

GROWTH AND DEVELOPMENT OF OIL PALM SHOOTS UNDER DIFFERENT LIGHT QUALITIES

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ABSTRACT

Light quality is one important factor that affects the growth and development of *in vitro* plants. This study examines the influence of different light qualities on the *in vitro* growth and development of oil palm shoots which were cultured in Murashige & Skoog medium under white fluorescent lamp, white light-emitting diode (LED), red LED, blue LED, combination of red and blue LED, and in darkness. The results showed that the oil palm shoots grew and developed differently under different light qualities. Root initiation and shoot elongation progressed well under red light, while chlorophyll and sugar content were better produced under white and blue light than under the red light. Both white fluorescent lamp and the combination of red and blue LED resulted in higher growth parameter compared to other light qualities. However, the results were not significantly different.

Keywords: chlorophyll, *Elaeis guineensis*, hormone, *in vitro*, LED light

INTRODUCTION

In the production of oil palm (*Elaeis guineensis*) clones, the use of tissue culture through somatic embryogenesis is widely applied. However, the efficiency of tissue culture in oil palm is very low (Rohani *et al.* 2000; Kushairi *et al.* 2010). Hence, improvement on the efficiency of this technology on oil palm is important for its mass production.

Plant growth and development in tissue culture are regulated by various environmental factors, wherein light is one of the most important. Undoubtedly, light is necessary for photosynthesis and photomorphogenesis, with certain specific wavelengths playing important roles in plant tissue culture efficiency (Fujiwara & Kozai 1995).

The lighting system generally used for maintaining plant tissue culture is the tubular fluorescent lamps (TFL). However, TFL has a wide spectrum and contain unnecessary

wavelengths for plant growth. The light-emitting diode (LED), an alternative light source for plant tissue culture, has several advantages in comparison to other lighting systems. LED has wavelength specificity, durability, longer operating lifetime, has the ability to select spectral composition, has a smaller size, and emits less heat (Gupta & Jatothu 2013). The disadvantage of LED is the establishment cost. However, LED can still be profitable in the long run, even though it may require a high capital investment, because the long-term operating costs of LED are generally lower than other lighting systems.

The LED lighting system using various light qualities in plant tissue culture has been applied to a variety of species, such as *Cymbidium* (Tanaka *et al.* 1998), strawberry (Nhut *et al.* 2003), grape (Poudel *et al.* 2008), *Anthurium* (Budiarto 2010), *Gossypium hirsutum* (Li *et al.* 2010), *Oncidium* (Mengxi *et al.* 2011), *Brassica napus* (Li *et al.* 2013), banana (Vieira *et al.* 2015), *Vanilla planifolia* (Bello-Bello *et al.* 2016), and sugarcane (Ferreira *et al.* 2017). The effect of

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light quality varied depending on the plant species yet little is known about LED utilization with different light qualities in oil palm shoot. Hence, the influence of various light qualities on *in vitro* growth and development of oil palm shoot was evaluated in this study.

MATERIALS AND METHODS

Plant Materials and Light Quality Treatments

The *in vitro* oil palm shoots used in this study were obtained through indirect somatic embryogenesis from young leaf explants following the Wong *et al.* (1997) method. The different lights used were white TFL (Phillips T5 TCH086 EV 28W, white 6500 K), white LED (Vanq VQ-GLT8020W-27, white 10000 K), red LED (Vanq VQ-GLT8020W-27, wavelength: 660 nm), blue LED (Vanq VQ-GLT8020W-27, wavelength: 460 nm) and red blue LED (Vanq VQ-GLT8020W-27, wavelength: 660:460 nm (3:1)).

Effect of Light Quality on Shoot Development

The 4-6 cm shoots, which have more than two leaves were selected and transferred onto a modified Murashige & Skoog (1962) medium without plant growth regulator. All cultures were maintained in a culture room at a temperature of 28 ± 2 °C, relative humidity $50 \pm 10\%$, and with 16/8 light/dark photoperiod under various light qualities. After two months of culture, the fresh weight, the number of new leaves, the shoot height, and its root formation were observed, and then whole shoot samples from each treatment were randomly collected for chlorophyll, sugar, and endogenous hormone analyses. The whole shoots were ground in liquid nitrogen and stored at -80 °C prior to analyses.

