Effects of LEDs (Light-Emitting Diodes) Lights on the *in vitro* Growth of *Erycina pusilla*

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Key word: *Erycina pussila*, LEDs light, *In vitro* cultivation, Chlorophyll fluorescence

Summary

The effects of different types of LEDs lights on the *in vitro* growth of *Erycina pusilla* were examined during 50 weeks of cultivation, and responses were compared with plantlets grown under fluorescent light. Four sets of light illumination were used, including: (1) RB+W+RB LEDs, (2) RB+W+RGB LEDs, (3) RGB+W+RGB LEDs (RB: red-blue, W: white, RGB: red-green-blue), and (4) fluorescent light. The plant materials were sorted into 3 groups, including: small size plants (4-5 leaves), medium size plants (6-7 leaves), and large size plants (more than 8 leaves).

The results indicated that compared with the fluorescent light and other LED treatments, RB+W+RGB LEDs significantly increased root and leaf number and plant high of *in vitro* plantlets of *Erycina pusilla*. Double sigmoid growth pattern of increase in numbers of leaves was observed in the *Erycina pusilla* grown *in vitro* under LEDs lights, especially in RB+W+RGB LEDs treatment.

RB+W+RB LEDs significantly increased the values of Fo, Fv, and Fv/Fm of *in vitro* plantlets after 25 weeks of cultivation, whereas significantly increased values of chlorophyll fluorescent parameters was found in the fluorescent light treatment after 50 weeks of cultivation. These results suggested RB+W+RGB LEDs and RB+W+RB LEDs could promoted the quality of *in vitro* cultivation of *Erycina pusilla*.

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Introduction

The Orchidaceae, known as the orchid family, consists of more than 21,000 known species, and many new species are described annually (Gustafsson *et al*., 2010). *Erycina pusilla* (*Erycina pusilla* (L.) N. H. Williams & M. W. Chase) used to be known as *Oncidium pusillum* or *Psygmorchis pusilla* is so unique appearance that it has miniature size with bright yellow flowers on a green fan of leaves (Williams *et al*., 2011). The chromosome number of *Erycina pusilla* is 2n = 12 (Felix and Guerra, 1999), and it's the smallest known in orchids. Pollination and production of *Erycina pusilla* seed capsules rarely occurs in nature (Damon *et al*., 2007). Taiwan's flora industry is booming because of its unique tropical, sub-tropical, and temperate climates and geographic location. In recent years, *Erycina pusilla* has gradually formed a promising flora industry in Taiwan. Use of *in vitro* cultivation to enhance the seedling quality characteristics of *Erycina pusilla* has become a vital tool for commercial production.

Light plays a very crucial role in the mass propagation of orchids through *in vitro* seed and shoot culture technology. Most orchid grower who grow orchids *in vitro* use white fluorescent lamps. They can be used alone, or supplied with plant growth fluorescent lamps (Sun *et al*., 2004). The fluorescent lamps have different spectral emissions composing of many wavelengths from 350 to 750 nm, and this main light source is used for maintaining tissue cultures. Light also effects on the growth of orchids, especially in the formation of root system (Toshinari *et al*., 2011). LEDs (Light-Emitting Diodes) have many advantages over fluorescent lamps including lower energy consumption, longer lifetime, and improved physical hardiness (Sun *et al*., 2004; Brown *et al*., 1995). Along the line of the LEDs technology improvements, LEDs become a promising light source for plant tissue culture.

Since the single-wavelength nature of LEDs, the LEDs enable grower to enhance the specific wavelengths of light in the lighting system, thus reducing the amount of energy required to power the plant growth lamps. The optimal light wavelength conditions for orchid micropropagation will vary from species to species. Whilst the green and yellow light wavelengths are reflected or transmitted by the leaves, red and blue lights are the best light sources for driving photosynthesis (Naichia *et al*., 2009). It has been well documented that red light is important for shoot or stem elongation and changes in plant anatomy (Komg *et al*., 2008; Schuerger *et al*., 1997). In contrast, blue light is important in chlorophyll biosynthesis, stomatal opening, enzyme synthesis, maturation of chloroplast and photosynthesis (Komg *et al*., 2008; Tibbitta *et al*., 1983).

Blue and red LEDs have been used for studies in many areas of photobiological research such as chlorophyll synthesis (Tripathy and Brown, 1995), photosynthesis (Tennessen *et al*.,

1994), and plant morphogenesis (Brown *et al*. 1995).

