# AN ADJUSTABLE LIGHT SOURCE FOR PHOTO–PHYTO RELATED RESEARCH AND YOUNG PLANT PRODUCTION

R. C. Jao, W. Fang

**ABSTRACT** *A flexible light source, entitled LEDSet, was developed suitable for the research on photobiological responses of plantlets in vitro and for the young plant production. The device allows the light intensity, light quality, duty ratio (0~100%) and frequency (0~5 kHz) to be controlled. The components of the device include super–bright light–emitting diodes (LEDs), mounting fixture, and driver. The LEDs are commercially available with peak emissions in the red (645±20 nm) and blue (460±20 nm) bands. In each LEDSet, there are four blue and nine red LEDs installed. This device can provide downward or sideward light source depends on the installation and 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ mean light intensity at a 10–cm distance when given 20 mA forward working current to each light–emitting diode of LEDSet. Experiments on the growth of Phalaenopsis (Phal. White Dream × Enshyu) and potato (*Solanum tuberosum *L. 'Kennebec') tissue culture plantlets were conducted using the device developed to demonstrate its applications on both production and research on photosynthesis and energy savings.*

***Keywords.*** *Light–emitting diode, Light source, Photosynthesis, Photomorphogenesis.*

Tubular fluorescent lamps (TFLs) are the most popular light source in micropropagation (Dooley, 1991). However, light–emitting diodes (LEDs) have many attractive features such as small mass and volume, easy manipulation, wavelength specific, solid state construction, reduced heat output, extremely rapid on/off switching (200 ns), and long life (Bula et al., 1991; Brown et al., 1995; Fang and Jao, 2000), thus making it a promising light source for plant growth in a confined environment especially in a space–based plant culturing system (Bula et al., 1991; Barta et al., 1992) and in photosynthesis research (Tennessen et al., 1994). Recently, LEDs have been used in many areas of plant photobiological research such as chlorophyll synthesis (Tripathy and Brown, 1995), photosynthesis (Tennessen et al., 1994), algal photo–bioreactor (Lee and Palsson, 1994) and photomorphogenesis (Hoenecke et al., 1992), etc. Several plant species have been reported to grow successfully under LEDs including lettuce (Bula et al., 1991; Hoenecke et al., 1992; Yanagi et al., 1996), pepper (Brown et al., 1995), cucumber (Schuerger and Brown, 1994), wheat (Tripathy and Brown, 1995), spinach (Yanagi and Okamoto, 1994), Cymbidium (Tanaka et al., 1998), strawberry (Nhut et al., 2000), potato (Iwanami et al., 1992; Miyashita et al., 1995; Jao and Fang, 2003a, 2003b), and Phalaenopsis (this study).

Plant growth chamber using red, blue, and infrared LEDs as a light source was developed (Okamoto et al., 1996). Red and blue LEDs manufactured by four companies (Everlight, Taiwan; Panasonic, Japan; Toshiba, Japan; and Rohm, USA) were examined by Ono et al. (1997) to find the optimum working forward current and voltage. A similar study was conducted by Fang and Jao (2000) on other four commercially available red and blue LEDs (Excellence, Taiwan; Everlight, Taiwan; Hewlett Packard, USA; and Nichia, Japan). The conversion factors among photometric and quantum units of above were also derived (Fang and Jao, 2000). Simulation models of light environment under red and blue LEDs were developed (Takita et al., 1996; Jao and Fang, 2000) to provide the capability of predicting the spatial distribution of light intensity and light quality [red vs. blue (R/B)] of various arrangements of multiple LEDs.

Experiments on photosynthesis in intermittent light started long ago. Brown and Escombe (1905) used a rotating sector and found that three–quarters of the light could be cut out without decreasing the rate of photosynthesis. They also reported that photosynthetic efficiency of light utilization in higher plants increased by more than 100% in intermittent light. Warburg (1919) found that Chlorella grew better under intermittent light than under continuous light, depending on the frequency of the flashing. With a frequency of 4 periods per minute (0.067 Hz) the improvement was 10%, and with a frequency of 8000 periods per minute (133 Hz) it was 100%. Emerson and Arnold (1932) used 50 flashes per second (50 Hz) and make the light period much shorter than the dark period. They were able to improve the yield by 400% and came to a conclusion that the improvement of yield (photosynthetic oxygen produced per amount of light) was not only a function of frequency of flashing but also was dependent on the relative duration of light and dark periods.

Time constant scale of photosynthetic processes can range from picosecond to minute and can be divided into three ranges according to the time constant: (1) primary photochemistry process, within picosecond to nanosecond (Diner,

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1986), (2) electron transport process, within microsecond to millisecond (Whitmarsh and Cramer, 1979; Harbinson and Hedley, 1988; Whitmarsh, 1992), and (3) carbon metabolism process, within second to minute (Kirschbaum and Pearcy, 1988; Sassenrath–Cole and Pearcy, 1992). Each process can be studied by providing appropriate range pulses of light. Advances in light–emitting diode technology have made them an excellent light source for these researches.

