Effects of Light-Emitting Diodes on Growth and Morphogenesis of Pineapples *in vitro*

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Summary

The effect of different light emitting diode (LED) lights on growth and morphogenesis of 'Tainung 17' (TN17) and 'MD-2' pineapples cultured *in vitro* was investigated. Plantlets regenerated from axillary buds of crown stems and meristematic globular bodies were used in this study.

The result showed that there were significant differences in leaf and root numbers of 'TN17' plantlets regenerated from axillary buds of crown stems among treatments. The lowest leaf and root numbers were obtained under infrared light (9IR) treatment. The density of stomata of 'TN17' plantlets regenerated from axillary buds of crown stems in the blue light (9B) treatment was the highest (147/mm^2). 'TN17' and 'MD-2' plantlets regenerated from meristematic globular bodies had significant differences in chlorophyll contents. Plantlets under 9IR treatment had the lowest chlorophyll contents in both 'TN17' and 'MD-2' cultivars. 'TN17' plantlets regenerated from meristematic globular bodies in 9B treatment had the lowest stomatal density. The stomatal density in CW treatments were the highest and that in 9IR treatment was the lowest in 'MD-2' plantlets regenerated from meristematic globular bodies.

In summary, different LED light sources had significant effects on growth and morphogenesis of 'TN17' and 'MD-2' pineapples cultured *in vitro*. Red and blue lights were more effective than IR light on promoting growth and development of pineapples.

Introduction

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Pineapple (*Ananas comosus*) is one of the most important tropical fruit in the world (Usman *et al*., 2013). Costa Rica, Brazil, Philippines, Thailand and Indonesia are the five leading producers of pineapple (FAO, 2014). Pineapple fruits are consumed fresh or processed into canned fruit, juice, and jam (Usman *et al*., 2013).

Traditional propagations of pineapple use slips, crowns and suckers (Hepton, 2003). Micropropagation has many advantages than traditional methods, which allows a rapid increase in a short time (Akbar *et al*., 2003; Zuraida *et al*., 2011). Light is an important factor for growth and development of pineapple plants *in vitro* (Huges, 1981). Fluorescent lamps is the common light source for *in vitro* culture of plants, but some research and commercial laboratories use metal halide, sodium or incandescent lamps (Shin *et al*., 2008). Recently, light emitting diode (LED) has been developed and is used as a new choice for the light source for *in vitro* culture of plants (Shin *et al*., 2008). People have an interest in LEDs because of their specific wavelengths and narrow bandwidth, small size, long life and cool light (Brown *et al*., 1995). LEDs have been used for research in many areas such as photosynthesis (Tennessen *et al*., 1994), chlorophyll content (Tripathy and Brown, 1995), growth and morphogenesis (Hoenecke *et al*., 1992; Brown *et al*., 1995).

The aim of this study was to evaluate the effect of LED lights on the growth and morphogenesis of ‘TN17’ and ‘MD-2’ pineapples *in vitro*.

Materials and methods

I. Growth of axillary buds of 'TN17' pineapple crowns

The stem of the crown was surface sterilized with 1% sodium hypochlorite containing 0.1% tween-20 for 20 minutes and then washed thrice in sterilized water. Axillary buds were excised from the crown stem and cultured in a basic medium containing 1/2 strength MS (Murashige and Skoog, 1962) supplemented with 30 g l^{-1} sucrose and 8 g l^{-1} agar. The medium pH was adjusted to 5.8 before autoclaved at 121℃ for 20 minutes. The explants were first grown under fluorescent light at 26 ± 1℃ with a 16-h photoperiod for 2 weeks and then cultivated under different LED light sources (Nano Bio Light Company) at 26 ± 1℃ with a 16-h photoperiod and intensity of 56 μmole/m^2/sec for 60 days. Five axillary buds in one bottle was one replicate and there were three replicates for each treatment.

Treatments of mono-wavelength light

9R – Red 100% (660 nm)

9B – Blue 100% (450 nm)

9IR –Infrared 100% (730 nm)

CW – Cool white (5500 K; Red 23%, Green 59%, Blue 18%)

Fluorescent light

II. Growth of meristematic globular bodies (MGB) of 'TN17' and 'MD-2' pineapples

The explants, with their leaves shortened were subcultured on the basic medium supplemented with 16 ppm BA and 1 ppm NAA for 45 days. MGB was induced and then cut off from the explants at a size about 1×1 cm and were used for experiments.