As a first step toward to manipulating the *in vitro* cultivation of *Erycina pusilla*, the effects of LEDs on the plant growth and morphogenesis as well as on the physiological responses of *in vitro* cultured *Erycina pusilla* plantlets were conducted. Red(R)/blue(B), red(R)/green(G)/ blue(B), white (W) LEDs were used in this study, and responses of growth and chlorophyll fluorescence were compared with the plantlets grown under fluorescent illumination.

Materials and methods

1. Plant materials

Erycina pusilla plants were purchased from Flower Space Orchids Company, Changhua, Taiwan. The seed capsules after self-pollination were collected and used as experimental materials.

2. Induction of protocorm-like bodies (PLBs) and proliferation

The self-pollinated seed capsules of *Erycina pusilla* were dissected aseptically. Mature seeds of *Erycina pusilla* were sterilized with a solution of NaOCl (1% available chlorine) that shaking by vertex 15 min, and then washed three to five time with sterilized distilled water. The seeds were sown in plastic petri dishes containing sterile ½ MS medium (2.2 g/L Murashige and Skoog salts, 30 g/L sucrose, 8 g/L agar, pH 5.7) (Murashige and Skoog, 1962). The plastic petri dishes were sealed with parafilm, and incubated in a growth chamber at 25 ºC with under 8 hours/16 hours dark/light photoperiod. After 2 months of germination, germinating plantlets were transferred from petri dishes to glass jars containing ½ MS medium. After 6 months of cultivation, the plantlets were sub-cultured onto fresh ½ MS medium, and ready for the experimental use. Plant materials were sorted into 3 groups based on number of leaves, including: (1). Small size plants (4-5 leaves), (2). Medium size plants (6-7 leaves), and (3). Large size plants (more than 8 leaves). And then the plantlets were incubated in a growth chamber illuminated with LEDs at 25 ºC with under 8 hours/12 hours dark/light photoperiod.

3. LEDs treatments

Three type of tubular LEDs lamps were used in the experiment included RB (red+blue), RGB (red+green+blue) and W (white). RB lamp contained 28 sets of 8R1B-type chip (8 red and 1 blue chips), totally 224 red and 28 blue chips were installed in one RB lamp. RGB lamp contained 28 sets of 7R1G1B-type chip (7 red, 1 green, and 1 blue chips), totally 196 red, 28 green and 28 blue chips were installed in one RGB lamp. W lamp contained totally 252 cool white (color temperature, CT: 5000 K) chips. Figure 1 show spectral distribution of RB (Fig.

1A), RGB (Fig. 1B), and white (Fig. 1C) LEDs lights. Peaks of wavelength for red, green, and blue LEDs chips used in this study were 660, 530, and 450 nm, respectively.

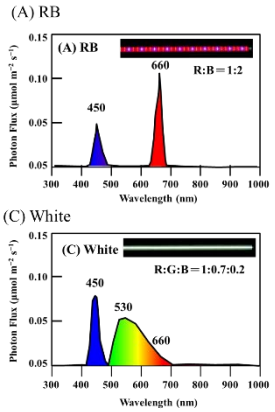
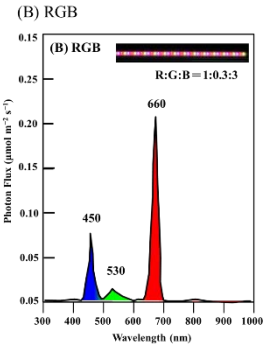


Fig. 1. Spectral power distribution of RB (A), RGB (B), and white (C) LEDs light.



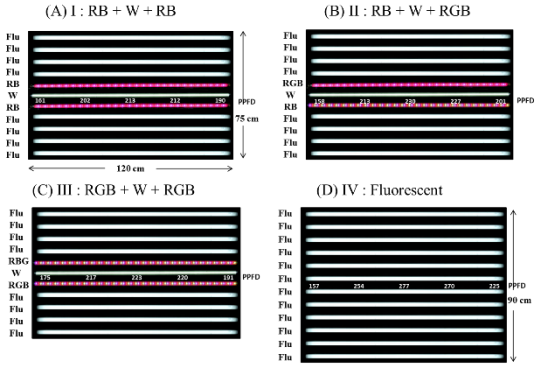


Fig. 2. Lamp arrangement in treatment I (RB+W+RB), II (RGB+W+RB), III (RGB+W+RGB) and IV (Fluoescen), and light intensity in middle row.

