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Effect of light-emitting diodes on somatic embryogenesis and tissue-cultured plantlet growth of arecanut (*Areca catechu*) dwarf hybrid VTLAH-2

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ABSTRACT

This study was carried out to evaluate the effects of LEDs on embryogenic callus proliferation, somatic embryo formation and tissue cultured plantlet growth of arecanut (*Areca catechu* L.) dwarf hybrid (VTLAH-2) at ICAR-CPCRI, Kasaragod, Kerala, during 2021-2022. Yellow, red, blue, white monochromatic LEDs, and a combination of red: blue and blue: yellow with different photosynthetic photon flux densities (PPFDs 10, 20 and 100 μmol $m^{-2}s^{-1}$) were used. Eeuwen's Y3 basal media supplemented with picloram 2.5 μM were used for multiplication of calli. Embryogenic calli were inoculated to multiplication media, and these plates were kept under different LED conditions to examine calli multiplication and somatic embryogenesis. To check the growth of plantlets, germinated somatic embryos were transferred to test tubes containing arecanut plantlet growing media (i.e., 0.5 mg/L BAP +0.5 mg/L NAA and +0.25 mg/L IBA with Y3 basal medium. Multiplication rate of arecanut embryogenic calli (0.051±0.008 $gg^{-1}d^{-1}$), somatic embryo formation (46.1±2.9), plantlet growth (RGR-wt.0.9±0.07 $gg^{-1}d^{-1}$; RGR-ht: 0.57±0.01 $cm.cm^{-1}d^{-1}$) and survival (61.4±4.3%) were found to be superior under a combination of red-yellow LED, which was followed by blue and yellow monochromatic LEDs. Whereas comparatively lower callus multiplication (0.008±0.001 $gg^{-1}d^{-1}$) and plantlet growth (RGR-wt: 0.72±0.03 $gg^{-1}d^{-1}$; RGR-ht: 0.24±0.12 $cm.cm^{-1}d^{-1}$) was noticed with white and red LEDs with a PPFD values of 100 μmol $m^{-2}s^{-1}$ and 20 μmol $m^{-2}s^{-1}$ consecutively. Thus, there was a positive effect of LED light source on arecanut somatic embryogenesis and plantlet growth.

Key Words: LED, Somatic embryogenesis, Callus multiplication, *In vitro* plantlet growth

Arecanut (*Areca catechu* L.) belong to the family Arecaceae. Traditionally, its seed is the only available propagules like other palm species. Tissue culture seems to be the only available vegetative propagation method applicable to this palm. Use of tissue culture technology for clonal multiplication of arecanut has been reported (Karun *et al*., 2004). However, in dwarfs and dwarf hybrid arecanut material the cultures have showed lower multiplication rate and prolonged regeneration cycle for plantlet development. Light, one of the key environmental factors, work as a signal and energy source, affects almost every aspect of plant life (Reuveni and Evenor, 2007; OuYang *et al*., 2015). As an alternative to the conventional sources of light, light-emitting diodes (LEDs) have emerged in recent years (Río-Álvarez *et al*., 2014). Moreover, LEDs emit wavelengths that are consistent with the absorption spectra of different plant species and can promote the growth effectively (Li *et al*., 2018). LEDs of different wavelengths can be used independently or in combinations to optimize the growth of plant cultures (Shengxin *et al*., 2016). Under *in vitro* conditions, lower light intensities are sufficient to regulate plant morphogenesis due to availability of sugar in medium which acts as a source of energy. Also, energy consumption levels of *in vitro* cultured plants are low, and they do not overheat due to maintenance of humidity in closed vessels (George and Davies, 2008). The PPFD requirement for *in vitro* grown plants varies, and PPFD range for herbaceous plants varies from 7 to 120 μmol m^{-2} s^{-1}, but for majority species optimal level is at 30-40 μmol m^{-2} s^{-1} (Alvarenga *et al*., 2015; Silva *et al*., 2017). LEDs with different wavelengths (colours) and intensities can be used to improve *in vitro* multiplication of tissue-cultured plants. A combination of red and blue LEDs (4:1) improved shoot multiplication (He *et al*., 2020). Use of a combination of red (70%) and blue (30%) LEDs (40-120 μ mol m^{-2} s^{-1}) improved shoot multiplication in gerbera, sugarcane, and rice under

