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DOI: 10.11598/btb……..

To appear in : BIOTROPIA Issue

Received date : 22 September 2017
Accepted date : 07 January 2020

GROWTH AND DEVELOPMENT OF OIL PALM SHOOTS UNDER DIFFERENT LIGHT QUALITIES

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ABSTRACT

Light is one of the most important factor in plant tissue culture. The growth and development of plant *in vitro* are affected by light quality. This study examines the influence of different light qualities on the *in vitro* growth and development of oil palm shoots. Oil palm shoots were cultured in Murashige & Skoog medium under either white fluorescent lamp, white light-emitting diode (LED), red LED, blue LED, combination of red and blue LED, or in darkness. The results show that the growth and development of oil palm shoots are influenced by the light quality. Root initiation and shoot elongation were good under red light, while chlorophyll and sugar content was better produced under white and blue light than red light. In addition to white fluorescent lamp, the combination of red and blue LED resulted in higher growth parameter compared to other light treatments, but the results were not significantly different.

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

INTRODUCTION

Tissue culture of oil palm (*Elaeis guineensis*) through somatic embryogenesis is widely used to produce oil palm clones. However, the efficiency in oil palm tissue culture process is very low (Rohani *et al.* 2000; Kushairi *et al.* 2010). Improvement in oil palm tissue culture efficiency is important for mass production of oil palm.

Plant growth and development in tissue culture are regulated by various environmental factors, and light is one of the most important factor when it comes to increasing plant growth and development in plant tissue culture. Light is a source of energy for photosynthesis and photomorphogenesis. Specific wavelengths are important for photosynthesis and photomorphogenesis efficiency in plant tissue cultures (Fujiwara & Kozai 1995).

The lighting system generally used for maintaining plant tissue culture is tubular fluorescent lamps (TFL). However, TFL has a wide spectrum and contain unnecessary wavelengths for plant growth. LED is an alternative light source for plant tissue culture. LED has several advantages in comparison to other lighting systems. LED has wavelength specificity, durability, longer operating lifetime, the ability to select spectral composition, is small in size, and emits less heat (Gupta & Jatothu 2013). One main disadvantage of LED is the cost. LED require high capital investment but

this high investment will be returned as a profit in long operation because the operating cost of LED is lower than other lighting systems.

The apllications of LED lighting system with various light qualities are used in plant tissue culture and numerous studies have been carried out on a variety of plant 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 light quality may vary depending on the plant species and there is no study about the utilization of LED with different light qualities in oil palm shoot. In this study, the influence of various light qualities on *in vitro* growth and development of oil palm shoot was evaluated.

MATERIALS AND METHODS

Plant materials and light quality treatments

Plant material used in this study was *in vitro* oil palm shoots. The shoots of oil palm used in this study were obtained through indirect somatic embryogenesis from young leaf explants (Wong *et al.* 1997). Different lights used in this study 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 shoots, 4-6 cm in height with 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 temperature 28±2 ºC, relative humidity 50±10%, 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. After observation, samples of shoots from each treatment were collected randomly for chlorophyll, sugar, and endogenous hormone analysis. The whole shoots were grounded in liquid nitrogen and stored at -80^{o}C prior to analysis.

Chlorophyll content analysis

The chlorophyll content was extracted from the sample in 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 a method described by Dubois *et al*. (1951). The sample (2 g) was homogenized in 10 mL of distilled water and kept in water bath of 95 ºC for 5 minutes. Afterward, the sample was kept overnight at 70 ºC. The sample was centrifuged at 5000 rpm for 10 minutes and 2 mL of the supernatant was transferred into another tube then added 0.02 mL of 80% phenol and 5 mL of sulfuric acid. The mixture was incubated at 30 ºC for 30 minutes. The optical density was measured using a spectrophotometer (BioTek Epoch microplate) at 490 nm. Fructose and glucose was 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 ºC. The 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 phase, 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 phase was evaporated in rotary evaporator at 50 ºC. The dry residue containing hormones was dissolved in 2 mL of methanol and filtered into a UPLC vial using a 0.2 µm milipore 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 times 3 minutes. The signal of the compounds was monitored by a photodiode array (PDA) detector at 208 nm. Indole acetic acid (IAA), GA_3, and ABA (100 ppm) were used as the standard. Compounds.