In treatment I (RB+W+RB), one W lamp was arranged in the middle of lamp base, one RB lamp on each side, and followed by four fluorescent lamps (Sylvania, F48T12/CW/VHO, 115W) on each side (Fig. 2A). In treatment II (RGB+W+RB), one W lamp was arranged in the middle of lamp base, one RGB and RB lamp on each side, respectively, and followed by four fluorescent lamps on each side (Fig. 2B). In treatment III (RGB+W+RGB), one W lamp was arranged in the middle of lamp base, one RGB lamp on each side, and followed by four fluorescent lamps on each side (Fig. 2C). In treatment IV, ten fluorescent lamps were arranged in the lamp base (Fig. 2D). Eighteen glass bottles of *Erycina pusilla* plantlet (3 plant size x 6 replication) were arrayed in a row under the middle of lamp base with 30 cm distance, and the glass bottles were tilted back 45 degrees. The light intensity was maintained at 150~250 μmole m^{-2} s^{-1} PPFD (photosynthetic photon flux density) as indicated in Figure 2.

4. Measurement and analysis

(1). Chlorophyll fluorescence

After a 25 weeks and 50 weeks period of LEDs and fluorescent lamps treatments, measurements of chlorophyll fluorescence were taken on mature and fully expanded leaves (n=

6) from the middle part of the rosette using Chlorophyll Fluorometer PAM-2100 (Heinz Walz GmbH, Effeltrich, Germany). The results were subjected to an analysis of variance and means separation using the Statistic version 8 test at $p \leq 0.05$.

(2). Plant growth parameters:

Plant height and width were measured (n= 6) by ruler every two weeks. The numbers of leaf and root were counted (n= 6) every two weeks.

5. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using statistic version 8 and mean separation was conducted using Least Significant Difference test at $p \leq 0.05$

Results

1. Effects of LEDs lights on the growth of *Erycina pusilla* with different size grown *in vitro*

(1). Small size (4-5 leaves) of *Erycina pusilla*

The growth and development of *Erycina pusilla* were significantly affected by different light treatments *in vitro*. The plantlets of *Erycina pusilla* were grown under RB+W+RB, RB+W+RGB, RGB+W+RGB LEDs and fluorescent light for a period of 50 weeks. After 50 days of cultivation, the numbers of roots, numbers of leaves, and plant height were shown in Table 1. Among 4 treatments of small size of *Erycina pusilla*, RB+W+RGB LEDs showed the highest promotive effect on the numbers of roots and leaves during the course of 50 weeks of growth (Fig. 3 and Fig. 4). After 50 weeks of cultivation, the numbers of roots and leaves were maximum (15.0, 15.7) under RB+W+RGB LEDs and the minimum (9.7, 13.3) were under RGB+W+RGB LEDs (Table 1 and Fig. 9). Whereas highest plant height (4.0 cm) was obtained from RGB+W+RGB LEDs and was significantly different with RB+W+RB and RB+W+RGB LEDs.

Increase in numbers of leaves exhibited a double sigmoid growth pattern in all treatments. An initial phase of rapid increase of leaves number (stage I, week 2~20), was followed by a lag phase (stage II, week 20~34). Finally, during stage III (weeks 34~50) additional increase of leaves number occurred, especially in RB+W+RGB LEDs treatment (Fig. 3). RB+W+RGB LEDs treatment had a profound effect on the increase of roots number, on average, increased by 75 percent. (Fig. 4).

(2). Medium size (6-7 leaves) of *Erycina pusilla*

The plantlets of medium size of *Erycina pusilla* were grown under similar treatments for a period of 50 weeks. Although there was a tendency for the RB+W+RGB to have a higher

numbers of roots and leaves during the course of 50 weeks of growth (Fig. 5, Fig. 6), there were no statistically significant differences in numbers of roots, leaves and plant height among the treatments of RB+W+RB, RB+W+RGB and RGB+W+RGB LEDs light after 50 weeks of cultivation (Table 1 and Fig. 9).

The minimum numbers of roots, leaves and plant height were 12.7, 10.3, and 2.7 cm, respectively, under fluorescent lamp (Table 1 and Fig. 9). Double sigmoid growth pattern of increase in numbers of leaves was also observed in the medium size of *Erycina pusilla* grown *in*

Table 1. Effects of LEDs light on the numbers of roots, numbers of leaves, and plant height of *Erycina pusilla* with different size grown *in vitro* for 50 weeks.