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in vitro (Cioc *et al*., 2019; Silva *et al*., 2014; YU Lan-lan *et al*., 2020). However, the best LED light source for palm tissue culture remains unknown. Therefore, monochromatic LEDs (white, red, blue, yellow) and their combinations (red: blue 1:1; red: yellow 1:1) with different PPFD values (10, 20 and 100 μmol m^{-2} s^{-1}) were used to study their influence on embryogenic callus multiplication, somatic embryogenesis, and tissue-cultured plantlet growth of arecanut.

MATERIALS AND METHODS

Embryogenic calli and germinated somatic embryos of *Areca catechu* dwarf hybrid VTLAH-2 were selected. The 0.5 g callus was weighed and incubated in callus multiplication media in a Petri plate containing Eeuwen's Y3 solid medium (pH 5.8) supplemented with 2.5 μM picloram. These plates were subjected to LED lights for observations on the multiplication and somatic embryo formation (formation of somatic embryos were observed on same multiplication media). Sub-culturing of multiplied material was carried out at monthly interval to the same multiplication media and somatic embryos formed were transferred to hormone free Y3 media (Aparna *et al*., 2022, Neema *et al*., 2022) for its growth and development. The observations on callus growth, other callus parameters and somatic embryo formation were recorded.

For checking the influence of LEDs on somatic embryo growth and plantlet development, germinated somatic embryos having a size of 0.8-1.0 cm were transferred to test tubes having Eeuwen'sY3 basal media supplemented with 0.5 mg/L BA, 0.5 mg/L NAA and 0.25 mg/L of IBA (pH 5.8) (Aparna *et al*., 2022). The selected somatic embryos were individually transferred to test tubes and were exposed to different LED light conditions to check the growth and development of plantlets. Observations on growth and development of somatic embryos were recorded at bimonthly intervals and sub-culturing was carried out into the same media for its growth and development immediately after recording the observations. A photoperiod of 16 h and a temperature of 27±2°C and R.H of 70% were maintained in the culture room.

A LED light fixtures (light strips of 100 cm long 1 cm width consisting of small lamps) were used. for the experiment. The light intensity values (PPFD) were measured with porometer.

The light quality treatments were:

T_1) dark (control-for callus multiplication and somatic embryogenesis)

T_2) W: 100% white LED (400-750) with PPFD of ≅10 μ mol m^{-2} s^{-1}

T_3) B: 100% blue with a wavelength of 450-495 nm and PPFD of ≅ 10 μ mol m^{-2} s^{-1}

T_4) Y: 100% yellow with a wavelength of 570-590 nm and PPFD of ≅ 10 μ mol m^{-2} s^{-1}

T_5) R: 100% red with a wavelength of 610-760 nm and PPFD of ≅ 10 μ mol m^{-2} s^{-1}

T_6) B: R=1:1 light: 50% blue LED light with a wavelength of 450-495 nm and 50% red LED light with a wavelength of 610-760 nm and PPFD of ≅ 20 μ mol m^{-2} s^{-1}

T_7) R: Y=1:1 light: 50% red LED light with a wavelength of 610-760 nm and 50% yellow LED light with a wavelength of 570-590 nm and PPFD of ≅ 20 μ mol m^{-2} s^{-1}

T_8) W: 100% white LED (400-750) with a PPFD of ≅ 100 μ mol m^{-2} s^{-1}

To check the influence of LEDs on callogenesis and somatic embryogenesis of arecanut, observations on increase in callus weight and somatic embryo formation, callus friability, tissue browning, pigment development and vitrification were recorded at monthly intervals continuously for a period of four months. Similarly, to check the growth and development of plantlet, observations on conversion of somatic embryos to plantlets, plantlet growth rate in terms of weight and height, survival, rooting, tendency of multiple shooting, and secondary callogenesis from somatic embryos were recorded at 60 days interval for duration of six months. The recorded values on callogenesis, somatic embryo formation, and plantlet growth and development were subjected to statistical analysis to find out the suitable light source for somatic embryogenesis and plantlet growth of arecanut under *in vitro*.