For cytokinin isolation, 1 g of sample was extracted in 20 mL 70% methanol for 4 hours at 4 ºC. The extracted sample was filtered and injected into a UPLC column as mentioned above. The mobile phases constituted of water: methanol (20:80) pH 2 with a flow rate of 0.35 mL/min and retention times 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

The growth and development of oil palm shoots are influenced by light quality. The growth of oil palm shoots under different light qualities is shown in Table 1. Dark treatment gave the lowest growth compared to other treatments. The number of new leaves and fresh weight under dark treatment were significantly lower than that under light treatments. The results among different light treatments were not significantly different. However, the highest shoot height and fresh weight were observed in shoots under the combination of red and blue LED. Meanwhile, the highest number of new leaves was observed in shoots under white 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 unnecessary for plant growth and development, while LED can emit light at specific wavelengths (Gupta & Jatothu 2013).

Different wavelength promoted different responses on plant growth and development. In this study, red blue LED gave the greatest growth compared to other color of LED (Table 1 & Figure 1). Red and blue light have the greatest impact on plant growth, because they are the major energy sources for photosynthesis and photomorphogenesis. Red and blue light, either alone or in combination induce different responses in plant growth. Red light seems to induce 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 give higher growth rate, due to the combined advantages of red and blue light for inducing plant growth and development. It has been observed that the combination of red and blue light enhanced plant growth of different 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	No. 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]

Note: Values are mean ± SE for n = 330. Different letters in the same column indicate significant difference at p = 0.05 by DMRT.

Figure 1 Representative of oil palm shoots under different light qualities. (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 affects plant growth and development by regulating synthesis of endogenous hormones. In this experiment, the roles of cytokinin, gibberellin, auxin and ABA as plant hormones in the treated shoot samples with different light qualities were investigated.

Endogenous hormone in oil palm shoot was varied in response to the different light qualities (Table 2). Cytokinin, gibberellin and ABA contents differed significantly from each of the light quality treatments indicating their photo-responses activity in certain wavelength produced by different lights. The highest cytokinin content was observed under white LED and lowest in dark treatment. Compared with white light, GA was higher when cultured under red, blue, or red blue LED. ABA was highest under red blue LED, and lowest in dark treatment. Auxin did not respond to any of the light quality treatments. Auxin content in all treatments did not differ significantly, but the highest was observed in red LED treatment.

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

Treatment	Cytokinin (mg/g)	GA (µg/g)	Auxin (µg/g)	ABA (µg/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]

Note: Values are mean ± SE for n = 6. Different letters in the same column indicate significant

177 difference at $p = 0.05$ by DMRT.
178
179 Synthesis of hormone in plant is regulated by plant photoreceptors, which include
180 phytochromes, cryptochrome, and phototropin (de Wit *et al*. 2016). Interaction between light quality
181 and endogenous hormone plays an important role in plant growth and development.
182 GA and auxin play a role in plant elongation. Ouyang *et al*. (2015) reported that stem
183 increment and GA levels of Norway spurce seedlings under red light were higher than blue light,
184 whereas IAA levels under red light were lower. Fukuda *et al*. (2016) showed different results, where
185 GA levels in petunia plants were higher under blue light compared to red light. Blue light enhanced
186 shoot elongation and red light inhibited shoot elongation in petunia plants. In this study, GA and
187 auxin content in oil palm shoots under LED light were not significantly different, but GA
188 concentration in shoots under blue LED was higher than that under other lights. The resulting
189 differences may be attributed to plant species, stage of plant growth, and other environment factors.
190 Shoot elongation seems to correlate with the interaction of endogenous hormones. In this
191 study, shoot elongation under red LED was higher than that under other lights (Table 1) and this
192 might have a correlation with the higher auxin concentration under red LED (Table 2). GA and
193 auxin are known to stimulate plant elongation through different mechanism. Auxin affects cell
194 elongation by promoting the release of hydrogen ions from the plant cell and which in turn reduces
195 the stress on the cell wall. GA requires cell wall plasticity and cytoplasmic protein synthesis for cell
196 elongation. To achieve this, cooperation with other hormones, such as auxin is required (Wang &
197 Irving 2011).
198 Cytokinin has different effect on plant elongation. Cytokinin inhibits plant elongation by
199 inhibiting auxin-promoted extension growth (Cohen *et al*. 1991). Cohen *et al*. (1991) reported
200 cytokinin and blue light induced inhibitory effects on hypocotyl growth of cucumber. In this study,
201 cytokinin content and shoot elongation under light treatments were not significantly different. This
202 results show that cytokinins might not have a correlation with elongation of oil palm shoots.
203 Auxin also plays a role in root initiation. Auxin concentration in oil palm shoots increased
204 under red LED (Table 2) and induced root initiation in oil palm shoot (Figure 2). The process of
205 root formation is considerably complex and involves interaction with other hormones. Fu &
206 Harberd (2003) reported that auxin regulates root growth by modulating the effect of GA induced
207 destabilization of DELLA protein growth repressor. In the presence of GA, DELLA are degraded
208 and then modulates other plant hormones, such as auxin.
209