Treatments	No, of roots	No. of leaves	Plant height (cm)
		Small size (4-5 leaves)	
RB+W+RB	12.7 ab[y]	13.3 b	2.4 c
RB+W+RGB[x]	15.0 a	15.7 a	2.5 bc
RGB+W+RGB	9.7 b	13.3 b	4.0 a
Fluorescent	13.3 ab	14.7 ab	2.8 b
		Medium size (6-7 leaves)	
RB+W+RB	16.3 a	17.0 a	3.4 a
RB+W+RGB	16.7 a	18.3 a	3.0 ab
RGB+W+RGB	14.7 a	20.0 a	3.3 a
Fluorescent	12.7 a	10.3 b	2.7 b
		Large size (> 8 leaves)	
RB+W+RB	15.0 a	14.7	3.4 a
RB+W+RGB	21.0 a	19.3	3.4 a
RGB+W+RGB	17.3 a	14.7	3.0 b
Fluorescent	16.0 a	15.0	3.1 ab

[x] RB: red and blue; RGB: red, green, and blue; W: white.

[y] Each mean represents 6 individual plant replicates. Means within a column of the same plant size group with different letters are significantly different at the 5% level as determined using the F-Protected Least Significant Difference.

vitro under LEDs lights. Increase six leaves was achieved in 22 weeks, followed by a lag phase of 12 weeks, and additional increase of 4 leaves in 16 weeks (Fig. 5).

(3). Large size (> 8 leaves) of *Erycina pusilla*

The plantlets of large size of *Erycina pusilla* were grown under similar treatments for a period of 50 weeks. Among 4 treatments of large size of *Erycina pusilla*, RB+W+RGB LEDs showed the highest promotive effect on the numbers of roots and leaves during the course of 50 weeks of growth (Fig. 7, Fig. 8). After 50 weeks of cultivation, the numbers of roots and leaves were maximum (19.3, 21.0) under RB+W+RGB LEDs and the minimum (14.7, 15.0) were under RB+W+RB LEDs (Table 1 and Fig. 9). Highest plant height (3.4 cm) was obtained from both RB+W+RGB LEDs and RB+W+RB LEDs, and was significantly different with RGB+W+RGB LEDs (3.0 cm). Double sigmoid growth pattern of increase in numbers of leaves was only observed in the large size of *Erycina pusilla* grown *in vitro* under RB+W+RGB LEDs and fluorescent lamp (Fig. 7). RB+W+RGB LEDs treatment had a profound effect on the increase of roots number, on average, increased by 30 percent. (Fig. 8).

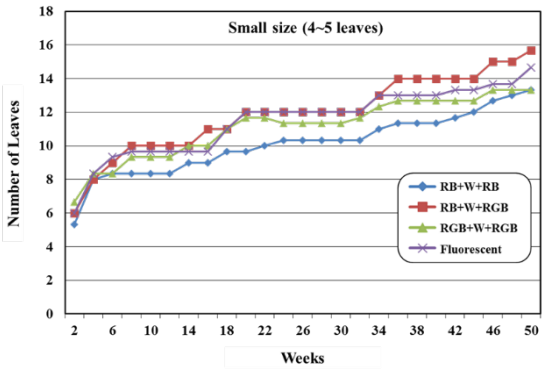


Fig. 3. Effects of LEDs light on the number of leaves of *Erycina pusilla* with small size (4~5 leaves) grown *in vitro* during 50 weeks of cultivation.

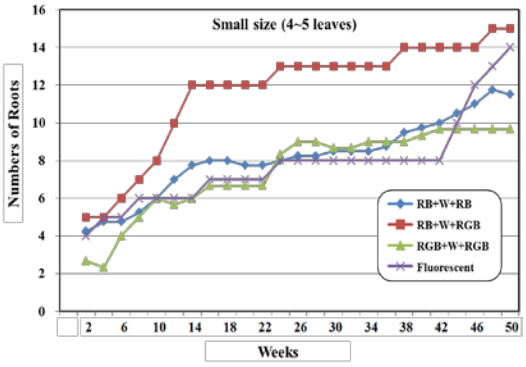


Fig. 4. Effects of LEDs light on the numbers of roots of *Erycina pusilla* with small size (4~5 leaves) grown *in vitro* during 50 weeks of cultivation.

