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Morphological response of tomato seedling under two periods of different red and blue photon flux ratio

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ABSTRACT

Previous researches demonstrated beneficial effects of the red (R) and blue (B) LED light for plant growth and development under the single format of the light spectrum. In these studies, the light spectra, intensities, and light hour controlled by growers in the growth chamber by supplying LED light with constant R/B ratio during the experimental period were indicated as important parameters. The purpose of this work is to investigate the effects of spectral distribution on the morphological growth of tomatoes seedling such as plant height, stem diameter, leaf area, leaf number, leaf thickness and leaf color, and to examine the effects of varying R/B LED light ratios between two periods of the young tomato. Tomatoes were soil-cultured with a 14-h photoperiod at 29/26°C, and 55%/75% relative humidity, under RB0.34+1.0 at 100-150 μmol $m^{-2}s^{-1}$ (varying between two periods), commercial LED growth light (RB1.75) at 100 μmol $m^{-2}s^{-1}$ (constant one period), and white LED at 100 μmol $m^{-2}s^{-1}$ (as a control) inside growth chambers for 25 days. The analysis of variance statistic was applied to determine the mean difference of data. The results found that the tomato seedling under (RB0.34+1.0) has the best PAR spectrum to support the highest stem diameter, leaf thickness, and leaf color (highest chlorophyll content). Moreover, the tomato seedling grown under commercial LED light is also acceptable. The advantage of RB0.34+1.0 treatment is very good for promoting high quality tomato seedlings, perfect leaf and stem, for reducing the stem damage on the transplants, and increasing the tomato production.

1. Introduction

Light is a major factor that contributes to the developmental processes of all living thing, including plant photosynthesis mechanisms. The light quality has a significant effect to the physiology of plants. Photosynthesis needs light within visible light spectrum (Kang et al., 2013). Plants that accept any wavelength of light in accordance to the PAR spectrum (400 to 700 nm) would lead to the improvement of photosynthesis and plants production (McCree, 1972). The blue light of 400 to 520 nm and red light of 600 to 720 nm contributed most to the photosynthesis process of the plants (Hogewoning et al., 2010; Trouwborst et al., 2016).

Most of the previous studies demonstrated the effect of R and B LED light in several different R/B ratios to promote plant growth. It can be concluded with the previous results that (1) if the ratio of R and B LED light is the same (R/B=1), there would be a positive effect to the vegetative growth of Lilium oriental hybrids (Lian et al., 2002), Wasabi japonica (Kim and You, 2013), Cos lettuce (Jishi et al., 2016) and could promote the leaf number of tomatoes (Hernandez and Kubota, 2016; Fan et al., 2013). (2) If the ratio of the B light is higher than the R LED light (R/B less than one), there could be a promotion in the shooting and seedling of very young plants such as lettuce (Lin et al., 2013), Orchidaceae (Godo et al., 2011), and tomatoes (Hernandez and Kubota, 2016). The effect of B light, which indicated the highest of chlorophyll content of micro-greens (Lobiuc et al., 2017), could also promote the leaf thickness of tomato plants. (3) If the ratio of the R light is higher than the B light, there would be contribution to the vegetative growth, flowering, and fruits yield. Plants that showed these results are, for instance, Chinese cabbage (Averchera et al., 2008), strawberries (namely the fruits yield) (Samuoliene

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et al., 2010; Yoshida et al., 2016), radish (Samuoliene et al., 2011), and tomato fruits (Xu et al., 2016).

Tomato is one of the most widely distributed plants in the world. Tomato seedlings are mainly produced in a controlled environment on a big scale to meet the increased production demands (Fan et al., 2013). This is an interesting point which motivated the authors in choosing the tomatoes for our study. The research question is whether there is a difference in the morphological response of tomato seedlings undergoing treatment by LED light in two periods to seedlings undergoing constant treatment of LED light in one period?.

This paper presents the morphological response of young tomato plants (25 days after sowing) to treatment by the LED light in two periods. These periods are 1st period supplying a higher ratio of B light to R, and the 2nd period supplying an equal ratio of B light to R. In addition, there is also a comparison of the young tomato plants under constant (R/B ratio 1.75) at a bit higher ratio of R light to B (supplied by the commercial LED grow light) in one period during 25 days. The objective of this study is to investigate the effects of spectral distribution on the morphological growth of tomatoes. To do so, the authors have examined the effects of different R and B light ratios during two periods of the young tomato.