As shown in figure 1, duty ratio of intermittent light within a light period can be defined as TH/(TH + TL). In Warburg's experiments, the duty ratios of the light source were always 50% (TH = TL). In Emerson and Arnold's experiments, various duty ratios were investigated, including 95, 90, 33, 22, and 17%. At the given frequency, the smaller the duty ratio, the better the growth. As shown in table 1, the yield was improved by 300% when the TL period increased from 3.8 to 16.6 ms. This trend continues until there is extra time remaining in the zero voltage period with no intermediate products formed in previous light flash to be removed. Emerson and Arnold (1932) suggested that at 25°C, the TL period of 40 ms is "adequate for the complete removal of the material remaining at the end of each light flash." Two steps are involved in the reduction of carbon dioxide: light and dark reactions. During light reaction, light was absorbed by chlorophyll pigment and induced an electron transport between reaction centers of photosystems II and I to generate ATP (material remaining at the end of each light flash) for dark reaction. The speed of light reaction (microseconds to milliseconds scale) is much faster than dark reaction (seconds scale) (Whitmarsh and Govindjee, 1999). If ATP generated in previous light reaction was not used in the following dark reaction then ATP will be consumed or lost by other chemical reactions. The durations of dark period (TL) decide if ATP can be generated and used efficiently. The TL period in the last row of table 1 is 7462 ms, which is too long, thus leading to an improvement of only 10%. Above mentioned experiments did not provide a long continuous dark period as shown in figure 1.

**Table 1. Improvement of yield under various intermittent light over continuous light.**

| $T_H$ Period (ms) | $T_L$ Period (ms) | Duty Ratio (%) | Frequency (Hz) | Yield Improvement (%) | Source |
|---|---|---|---|---|---|
| 3.8 | 3.8 | 50 | 132 | 100 | Warburg (1919) |
| 3.4 | 16.6 | 17 | 50 | 400 | Emerson & Arnold (1932) |
| 7,462 | 7,462 | 50 | 0.067 | 10 | Warburg (1919) |

### OBJECTIVE

The objective of this study was to develop a self–contained adjustable lighting device for plant photobiological related research and young plant production. This device provides the capability of altering blue (B) and red (R) light intensity separately, altering light quality (R/B ratio), duty ratio, and frequency through a control driver.

## MATERIALS AND METHODS

### MEASUREMENT OF LEDS

Table 2 shows the specifications of super–bright red and blue LEDs (TYNTEK Corp., Taiwan) used in this study. For the reliability and stability of the lighting device designed, only LEDs with 20–nm bandwidth and 10% variance of light intensity were used. The spectral and electrical data of LEDs were established using 30 red and 30 blue randomly selected qualified LED samples.

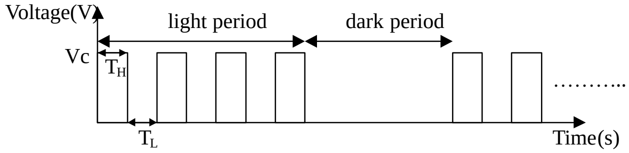


**Figure 1. Schematic diagram showing intermittent light within light period.**

A rectangular box (40 × 35 × 25 cm) was set up to hold one spectroradiometer (LI–1800, LI–COR Corp., USA) and one LED sample. LED samples were fixed at 10 cm above the luminance detector head of spectroradiometer. Four tunable knobs were installed at the bottom of the box to keep the box in level position. The box was sealed to keep out other indoor light during measurement. A DC power supply (GPC–3030DQ, Instec Corp., Taiwan) was used to provide the forward current (20 mA) to the LED. The spectral distributions of LEDs were measured using the spectroradiometer after the LEDs were lit for 10 min. The scanning range was from 300 to 1100 nm and the scanning interval was 5 nm.

The electrical properties such as light intensity of LEDs versus forward current and voltage provided, and the upper limit of the working current are of great importance in design. Due to the different materials made of red (AlGaInP) and blue (InGaN) LEDs, different electrical properties can be expected. Photometer (J–17, TekLumaColor Inc., USA) with LED head (J1805, TekLumaColor Inc., USA) and luminance head (J1811, TekLumaColor Inc., USA) were used to measure the mean light intensity of sampled LEDs at given current and voltage as shown in figure 2. Only one LED can be measured each time. The unit of light intensity measured by J–17 is in mcd.