MGB was cultured on the basic medium. The culture was kept under different LED light sources at 26 ± 1℃ with a 16-h photoperiod and intensity of 56 μmole/m^2/sec for 30 days. Ten MGBs in one bottle was one replicate and there were three replicates for each treatment. The light sources were the same as 1.

III. Measurement and analysis

1. Plant growth parameters:

A. Fresh weight: fresh weight of leaf, root and whole plant were measured.

B. The number of leaf and root were counted.

C. Primary root lengths were measured by a ruler.

D. Leaf area was measured by a Leaf Area Meter (AM 300, ADC BioScientific Ltd.)

E. Dry weight: plants were dried at 70℃ for 72 hour and dry weight were measured.

2. Plant tissue analysis

A. Stomata observation:

Use clear nail polish on the lower surface of the leaf. The epidermal tissue of leaf was peeled off carefully by tweezers. The stomatal densities and size were recorded under a microscope.

B. Chlorophyll analysis:

The leaf sample was ground with quartz sand and 5 ml of 95% acetone. The sample was then poured into a tube and 5 ml of 80% acetone was used to wash the mortar and then poured into the same tube. The sample was kept in the dark for 24 hours. The sample was then centrifuged with a centrifugal force 6,000 ×g for 10 minute. After that, the supernatant was adjusted with 80% of acetone to a total volume of 20 ml. The sample was then measured with a spectrophotometer (GENESYS 20 or Helios Epsilon Spectrophotometer) at 663 nm for chlorophyll a and at 645 nm for

chlorophyll b. Total chlorophyll, chlorophyll a and chlorophyll b contents were calculated using the following equation (Arnon, 1949):

1) Chlorophyll a (mg/g) = $(12.7 \times OD_{663} - 2.69 \times OD_{645})$

2) Chlorophyll b (mg/g) = $(22.9 \times OD_{645} - 4.68 \times OD_{663})$

3) Total chlorophyll (mg/g) = $(8.02 \times OD_{663}) + (20.21 \times OD_{645})$

4. Statistical analysis

All data were subjected to analysis of variance (ANOVA) analysis using the Statistical Analysis System (SAS) version 9.4. A probability of $p \leq 0.05$ was considered as significant differences.

Results

There were significant differences in the fresh weight and dry weight of 'TN17' pineapple plantlets regenerated from axillary buds of crown stems among different LEDs (Table 1). The highest whole plant fresh weight and dry weight were 612.63 mg and 63.15 mg under the 9B treatment, respectively. The lowest whole plant fresh weight and dry weight (217.81 mg and 16.14 mg, respectively) were obtained in the treatment of 9IR. The leaf fresh and dry weight in the 9B treatment was the highest (552.47 mg and 56.11 mg, respectively) and that in the 9IR treatment was the lowest (204.61 mg and 14.55 mg, respectively). The lowest root fresh weight and dry weight were obtained in the treatment of 9IR (10.17 mg and 1.53 mg, respectively).

The highest leaf and root number were obtained in the CW and 9B treatment, respectively (Table 2). The lowest leaf and root numbers were 6.93 and 3.53 under 9IR treatment. Different LEDs did not significantly affect primary root length, total leaf area, leaf length and leaf width of 'TN17' pineapple plantlets regenerated from axillary buds of crown stems.

Table 1. Effects of different light sources on fresh weight and dry weight of 'TN17' pineapple plantlets regenerated from axillary buds of crown stems after 60 days of culture *in vitro*.

Light source[z]	Whole plant	
	Fresh weight (mg)	Dry weight (mg)
9R	567.25 ± 143.55[y] a[x]	52.52 ± 13.27 a
9B	612.63 ± 253.15 a	63.15 ± 30.55 a
9IR	217.81 ± 17.06 b	16.14 ± 1.18 b
CW	416.08 ± 108.92 ab	39.95 ± 9.74 ab
Fluorescent	587.25 ± 38.96 a	58.35 ± 7.63 a

