Effect of Light-Emitting Diodes Lighting on Growth and Morphogenesis of 'Tainung No. 2' Papaya *in vitro*

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Summary

The growth and morphology of plants are affected by light qualities. The objective of this study was to investigate the effects of red and blue lights on the growth and morphogenesis of 'Tainung No. 2' papaya seedlings *in vitro*. Germinated papaya seeds were cultured on ½ MS medium and placed under different red and blue light ratios including 100R, 72R28B, 25R75B, and 100B at intensity of 60 μmole m^{-2} s^{-1} for 14 days. Papaya seedlings grown under monochromatic red light (100R) showed undesirable plant height by producing the longest slender shoots. Papaya seedlings in the treatment of combined LED lights (72R28B and 25R75B) and monochromatic blue light (100B) had higher leaf number, SPAD value, and stomata number than monochromatic red light. Vigorous papaya seedlings were obtained in the treatment of combined lights, especially the combination of high red ratios and blue light treatment (72R28B), which had greater fresh and dry weight and higher ratios of plant fresh and dry weight to plant height than monochromatic red or blue light. In summary, combined LED light was an effective spectrum to improve and enhance the quality of papaya seedlings *in vitro*.

Introduction

Papaya (*Carica papaya* L.) is one of the most important fruit trees in tropical and subtropical regions (Fuentes and Santamaria, 2014). The papaya plant is a fast-growing semi-woody herb (Morton, 1987). Papaya is a trioecious plant that has three kinds of sex forms including female,

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male, and hermaphrodite (Ming *et al*., 2007). In commercials, hermaphrodite fruit is more desired than female fruit because of its shape and flavor (Wu *et al*., 2012). According to the statistics of the Food and Agriculture Organization of the United Nations (FAO), world papaya production increased 34.3% from 10,114.7 thousand tons in 2008 to 13,894.7 thousand tons in 2020.

Light plays an important role throughout the whole life cycle of plants (Yang and Liu, 2020). Fluorescent lamps, high-pressure sodium lamps, metal halide lamps, and incandescent lamps are traditional artificial light sources that have a wide range of wavelengths (Gupta and Jatothu, 2013; Deram, 2014). Recently, light-emitting diodes (LEDs) are continuously expanding for use in horticulture due to their advantages, such as longevity, high efficiency, specific wavelength, small size, and cool emitting surface (Bourget, 2008; Massa, 2008; Gupta and Jatothu, 2013).

It has been reported that red light can promote shoot elongation in many plants (Wollaeger and Runkle, 2014). Red light can also promote root growth in grapes (Poudel *et al*., 2008). Whereas, blue light can promote leaf growth, stomatal number, SPAD value, and chlorophyll contents (Poudel *et al*., 2008; Son and Oh, 2013; Liu *et al*., 2018). High ratios of blue light combined with red light decreased plant height in many plants, such as cucumber (Hernandez and Kubota, 2016), tomato (Hernandez *et al*., 2016), and papaya seedlings (Teixeira da Silva, 2014). The objective of this study was to investigate the effects of red and blue lights on growth and morphogenesis of 'Tainung No. 2' papaya seedlings *in vitro*.

Materials and Methods

Papaya seeds cv. 'Tainung No. 2' from mature fruit were washed with running tap water and tested by simple floatation for determining seed viability. Only sunk and non-split seeds were used. Seeds were gently scraped against a kitchen sieve to remove sarcotesta. Seeds were dabbed with kitchen paper towels to remove excess water (Giang *et al*., 2011). Naked seeds were surface sterilized in 1% sodium hypochlorite (NaOCl) containing 0.1% tween-20 in an ultrasonic cleaner (B-2210, Branson) for 20 minutes then were rinsed 3 times with sterilized distilled water in a laminar hood.