The forward current of LED was set to 10 mA and a variable resistor (R1212N, Song Hui Electric Corp., Taiwan) was used to increase forward current by 10 mA for each measurement until saturated. Besides light intensity, the forward voltage and forward current were recorded at the same time by a voltage meter (TES2800, TES Electrical Electronic Corp., Taiwan) and an ammeter (TES2800, TES Electrical Electronic Corp., Taiwan), respectively.

### DRIVER DESIGN AND SENSING DEVICE

Figure 3 shows four blue and nine red LEDs installed on a 3– × 3–cm IC board. LEDs are spaced 0.5 cm apart. Due to different electrical properties of blue and red LEDs, two sets of circuits were needed in the driver. Two variable resistors (R1212N, Song Hui Electric Corp., Taiwan) were used to adjust the working current passing through the red and blue

**Table 2. Specifications of super–bright red and blue LEDs used in this study.**

| Color | Model | Dominant Wavelength (nm) | Viewing Angle (degree) | Min/Typ Luminous Intensity (mcd) | Material |
|---|---|---|---|---|---|
| Blue | MVL–514UB | 460 | 15 | 765/4575 | InGaN |
| Red | MVL–5A4UOL | 645 | 6 | 3600/13800 | AlGaInP |

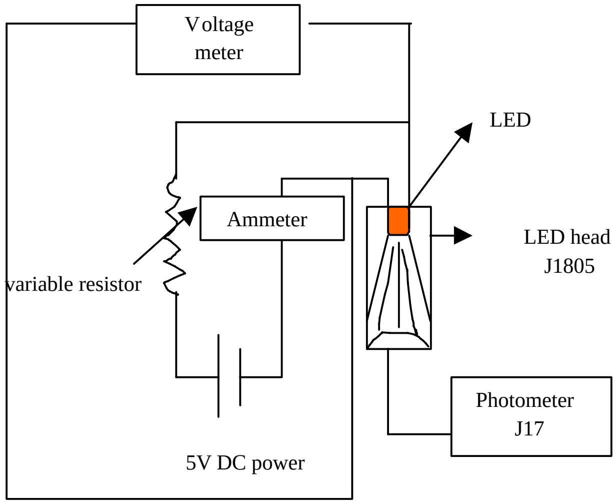


**Figure 2. Schematic diagram showing the measurement of LED.**

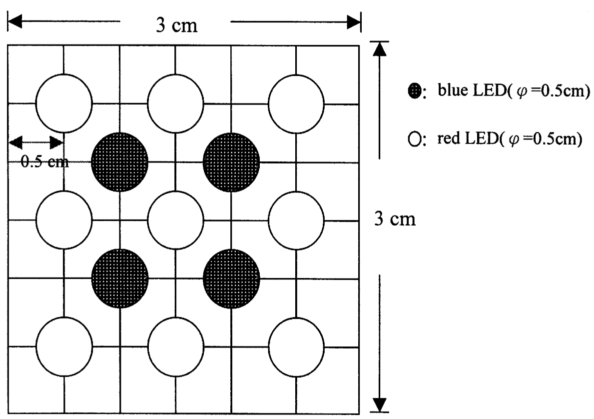


**Figure 3. Schematic diagram of LEDSet layout.**

LEDs, therefore light intensities of red and blue light can be adjusted separately.

As shown in figure 1, the frequency of intermittent light is defined as 1/(TH+TL) and the duty ratio of intermittent light is defined as TH/(TH+TL). By adjusting the length of TH and TL, the frequency and duty ratio can be adjusted accordingly. One 555 IC (HA1755, Hitachi Corp., Malaysia) with two external resistors ($R_A$ and $R_B$) and an external capacitor (C) were used to provide this function as shown in figure 4. The device produces a square waveform signal at the output $V_0$. The values of TH and TL can be determined as follows (Sedra, 1991):