Light source	Leaf	
	Fresh weight (mg)	Dry weight (mg)
9R	498.39 ± 131.86 a	46.79 ± 11.98 a
9B	552.47 ± 226.74 a	56.11 ± 27.78 a
9IR	204.61 ± 19.61 b	14.55 ± 1.14 b
CW	377.27 ± 79.49 ab	34.91 ± 8.10 ab
Fluorescent	505.09 ± 57.77 a	51.76 ± 5.95 a

Light source	Root	
	Fresh weight (mg)	Dry weight (mg)
9R	53.27 ± 16.94 a	5.63 ± 1.22 a
9B	48.27 ± 22.89 a	7.01 ± 2.73 a
9IR	10.17 ± 3.80 b	1.53 ± 0.60 b
CW	38.51 ± 10.49 a	4.94 ± 1.67 a
Fluorescent	50.53 ± 13.05 a	6.37 ± 1.62 a

[z]9R: Red 100%, 9B: Blue 100%, 9IR: Infrared 100%, CW: Cool white.

[y]Mean ± standard deviation.

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

Table 2. Effects of different light sources on leaf and root growth of 'TN17' pineapple plantlets regenerated from axillary buds of crown stems after 60 days of culture *in vitro*.

Light source[z]	Leaf no.	Root no.	Primary root length (cm)
9R	11.87 ± 0.64[y] ab[x]	6.93 ± 0.76 ab	4.21 ± 0.32 a
9B	10.47 ± 4.31 ab	9.27 ± 3.53 a	3.27 ± 1.48 a
9IR	6.93 ± 1.10 b	3.53 ± 2.34 b	3.64 ± 2.22 a
CW	12.73 ± 0.50 a	8.20 ± 1.59 a	3.57 ± 0.48 a
Fluorescent	12.00 ± 4.93 ab	7.67 ± 2.80 ab	3.38 ± 1.20 a

Light source	Total leaf area (mm^2)	Leaf length (mm)	Leaf width (mm)
9R	164.40 ± 143.41 a	23.01 ± 20.37 a	5.81 ± 5.08 a
9B	183.13 ± 143.18 a	23.61 ± 16.49 a	6.62 ± 4.68 a
9IR	86.80 ± 107.32 a	18.81 ± 21.10 a	3.13 ± 3.31 a
CW	194.87 ± 57.97 a	30.05 ± 8.38 a	7.53 ± 1.63 a
Fluorescent	262.67 ± 77.53 a	40.35 ± 10.75 a	8.79 ± 1.39 a

[z]9R: Red 100%, 9B: Blue 100%, 9IR: Infrared 100%, CW: Cool white.

[y]Mean ± standard deviation.

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

The chlorophyll contents were not significantly affected by different light sources (Table 3). The result showed that plantlets under fluorescent light treatment had the highest contents of chlorophyll a (1.30 mg/g), chlorophyll b (0.84 mg/g) and total chlorophyll (2.14 mg/g). Plantlets in the treatment of 9IR had the lowest chlorophyll a (0.49 mg/g) and total chlorophyll (0.96 mg/g) contents.

There were significant differences in the stomatal number and stomatal width of 'TN17' pineapple plantlets (Table 4). The highest and lowest stomatal density were obtained in the 9B (147.00/mm^2) and 9IR (88.33/mm^2) treatments, respectively. Stomatal width in the treatment of 9R was the highest (233.68 µm) and the lowest (203.71 µm) one was in the treatment of 9IR.

Table 3. Effects of different light sources on chlorophyll content of 'TN17' pineapple plantlets regenerated from axillary buds of crown stems after 60 days of culture *in vitro*.

Light source[z]	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
9R	0.69 ± 0.62[y] a[x]	0.45 ± 0.40 a	1.15 ± 1.02 a
9B	0.67 ± 0.54 a	0.39 ± 0.29 a	1.05 ± 0.83 a
9IR	0.49 ± 0.43 a	0.47 ± 0.41 a	0.96 ± 0.84 a
CW	0.96 ± 0.34 a	0.58 ± 0.22 a	1.53 ± 0.56 a
Fluorescent	1.30 ± 0.17 a	0.84 ± 0.15 a	2.14 ± 0.26 a

[z]9R: Red 100%, 9B: Blue 100%, 9IR: Infrared 100%, CW: Cool white.

[y]Mean ± standard deviation.

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

Table 4. Effects of different light sources on stomatal number and stomatal length and width of 'TN17' pineapple plantlets regenerated from axillary buds of crown stems after 60 days of culture in vitro.