Chlorophyll Content Analysis

The chlorophyll content was extracted from the sample using 10 mL of 100% acetone. The optical density was measured using a spectrophotometer (Hitachi U-2900) at 662 nm

for chlorophyll a and at 645 nm for chlorophyll b. The chlorophyll content was calculated according to Lichtenthaler (1987).

Sugar Content Analysis

The sugar content was measured, according to the method described by Dubois *et al.* (1951). The 2 g sample was homogenized in 10 mL of distilled water and kept in water bath at 95 °C for 5 min. The sample was then stored overnight at 70 °C. Subsequently, the sample was centrifuged at 5,000 rpm for 10 min and 2 mL of the supernatant was transferred into another tube then added with 0.02 mL of 80% phenol and 5 mL of sulfuric acid. The mixture was then incubated at 30 °C for 30 min. The optical density was measured using a spectrophotometer (BioTek Epoch microplate) at 490 nm. Fructose and glucose were used as the standard.

Endogenous Hormone Content Analysis

The endogenous hormone content was measured, according to Kelen *et al.* (2004), with some modifications using Ultra Performance Liquid Chromatography (Water UPLC).

Auxin, gibberellic acid (GA), and abscisic acid (ABA) were extracted from 5 g of sample and soaked overnight in 30 mL 80% methanol at 4 °C. The 5 g extracted sample was dissolved with 30 mL 0.1 M phosphate buffer (pH 8.5) and then partitioned with ethyl acetate two times. After removal of the ethyl acetate, the pH of the aqueous phase was adjusted to 2.5 with 1 N HCl. The sample was partitioned again with ethyl acetate 2 times and passed through anhydrous sodium sulphate. The ethyl acetate was evaporated in rotary evaporator at 50 °C. The dry residue containing hormones was dissolved in 2 mL of methanol and filtered into a UPLC vial using a 0.2 µm Millipore syringe filter. An injection volume of 1 µL was used for each analysis. The column used was Water Acquity UPLC BEH C18 1.7 µm (2.1 x 50 mm). The mobile phases constituted of acetonitrile: water (26:74) pH 4 with a flow rate of 0.2 mL/min and retention time of 3 minutes. The signal of the compounds was monitored by a photodiode array (PDA) detector at 208 nm. Indole Acetic Acid (IAA), GA₃, and ABA (100 ppm) were used as the standard compounds.

For cytokinin isolation, 1 g of sample was extracted using 20 mL 70% methanol for 4 hours at 4 °C. The extracted sample was filtered and injected into a UPLC column as previously mentioned. The mobile phases constituted of water: methanol (20:80) pH 2 with a flow rate of 0.35 mL/min and retention time of 0.6 minutes. The signal of the compounds was monitored by a PDA detector at 210 nm. Zeatin (100 ppm) was used as the standard compound.

Statistical Analysis

This experiment used randomized complete block design with three block and six treatments. Data were analyzed statistically using analysis of variance (ANOVA), and the differences among the treatment means were tested using Duncan's multiple range test (DMRT) at $P = 0.05$. Statistical analyses were conducted using SAS version 9.1.3.

RESULTS AND DISCUSSION

Shoot Growth and Development

The growth and development of oil palm shoots were influenced by the light quality (Table 1). Compared with other treatments, the no light (darkness) treatment gave the lowest growth. When under darkness, the number of new leaves and fresh weight were significantly lower than those under light treatments, while the results among different light treatments did not significantly differ from each other.

However, the highest shoot height and fresh weight were observed in shoots growing under the combination of red and blue light-emitted diode (LED). Meanwhile, the highest number of new leaves was observed in shoots under white tubular fluorescent lamp (TFL) (Table 1). White TFL is the most widely used light in plant tissue culture. However, white TFL produces a wide range of wavelengths that contain unnecessary wavelengths for plant growth and development, while LED can emit light at specific wavelengths (Gupta & Jatothu 2013).

Different wavelengths generally bring about different responses on plant growth and development. In this study, the red blue LED gave the greatest growth compared to other LED colors (Table 1 & Fig. 1). Red and blue lights remarkably impacted plant growth, probably because these are the major energy sources for photosynthesis and photomorphogenesis. Red and blue lights, either alone or in combination produce different responses in plant growth. Red light induces shoot elongation (Nhut *et al.* 2003; Cybularz-Urban *et al.* 2007; Poudel *et al.* 2008), while blue light induces chlorophyll and stomata formation (Poudel *et al.* 2008; Muneer *et al.* 2014). The combination of these two lights may have produced higher growth rate, due to the combined advantages of red and blue lights for inducing plant growth and development. The combination of red and blue lights also enhance the growth of other plant species, such as strawberry (Nhut *et al.* 2003), cotton (Li *et al.* 2010), ginseng (Nhut *et al.* 2015), and vanilla (Bello-Bello *et al.* 2016).