Surface-sterilized seeds were cultured on a basic medium containing ½ MS (Murashige and Skoog basal salt with mineral organics, Sigma) supplemented with 3% sucrose and 0.8% agar. The medium was adjusted to pH 5.8 prior to autoclaving at 121 °C for 20 minutes. Germinated seeds were cultured on the basic medium and placed under different red and blue light ratios (Nano Bio Light Company) at 26 ± 1 °C with a 16-h photoperiod and intensity of 60 μmole m^{-2} s^{-1} for 14 days. The peak wavelengths of red and blue LEDs were 660 nm and 450 nm, respectively.

There were five germinated seeds in one bottle as one replicate. There were three replicates for each treatment. The experiment was repeated three times.

1. Treatments of different red and blue light ratios

(1). 100R: Red (660 nm) 100%

(2). 72R28B: Red 72% and Blue (450 nm) 28%

(3). 25R75B: Red 25% and Blue 75%

(4). 100B: Blue 100%

2. Measurement and analysis

(1). Plant growth and morphogenesis parameters

A. Plant height (cm) was measured by a ruler.

B. The number of leaves was counted.

C. Internode length (cm) was calculated by height (cm)/ the number of nodes.

D. The number of primary roots was counted.

E. Primary root length (cm) was measured by a ruler.

F. Stem diameter (mm) was measured by a vernier caliper at 1 cm height from the base of the shoot.

G. SPAD value was measured by chlorophyll content meter (Model CL-01, Hensatech Instrument) at the 3^{rd} and 4^{th} leaves counted from the shoot top.

H. Total leaf area (cm^2) was analyzed with Java-based image processing program ImageJ (National Institutes of Health, USA).

I. Fresh weight (mg) of leaf, stem, and root was measured.

J. Dry weight (mg) of leaf, stem, and root was measured. Leaf, stem, and root were dried at 60 °C for 72 hours and then weighed.

K. Whole plant fresh weight/ height (mg/cm) was calculated.

L. Whole plant dry weight/ height (mg/cm) was calculated.

M. Water content (%): ((fresh weight - dry weight)/ fresh weight) × 100%

2. Plant tissue analysis

(1) Stomata observation

The abaxial surface of leaf was stuck by transparent tape. The adaxial surface of leaf was gently scraped until the abaxial surface of leaf was seen. The leaf was cut into 5×5 cm, stained with safranin, and then rinsed 3 times with sterilized distilled water. The stained leaf was placed onto a microscopic glass slide. The number of stomata per mm^2, stomata width (μm), and stomata length (μm) were measured under a microscope by using ImageView software. Stomatal area was measured by using Java-based image processing program ImageJ (National Institutes of Health, USA).

(2). Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Enterprise Guide) version 7.1. The data were analyzed with the least significant difference (LSD) test at $p \leq 0.05$.

Results

There were significant differences in plant height and internode length among different red and blue light ratios (Table 1; Figure 1). The highest plant height and internode length were 12.98 cm and 2.66 cm in the treatment of 100R, respectively. However, there were no significant differences in plant height and internode length among blue light treatments (72R28B, 25R75B, and 100B). Stem diameter of papaya seedlings was not significantly affected by different red and blue light ratios (Table 1).

Different red and blue light ratios significantly affected the number of leaves and SPAD values of 'Tainung No. 2' papaya seedlings *in vitro* (Table 2). The highest and lowest leaf numbers were obtained in the 72R28B (6.16) and 100R (5.03) treatments, respectively. The lowest SPAD value was found in the treatment of 100R (9.51). Nevertheless, there were no significant differences between the combined red and blue light treatments (72R28B and 25R75B) and the

Table 1. Effect of red and blue lights on morphology of 'Tainung No. 2' papaya seedlings *in vitro*.