2. Material and methods

2.1. Plant and growth conditions

Seed of tomato "Sida Kam-mam 333" (Known-you Seed Co., ltd, Thailand) were sown into rectangle growing tray with 15 pots(pot diameter is 10 cm) containing loamy soil, compost, paddy husk charcoals, and coconut dust in the same quantify and placed in the growth chamber (60 cm × 60 cm × 80 cm). The growth chamber is placed in the temperature control room. The temperature is maintained at 29/26°c (day/night) and the humidity is at 55±7%/73±3% (day/night). There is one control group and two experimental groups, each group consisting of 15 pots per tray, and within one pot there are 3 tomato seeds. Twenty milliliter of tap water was supplied to each pot once a day in the morning.

2.2. Treatments and LED lighting system

1. Ctrl is the control group. This group receives no light treatment on 1st to 3rd day after sowing. During the 4th to 25th day warm white LED light was supplied on the top of growing tray at PPFD 100 µmol m^{-2}s^{-1}; the light/dark hour is 14/10 (Fig. 1A). The PAR spectrum of the Ctrl treatment is shown in Fig. 1B.

2. RB0.34+RB1.0 are the first experimental groups, (Figs. 1A and 2B) with treatments of different R and B LED light in two periods. The first ten day the supplied R/B ratio is 0.34 (R 25%, B 75% of PPFD) at PPFD 100 µmol m^{-2}s^{-1}. The PAR spectrum of RB0.34 treatment is shown in Fig. 1C. After that, from day 11st to 25th the R/B ratio applied is 1.0(R 50%, B 50% of PPFD) at PPFD 150 µmol m^{-2}s^{-1}.ThePAR spectrum of RB1.0 treatment is shown in Fig. 1C.

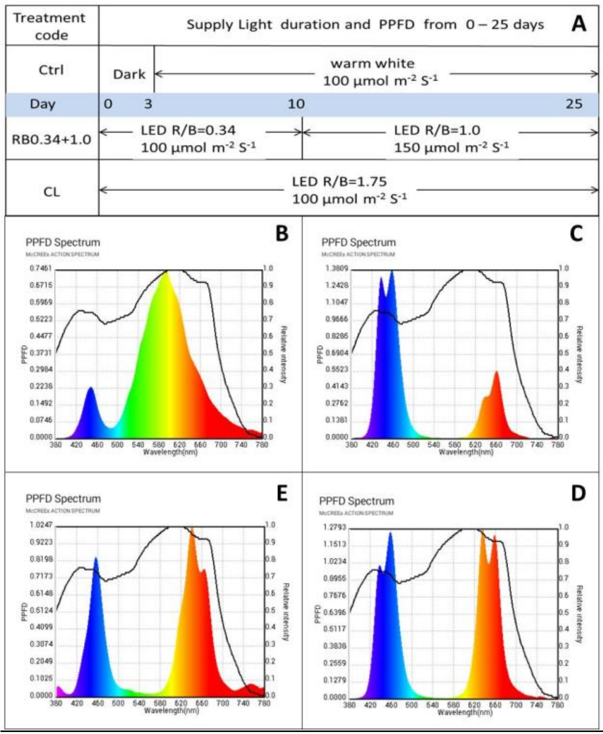

Fig. 1: (A) Experimental diagram of all experimental groups. (B) Spectral distribution of the light treatment of the Ctrl group. (C) Spectral distribution of the light treatment of the RB0.34+1.0 group (day 1 to 10), and (D) on day 11 to 25. (E) Spectral distribution of the light treatment of the commercial LED light source (CL) (day 1 to 25)

3. CL is the second experimental group (Fig. 2C), with light treatment from commercial 180W model UFO180 LED grow light (BOSSLED Shenzhen, China). The author measured the R/B ratio of the LED grow light and obtained the result of R/B = 1.75. The spectrum distribution is shown in Fig. 1D and Fig. 2C. The CL group was treated from day 1st to 25th of tomato seedling. The light/dark hour of the second and the third group was applied in the same hour as the first experimental group.