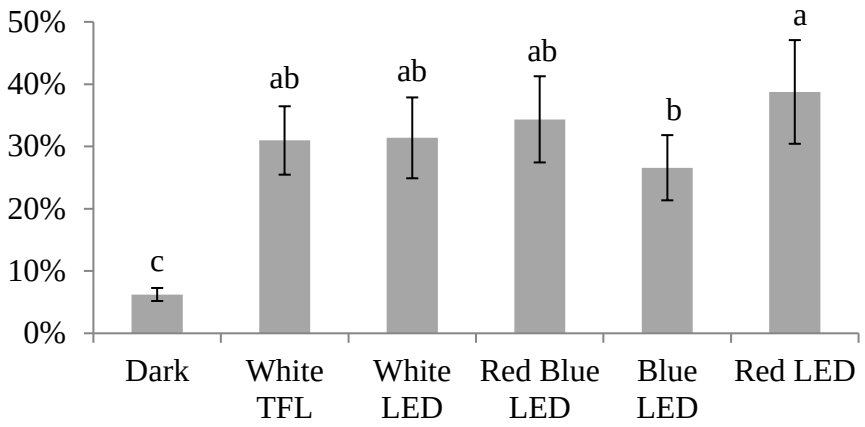

Figure 2 Percentage of rooting in shoots under different light qualities. Vertical bars indicate ± SE of the means for n = 6. Different letter indicate significant difference at p = 0.05 by DMRT.

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 oil palm shoots was significantly affected by light (Figure 3 & 4). Chlorophyll and sugar content in shoots under light treatment were higher than that under dark treatment. Light is an important factor that regulates chlorophyll biosynthesis.

Shoots under white, red blue, and blue light accumulated higher chlorophyll, compared to that under red or dark treatment (Figure 3). Several studies have shown that blue light is a good light for chlorophyll synthesis and that red light decrease chlorophyll content (Tanaka *et al.* 1998; Li *et al.* 2010). This was also observed in this study, that chlorophyll content in oil palm shoots decreased under red LED, and increased under white and blue LED (Figure 3).

Blue light is important in chlorophyll synthesis. Blue light stimulates chlorophyll synthesis by inducing 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 dark treatment (Figure 3) and this may be stimulated by high cytokinin content under light treatments (Table 2). Cytokinin affects chlorophyll biosynthesis by promoting 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 oil palm shoots under different light treatments were not significantly different (Figure 4). The different light quality did not give differential effect on sugar content because the *in vitro* shoots are mainly heterotrophic, where the shoots were grown on 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 reduces the need for sugar production and limits photosynthesis activity.

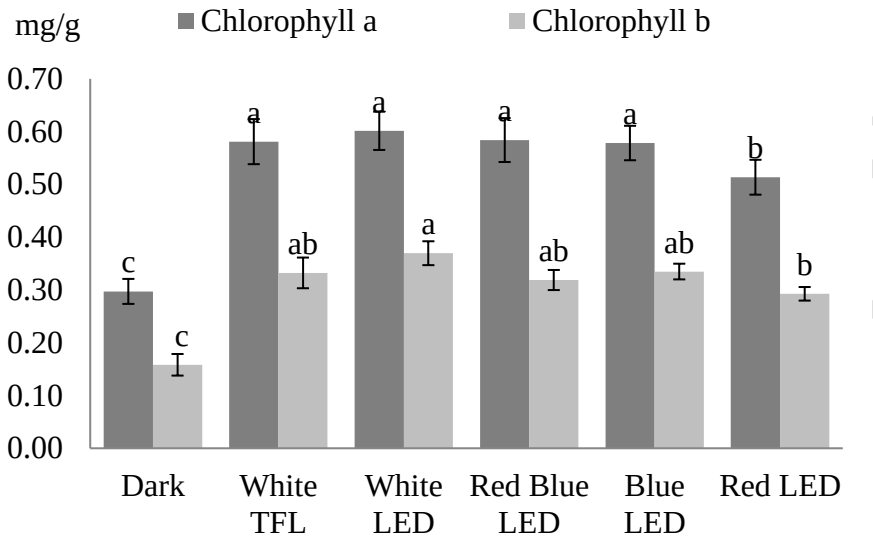

Figure 3 Chlorophyll content in oil palm shoots under different light qualities. Vertical bars indicate ± SE of the means for $n = 6$. Different letter indicate significant difference at $p = 0.05$ by DMRT.