Light source[z]	No. of stomata/mm^2	Stomatal length (µm)	Stomatal width (µm)
9R	137.00 ± 12.17[y] ab[x]	272.70 ± 12.16 a	233.68 ± 6.07 a
9B	147.00 ± 4.58 a	272.89 ± 2.14 a	220.71 ± 3.20 b
9IR	88.33 ± 7.02 c	299.99 ± 26.86 a	203.71 ± 2.08 c
CW	124.00 ± 3.61 b	289.47 ± 37.29 a	217.23 ± 4.36 b
Fluorescent	129.00 ± 5.29 b	272.87 ± 24.33 a	217.92 ± 8.09 b

[z]9R: Red 100%, 9B: Blue 100%, 9IR: Infrared 100%, CW: Cool white.

[y]Mean ± standard deviation.

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

The contents of chlorophyll a chlorophyll b and total chlorophyll were significant differences among treatments of 'TN17' pineapple plantlets regenerated from MGB (Table 5). The result

showed plantlets under 9IR treatment had the lowest contents of chlorophyll a (0.17 mg/g) and chlorophyll b (0.10 mg/g) and total chlorophyll (0.27 mg/g). The chlorophyll contents were higher in the treatment of CW and fluorescent light.

There were significant differences in the number of stomatal, stomatal length and stomatal width of 'TN17' pineapple plantlets regenerated from MGB among different light sources (Table 6). The highest stomatal number was obtained in the CW treatment (138.87/mm^2). Plantlets in the treatment of 9B was the lowest stomatal number (86.02/mm^2), but those was the highest stomatal length (402.12 µm) and stomatal width (268.50 µm). Plantlets under 9R, 9IR and CW treatments had lower stomatal width compare with others treatments.

Chlorophyll contents were significantly different among treatments of 'MD-2' pineapple plantlets regenerated from MGB (Table 7). The highest chlorophyll a (0.79 mg/g) and chlorophyll b (0.28 mg/g) and total chlorophyll (1.06 mg/g) contents were obtained in the 9R treatment (Table 7). Plantlets in the treatment of 9IR had the lowest contents of chlorophyll a (0.33 mg/g), chlorophyll b (0.18 mg/g) and total chlorophyll (0.51 mg/g).

Stomatal number and stomatal width of 'MD-2' pineapple plantlets regenerated from MGB were significantly different among treatments (Table 8). Plantlets in the 9IR treatment had the lowest stomatal number (60.02/mm^2), stomatal length (281.52 µm) and stomatal width (200.51 µm). Stomatal length was not significantly affected by different light sources.

Table 5. Effects of different light sources on chlorophyll contents of 'TN17' pineapple plantlets regenerated from meristematic globular bodies after 30 days of culture *in vitro*.

Light source[z]	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
9R	0.59 ± 0.06[y] a[x]	0.28 ± 0.02 a	0.87 ± 0.05 ab
9B	0.40 ± 0.05 b	0.23 ± 0.05 ab	0.62 ± 0.10 b
9IR	0.17 ± 0.14 c	0.10 ± 0.09 b	0.27 ± 0.23 c
CW	0.64 ± 0.08 a	0.32 ± 0.06 a	0.96 ± 0.13 a
Fluorescent	0.72 ± 0.10 a	0.33 ± 0.10 a	1.05 ± 0.18 a

[z]9R: Red 100%, 9B: Blue 100%, 9IR: Infrared 100%, CW: Cool white.

[y]Mean ± standard deviation.

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

Table 6. Effects of different light sources on stomatal number and stomatal length and width of 'TN17' pineapple plantlets regenerated from meristematic globular bodies after 30 days of culture in vitro.

Light source[z]	No. of stomata/mm^2	Stomatal length (µm)	Stomatal width (µm)
9R	123.67 ± 10.89[y] b[x]	316.45 ± 12.73 b	207.50 ± 2.82 c
9B	86.20 ± 2.08 c	402.12 ± 11.43 a	268.50 ± 2.91 a
9IR	114.07 ± 7.25 b	308.54 ± 25.54 b	210.49 ± 7.31 c
CW	138.87 ± 6.15 a	315.97 ± 10.54 b	213.00 ± 3.45 c
Fluorescent	118.87 ± 7.17 b	316.95 ± 15.20 b	223.51 ± 2.34 b

[z]9R: Red 100%, 9B: Blue 100%, 9IR: Infrared 100%, CW: Cool white.