$$TH = C \times (R_A + R_B) \times \ell n(2) \quad (1)$$

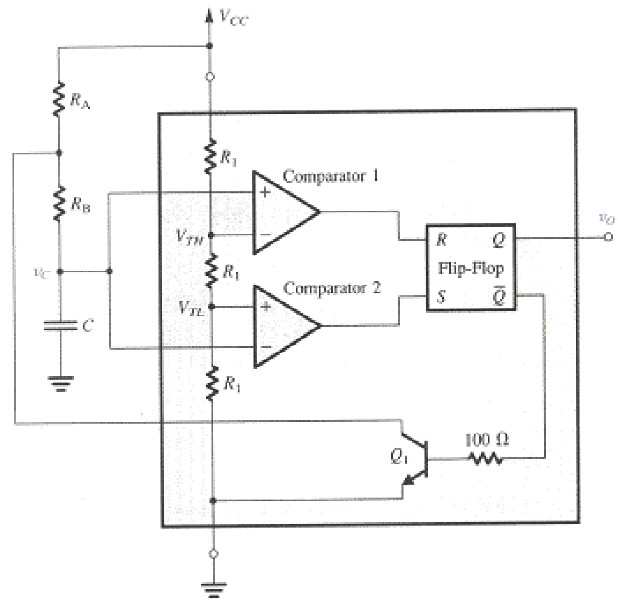


**Figure 4. The 555 IC related circuit.**

$$T_L = C \times R_B \times \ell n(2) \quad (2)$$

A control box was built to contain not only the driver mentioned above, but also to contain on–line sensing devices such as a self–designed duty ratio meter and a commercially available frequency meter (G35AAPOE, JY–TECK Corp., Taiwan) as shown in figure 5.

### Mounting Fixture Design

A commercially available electrical track (1 m in length) and associated electrical box (MR–16–608, Mental Corp., Taiwan) were modified as a mounting fixture. One set of 3–× 3–cm IC board with LEDs was attached to one electrical box (fig. 6). Together, it was named LEDSet. The LEDSet can be attached to and detached from the electrical track by a locking element. One electrical track can hold at most 10 LEDSets and they were electronically connected in parallel. The commercially available two–wire electrical track was changed to three–wire to allow two separate circuits in LEDSets with one common ground.

## Results

### Measurement of LEDs

Figure 7 shows the relative spectral distributions of red and blue LEDs between 300 to 1100 nm. The dominant


**Figure 5. Photos of duty ratio meter (left) and frequency meter (right).**

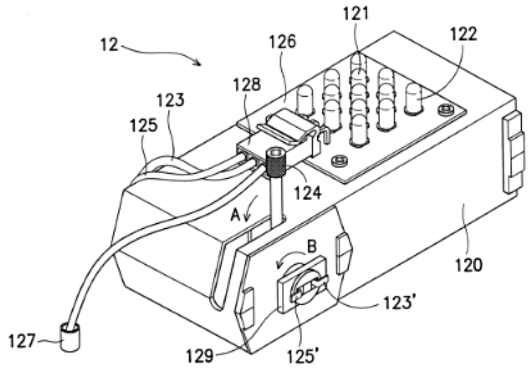


**Figure 6. Schematic diagram of the LEDSet.**

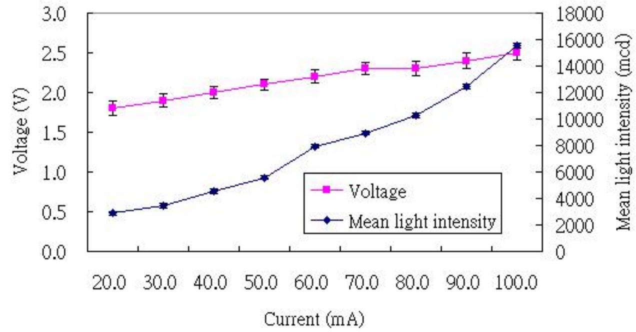


**Figure 8. Characteristics of red LEDs.**

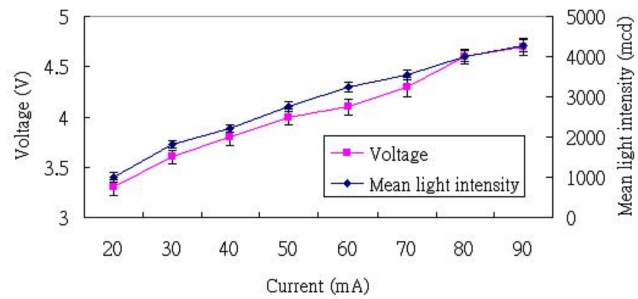


**Figure 9. Characteristics of blue LEDs.**

**Table 3. Designed upper limits of voltage and current provided to LEDs.**

| Color | Upper Limit Voltage (V) | Upper Limit Current (mA) |
|---|---|---|
| Blue | 4.5 | 80.0 |
| Red | 2.2 | 90.0 |

wavelength is 460 nm for blue LEDs and 645 nm for red LEDs, respectively.

Figures 8 and 9 show the relationship between electrical properties and light intensity of 30 red and 30 blue sampled LEDs. It shows that higher forward voltage or forward current will lead to higher intensity. Providing the same current for each blue and red LED, for example 40 mA, the voltage over blue LED will be 3.7 V and the mean light intensity will be 2300 mcd, but for red LED it will be 2.1 V and the intensity will be 5000 mcd. Therefore, two sets of circuits were required to provide the desired working current. The designed upper limits of forward voltage and current for red and blue LEDs were listed in table 3. At this point, the maximum light intensity of red and blue LEDs were 13560+123 mcd (at 90 mA) and 4277+154 mcd (at 80 mA), respectively.