4. The LED arrays used for the control group are custom made; the light panel (16cm long × 12.5cm wide) consists of 16 of 10W, 900lm of warm white. (Chanzon Company, China) The LED circuit was connected in two series and eight parallel with 24V DC power source. The PPFD of the LED panel was controlled by 24V LED driver with PWM dimming (Fig. 2A). The custom-made LED panel for the 1st experiment group (26 cm long×12.5 cm wide) consist of high power LED bleed type of 3W 8mm diameter. The number of R LED is 45 (25×420nm+20×460nm), and B LED is 45 (25×620nm + 20×660nm). The ratio of R/B was tunable by micro-controller (Arduino). There are

0.33, 1.0, and 3.0 of R/B quantum light ratio. The highest PPFD is about 250 μmol $m^{-2}s^{-1}$ (Fig. 2B).

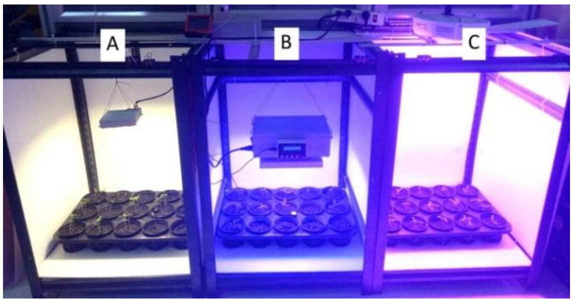

Fig. 2: An experimental set up of three growth chamber under (A) Ctrl treatment, (B) RB0.34+RB1.0 treatment, and (C) CL treatment

2.3. Measurements

The PPFD spectrum distribution of the LED light sources was measured by the spectroradiometer from Lighting Passport Pro Essence (Asensetek Incorporation, Taiwan). The growth of tomatoes was recorded at the first day of the treatment. The morphology of the tomatoes was investigated and measured, including leaf number, plant height, stem diameter, leaf thickness, leaf area, and leaf color. The root system was not focus in this study because it is very difficult to remove the soil from the root, and by doing so, the tomatoes' roots may be damaged.

The plant height was recorded every five days, measuring from the main stem base to the top of the plants (Fan et al., 2013). The leaf area was measured at day 25th, but for some study the experiment took place in 24 days (Matsuda et al., 2016). The leaf area was measured by the digital camera of the smart phone OPPO-A39, and analyzed by the software ImageJ (Schneider et al., 2012). The leaf area was analyzed from the 1st to the 4th of leaves of the nine plants (average area). The leaf thickness was measured from the 1st to 4th of leaves of the nine plants; the stem was measured from the same nine plants. The stem diameter and leaf thickness were measured by digital venire caliper 0-200 mm (Mitutoyo Crop., Kanagawa, Japan). The leaf color was measured from nine leafs of three plants (three leafs per one plant), selected from the center pot of each growing tray. The leaf color was analysed by the RGB histogram of the ImageJ software (Schneider et al., 2012). The RGB histogram showed 0-255 levels of color: the 0 level meant a very dark green and 255 level indicated the very light green color. If the leaf color shows the small RGB number, this means that the leaf contained a high concentration of chlorophyll.

2.4. Statistical analyses

Nine tomato plants with specific sampling from the 3 pots at the center of each treatment group was selected for the investigation the leaf number, plant height, stem diameter, leaf thickness, leaf area, and leaf color. The significant difference were analyzed by ANOVA ($p=0.05$). The mean separations were analyzed by the Tukey post hoc test ($p=0.05$). The IBM SPSS statistics was used for analysis (SPSS an IBM Company, New York, USA).

3. Results and discussion

3.1. Effect of R/B in two periods on the leaf number

In this study, the leaf number of tomato "Sida Kam-mam 333" grown under CL (RB1.75) treatment was highest with 17.666 leafs, and under RB0.34+1.0 treatment was 17.111, with no significant difference at $p<0.05$ (Table 1). However, the leaf number of tomato from ctrl group was lowest (14.777) and shown significant difference at $p<0.05$ (Table 1) with the leaf number under the RB0.34+1.0 and CL (Fig. 3A). This is in accordance with the study of Hernandez and Kubota as shown that the tomato leaf number (21 days) that received treatment by R and B LED light (100μmol m^{-2} s^{-1}) at 50B: 50R (R/B=1) and 30B:70R (R/B=2.33) are higher than other treatments and they are not significantly different at $p<0.05$ (Hernandez and Kubota, 2016). Moreover, the leaf number developed slowly during the first ten days after sowing, and the development was faster after day 10 to day 25 (Fig. 3A). That showed the normal tomato leaf developments; there are 1 or 2 leaves after 5 to 7 day after sowing (Lin et al., 2015). These showed the same effects with the leaf number of the "Lettuce Leaf" basil under the RB light (28days) of 61R12B (R/B=5), 65R8B(R/B=8) and 53R24B(R/B=2.5) with no significant difference. This indicated that the leaf number under R light more than B light were better than under R less than B light (Bantis et al., 2016).