[y]Mean ± standard deviation.

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

Table 7. Effects of different sources on chlorophyll content of 'MD-2' pineapple plantlets regenerated from meristematic globular bodies after 30 days of culture *in vitro*.

Light source[z]	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
9R	0.79 ± 0.09[y] a[x]	0.28 ± 0.04 a	1.06 ± 0.12 a
9B	0.68 ± 0.06 ab	0.23 ± 0.06 ab	0.92 ± 0.11 a
9IR	0.33 ± 0.03 c	0.18 ± 0.02 b	0.51 ± 0.05 b
CW	0.67 ± 0.06 b	0.27 ± 0.03 a	0.94 ± 0.09 a
Fluorescent	0.72 ± 0.05 ab	0.27 ± 0.01 a	0.99 ± 0.06 a

[z]9R: Red 100%, 9B: Blue 100%, 9IR: Infrared 100%, CW: Cool white.

[y]Mean ± standard deviation.

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

Table 8. Effects of different light sources on stomatal number and stomatal length and width of 'MD-2' pineapple plantlets regenerated from meristematic globular bodies after 30 days of culture in vitro.

Light source[z]	No. of stomata/mm^2	Stomatal length (µm)	Stomatal width (µm)
9R	104.40 ± 8.56[y] ab[x]	294.38 ± 13.25 a	234.95 ± 2.19 a
9B	112.93 ± 1.53 a	284.22 ± 7.75 a	226.76 ± 3.16 b
9IR	60.07 ± 8.02 c	281.52 ± 13.73 a	200.51 ± 2.93 c
CW	114.27 ± 5.11 a	287.98 ± 17.02 a	225.38 ± 3.84 b
Fluorescent	100.93 ± 3.61 bc	285.99 ± 15.06 a	225.49 ± 3.84 b

[z]9R: Red 100%, 9B: Blue 100%, 9IR: Infrared 100%, CW: Cool white.

[y]Mean ± standard deviation.

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

Discussion

Light quality affectes growth and morphology of plants (Kim *et al*., 2004). The fresh weight and dry weight were higher under red and blue light than under green and white light in *Hydrilla* plants (Van *et al*., 1997). The highest dry weight was obtained in the treatment of blue LED of *Rehmannia glutinosa* plantlets (Hahn *et al*., 2000). Marigold seedlings were significantly increased in dry weight in monochromic red light, fluorescent light plus red LED or fluorescent light (Heo *et al*., 2002). Salvia had significantly greater dry weight under fluorescent light plus blue LED, fluorescent light plus red LED and fluorescent light plus far-red LED (Heo *et al*., 2002). Shoot fresh weight and dry weight were increased under fluorescent lighting, red light, or a mixture of blue plus red light, but was not affected under blue light alone (Heo *et al*., 2006). The effect of light quality tends to be different to different plant species, stages of growth and environmental conditions (Schuerger *et al*., 1997; Hahn *et al*., 2000). In this study, it was found that fresh weight and dry weight were the lowest under IR treatment in 'TN17' pineapples examined (Table 1). Similarly, Johnson *et al*., (1996) examined effects of infrared (IR) LED on etiolated oat seedlings. The

proportion of coleoptile tissue was significantly lower under IR source or dark-grown treatments (Johnson *et al*., 1996). Too much infrared light may reduce plants health (Johnson *et al*., 1996).

Different LEDs also significantly affected leaf and root numbers of 'TN17' pineapple plantlets (Table 2). The highest leaf and root numbers were obtained in the CW treatment of 'TN17' pineapple plantlets. Leaf and root numbers and total leaf area of 'TN17' pineapple plantlets were significantly reduced under IR treatment. LED light could affect *in vitro* rooting of plantlets (Gupta and Jatothu, 2013). The rooting of plantlets was the greatest under red LED light (Li *et al*., 2010; Moon *et al*., 2006). In this study, it was also found that primary root length was the greatest in the red light (9R) treatment of 'TN17' pineapple plantlets (Table 2). The effectiveness of light on rooting is dependent on genotypes (Gupta and Jatothu, 2013). The leaf area under red LED was the largest and significantly different among treatments of pea seedlings (Wu *et al*., 2007). In cherry tomato plants, there was no significant difference in leaf area under different LED light sources (Xiaoying *et al*., 2012). Tobacco leaf area was decreased under far red light (Kasperbauer and Peaslee, 1973). The quality of light affected structure of leaf and the response largely depends on the species analyzed (Stefano and Rosario, 2003).