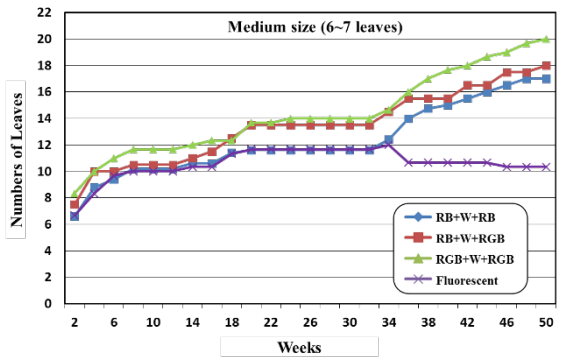


Fig. 5. Effects of LEDs light on the number of leaves of *Erycina pusilla* with medium size (6~7 leaves) grown *in vitro* during 50 weeks of cultivation.

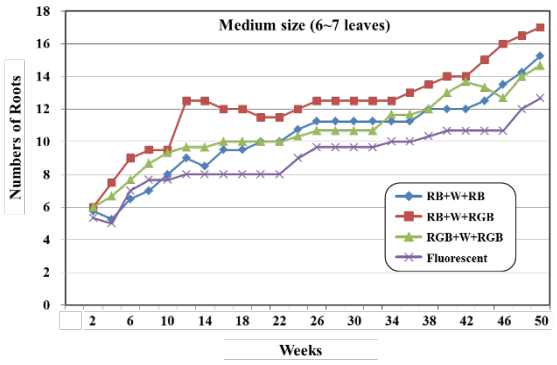


Fig. 6. Effects of LEDs light on the number of roots of *Erycina pusilla* with medium size (6~7 leaves) grown *in vitro* during 50 weeks of cultivation.

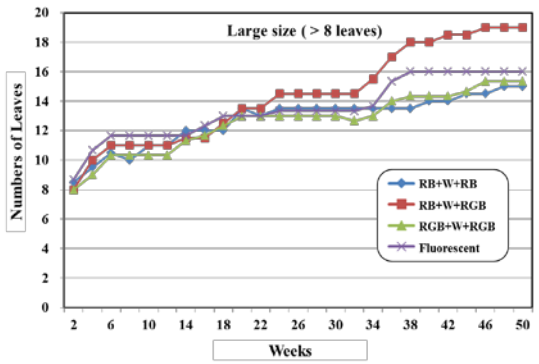


Fig. 7. Effects of LEDs light on the number of leaves of *Erycina pusilla* with large size (>8 leaves) grown *in vitro* during 50 weeks of cultivation.

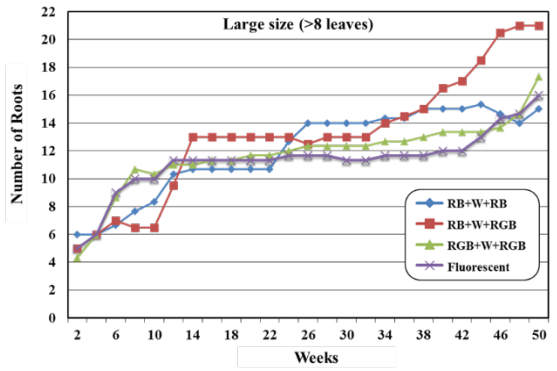


Fig. 8. Effects of LEDs lights on the number of roots of *Erycina pusilla* with large size (>8 leaves) grown *in vitro* during 50 weeks of cultivation.