## Driver Design

A 5V DC power is needed for the designed control driver as shown in figure 10. Two variable resistors (R2 and R3) were used to adjust the TH and TL. Another two variable resistors (R8 and R9) were used to control the light intensity of red and blue LEDs, separately. Besides the functions mentioned above, other components shown in figure 10 were designed for precautionary purposes such as to prevent LED saturation due to high voltage or large current.

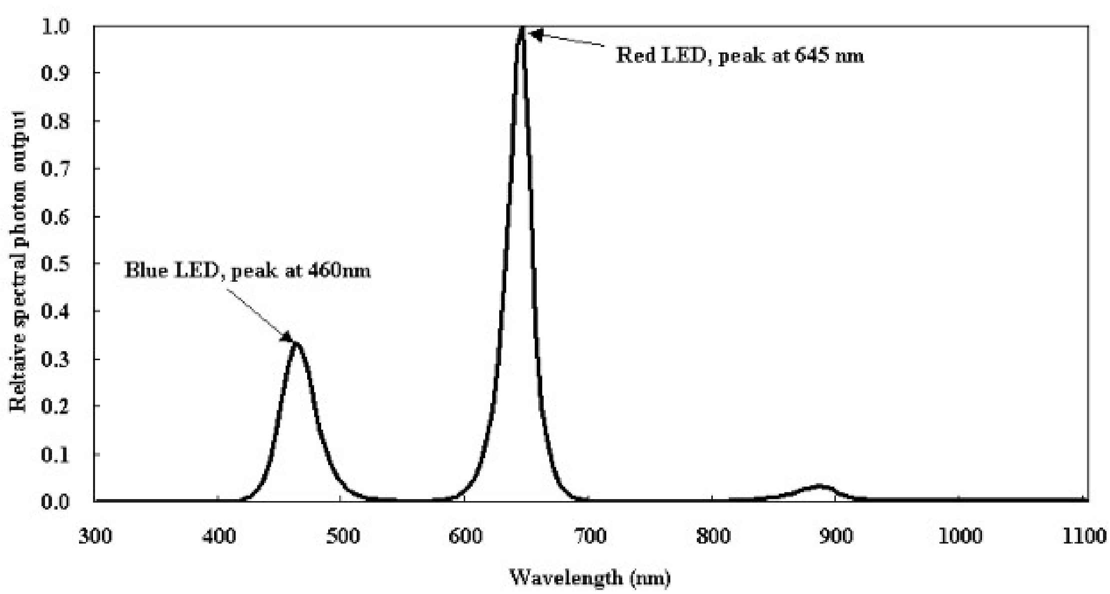


**Figure 7. Relative spectral distributions of super–bright red and blue LEDs used in this study.**

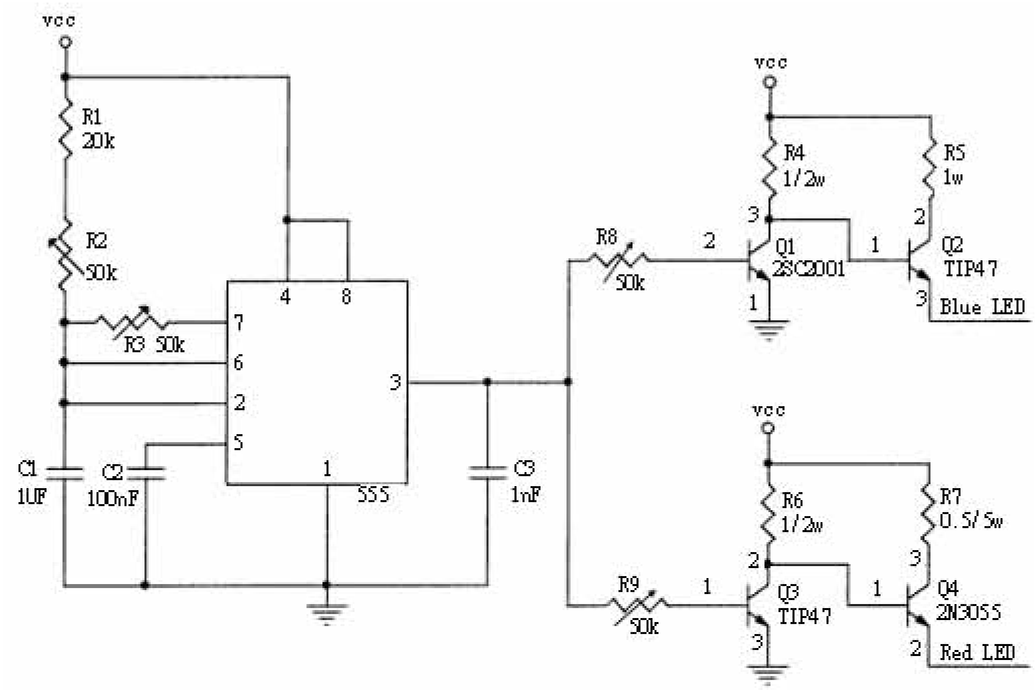


**Figure 10. LEDSet control circuits designed in this study.**

Figure 11 shows the prototype of the driver designed in this study. The capability of adjusting light intensity, light quality, duty ratio, and frequency is provided by the driver through four knobs. Several wires connected with the driver (as shown in the lower right corner of fig. 11) are designed for the user to connect with devices shown in figure 5 for on–line sensing of frequency and duty ratio.