Completely randomized design (CRD) was used. For testing callus multiplication and somatic embryogenesis four replications were calculated and each Petri plate was considered as one replication. To test the growth and development of plantlets 18 test tubes containing germinated somatic embryos were used for each treatment. The average values of six tubes were considered as one replication. For analysis multiple comparisons of treatments were determined by means of Fisher-least square difference (LSD) test and followed by grouping of treatments using

"agricolae" package in R (Mendiburu, 2020). The level by alpha is 0.05 and p-values were adjusted using bonferroni criteria.

RESULTS AND DISCUSSION

Data on relative growth rate of calli revealed the highest multiplication (0.051 $gg^{-1}d^{-1}$) of embryogenic calli under a combination of red: yellow (1:1) LEDs. This was followed by yellow, white monochromatic LEDs with a callus growth rate of 0.03 $gg^{-1}d^{-1}$ and 0.028 $gg^{-1}d^{-1}$ LEDs subsequently (Table1; Fig.2). Callus multiplication is almost doubled under a combination of red: yellow (1:1) LEDs as compared to the control, i.e., dark incubation. The positive effect of yellow and red LEDs on improving calli weight and somatic embryo formation were observed in *Panax vietnamensis* (Nhut *et al*., 2015) and *Fritillaria cirrhosa* (Chen *et al*., 2020) plants respectively.

Similarly, Soni and Swarnkar (1996) reported callusing and shoot bud formation from leaf cultures of *Vigna aconitifolia* using blue and yellow spectra. In our study, white LEDs with lower light intensity gave better results for multiplication over higher intensity (100 μmol $m^{-2}s^{-1}$). Callus type (friability, compactness, embryonic or organellar calli) decides its multiplication rate. Calli with no apparent organ formation is typically called friable or hard calli (Ikeuchi *et al*., 2013). If calli displays some degrees of organ regeneration it is called rooty, shooty or embryonic callus depending upon the type of organs they develop (Frank *et al*., 2000).

Friable calli of arecanut was found to multiply faster as compared to the compact and organellar calli. The friable and compact organellar callus measured under different LEDs recorded highest friable calli (65%) under a combination of red: yellow (1:1) followed

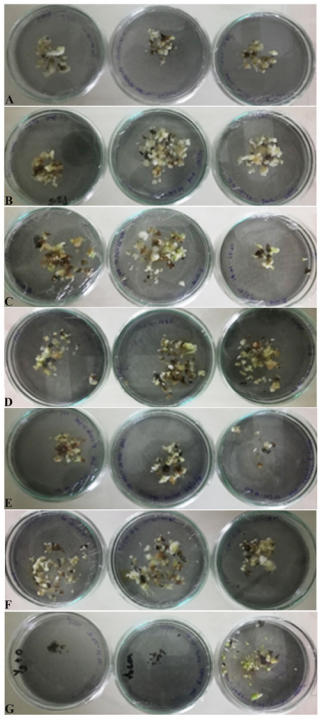

Fig. 1. Embryogenic calli multiplication and somatic embryogenesis in different LED treatments; T_1: dark; T_2: white Lower Intensity; T_3:blue; T_4:yellow; T_5: red; T_6: red: yellow 1:1; T_7: Blue: red 1:1; T_8: White High Intensity

Table 1. Effect of LEDs on callogenesis and somatic embryo formation in Dwarf hybrid line VTLAH-2

Treatment	PPFD (μ mol m^{-2} s^{-1})	Calli wt. (RGR $gg^{-1}d^{-1}$)	Pigmented calli (%)	Somatic embryos / month
T_1-Dark	-	0.026(0.160^b)	0.0 (0.00^c)	30.33(28.33^a)
T_2-White LI	10	0.028(0.168^{ab})	0.0 (0.00^c)	29.00(14.16^a)
T_3- Blue	10	0.025(0.159^b)	25.0(4.98^a)	36.70(29.88^a)
T_4-Yellow	10	0.030(0.169^{ab})	0.00(0.00^c)	38.25(38.25^a)
T_5-Red	10	0.019(0.135^{bc})	8.33(2.85^b)	24.00(16.71^a)
T_6-Red + yellow	20	0.051(0.225^a)	0.00 (0.00^c)	46.1(46.11^a)
T_7-Blue + red	20	0.022(0.148^{bc})	3.33(0.00^c)	18.66 (4.30^a)
T_8-White HI	100	0.008(0.089^c)	0.00 (1.49^b)	6.00 (2.34^a)
Mean		0.026(0.157)	4.58(1.16)	28.63(22.51)
CV (%)		33.40(12.01)	49.79(40.43)	37.03(66.38)
CD (0.05)		0.015(0.03)	3.98(0.83)	18.51(26.17)
S Em		0.005(0.00035)	1.31(0.222)	6.12(223.31)