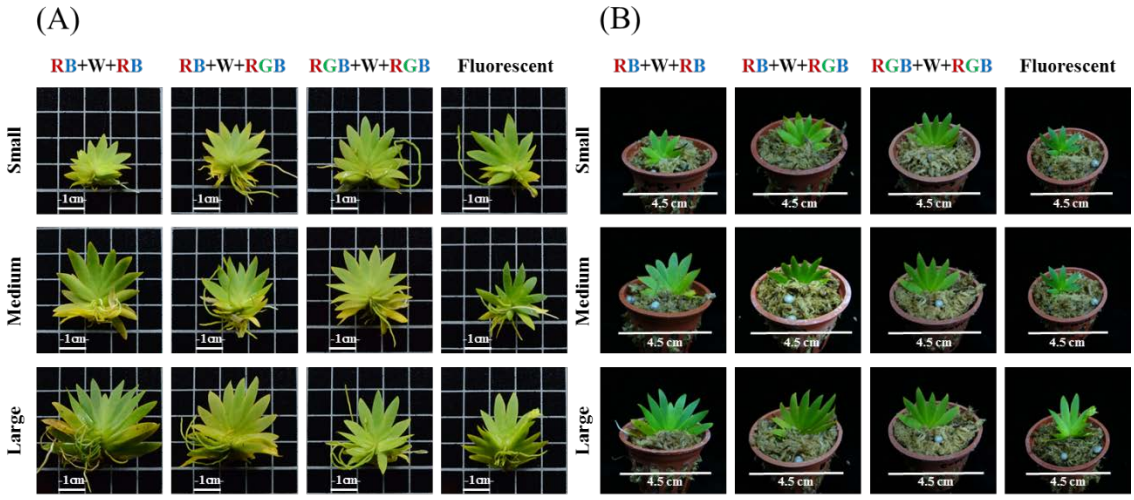


Fig. 9. Appearances of *Erycina pusilla* with different size grown *in vitro* illuminated with different light source after 50 weeks of cultivation (A) and followed by transplanting into pot for 3 weeks (B).

2. The effects of LEDs lights on the chlorophyll fluorescent parameter of *Erycina pusilla* with different size grown *in vitro*

(1). Small size (4-5 leaves) of *Erycina pusilla*

The results of chlorophyll fluorescence analysis showed that the maximum quantum yield of PSII, as revealed by Fv/Fm ratio, was affected by LEDs lights (Table 2). After 25 weeks of *in vitro* cultivation, the maximum fluorescence (Fm) and maximum quantum yield (Fv/Fm) of leaves of small sized plantlets were higher under RB+W+GB and RB+W+RGB than those of under RGB+W+RGB and fluorescent lamps. Whereas after 50 weeks of *in vitro* cultivation, the Fo, Fm and Fv/Fm were maximum (0.5447, 1.4847, 0.6330) under fluorescent lamps and the minimum were under three LEDs lights treatments (Table 2). In general, there was a tendency to decrease in maximum quantum yield (Fv/Fm) of leaves of small sized plantlets after 50 weeks of *in vitro* cultivation in all tree LEDs lights treatments.

(2). Medium size (6-7 leaves) of *Erycina pusilla*

The results of chlorophyll fluorescent analysis of medium size of *Erycina pusilla* during *in vitro* cultivation at 25 and 50 weeks were presented in Table 2. After 25 weeks of *in vitro* cultivation, the highest Fo, Fm and Fv/Fm was observed under RB+W+RB LEDs light with values of 0.3980, 1.0130 and 0.6073, respectively. Whereas after 50 weeks of *in vitro* cultivation, highest Fo, Fm and Fv/Fm was observed under fluorescent light with values of

0.4750, 1.0130 and 0.5310, , respectively (Table 2). In general, there was a tendency to decrease in maximum quantum yield (Fv/Fm) of leaves of medium sized plantlets after 50 weeks of *in vitro* cultivation in all tree LEDs lights treatments.

(3). Large size (> 8 leaves) of *Erycina pusilla*

The results of chlorophyll fluorescent analysis of large size of *Erycina pusilla* during *in vitro* cultivation at 25 and 50 weeks were presented in Table 2. Highest Fo, Fm and Fv/Fm of leaves of large sized plantlets was observed under RB+W+RB LEDs light both after 25 weeks and 50 weeks of *in vitro* cultivation, with values of 0.4340, 0.4223, 1.0390, 0.9783, 0.5823 and 0.5677, respectively (Table 2). In general, there was a tendency to small decrease in maximum quantum yield (Fv/Fm) of leaves of large sized plantlets after 50 weeks of *in vitro* cultivation in all tree LEDs lights and fluorescent lamps treatments.

Discussion

Orchidacaea is a large family in the plant kingdom with wide range of biodiversity. *Oncidium* is one of the most popular and important cut flower especially in Asian. Growth and development of *in vitro* grown plants are regulated by various micro-environmental factors. Light quality is a one of the most important factors that affects the growth and morphogenesis of *in vitro* plants (Huges, 1981). In recent years, with the technological advancement of LEDs, numerous studies have been carried out on a variety of plant species to investigate the adaptation of LEDs as an alternative light source for *in vitro* cultivation. The aim of these studies was mainly to investigate the effects of LEDs applied singly or combination spectra of LEDs on *in vitro Erycina pusilla* growth morphogenesis, and physiological responses.