### Mounting Fixture Design

Figure 6 shows the diagram of LEDSet. The LEDSet consists of an electric box and a circuit board with 13 LEDs installed. Super bright red LEDs and blue LEDs are alternately mounted on the circuit board. The LEDs are spaced apart by the same distance as described in figure 3. Lighting the red and blue LEDs requires different voltages, with a common ground wire, the number of wires required is reduced to three. A three–wire connector is attached to the circuit board.

Figure 12 is a perspective diagram of the three–wire electrical track which was modified from commercially available two–wire electrical track. A portion of the electrical track is cut away to show the inside. The electrical track has an elongated body and a pair of longitudinal engaging grooves and receiving grooves provided in the body. Bare copper wires are received in the receiving grooves. An elongated cover is fixed to the body. The common ground wire is received in the cover and laterally connected to a plurality of connectors outside the cover. Figure 13 depicts the electrical track with LEDSets mounted thereon.

### System Integration and Architecture

Figure 14 is a schematic diagram of a plant growth bench equipped with lighting system developed and related control devices. The plant growth bench consists of multiple layers. At the ceiling of each layer mounted with several three–wire electrical tracks with LEDSets attached. Each LEDSet is a mixed blue and red light source. Cultured plantlets are provided in tissue culture vessels placed under the LEDSet. Power on–off switch, AC/DC converter, timer, and several drivers are mounted on the sideboard of the plant growth bench. The switch is used for manually turning on/off the power. An AC/DC converter which supplies the LEDSets with DC power through the three–wire electrical track to

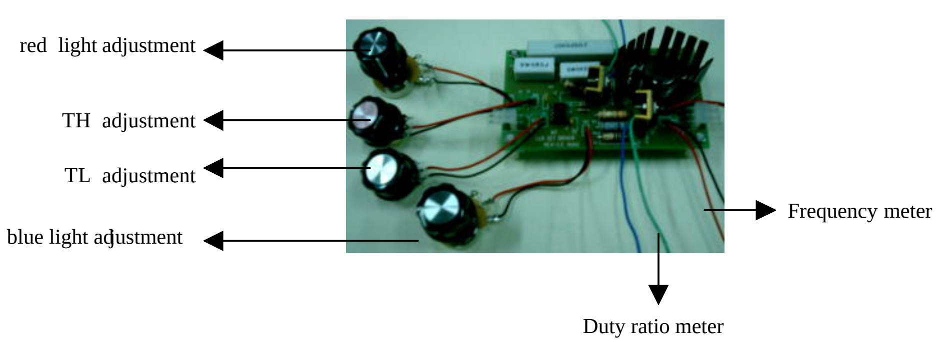


**Figure 11. Prototype of the driver.**

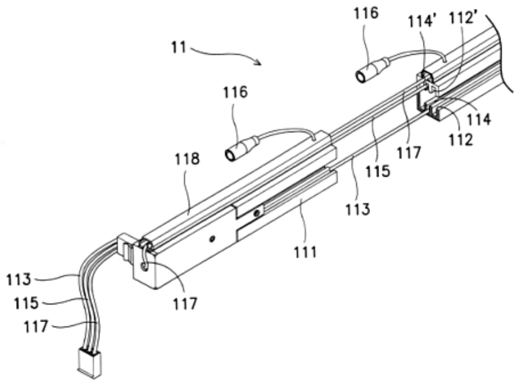


**Figure 12. A perspective diagram of an electrical track modified from a two–wire electrical track.**

illuminate the plantlets in the vessel was used in this study. When the power is on, the timer is used to control the photoperiod. The integrated control of timer and driver makes the LEDSet a flexible lighting device capable of providing continuous light, intermittent light at high/low frequency, and both with adjustable duration of light/dark period.

The adjustable ranges of duty ratio is 0~100% and frequency is 0~5 kHz, respectively. When TL equals 0, duty ratio reaches 100%, the LEDSet provides continuous non–fluctuating light. When TL is greater than 0, LEDSet provides intermittent/pulse light. Ten minutes of tune–up time is required after adjustment on frequency or duty ratio. The error within the first 10 min after adjustment is about 2.5% on duty ratio and 1.02% on frequency, respectively. The errors reduced to 0.08% on duty ratio and 0.02% on frequency after 10 min.