Table 1 Effect of different light qualities on growth and development of oil palm shoots

Treatment	Number of new leaves	Fresh weight increase (mg)	Shoot height increase (cm)
Dark	0.51 ± 0.03 ^b	47.53 ± 1.61 ^b	1.76 ± 0.07 ^c
White TFL	0.78 ± 0.03 ^a	82.40 ± 3.10 ^a	2.03 ± 0.07 ^{ab}
White LED	0.69 ± 0.03 ^a	80.15 ± 2.98 ^a	1.88 ± 0.08 ^{bc}
Red blue LED	0.76 ± 0.03 ^a	85.17 ± 3.41 ^a	2.18 ± 0.09 ^a
Blue LED	0.76 ± 0.04 ^a	82.35 ± 3.21 ^a	1.96 ± 0.07 ^{abc}
Red LED	0.70 ± 0.03 ^a	81.42 ± 3.49 ^a	2.11 ± 0.08 ^{ab}

Notes: Values are mean ± SE for $n = 330$. Different superscripts on the same column indicate significant differences at $P = 0.05$ by DMRT.



Figure 1 Representative of oil palm shoots under different light qualities
Notes: D = dark; W TFL = white TFL; W LED = white LED; RB LED = combination red blue LED; B LED = blue LED; R LED = red LED.

Light Quality and Endogenous Hormones

Light quality affects plant growth and development by regulating the synthesis of endogenous hormones: cytokinin, gibberellic acid (GA), auxin and abscisic acid (ABA).

The endogenous hormone in the oil palm shoot showed varied responses to different light qualities (Table 2). Cytokinin, GA and ABA contents differed significantly from each other when exposed under the different light quality treatments, indicating their different photo-responses to certain wavelengths. The highest cytokinin content was observed under the white LED and lowest in the darkness. Gibberellic Acid (GA) content was higher when the shoots were cultured under red, blue, or red and blue LED when compared with white light. ABA content was the highest under red blue LED, and the lowest in dark treatment. Auxin did not respond to any of the light quality treatments. Although the auxin content did not differ significantly among treatments, the highest was observed in red LED treatment.

Interaction between Endogenous Hormones and Shoot Growth

Synthesis of hormone in plant is regulated by plant photoreceptors, which include phytochromes, cryptochrome, and phototropin (de Wit *et al.* 2016). The interaction between light quality and endogenous hormone plays an important role in plant growth and development.

GA and auxin are key players in plant elongation. The stem increment and GA content of Norway spruce seedlings under red light were higher than under blue light, whereas IAA content under red light were lower (Ouyang *et al.* 2015). In contrast, GA content in petunia plants were higher under blue light compared to red light (Fukuda *et al.* 2016). Blue light enhances shoot elongation and red light inhibites shoot elongation in petunia plants. In this current study, GA and auxin contents in oil palm shoots under LED light were not significantly different, but GA content in shoots under blue LED was higher than those under other lights. The resulting differences may be attributed to plant species, stage of plant growth, and other environmental factors.

Table 2 Endogenous hormones content in oil palm shoots under different light qualities

Treatment	Cytokinin (mg/g)	GA (μ g/g)	Auxin (μ g/g)	ABA (μ g/g)
Dark	0.48 ± 0.04^c	3.39 ± 0.33^a	3.51 ± 0.68^a	8.13 ± 5.63^c
White TFL	0.61 ± 0.04^b	2.50 ± 0.32^b	3.83 ± 0.55^a	35.81 ± 10.41^{ab}
White LED	0.70 ± 0.03^a	2.24 ± 0.35^b	4.59 ± 0.65^a	29.15 ± 10.34^{ab}
Red blue LED	0.64 ± 0.02^{ab}	2.89 ± 0.09^{ab}	4.37 ± 0.60^a	42.08 ± 12.11^a
Blue LED	0.64 ± 0.06^{ab}	3.06 ± 0.26^{ab}	3.99 ± 0.78^a	19.77 ± 9.70^{bc}
Red LED	0.67 ± 0.03^{ab}	2.67 ± 0.29^{ab}	4.79 ± 0.93^a	30.40 ± 8.51^{ab}

Notes: Values are mean \pm SE for $n = 6$. Different letters in the same column indicate significant difference at $P = 0.05$ by DMRT.