(The values in parentheses are square root transformed values)

by the control (dark condition) (61%) and yellow LEDs (61%) (Fig.1a), which supports the positive role of a red: yellow LEDs for calli multiplication. Compact organellar calli was found more under red and white (LI) monochromatic LEDs, subsequently resulting in suppression of calli multiplication under these LEDs. Callus browning or necrosis varied from 1.6 to 63% under different LEDs (Fig.1b). Least browning of calli was observed under white coloured LED (10 µmol m^{-2} s^{-1}) with lower intensity i.e.,1.6%, followed by the control (dark) and blue LED with 3% and 5% respectively. Maximum browning (63%) followed by cell death was observed in cultures kept under high light intensity, i.e., white LED with a PPFD of 100 µmol $m^{-2}s^{-1}$. Even though browning of the calli was comparatively high (21%) under yellow LED, regeneration of new calli was found from browned regions. Calli and somatic embryo vitrification was also observed in cultures, and it varied from 1-13%. The vitrified calli and somatic embryos of arecanut were observed to give secondary embryonic calli on same multiplication media.

The data on transformations of calli into somatic embryos was also done. Under certain stress conditions callus cells undergo the process of somatic embryogenesis in which embryos are formed from the adult somatic cells. Number of somatic embryos (SEs) formed from embryogenic calli on callus multiplication media were counted under different treatments showed maximum SE from a combination of red: yellow (46), which was followed by yellow (38) and blue (36) monochromatic LEDs separately, while least embryo formation was seen in white LED with a PPFD of 100 µmol $m^{-2}s^{-1}$ (Table1). Similarly, Chen *et al*. (2020) also reported the highest number of somatic embryos under red LEDs in *Fritillaria cirrhosa*.

Survival percentage of germinated somatic embryos under different LEDs varied from 40 -76%. Significant variability among treatments was not observed for survival of embryos. Relative growth rate of plantlets developed from somatic embryos observed under LEDs ranged from 0.17 - 0.025 $gg^{-1}d^{-1}$. Comparatively higher RGR values were seen in red: yellow (1:1) and white LI (LEDs) as compared to others (Table 2). Arecanut plantlet growth was sluggish in almost all treatments irrespective of LED colour and intensity. A combination of red and blue LEDs at appropriate ratios and optimal intensities enhanced the growth and development of rice (red 50%: blue 50%; 300 µmol $m^{-2}s^{-1}$) *Panax vietnamensis* (red 60%: blue 40%; 25µmol $m^{-2}s^{-1}$), and *Camellia oleifera* (red 80%: blue 20%, 50 ± 5 µmol $m^{-2}s^{-1}$) (Nhut *et al*., 2015; He *et al*., 2020). But in our study, tested LED treatments did not show significant differences (Table 2).

Variation in rooting percentage among different LEDs ranged from 10-46%. Highest rooting percentage (46%) was observed in plantlets maintained under red: yellow (1:1) and white LEDs with high intensity (100 µmol $m^{-2}s^{-1}$), while lowest rooting was (10%) observed under yellow LED. Influences of LEDs on rooting of *in vitro* grown plants were noticed in several species. Blue coloured LED stimulated the rooting in *Vanilla planifolia*