The system developed can provide 120 ± 3.48 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity at 10–cm distance covering a total cross–section of the culturing vessel (10 cm in diameter) when given 20–mA working current to each light–emitting diode of LEDSet. A quantum sensor (LI–190SA, LICOR, Inc., USA) and a datalogger (21X, Campbell Scientific, Inc., USA) were used to record the time course changes of light intensity. As shown in figure 15, the device requires 10–min of tune up before reaching steady state. To reach other level of light intensity, users can adjust the distance between light source and plant. It is easier to adjust the forward current related knobs than to adjust the intensity of red and/or blue light separately.

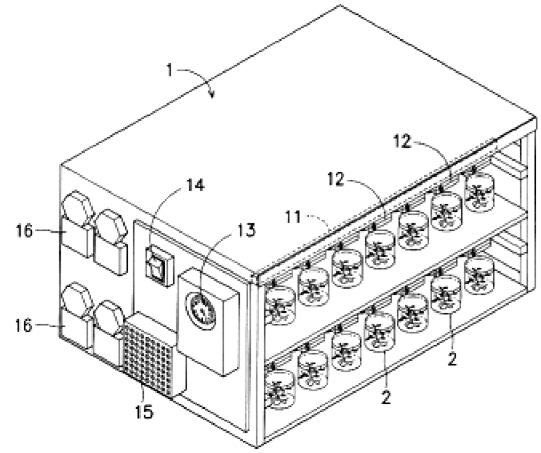


**Figure 14. Schematic diagram of the integrated system and control panel.**

## Applications

### Effects of Continuous Non–Fluctuating LED Light vs. Continuous Fluctuating TFL Light on the Growth of Phalaenopsis Plantlets *In Vitro*

Phalaenopsis plantlets (Phal. White Dream × Enshyu) *in vitro* were grown photomixtrophically with MS medium (Murashige and Skoog, 1962), 3 g/L HYPONEX 1, 3 g/L Tryptone+30 mg/L DL–Malic acid, 1 g/L charcoal, 20 g/L sucrose, and 7 g/L agar. The pH is 5.5 before autoclave. The purpose of this experiment was to study the feasibility of culturing Phalaenopsis plantlets *in vitro* using LEDSet. TFL (FL48D/38,TFC Corp., Taiwan) was used for comparsion. Mean fresh weight per plantlet was 128.32 ± 28.2 mg (20 samples). Both light sources were kept at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and plantlets were grown under 16/8 (light/dark) hours per photoperiod. Quantum ratios of blue (400 to 500 nm) and red (600 to 700 nm) light of LEDSet and TFL are 0.45 and 1.56, respectively. The temperature of the culture room was controlled at 25 + 2°C. The fresh weight, dry weight, number of leaves, leaf length, leaf width, and number of roots data were measured after 90 days of growth.