Shoot elongation seems to positively correlate with the interaction of endogenous hormones. In this study, shoot elongation under red LED was higher than those under other lights (Table 1) and this might be correlated with the higher auxin content under red LED (Table 2). GA and auxin are known to stimulate plant elongation through different mechanisms. Auxin affects cell elongation by promoting the release of hydrogen ions from the plant cell and which in turn reduces the stress on the cell wall. GA requires cell wall plasticity and cytoplasmic protein synthesis for cell elongation. To achieve this, cooperation with other hormones, such as auxin is required (Wang & Irving 2011).

Cytokinin has a different effect as it inhibits plant elongation by inhibiting auxin-promoted extension growth (Cohen *et al.* 1991). Cytokinin and blue light induced inhibitory effects on hypocotyl growth of cucumber (Cohen *et al.* 1991). In this study, cytokinin content and shoot elongation under light treatments were not significantly different. This results showed that cytokinins might not be correlated with the elongation of oil palm shoots.

Auxin also plays a key role in root initiation. In this study, auxin content in the oil palm shoots increased under the red LED (Table 2) and it induced the root initiation in the shoots (Fig. 2). The process of root formation is

considerably complex and involves interaction with other hormones. Auxin regulates root growth by modulating the effect of GA-induced destabilization of DELLA protein growth repressor (Fu & Harberd 2003). In the presence of GA, DELLA is degraded and thereby modulates other plant hormones, such as auxin.

Light Quality and Photosynthesis

Light quality also influences photosynthesis activity. In this study, the effect of light quality on photosynthesis was determined by the accumulation of photosynthetic pigments (chlorophyll a and b) and primary photosynthesis products (fructose and glucose). Chlorophyll and sugar content of the oil palm shoots were significantly higher under light treatment than those under dark treatment (Figs. 3 & 4). Light is an important factor that regulates chlorophyll biosynthesis.

The shoots under white, red blue, and blue light accumulated higher chlorophyll, compared to those under the red or dark treatment (Fig. 3). Several studies showed that blue light is a good light for chlorophyll synthesis and that red light decreases chlorophyll content (Tanaka *et al.* 1998; Li *et al.* 2010). Similarly, in this study, the chlorophyll content in oil palm shoots decreased under red LED, and increased under white and blue LED (Fig. 3).

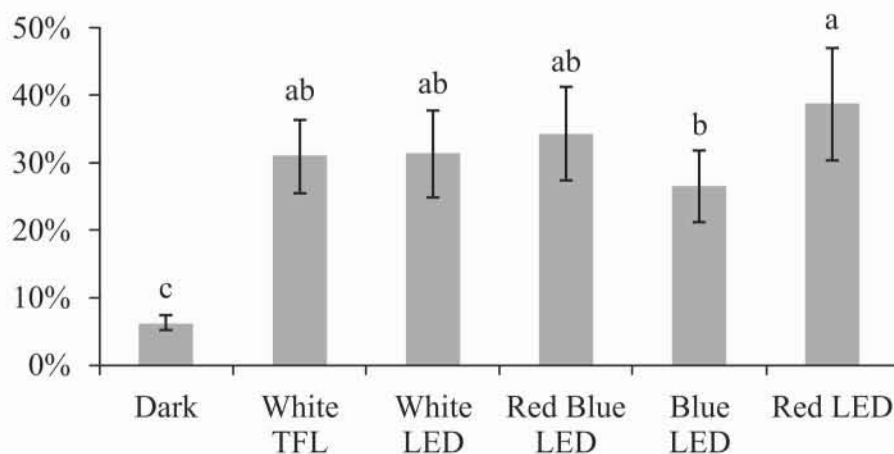


Figure 2 Percentage of rooting in shoots under different light qualities

Notes: Vertical bars indicate \pm SE of the means for $n = 6$. Different letters indicate significant differences at $P = 0.05$ by DMRT.