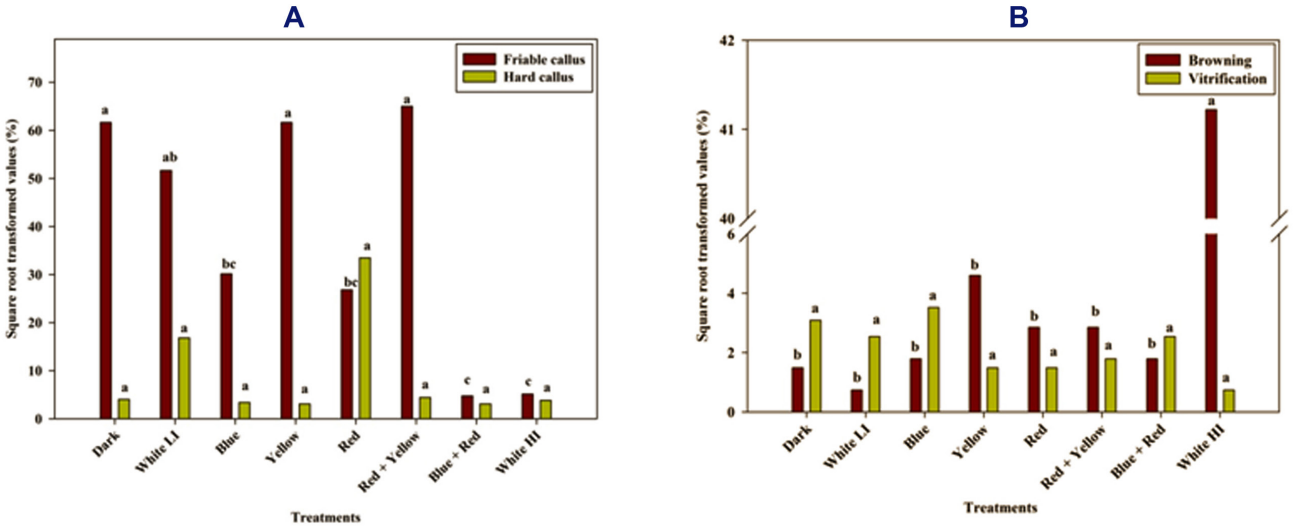

Fig. 2. Influence of different LEDs on (a), arecanut calli type and its multiplication, (b), calli browning and vitrification

Table 2. Effect of LEDs on tissue cultured plantlet growth and development

Treatment	PPFD (μ mol m^{-2} s^{-1})	Survival (%)	RGR-ht. ($cm.cm^{-1}d^{-1}$)	RGR-wt. ($g.g^{-1}d^{-1}$)	Rooting (%)	(%)Calli formation	Somatic embryogenesis (%)	Multiple shooting(%)
T_1-White LI	10	52.59 (27.80^a)	0.001 (0.032^a)	0.024 (0.152^a)	26.98(26.9^{ab})	59.67 (37.56^a)	62.45 (17.48^a)	38.99 (34.70^a)
T_2-Blue	10	66.74 (44.62^a)	0.004 (0.063^a)	0.017 (0.129^a)	15.87(12.36^b)	48.23(27.61^a)	45.45 (45.45^a)	44.44 (40.24^a)
T_3-Yellow	10	63.01 (38.27^a)	0.006 (0.068^a)	0.017 (0.131^a)	10.31 (2.62^b)	63.56 (63.56^a)	52.45 (52.45^a)	38.88 (38.88^a)
T_4-Red	10	40.47 (34.80^a)	0.003 (0.041^a)	0.016 (0.125^a)	15.15 (3.14^b)	59.36 (37.24^a)	56.58 (26.37^a)	22.22 (22.22^a)
T_5-Red + yellow	20	76.66 (27.19^a)	0.010 (0.105^a)	0.025 (0.157^a)	46.94 (46.94^a)	38.88(33.21^a)	36.10 (30.43^a)	31.11 (28.83^a)
T_6-Blue + red	20	52.85 (52.85^a)	0.004 (0.063^a)	0.019 (0.136^a)	18.253 (3.48^b)	47.29(47.29^a)	44.52 (37.85^a)	38.88 (34.69^a)
T_7-White HI	100	55.29 (55.29^a)	0.005 (0.069^a)	0.018 (0.134^a)	46.94 (46.94^a)	74.67(47.31^a)	71.95 (47.22^a)	55.55 (55.55^a)
Mean		58.23 (40.12)	0.0047(0.062)	0.0194 (0.138)	25.78 (20.35)	55.95(55.72)	52.79 (36.75)	13.18 (36.44)
CV (%)		30.81 (41.68)	62.11 (41.26)	28.34 (14.37)	56.33 (51.42)	29.53 (55.73)	33.18 (65.00)	62.00 (69.74)
CD (0.05)		N/A (29.75)	N/A (0.05)	N/A (0.04)	25.68 (18.62)	N/A (41.61)	N/A (42.50)	N/A (45.22)
S.Em		10.36(279.69)	0.002(0.0006)	0.003 (0.0003)	8.386 (109.55)	9.54 (547.13)	10.11(570.77)	13.81(646.08)