3.2. Effect of R/B ratio in two periods on tomato plant height

This study found that the tomato plant's stem rises the highest under the control treatment. As observed in the dark treatment, in the first three days after sowing the tomato seedling was seen to have longer stem. That means that the tomato tried to extend its stem as high as possible in order to be able to reach the light source. After day 3rd to day 25th of control group treatment the light at PPFD 100μmol $m^{-2}s^{-1}$, which is lower than two experiment groups (Fig. 3E). From these situations, the effect of the light to the tomato seedling height can be seen in (Fig. 3B). The tomato plant that received treatment under low PPFD will be higher than under higher PPFD (Fan et al., 2013; Matsuda et al., 2016). Moreover, the tomato plant height under Ctrl treatment and under CL treatment were not significantly different at $p<0.05$, but they are significantly different ($p<0.05$) to the tomato plant under RB0.34+1.0 treatment (Table 1). The tomato plant height under two periods (RB0.34+1.0) of treatments was lower (18.044 cm) than the control tomato plant (20.60 cm), and CL (19.586 cm) tomato plant (Table 1 and Fig. 3B).

Table 1: Parameters measurement of the morphological response of Tomato "Sida Kam-mam" seedling in the growth chamber with LED light treatment 14/10 (light/dark) hours in 25 days

Light treatment	Plant height (cm)	Stem diameter (mm)	Leaf number	Leaf thickness (mm)	Leaf color	Leaf area (cm^2)
Ctrl	20.600 ± 1.807[a]	2.761 ± 0.320[b]	14.777 ± 1.394[b]	0.388 ± 0.088[b]	125.751 ± 14.77[a]	9.199 ± 6.68[a]
RB0.34+1.0	18.044 ± 1.135[b]	3.940 ± 0.65[a]	17.111 ± 0.927[a]	0.592 ± 0.077[a]	98.942 ± 17.23[b]	10.036 ± 7.36[a]
CL(RB1.75)	19.588 ± 0.523[a]	3.986 ± 0.501[a]	17.666 ± 0.866[a]	0.438 ± 0.063[b]	131.578 ± 16.32[a]	10.434 ± 6.56[a]

[ab]: Followed by the same letter are not significantly different at $p<0.05$(mean ± standard deviation)

3.3. Effect of R/B ratio in two period lighting supplies on tomato stems diameter and leaf thickness

The stem diameter of the tomato seedlings shows strong characteristics of the plant. If the tomato plants have a thicker stem, that mean this plant will be appropriate for transplanting and it could reduce the stem breakage (Hernandez and Kubota, 2016). In this study, it is shown that the stem diameter under RB0.34+1.0 (3.940 mm) has good thick stem and not significantly different *($p<0.05$)* to the tomato stem under CL (3.986 mm), but they were significantly different *($p<0.05$)* in control stem diameter (2.761 mm) (Table 1). Fig. 3C shows the stem diameter distribution data from nine tomato plants. It was shown that the stem of the control plant was thinner than others. That shows the young tomato plants were unhealthy. This study shows the stem diameter in accordance with the study of Hernandez and Kubota, that shows that under treatments 30B:70R (R/B=2.33) and 50B:50R (R/B=1), the stem diameters were 3.4 mm and 3.7 mm, respectively (Hernandez and Kubota, 2016). These are thinner than our study (3.986 mm, 3.94 mm), because the experimental period of the previous study was 21 days whereas the experimental period of this study was 25. Moreover, this result was confirmed by the study of Li et al. (2017).

This result found that the leaf thickness under RB0.34+1.0 treatment was thickest (0.592 mm); it was significantly different at $p<0.05$ to the leaf thickness under CL treatment (0.438 mm) (Fig. 3D and Table 1), and the leaf thickness under control plant (0.388 mm). However, the leaf thickness under CL and Ctrl treatment was not significantly different at $p<0.05$ (Table 1). This mean that the leaf thickness was highest when supplied two periods of R/B photon flux such as under RB0.34 in the first ten days and RB1.0 from 11[st] to 25[th] day. Effect of B light at the first period could promote the leaf thickness and leaf color of the tomato seedling stage.