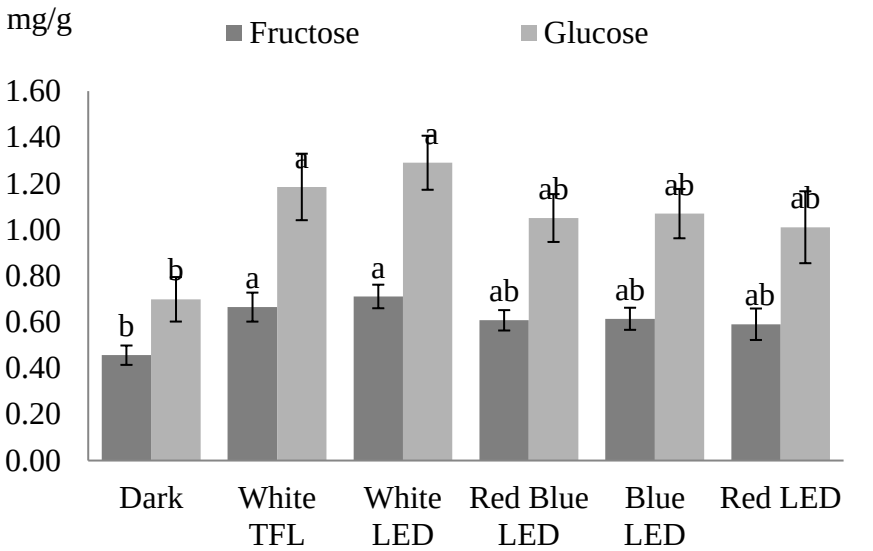

Figure 4 Sugar content in oil palm shoots under different light qualities. Vertical bars indicate ± SE of the means for $n = 6$. Different letter indicate significant difference at $p = 0.05$ by DMRT.

CONCLUSION

Light quality influenced growth and development of oil palm shoot. The different light quality produce a differential effect on growth and development of oil palm shoot. Root initiation

and shoot elongation were good under red light, while chlorophyll and sugar content was better produced under white and blue light than red light. The combination of red and blue LED resulted in higher growth parameter compared to other light treatments, but the results were not significantly different. In addition to white light, the combination of red and blue light provides good lighting for promoting growth and development of oil palm shoots. Red blue LED may be used as an alternative to replaces white TFL for oil palm tissue culture, but further study to analyze the ratio of red and blue LED may be needed to optimize the protocols for oil palm tissue culture.

ACKNOWLEDGEMENTS

This study was supported by Plant Production and Biotechnology Division of PT SMART Tbk. We would like to thank all technicians in tissue culture and biotechnology laboratory of SMART Biotechnology Center, who have helped with this study.

REFERENCES

Bello-Bello JJ, Martínez-Estrada E, Caamal-Velázquez JH, Morales-Ramos V. 2016. Effect of LED light quality on *in vitro* shoot proliferation and growth of vanilla (*Vanilla planifolia* Andrews). Afr J Biotechnol 15(8):272-277.

Budiarto K. 2010. Spectral quality affects morphogenesis on *Anthurium* plantlet during in vitro culture. Agrivita 32:234–240.

Cohen L, Gepstein S, Horwitz BA. 1991. Similarity between cytokinin and blue light inhibition of cucumber hypocotyl elongation. Plant Physiol 95: 77-81.

Cortleven A, Schmulling T. 2015. Regulation of chloroplast development and function by cytokinin. J Exp Bot 66(16):4999-5013.

Cybularz-Urban T, Hanus-Fajerska E, Swiderski A. 2007. Effect of light wavelength on in vitro organogenesis of a *Cattleya* hybrid. Acta Biol Cracov 49(1):113-118.

de Wit M, Galvão VC, Fankhauser C. 2016. Light-mediated hormonal regulation of plant growth and development. Annu Rev Plant Biol. 67:513-37.

Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. 1951. A colorimetric method for determination of sugars. Nature 168: 167.

Ferreira LT, de Araújo Silva MM, Ulisses C, Camara TR, Willadino L. 2017. Using LED lighting in somatic embryogenesis and micropropagation of an elite sugarcane variety and its effect on redox metabolism during acclimatization. Plant Cell Tiss Organ Cult 128(1):211–221.