Light source[z]	Plant height (cm)	Stem diameter (mm)	Internode length (cm)
100R	12.98±1.26[y] a[x]	1.62±0.13 a	2.66±0.29 a
72R28B	3.90±0.31 b	1.42±0.02 a	0.63±0.04 b
25R75B	3.33±0.25 b	1.50±0.17 a	0.52±0.02 b
100B	3.08±0.30 b	1.24±0.05 a	0.59±0.03 b

[z]Germinated papaya seeds were cultured under different red and blue light ratios for 14 days. 100R: Red 100%, 72R28B: Red 72%, Blue 28%, 25R75B: Red 25%, Blue 75%, and 100B: Blue 100%.

[y]Mean ± standard error of three repeats.

[x]Means within each column followed by different letters are significantly different at $p \leq 0.05$ by the LSD test.

monochromatic blue treatment (100B). There were no significant differences in total leaf area among different LEDs (Table 2). There were not significantly different in the primary root number among different red and blue light ratios (Table 3). However, 100R treatment showed the highest primary root length (9.86 cm), but there were no significant differences between the combined red and blue light and the monochromatic blue treatments (Table 3).

Table 2. Effect of red and blue lights on leaf growth of 'Tainung No. 2' papaya seedlings *in vitro.*

Light source[z]	Leaf no.	SPAD value	Total leaf area (cm^2)
100R	5.03±0.10[y] b[x]	9.51±0.08 b	1.82±0.34 a
72R28B	6.16±0.15 a	18.91±0.89 a	2.14±0.49 a
25R75B	5.77±0.23 ab	21.71±1.25 a	1.39±0.36 a
100B	5.63±0.23 ab	20.28±1.87 a	1.43±0.36 a

[z]Germinated papaya seeds were cultured under different red and blue light ratios for 14 days. 100R: Red 100%, 72R28B: Red 72%, Blue 28%, 25R75B: Red 25%, Blue 75%, and 100B: Blue 100%.

[y]Mean ± standard error of three repeats.

[x]Means within each column followed by different letters are significantly different at $p \leq 0.05$ by the LSD test.

Table 3. Effect of red and blue lights on root growth of 'Tainung No. 2' papaya seedlings *in vitro.*

Light source[z]	Primary root no.	Average primary root length (cm)
100R	5.86±0.36[y] a[x]	9.86±0.26 a
72R28B	6.92±0.41 a	6.47±0.66 b
25R75B	6.07±0.17 a	5.17±0.60 b
100B	5.92±0.10 a	4.93±0.84 b

[z]Germinated papaya seeds were cultured under different red and blue light ratios for 14 days. 100R: Red 100%, 72R28B: Red 72%, Blue 28%, 25R75B: Red 25%, Blue 75%, and 100B: Blue 100%.

[y]Mean ± standard error of three repeats.

[x]Means within each column followed by different letters are significantly different at $p \leq 0.05$ by the LSD test.

There were significant differences in the stomata number, but there were no significant differences in stomatal width, stomatal length, and stomatal area among different light ratios (Table 4; Figure 2). Monochromatic red light showed the lowest stomatal density (267.33/mm2). There were no significant differences in stomatal number among 72R28B, 25R75B, and 100B treatments (Table 4). Fresh weight and dry weight were affected by different light ratios (Table 5; Figure 1). Papaya seedlings in the treatment of 72R28B had the highest whole plant fresh weight (1194.30 mg) and dry weight (94.22 mg); leaf fresh weight (113.41 mg) and dry weight (21.17 mg); root fresh weight (1010.40 mg) and dry weight (65.09 mg). The lowest whole plant fresh and dry weight (720.30 mg and 65.99 mg); leaf fresh weight and dry weight (73.47 mg and 13.55 mg) were obtained in the treatment of monochromatic blue (100B). Monochromatic red treatment (100R) showed the highest shoot fresh weight (247.88 mg) and dry weight (21.13 mg).

The highest whole plant fresh weight to height ratio (308.25 mg/cm) was found in the treatment of 72R28B, but there were no significant differences with 25R75B treatment (Table 6). Monochromatic red treatment had the lowest whole plant fresh weight to plant height ratio (80.42 mg/cm) and whole plant dry weight to plant height ratio (6.92 mg/cm). The highest and lowest water content were observed in 72R28B treatment (92.11%) and 100B treatment (90.80%), respectively (Table 6).