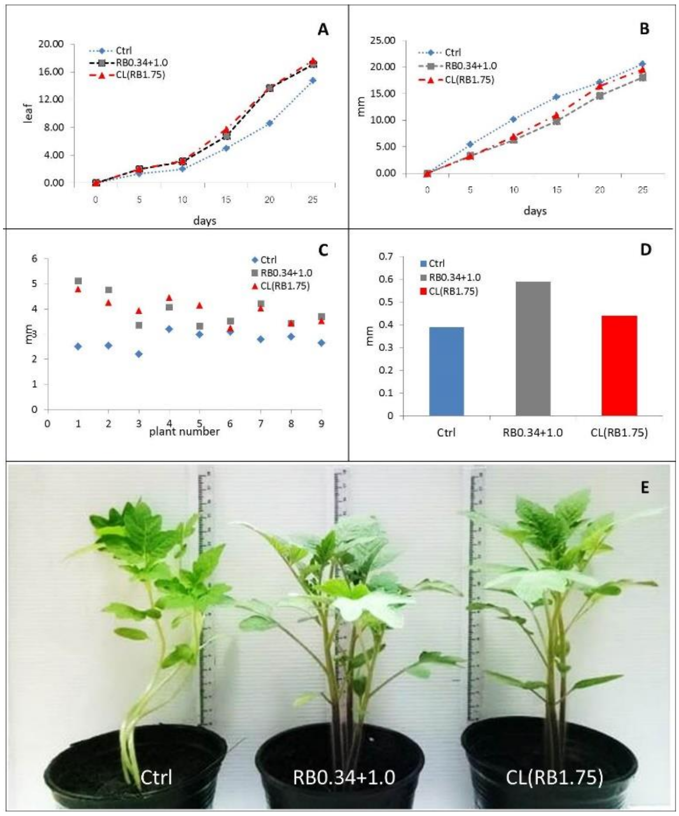

Fig. 3: The measured results of morphological of Tomato during treatment. (A) Leaf number measure every five days. (B) Plant height (cm) measure every five days. (C) Stem diameter (mm) of Tomato seedling average from the sample plants. (D) Leaf thickness (mm) average from the sample plants. (E) Tomato growth and development on day 21

3.4. Effect of R/B ratio in two periods light supplies on tomato leaf area and leaf colour

This study found that the leaf area of tomatoes under Ctrl treatment was smallest (9.199 cm^2), under RB 0.34+1.0 is 10.036cm^2 and under CL is 10.434cm^2. The statistical result shows no significant different *($p<0.05$)* in all treatments (Table 1). This is in accordance to the study of the tomato "Komett" (21 days) under 30B:10R (R/B=2.3) and 50B:50R (R/B1.0) was show the leaf area per plant were not significant different *($p<0.05$)* (Hernandez and Kubota, 2016).

Moreover, many studies show that the effect of R light could promote the large leaf area of others plant types such as; in "Lettuce Leaf" basil and "Red Rubin" basil cultivars in 28 days under the percentage of R light more than B light was significantly different from the leaf area under the percentage of B more than R light (Bantis et al., 2016). In Phalaenopsis, greater leaf area was observed with increasing R light (Ouzounis et al., 2014b) which has also been reported for cucumber (Hogewoning et al., 2010). In cucumber seedling in 14 days, there was greater leaf area when the increasing of the R light (Hernández and Kubota, 2016). In roses, greater leaf area was observed with increasing R light, while in chrysanthemums and campanulas greater values were observed under 20B80R (R/B=4) and white light respectively (Ouzounis et al., 2014a).