It is well known that red (R) and blue (B) lights enhances plant growth and development by increasing net photosynthetic rate since the spectral energy distribution of RB consisted with that of chlorophyll absorption (Goins *et al*., 1997; Kim *et al*., 2004). Our results indicated that RB+W+RGB and RB+W+RB LEDs had highest promotive effect on the numbers of roots and leaves during the course of 50 weeks of growth (Fig. 7, Fig. 8). This results was in accordance with previous reports that red light can promoted the induction rate of PLBs of *Oncidium* appeared in the RR treatment (Mengxi *et al*. 2011), and induction of callus in monocots such as *Phalaenopsis* (Park *et al*. 2002), *Cymbidiun* (Huan and Tanaka 2004), *Saccharum sinense* (Liang and Chen, 2006) and *Cattleya* (Cybularz-Urban *et al*., 2007). In *Cymbidium*, the highest of PLB formation was obtained with 25 % red LEDs with 75 % blue LEDs (Huan and Tanaka, 2004). Sivakumar *et al*. (2006) showed that the continuous red light significantly stimulated shoot elongation of sweet potato plantlets *in vitro*.

Table 2. Effects of LEDs light on the chlorophyll fluorescent parameter of *Erycina pusilla* with different size grown *in vitro* for 25 and 50 weeks.

Treatments	25 weeks	50 weeks	25 weeks	50 weeks	25 weeks	50 weeks
	Fo		Fm		Fv/Fm	
			Small size (4-5 leaves)			
RB+W+RB	0.3413 a [y]	0.3817 b	0.9060 a	0.6120 b	0.6223 a	0.3763 c
RB+W+RGB [x]	0.3590 a	0.3560 c	0.9013 a	0.6010 b	0.6017 a	0.4070 b
RGB+W+RGB	0.3553 a	0.3500 c	0.6897 b	0.5640 b	0.4817 b	0.3790 c
Fluorescent	0.3830 a	0.5447 a	0.7427 b	1.4847 a	0.4830 b	0.6330 a
			Medium size (6-7 leaves)			
RB+W+RB	0.3980 a	0.3487 b	1.0130 a	0.6533 b	0.6073 a	0.4657 b
RB+W+RGB	0.3813 a	0.4167 ab	0.8533 b	0.7293 b	0.5527 b	0.4243 b
RGB+W+RGB	0.3643 a	0.3340 b	0.7077 c	0.5327 b	0.4853 c	0.3737 c
Fluorescent	0.2770 b	0.4750 a	0.6403 c	1.0130 a	0.5667 b	0.5310 a
			Large size (> 8 leaves)			
RB+W+RB	0.4340 a	0.4223 a	1.0390 a	0.9783 a	0.5823 a	0.5677 a
RB+W+RGB	0.4223 ab	0.3280 b	0.9530 ab	0.5237 c	0.5567 a	0.3740 c
RGB+W+RGB	0.3563 b	0.3313 b	0.6580 c	0.5137 c	0.4583 b	0.3553 c
Fluorescent	0.4470 a	0.4127 a	0.8273 b	0.7413 b	0.4597 b	0.4427 b

[x] RB: red and blue; RGB: red, green, and blue; W: white.

[y] Each mean represents 6 individual plant replicates. Means within a column of the same plant size group with different letters are significantly different at the 5% level as determined using the F-Protected Least Significant Difference.

Moreover, double sigmoid growth pattern of increase in numbers of leaves was observed in the *Erycina pusilla* grown *in vitro* under LEDs lights, especially in RB+W+RGB LEDs treatment (Fig 3, Fig 5, Fig 7). In general, increase six leaves was achieved in 22 weeks, followed by a lag phase of 12 weeks, and additional increase of 4 leaves in 16 weeks. Double sigmoid growth pattern was reported in grape berry and peach, like those of many berry and drupaceous fruits (Kuhn *et al*., 2014), but not in orchid plants. It's interesting to verify the phenomena of double sigmoid growth pattern existed in the *Erycina pusilla*.

Our results showed that the mixture of RB and RGB (RB+W+RGB) LEDs enhanced plant growth (Table 1). Previous studies had attempted to find the relationship between the ratios of red and blue light on the regulation of plant growth and morphogenesis. In some instances, plant growth was found to be higher under 10 % blue LEDs, whereas in some cases plant growth was enhanced under 30 % blue LEDs in a mixed circuit of blue and red (Nhut and Nam, 2010; Gupta and Jatothu, 2013). Maximum elongation of stem and internode length of *Chrysanthemum* were achieved under red LEDs light (Kim *et al*., 2004). Stem length of upland cotton was stimulated when cultured under BR (1:1) LEDs, whereas found that the stem length in marigold was highest under monochromatic blue LEDs light.