Fujiwara K, Kozai T. 1995. *Physical Microenvironment and Its Effects*. In: Aitken-Christie J, Kozai T, Smith ML, editors. Automation and Environmental Control in Plant Tissue Culture. Netherlands: Kluwer Academic Publishers. P. 319-369.

Fukuda N, Ajima C, Yukawa T, Olsen JE. 2016. Antagonistic action of blue and red light on shoot elongation in petunia depends on gibberellin, but the effects on flowering are not generally linked to gibberellin. Environ Exp Bot 121:66-74.

Fu X, Harberd NP. 2003. Auxin promotes *Arabidopsis* root growth by modulating giberellin reponse. Nature 421:740-743.

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

Kelen M, Demiralay EC, Sen S, Ozkan G. 2004. Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (*Vitis berlandieri x Vitis rupestris*) and rose oil (*Rosa damascena* Mill.) by reversed phase liquid chromatography. Turk J Chem 28:603 -610.

Kushairi A**,** Tarmizi AH, Zamzuri I, Ong-Abdullah M, Samsul Kamal R, Ooi SE, Rajanaidu N. 2010. Production, Performance and Advances in Oil Palm Tissue Culture. In: International Seminar on Advances in Oil Palm Tissue Culture. Proceedings: 2010 May 29; Yogyakarta: International Society for Oil Palm Breeders (ISOPB). p. 1-23.

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

Li H, Tang C, Xu Z. 2013. The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis in vitro. Sci Hortic 150:117–124.

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350-382.

Matters GL, Beale SI. 1995. Blue-light-regulated expression of genes for two early steps of clorophyll biosynthesis in *Chlamydomonas reinhardtii*. Plant Physiol 109:471-479.

Mengxi L, Zhigang X, Yang Y, Yijie F. 2011. Effects of different spectral lights on *Oncidium* PLBs induction, proliferation and plant regeneration. Plant Cell Tiss Organ Cult 106(1):1–10.

Muneer S, Kim EJ, Park JS, Lee JH. 2014. Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (*Lactuca sativa* L.). Int J Mol Sci 15(3):4657-4670.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497.

Nhut DT, Takamura T, Watanabe H, Okamoto K, Tanaka M. 2003. Responses of strawberry plantlets cultured in vitro under superbright red and blue light-emitting diodes (LED). Plant Cell Tiss Organ Cult 73:43–52.

Nhut DT, Huy NP, Tai NT, Nam NB, Luan VQ, Hien VT, Tung HT, Vinh BT, Luan TC. 2015. Light-emitting diodes and their potential in callus growth, plantlet development and saponin accumulation during somatic embryogenesis of Panax vietnamensis Ha et Grushv. Biotechnol Biotechnol Equip 29(2):299-308.

Ouyang F, Mao JF, Wang J, Zhang S, Li Y. 2015. Transcriptome analysis reveals that red and blue light regulate growth and phytohormone metabolism in Norway spruce [*Picea abies* (L.) Karst.]. PloS One 10(8): e0127896. doi: 10.1371/journal.pone.0127896.

Poudel PR, Kataoka I, Mochioka R. 2008. Effect of red-and blue-light-emitting diodes on growth and morphogenesis of grapes. Plant Cell Tiss Organ Cult 92(2):147-153.

Rohani O, Sharifah SA, Rafii MY, Ong M, Tarmizi AH, Zamzuri I. 2000. Tissue culture of oil palm. In: Basiron Y, Jalani BS, Chan KW, editors. Advances in Oil Palm Research. Bangi: Malaysian Palm Oil Board. p. 238-283.

Tanaka M, Takamura T, Watanabe H, Endo M, Yanagi T, Okamoto K. 1998. In vitro growth of *Cymbidium* plantlets cultured under super bright and blue light emitting diodes (LEDs). J Hortic Sci Biotechnol 73:39–44.

Vieira LN, Fraga HPF, Anjos KG, Puttkammer CC, Scherer RF, Silva DA, Guerra MP. 2015. Light-emitting diodes (LED) increase the stomata formation and chlorophyll content in

Musa acuminata (AAA) 'Nanica˜o Corupa′' in vitro plantlets. Theor Exp Plant Physiol 27: 91–98.

Wang YH, Irving R. 2011. Developing a model of plant hormone interactions. Plant Signal Behav 6(4):494-500.

Wong G, Tan CC, Soh AC. 1997. Large scale propagation of oil palm clones – experiences todate. Acta Hort 447: 649-658.

Yaseen M, Ahmad T, Sablok G, Standardi A, Hafiz IA. 2013. Review: role of carbon sources for *in vitro* plant growth and development. Mol Biol Rep 40:2837–2849.