Table 4. Effect of red and blue lights on stomata characteristics of 'Tainung No. 2' papaya seedlings *in vitro*.

Light source[z]	Number of stomata /mm^2	Stomata width (μm)	Stomata length (μm)	Stomatal area (μm^2)
100R	267.33±55.52[y] b[x]	18.69±0.72 a	22.81±1.21 a	299.74±19.59 a
72R28B	496.89± 6.16 a	20.40±0.43 a	23.18±0.88 a	327.58±19.03 a
25R75B	566.22±32.88 a	18.74±0.20 a	22.31±0.22 a	295.26± 9.59 a
100B	509.56±48.04 a	18.48±0.52 a	22.78±0.92 a	295.89±13.71 a

[z]Germinated papaya seeds were cultured under different red and blue light ratios for 14 days. 100R: Red 100%, 72R28B: Red 72%, Blue 28%, 25R75B: Red 25%, Blue 75%, and 100B: Blue 100%.

[y]Mean ± standard error of three repeats.

[x]Means within each column followed by different letters are significantly different at $p \leq 0.05$ by the LSD test.

Fig. 1. Growth of papaya seedlings under red and blue lights after 14 days of culture *in vitro*. 100R: Red 100% (A), 72R28B: Red 72%, Blue 28% (B), 25R75B: Red 25%, Blue 75% (C), and 100B: Blue 100% (D). Scale bar is 5 cm.

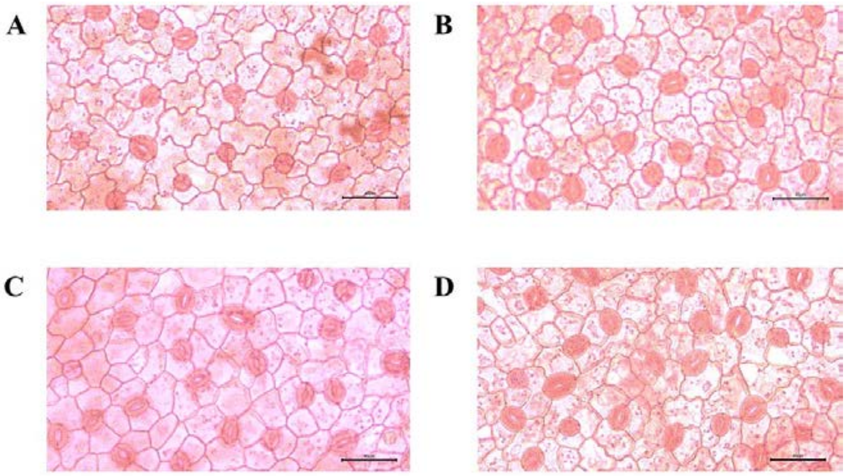

Fig. 2. Stomata characteristics of papaya leaves under red and blue lights after 14 days of culture *in vitro*. 100R: Red 100% (A), 72R28B: Red 72%, Blue 28% (B), 25R75B: Red 25%, Blue 75% (C), and 100B: Blue 100% (D). Scale bar is 40 μm.

Table 5. Effect of red and blue lights on fresh weight and dry weight of 'Tainung No. 2' papaya seedlings *in vitro*.