Blue light is also important in chlorophyll synthesis. Blue light stimulates chlorophyll synthesis by inducing the expression of genes encoding the enzyme for two early step of chlorophyll biosynthesis, glutamate-1-semialdehyde aminotransferase and 5-aminolevulinic acid dehydratase (Matters & Beale 1995). Chlorophyll content were also correlated to cytokinin content in oil palm shoots. Chlorophyll content under light treatments was significantly higher than that in dark treatment (Fig. 3) and this may be stimulated by high cytokinin content under light treatments (Table 2). Cytokinin affected chlorophyll biosynthesis by promoting the synthesis of 5-aminolevulinic acid, a precursor

of chlorophyll synthesis, and enhancing activity of Protochlorophyllide Oxidoreductase, an enzyme in chlorophyll synthesis (Cortleven & Schmulling 2015).

Sugar content in the oil palm shoots under different light treatments did not significantly differ (Fig. 4). Apparently, different light qualities did not affect sugar content because the *in vitro* shoots are mainly heterotrophic, where the shoots were grown in culture media with sugar. Sugar is the main carbon source for heterotrophic growth (Yasseen *et al.* 2013). The presence of sugar in culture media might reduce the need for sugar production and limits photosynthetic activity.

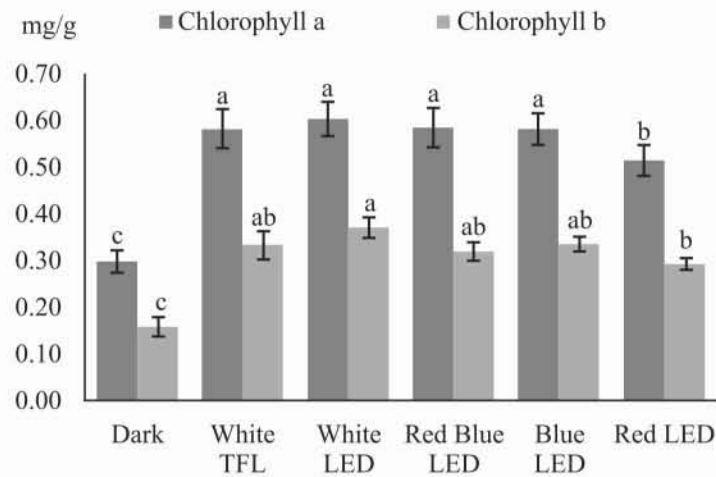


Figure 3 Chlorophyll content in oil palm shoots under different light qualities
 Notes: Vertical bars indicate \pm SE of the means for $n = 6$. Different letters indicate significant differences at $P = 0.05$ by DMRT.

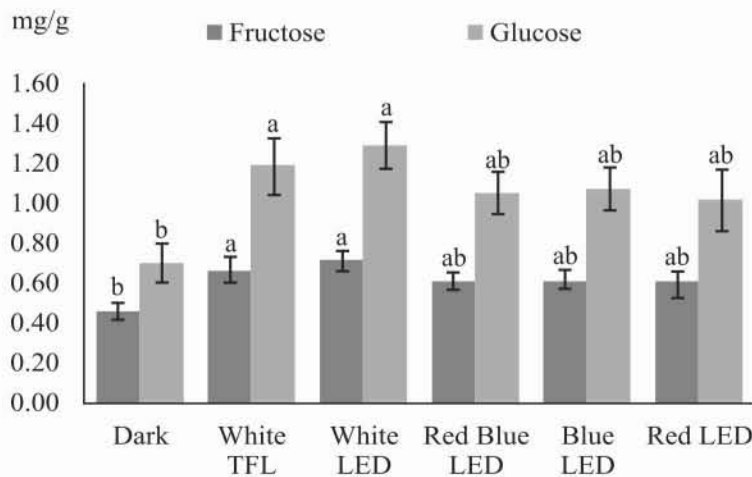


Figure 4 Sugar content in oil palm shoots under different light qualities
 Notes: Vertical bars indicate \pm SE of the means for $n = 6$. Different letters indicate significant differences at $P = 0.05$ by DMRT.

CONCLUSIONS

Light quality influenced the growth and development of the oil palm shoots. The different wavelengths produced different effects on the root and shoot activities. Root initiation and shoot elongation were well-produced under red light, while chlorophyll and sugar contents were better produced under the white and blue light. Although the results were not significantly different, the combination of red and blue LED resulted in higher growth than the other light treatments. Besides the white light, the red and blue light combination provides good lighting for promoting the growth and development of oil palm shoots. Hence, the red blue LED may be used as an alternative for the white TFL used in oil palm tissue culture. However, further study may be needed to analyze the ratio of red and blue LED in order to optimize the protocols for oil palm tissue culture.

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