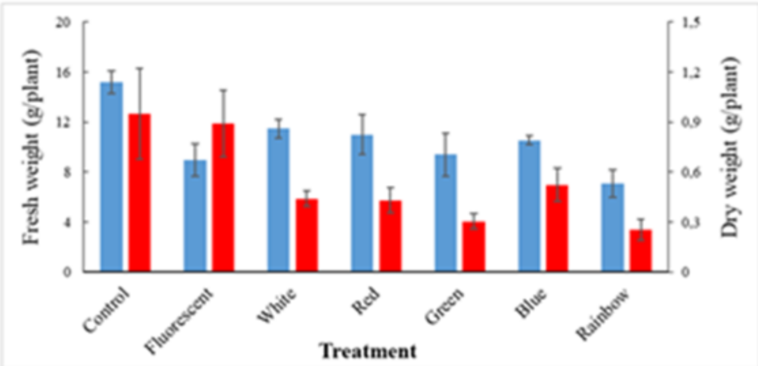

Figure 9: Fresh and dry weight of sunny lettuce plants grown under different light source.

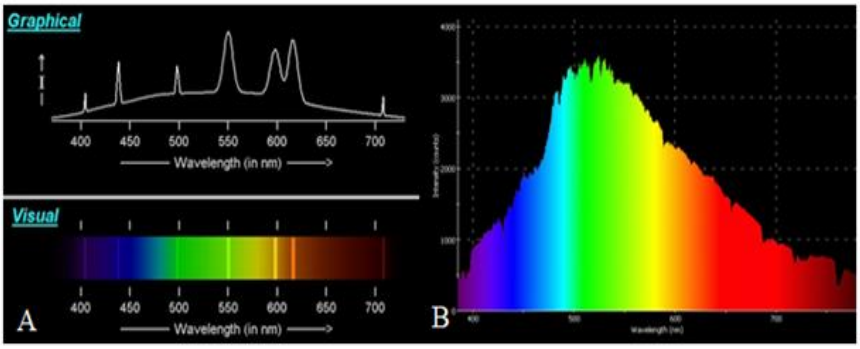

Figure 10: Light spectra of cool fluorescent lamb (A) and natural sunlight in visible region (B).

Conclusion

Through this research, it is confirmed that the effects of different single color LED lights on plants are the results of complicated interactions and mechanisms. Light absorption of specific colors by leaf seemed not to contribute much to photosynthetic rate under different light wavelength. However, high correlation between photosynthetic rate and transpiration rate were found, suggesting a relationship between these two processes. Plant growth and dry matter accumulation seems to be higher in fluorescent illuminated plants than LED illuminated plants, which may be due to a suitable light spectrum by fluorescent lamb. In this research, it is confirmed that green light can contribute significantly to photosynthesis and growth, although the photosynthetic rate and growth under green light is still lower than that under red or blue light. More studies are needed to find a suitable combination of LED lights of different colors to achieve best yield of plant.

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