Light	Whole plant		Leaf	
source[z]	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
100R	1012.20± 53.63[y] ab[x]	87.09±6.07 ab	104.93±4.55 ab	15.72±0.69 b
72R28B	1194.30± 93.07 a	94.22±7.55 a	113.41±7.94 a	21.17±1.43 a
25R75B	899.70±106.80 ab	75.87±6.06 ab	95.64±9.90 ab	17.85±1.22 ab
100B	720.30± 52.96 b	65.99±3.72 b	73.47±9.61 b	13.55±1.50 b

Light	Shoot		Root	
source[z]	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
100R	247.88±36.90[y] a[x]	21.13±2.92 a	659.40± 40.70 b	50.24±3.94 a
72R28B	70.48± 4.33 b	7.95±0.41 b	1010.40± 86.32 a	65.09±6.35 a
25R75B	53.72± 4.59 b	6.67±0.38 b	750.30±106.38 ab	51.34±5.26 a
100B	47.36± 2.99 b	5.87±0.46 b	599.50± 43.97 b	46.57±2.42 a

[z]Germinated papaya seeds were cultured under different red and blue light ratios for 14 days. 100R: Red 100%, 72R28B: Red 72%, Blue 28%, 25R75B: Red 25%, Blue 75%, and 100B: Blue 100%.

[y]Mean ± standard error of three repeats.

[x]Means within each column followed by different letters are significantly different at $p \leq 0.05$ by the LSD test.

Discussion

In this study, 'Tainung No. 2' papaya seedlings were treated with different LED lights *in vitro*. It was found that the treatment of monochromatic red light had the highest plant height and internode length, but there were no significant differences in plant height and internode length among blue light treatments (Table 1; Figure 1). Red light promoted shoot elongation in inpatient, petunia, salvia, and tomato seedlings (Wollaeger and Runkle, 2014). Poudel *et al*. (2008) reported that the greatest shoot elongation and internode length was obtained under monochromatic red light in grapevines. Plant height decreased when plants were placed under an increase of blue light ratios in combination with red light in many plants, for instance, cucumber (Hernandez and Kubota, 2016), tomato (Hernandez *et al*., 2016), pepper (Li *et al*., 2016), and papaya seedlings

Table 6. Effect of red and blue lights on plant fresh and dry weight to plant height ratios and water content of 'Tainung No. 2' papaya seedlings *in vitro*.

Light source[z]	Plant fresh weight/height (mg/cm)		Plant dry weight/height (mg/cm)	Water content (%)
100R	80.42± 9.11[y]	c[x]	6.92±0.91 b	91.42±0.16 ab
72R28B	308.25±18.16	a	24.38±0.18 a	92.11±0.12 a
25R75B	295.88±33.23	ab	24.94±1.96 a	91.45±0.37 ab
100B	219.57±17.78	b	20.24±0.90 a	90.80±0.20 b

[z]Germinated papaya seeds were cultured under different red and blue light ratios for 14 days. 100R: Red 100%, 72R28B: Red 72%, Blue 28%, 25R75B: Red 25%, Blue 75%, and 100B: Blue 100%.
[y]Mean ± standard error of three repeats.
[x]Means within each column followed by different letters are significantly different at $p \leq 0.05$ by the LSD test.

(Teixeira da Silva, 2014). Blue light is attributed to the stimulated cryptochrome photoreceptors leading to plant height reduction (Sellaro *et al*., 2009; Hernandez *et al*., 2016), consistent with this study. Papaya seedlings showed plant height reduction in the blue light treatments (Table 1; Figure 1).

Different plants might respond differently to light quality. Hernandez *et al*. (2016) reported that tomato seedlings under combined red and blue light had greater leaf number and leaf area than under monochromatic red light, but there were no significant differences between combined light sources. Whereas, leaf area of cucumber seedlings decreased with an increase of blue light ratios combined with red light (Hernandez and Kubota, 2016). Monochromatic red light promoted leaf expansion and elongation in lettuces (Son and Oh, 2013). In this study, the combination of red and blue light showed higher leaf number and SPAD value than monochromatic red light (Table 2), which could promote light interception and photosynthesis. Similar to the previous studies, the combination of red and blue light promoted chlorophyll contents more than monochromatic light in tomatoes (Hernandez and Kubota, 2016; Liu *et al*., 2018), cucumbers (Hernandez *et al*., 2016), papayas (Teixeira da Silva, 2014), and strawberries (Choi *et al*., 2015). Hernandez and Kubota (2016) stated that chlorophyll biosynthesis could be dependent on the synergism between cryptochromes and phytochromes leading to lower chlorophyll biosynthesis under monochromatic red light.