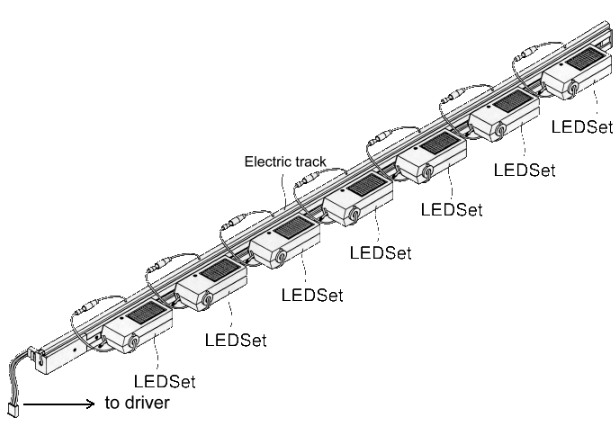


**Figure 13. Schematic diagram of the LEDSets mounted on an electrical track.**

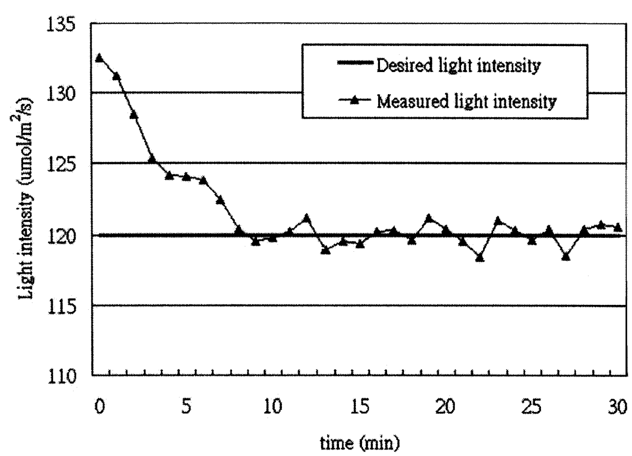


**Figure 15. Time course change of light intensity for LEDSet.**

**Table 4. The results of Phalaenopsis plantlets *in vitro* grown 90 days under LEDSets and TFLs.**

| | LED Sets | TFLs | t–test |
|---|---|---|---|
| Fresh weight (g) | 3.19±1.02 | 3.49±1.05 | ns[a] |
| Dry weight (g) | 1.22±0.44 | 1.20±0.44 | ns |
| Number of leaves | 3.50±0.61 | 3.44±0.62 | ns |
| Length of leaves (cm) | 4.47±0.63 | 3.87±0.50 | s[b] |
| Width of leaves (cm) | 1.87±0.28 | 2.00±0.23 | ns |
| Number of roots | 7.25±1.29 | 7.61±1.29 | ns |
| Length of root (cm) | 3.92±0.74 | 3.84±0.78 | ns |

[a] ns: no significant difference, $p < 0.05$.
[b] s: significant difference.

As shown in table 4, no significant differences were found between groups under LEDSet (providing continuous non–fluctuating light) and TFLs (providing continuous fluctuating light) on fresh/dry weight, number/ width of leaves and number/length of roots. Length of leaves is the only one observed with significant difference among treatments. This is mainly due to the large difference in R/B ratio of light sources. The leaves of plants grown under LEDsets are longer.

#### Effects of Frequency and Duty Ratio on the Growth of Potato Plantlets *In Vitro*

Effects of intermittent light on photomixotrophic growth of potato plantlets *in vitro* and the electrical savings that could be realized by adjusting the frequency and duty ratio of LEDSet were investigated and compared to the use of TFLs. TFLs provide continuous fluctuating light at 60 Hz and LEDs provide continuous non–fluctuating or intermittent/ pulse light depend on the preset frequency and duty ratio. Totally, eight treatments were investigated with varying light source, frequency, duty ratio, and photoperiod. Results indicated that the continuous fluctuating light of TFLs has no significant difference with continuous non–fluctuating light of LEDs. This result is consistent with the previously mentioned Phalaenopsis–experiment. However, continuous non–fluctuating light of LEDs have significant difference with intermittent light of LEDs on the growth of potato plantlets *in vitro*. In addition, if growth rate is the only concern, LEDs at 720 Hz (1.4 ms) and 50% duty ratio with 16/8–h photoperiod stimulated plant growth the most. However, if energy consumption is the major concern, due to the fact that higher frequency required more energy to generate, using LEDs at 180 Hz (5.5 ms) and 50% duty ratio with 16/8–h photoperiod would be the best choice in terms of energy savings for illuminating potato plantlets *in vitro* without sacrificing plant growth (Jao and Fang, 2003a).

#### Providing Concurrent vs. Alternating Red and Blue Light Photoperiods

Effects of concurrent versus alternating blue and red light using LEDSet on the photomixotrophic growth of potato plantlets *in vitro* were investigated. All seven treatments had the same daily light integral (DLI, 5.53 $mol \cdot m^{-2}$), photoperiod (16/8 h), and similar proportion of red light (45%) and blue light (55%). Results showed that the fresh/dry weight accumulation of potato plantlets *in vitro* under the concurrent blue and red light was superior than that under the alternating blue and red light, indicating that the simultaneous coexistence of blue and red light are necessary for optimum plantlet growth. Low PPF with long duration was better than high PPF with short duration. Within the concurrent blue and red light treatments, when the duration of blue light was shorter than that of red light, timing of the blue light affected the growth of potato plantlets *in vitro*. Providing red and blue light together at the beginning of the photoperiod resulted in better growth. Illuminating plantlets with alternately red and blue light had significantly less fresh/dry weight accumulation compared with concurrent red and blue light (Jao and Fang, 2003b).

## Conclusion

LEDs have many advantages that make it a promising light source for plant growth and research. At present, cost on the blue LED is the major drawback. In this study, a lighting system capable of control light quality, quantity, duty ratio, and frequency was designed. Experiments to demonstrate its applications were conducted. The lighting system is easy to use, to install, and does not require much changes on existing cultural bench. It is self contained and no special equipment is required to perform the experiments. LEDSets can be attached to and detached from the three–wire electrical track easily. Distance among LEDSets can be adjusted freely.

The lighting system developed can be applied to photosynthesis, energy savings, photomorphogenesis of plants, and photobiological related research of cells, etc. The effects of plant growth under different light intensity, quality, frequency, duty ratio, and any combination of the above could be studied using LEDSet.

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