The chlorophyll contents were not significantly affected by different light sources in 'TN17' pineapple plantlets (Table 3). Similarly, chlorophyll contents of pea seedlings were not significantly different among different light radiations (Wu *et al*., 2007). In cabbage and lettuce, there were no significant differences in chlorophyll contents among LED treatments (Mizuno *et al*., 2011; Lin *et al*., 2013). Tobacco leaves had lower chlorophyll contents under far red light (Kasperbauer and Peaslee, 1973). Far red LED supplemental cool white fluorescent lamps decreased chlorophyll concentration in lettuce as compared to white fluorescent lamps (Li and Kubota, 2009). In this study, it was found that chlorophyll contents in the pineapple plantlets under mono wavelength lights tended to be lower, compared with that under fluorescent light.

Stomata of pineapples were found mainly on the underside leaves (Bartholomew and Kadzimin, 1977, Krauss, 1949; Malézieux *et al*. 2003). In this study, there were significant differences in stomatal density in ‘TN17’ and ‘MD-2’ pineapple plantlets regenerated from axillary buds of crown stems and from meristematic globular bodies (Table 4; Table 6; Table 8). 'TN17' pineapple plantlets regenerated from axillary buds of crown stems had the highest stomatal density under blue light (9B) treatment (Table 4), but was the lowest one in plantlets from meristematic globular bodies (Table 6). The result suggested that different explants had different responses to the same light source. Plantlets under red and blue light had longer stomatal width than under other

light sources in 'TN17' and 'MD-2' pineapple plantlets (Table 4; Table 6; Table 8). Previous studies reported that stomatal shape under red and blue light were predominantly circular shape and under white light, stomatal shape was elliptical (Muleo and Morini, 1990). 'TN17' and 'MD-2' pineapple plantlets under IR treatment had the lowest stomatal density (Table 4; Table 6; Table 8). It had been showed that tobacco plants under far red light had lower stomatal density (Kasperbauer and Peaslee, 1973). In contrast, there was no significant difference in stomatal size of marigold under different light sources (Heo *et al*., 2002). However, the impact of light quality on stomatal characteristics have not yet been clearly determined, which may be involved in plant photosynthetic activity and plant growth (Gupta and Jatothu, 2013).

Red and blue lights are the most effective ight sources for photosynthesis in plants. Red light is needed for the development of photosynthetic apparatus and photosynthesis, while blue light is mainly for chlorophyll synthesis, chloroplast development, stomatal opening and photomorphogenesis (Senger, 1982; Akoyunoglou and Anni, 1984; Sæbø *et al*., 1995). However, the effect of different light sources on plant growth and morphogenesis is dependent on species and developmental stages. The highest stomata density was obtained in blue light (9B) treatment of 'TN17' pineapple plantlets (Table 4). Blue light increased stomatal number compared to red and white light. Red and blue lights were more effective than IR light on growth and morphogenesis of 'TN17' and 'MD-2' pineapples cultured *in vitro*.

References

Akbar, M. A., B. K. Karmakar, and S. K. Roy. 2003. Callus induction and high-frequency plant regeneration of pineapple (*Ananas comosus* (L.) Merr.). Plant Tiss. Cult. 13: 109-116.

Akoyunoglou, G. and H. Anni. 1984. Blue light effect on chloroplast development in higher plants. pp. 397-406. In: Senger, H. (eds.). Blue Light Effects in Biological Systems. Springer, Berlin, Heidelberg.

Arnon, D. L. 1949. Copper enzyme in isolated chloroplast polyphenol oxidase in *Bata vulgaris*. Plant Physiol. 24: 1-15.

Bartholomew, D. P. and S. B. Kadzimin. 1977. Pineapple. pp. 113-156. In: Alvim, P. D. T. and T. T. Kozlowski (eds.). Ecophysiology of Tropical Crops. Academic Press, Bahia.