Fig. 4A indicated the tomato's leaf color from two of each sample groups; as can be observed, there was a difference in the green color. The author found that the leaf color of tomato seedling under RB0.34+1.0 indicated the dark green pigment in color index 98.942 (index range 0-255), it was significantly different (*$p<0.05$*) from under CL (color index is 131.578) and Ctrl treatment (color index is 125.751) were show the green color pigment (Table 1 and Fig. 4B). However, the leaf color index under CL and Ctrl treatment was not significantly different *($p<0.05$)*. This result could demonstrate the leaf color (dark green) from the two periods of LED light treatment (RB0.34+RB1.0), which could represent the promotion of the highest chlorophyll concentration, more than other treatments. This is in accordance to the tomato "Komett" seedling under 30B:70R(R/B=2.3) and 50B:50R (R/B=1), showing the highest chlorophyll per leaf area more than 100R, 100B and 75B:25R treatment (Hernández and Kubota, 2016). The similar results were show on the Micro-greens under 1B:1R(R/B=1) and 1B:2R(R/B=2), indicating the highest chlorophyll a and chlorophyll b. On the other hand, Micro-greens under 2B:1R(R/B=0.5) and white LED was show low chlorophyll contents (Lobiuc et al., 2017).

4. Conclusion

In summary, tomato seedling under two periods of different R and B light supplements (RB0.34+1.0) at PPFD 100-150 μmol m^{-2} s^{-1} was the best PAR spectrum to support the highest stem diameter, leaf thickness, and leaf color (highest chlorophyll

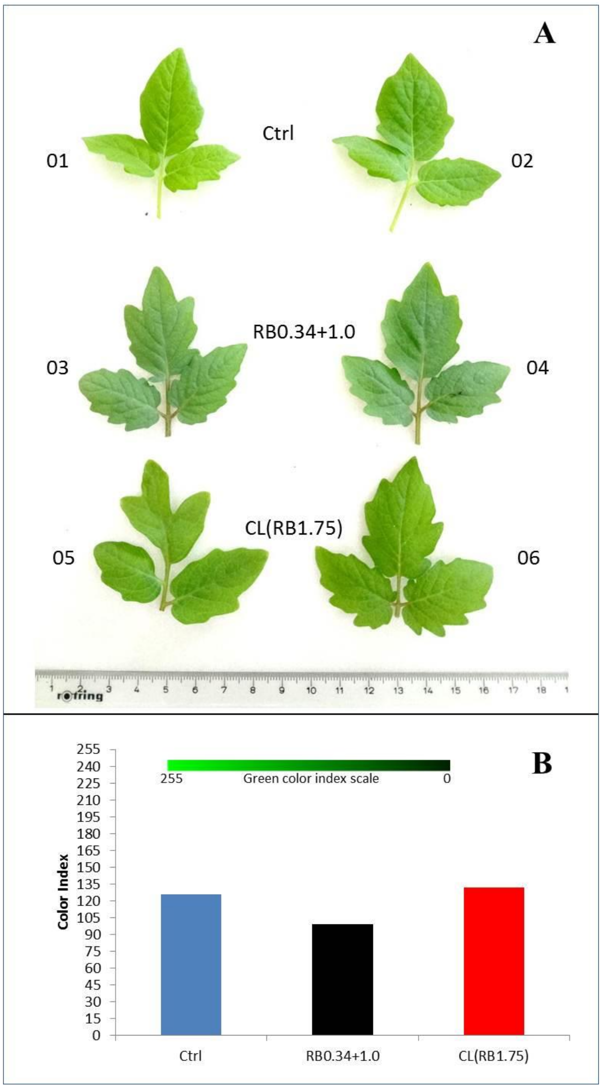

Fig. 4: The measured results of leaf color of Tomato from the sample leaves. (A) Tomato's leaf color (two of sample leaves) from the Ctrl treatment (01-02), RB0.34+1.0 treatment (03-04), and CL treatment (05-06). (B) Comparative of the leaf color analyses by RGB histogram

However, the young tomato growth under the one period of R and B light treatment (CL at PPFD 100 μmol m^{-2} s^{-1}) is also acceptable. Our results suggested that supplying more of the B light than the R (RB0.34) in the first ten days would result in a positive morphological response of tomato seedling plants better than less than one period of CL treatment.

The RB0.34+1.0 treatment could be applied to promote high quality tomato seedlings, reduce the stem damage on the transplants, and increase the tomato production. Additional study will be needed to extend the experiment for a complete investigate the tomato life cycle (sowing, seedling and fruit yield) under three periods of the different R/B photo flux ratio.

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List of symbols

LED	Light emitting diode
CL	Commercial LED grow light
R/B	Ratio of red and blue photon flux
R	Red LED light
B	Blue LED light
PAR	Photosynthesis active radiation
PPFD	Photosynthesis photon flux density

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