Light quality could affect plant growth by regulating stomatal movement through stomatal opening and stomata density (Wang *et al*., 2014; Liu *et al*., 2018). This study showed that stomata number was influenced by different LED lights (Table 4). Blue light alone or combined light sources had double stomata number of monochromatic red light (Table 4; Figure 2). A combination of blue and red lights had higher stomata numbers than monochromatic red light in cherry tomatoes (Liu *et al*., 2018), lettuces (Muneer *et al*., 2014), and peppers (Claypool and Lieth, 2020). Stomata development was influenced by blue light through the additive function of CRYPTOCHROME 1 and 2 (CRY1 and CRY2), which are photoreceptors of blue light (Pillitteri and Torii, 2012).

The treatment of high red light ratios combined with blue light had the highest fresh and dry weight in whole plants, leaves, and roots (Table 5). However, the treatment of monochromatic red light had the highest shoot weight because of their longest shoots (Table 5). Fresh and dry weight of plants under different light quality varied in the literatures. Cucumber seedlings showed the greater shoot fresh and dry mass under monochromatic blue light (Hernandez and Kubota, 2016). In contrast, the treatment of monochromatic red light had the higher fresh and dry weight in lettuces (Son and Oh, 2013) and tomatoes (Wollaeger and Runkle, 2014; Yang *et al*., 2019). The treatment of a combination of red and blue light had higher fresh and dry mass than monochromatic red or blue light in tomato (Hernandez *et al*., 2016) and pepper seedlings (Claypool and Lieth, 2020). Plant compactness, which is the relationship between dry mass and plant height determines seedling quality (Hernandez *et al*., 2016). In this study, papaya seedlings under combined light sources showed greater plant fresh weight or dry weight to plant height ratios than monochromatic light (Table 6). These findings further confirmed that the combined LED light was favorable for the growth of papaya seedlings.

Papaya seedlings grown under monochromatic red light showed lower growth rate and undesirable plant height. In addition, vigorous papaya seedlings were obtained in the treatments of blue lights which had higher leaf number, SPAD value, and stomata number than in the treatment of monochromatic red light. However, papaya seedlings grown under combined lights, especially in the treatment of a combination of high red ratios and blue light, had greater fresh and dry weight and higher ratios of plant fresh and dry weight to plant height than monochromatic red or blue light. In summary, combined LED light could enhance the growth and morphogenesis performance of 'Tainung No. 2' papaya seedlings *in vitro*.

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LED 光源對組織培養之'台農 2 號'番木瓜
生長及形態之影響

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關鍵字：番木瓜、LED 光源、光質

摘要：植物的生長與形態發生受到光質的影響。本研究調查紅藍光源對組織培養之'台農 2 號'番木瓜生長及形態發生之影響。將發芽的木瓜種子移至½ MS 培養基中，之後在不同比例的紅光和藍光(100R、72R28B、25R75B 和 100B)下以 60 μmole m^{-2} s^{-1} 的光強度培養 14 天。在單紅光處理(100R)下之番木瓜幼苗會產生過長的纖細枝條。組合 LED (72R28B 和 25R75B)光源和單藍光(100B)下之番木瓜幼苗葉片數、SPAD 值和氣孔數顯著高於單紅光之處理組，此外組合 LED 光源，尤其是高比例的紅光結合藍光處理(72R28B)下的番木瓜幼苗生長旺盛，植株鮮重和植株乾重、株鮮重與株高比及株乾重與株高比均顯著高於單紅光或單藍光之處理組。總結而言，組合 LED 光源能有效提升組織培養番木瓜幼苗之品質。

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