Mengxi *et al*. (2011) reported that the shortest *Oncidium* plantlets were observed in the mixed BB LEDs light treatment, and they concluded that stem elongation was inhibited by blue light. A similar result was present *in vitro* for pepper plantlets (Schuerger *et al*. 1997), *Chrysanthemum* (Kim *et al*. 2004) and *Zantedeschia* (Jao *et al*. 2005). Jao *et al*. (2005) reported that shorter plant height and higher chlorophyll content was present in the RB light treatment, and blue light may be involved in both plant height and chlorophyll development control. According to Nhut *et al*. (2003), the growth of strawberry plantlets under blue LEDs light was inhibited. Lin *et al*. (2010) reported that blue LEDs light was found to be the best condition for promoting shooting of PLBs of *Dendrobium officinale in vitro*. Therefore, it seems that a light treatment that includes blue LEDs light or more blue LEDs light is favorable for the vigorous growth of plantlets. Whereas some research present inhibition of shoot elongation was observed under red light in herbaceous flowering plants such as *maringold* and *salivia* (Heo *et al*., 2002). A different kind of synergistic interactions of the red and blue light receptors (cryptochromes and phytochromes, respectively) on the promotion or inhibition of stem elongation may account for such a difference and may vary with the species (Kim *et al*., 2004).

The Fv/Fm ratio is often used to indicate the maximum quantum efficiency of Photosystem II and is also defined as the maximum photochemical yield of photosystem II in dark-adapted state (Wojciechowska *et al*., 2013). Healthy plant samples achieve a maximum Fv/Fm of 0.850 (Kalaji and Guo, 2008) or 0.830 according to other researchers (Hall and Rao, 1999). In our experiment, highest values of Fv/Fm in *Erycina pusilla* plantlets grown under RB+W+RB LEDs after 25 weeks of cultivation in consistence the vigorous growth of plant (Table 1, Table 2).

The result implies that the composite spectra of red (R), green (G) and blue (B) LEDs light has excellent energy efficiency when used for the growth of plantlets *in vitro*. To control plant photomorphogenesis, LEDs light can be successfully applied for growing plantlets *in vitro* lower cost (Kozai *et al*., 1997 and Mengxi *et al*., 2011). The composite spectra of red (R) and blue (B) LEDs resulted in many positive effects on growth and root activity (Tanaka *et al*., 1998;

Nhut *et al*., 2003; Sivakumar *et al*., 2006; Cybularz-Urban *et al*., 2007; Mengxi *et al*., 2011), which were supported by our results.

However, the effect of light quality is likely to differ according to the plant species, developmental stage of the plant, and environmental conditions such as PPF (Kurilcik *et al*., 2008), medium composition (Schuerger *et al*., 1997) and ventilation (Hahn *et al*., 2000). Our results suggest that LEDs light are an appealing alternative light source for *Erycina pusilla* tissue culture and large-scale micropropagation. Therefore, further studies are needed to elucidate the efficiency of *in vitro* culture techniques and find to correlations between light quality and growth conditions for *in vitro* cultivation of *Erycina pusilla*.

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LEDs 燈對扇形文心蘭(*Erycina pusilla*)組培苗生長與發育之影響

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關鍵字：扇形文心蘭、發光二極體、試管內培養、葉綠素螢光

摘要：本研究調查不同 LED 光源對扇形文心蘭(*Erycina pusilla*)在試管內培養 50 週之生長反應的影響，並以日光燈作為對照，四種光照組合如下：(1) RB+W+RB、(2) RB+W+RGB、(3) RGB+W+RGB (RB：紅藍光；W：白光；RGB：紅綠藍光)、和(4)日光燈。植物材料依照葉片數分為三組：小植株(4-5 片葉)，中植株(6-7 片葉)大植株(>8 片葉)。試驗結果顯示，相較於日光燈及其他 LED 燈處理，在 RB+W+RB 處理下的根數、葉片數和株高有顯著增加。在 LED 燈處理下觀察到葉片數增加呈現雙 S 生長模式，特別是在 RB+W+RGB 的處理組中。在 25 週的培養後，RB+W+RGB 處理組的葉綠素螢光參數 Fo，Fm 和 Fv/Fm 數值有顯著增加，但培養 50 週後，則以日光燈處理的葉綠素螢光參數數值較高。試驗結果顯示 RB+W+RGB 和 RB+W+RB 處理促進扇形文心蘭在試管內之生長與發育。

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