Brown, C. S., A. C. Schuerger, and J. C. Sager. 1995. Growth and photomorphogenesis of pepper under red light-emitting diodes with supplemental blue or far-red lighting. J. Am. Soc. Hortic. Sci. 120: 808-813.

FAO. 2014. Faostat.fao.org/.

Gupta, S. D. and B. Jatothu. 2013. Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. Plant Biotech. Rep. 7: 211-220.

Hahn, E. J., T. Kozai, and K. Y. Paek. 2000. Blue and red light-emitting diodes with or without sucrose and ventilation affect *in vitro* growth of *Rehmannia glutinosa* plantlets. J. Plant Biol. 43: 247-250.

Heo, J., C. Lee, D. Chakrabarty, and K. Paek. 2002. Growth responses of marigold and salvia bedding plants as affected by monochromic or mixture radiation provided by a light-emitting diode (LED). Plant Growth Regul. 38: 225-230.

Heo, J. W., K. S. Shin, S. K. Kim, and K. Y. Peak. 2006. Light quality affects *in vitro* growth of grape 'Teleki 5BB7'. J. Plant Biol. 49: 276-280.

Hepton, A. 2003. Cultural system. pp. 109-142. In: Bartholomew, D. P., R. E. Pauli, and K. G. Rohrbach (eds.), The Pineapple: Botany, Production and Uses, CAB International, Wallingford.

Hoenecke, M., R. J. Bula, and T. W. Tibbitts. 1992. Importance of ‘blue’ photon levels for lettuce seedlings grown under red light-emitting diodes. Hort Sci. 27: 427-430.

Huges, K. W. 1981. *In vitro* ecology: exogenous factor effecting growth and morphogenesis in plant culture system. Environ. Exp. Bot. 21: 281-288.

Johnson, C. F., C. S. Brown, R. M. Wheeler, J. C. Sager, D. K. Chapman, and G. F. Deitzer. 1996. Infrared light-emitting diode radiation causes gravitropic and morphological effects in dark-grown oat seedlings. Photochem. Photobiol. 63: 238-242.

Kasperbauer, M. J. and D. E. Peaslee. 1973. Morphology and photosynthetic efficiency of tobacco leaves that received end-of-day red or far red light during development. Plant Physiol. 52: 440-442.

Kim, S. J., E. J. Hahn, J. W. Heo, and K. Y. PaeK. 2004. Effects of LEDs on net photosynthetic rate, growth and leaf stomata of *Chrysanthemum* plantlets *in vitro*. Scientia Hort. 101: 143-151.

Krauss, B. H. 1949. Anatomy of the vegetative organs of the pineapple, *Ananus comosus* (L.) Merr. II. The leaf. Botanical Gazette (Chicago). 110: 333-404.

Li, H., Z. Xu, and C. Tang. 2010. Effect of light emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets *in vitro*. Plant Cell, Tiss. Org. Cult. 103: 155-163.

Li, Q. and C. Kubota. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67: 59-64.

Lin, K. H., M. Y. Huang, W. D. Huang, M. H. Hsu. Z. W. Yang, and C. M. Yang. 2013. The effect of red, blue, and white light-emitting diodes on the growth, development and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. capitata). Scientia Hort. 150: 86-91.

Malézieux, E., F. Côte, and D. P. Bartholomew. 2003. Crop environment, plant growth and physiology. pp. 69-107. In: Bartholomew, D. P., R. E. Pauli, and K. G. Rohrbach (eds.), The Pineapple: Botany, Production and Uses, CAB International, Wallingford.

Mizuno, T., W. Amaki, and H. Watanabe. 2011. Effects of monochromatic light irradiation by LED on the growth and anthocyanin contents in leaves of cabbage seedlings. Acta Hort. 907: 179-184.

Moon, H. K., S. K. Park, Y. W. Kim, and C. S. Kim. 2006. Growth of Tsuru-rindo (*Tripterospermum japonicum*) cultured *in vitro* under various sources of light-emitting diodes (LED) irradiation. J. Plant Biol. 49: 174-179.

Muleo, R. and S. Morini. 1990. Effect of light quality on regeneration from callus of *Actinidia deliciosa*. Acta Hort. 280: 155-158.

Murashige, T. and F. Skoog. 1960. A revised medium for rapid growth and biossays with tobacco tissue culture. Plant Physiol. 15: 472-497.

Sæbø, A., T. Krekling, and M. Appelgren. 1995. Light quality affects photosynthesis and leaf anatomy of brich plantlets *in vitro*. Plant Cell, Tiss. Org. Cult. 41: 177-85.

Schuerger, A. C., C.S. Brown, and E. C. Stryjewski. 1997. Anatomical features of pepper plants (*Capsicum annuum* L.) grown under red light-emitting diodes supplemented with blue or far-red light. Ann. Bot. 79: 273-282.

Senger, H. 1982. The effect of blue light on plants and microorganisms. Photochem. Photobiol. 35: 911-20.

Stefano, M. and M. Rosario. 2003. Effects of light quality on micropropagation of woody species. pp. 3-35. In: Jain, S. M., and K. Ishii (eds.). Micropropagation of woody trees and fruits. Kluwer Academic Publishers, Netherland.

Shin, S. K., N. H. Murthy, W. J. Heo, J. E. Hahn, and Y. K. Paek. 2008. The effect of light quality on the growth and development of *in vitro* cultured *Doritaenopsis* plants. Acta Physiol. Plantae. 30: 339-343.

Tennessen, D. J., Singsaas E. L., and Sharkey T. D. 1994. Light-emitting diodes as a light source for photosynthesis research. Photo. Res. 39: 85-92.

Tripathy, B. C. and C. S. Brown. 1995. Root-shoot interaction in the greening of wheat seedlings grown under red light. Plant Physiol. 107: 407-411.

Usman, I. S., M. M. Abdulmalik, L. A. Sani, and A. N. Muhammad. 2013. Development of an efficient protocol for micropropagation of pineapple (*Ananas comosus* L. var. smooth cayenne). Afr. J. Agric. Res. 8: 2053-2056.

Van, T. K., W. T. Haller, G. Bowes, and L. A. Garrard. 1997. Effect of light quality on growth and chlorophyll composition in *Hydrilla*. J. Aquat. Plant Manage. 15: 29-31.

Xiaoying, L., G. Shirong, C. Taotao, X. Zhigang, and T. Tezuka. 2012. Regulation of the growth and photosynthesis of cherry tomato seedlings by different light irradiations of light emitting diodes (LED). Afr. J. Biotech. 11: 6169-6177.

Wu, M. C., C. Y. Hou, C. M. Jiang, Y. T. Wang, C. Y. Wang, H. H. Chen, and H. M. Chan. 2007. A novel approach of LED light radiation improves the antioxidant activity of pea seedling. Food Chem. 101: 1753-1758.

Zuraida, A. R., A. H. Nurul Shahnadz, A. Harteeni, S. Roowi, C. M. Z. Che Radziah, and S. Sreeramanan. 2011. A novel approach for rapid micropropagation of maspine pineapple (*Ananas comosus* L.) shoot using liquid shake culture system. Afr. J. Biotech. 10: 3859-3866.

LED 光源對組織培養之鳳梨生長與形態之影響

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關鍵字：發光二極體、光質、鳳梨

摘要：本研究以果冠之腋芽及芽球培養而來之鳳梨小苗為試驗材料，調查不同 LED 光源對台農 17 號('TN17')及'MD-2'鳳梨小苗瓶內生長及形態發生的影響。結果顯示，不同光源處理之'TN17'果冠腋芽之植株的葉片數及根數有顯著差異，在 9IR (100% 遠紅光)處理下之葉片數及根數最低。經 9B (100 % 藍光) 處理之植株葉片氣孔密度最高(147/mm^2)。來自'TN17'和'MD-2'芽球之植株的葉綠素含量在不同處理間有顯著差異。經 9IR (100% 遠紅光)處理下之'TN17'和'MD-2'植株的葉綠素含量最低。經 9B (100% 藍光)處理之'TN17'芽球之植株的植株葉片氣孔密度最低。經 CW (冷白光)處理之'MD-2'芽球之植株的葉片氣孔密度最高，而經 9IR (100% 遠紅光)最低。總結而言，不同 LED 光源對'TN17'和'MD-2'鳳梨瓶內生長及形態發生有顯著的影響。相較於遠紅光，紅光及藍光對促進鳳梨生長與發育的影響較為顯著。

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