The values in parentheses are square root transformed values

(Ramírez-Mosqueda *et al*., 2017). In gerbera, blue and red monochromatic LEDs had positive effect on rooting and plant survival (Pawłowska *et al*., 2018). A composite light of red-blue-purple-green (8:1:1:1) was optimal for getting better rooting rate, root activity and root growth of *Cunninghamia lanceolata* tissue culture seedlings (Xu *et al*., 2020). LEDs were found to have influence on multiple shooting from the developed somatic embryos. Even though 22-55% cultures had exhibited multiple shooting in arecanut, significant differences were not noticed between the LED treatments. This could be due to the addition of cytokinin (6-BAP) to the media to improve the shoot growth. May be the addition of cytokinin along with auxins might have disturbed the cytokinin and auxin balance within the plant system which led to outgrowth of axillary meristems from the plantlet. Similar phenomenon was used in coconut to induce the multiple shoots by suppressing apical meristem using a PGR Tidiazuron (TDZ) (Wilms *et al*., 2021).

Formation of secondary calli was observed from the basal portions of germinated somatic embryos. These secondary calli suppressed the plantlet growth and accelerated the process of secondary somatic embryogenesis. Formation of embryogenic calli was noticed in all the treatments. In this culture 38-74% cultures maintained under different LEDs exhibited secondary calli formation. The calli formed at the distal end of the plantlets subsequently gave rise to the somatic embryos and this process (secondary somatic embryogenesis) continued in all the treatments throughout experimental duration. Significant differences were not noticed among the LED treatments for secondary callogenesis and somatic embryogenesis in arecanut.

CONCLUSION

Thus, there was positive influence of LEDs in arecanut somatic embryogenesis. A combination of red: yellow (1:1) LEDs positively influenced embryogenic calli multiplication and somatic embryogenesis. But the tested monochromatic LEDs and its combinations did not show much influence on subsequent growth and development of somatic embryo originated plantlets. Testing additional combinations along with increased intensity levels may help us to identify the right LEDs suitable for growth of juvenile arecanut plants.

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REFERENCES

Alvarenga I C A, Pacheco F V, Silva S T, Bertolucci S K V and Pinto J E B P. 2015. *In vitro* culture of *Achillea millefolium* L. Quality and intensity of light on growth and production of volatiles. *Plant Cell, Tissue Organ Cult* **122**: 299-308.

Aparna V, Neema M, Devakumar K, Muralikrishna K S and Rajesh M K. 2022. Effects of light-emitting diodes on somatic embryogenesis and tissue cultured plantlet growth of arecanut (*Areca catechu* L.). 2nd Indian Horticulture Summit-2022 At: Navsari Agricultural University, Navsari (Gujarat), India, PP.123-24.

Aparna V, Neema M, Chandran K P, Muralikrishna K S and Karun A. 2022. Enhancement of callogenesis from plumular explants of coconut (Cocos nucifera) via exogenous supplementation of amino acids and casein hydrolysate. *Current Horticulture* **11**(1): 40-43.

Chen C C, Lee M R, Wu C R, Ke H J, Xie H M, Tsay H S, Agarwal D C and Chang H C. 2020. LED lights affecting morphogenesis and isosteroidal alkaloid contents in *Fritillaria cirrhosa* D. Don. an important Chinese medicinal herb. *Plants* **9**: 1351.

Cioc M, Kalisz A, Zupnik M and Pawłowska B. 2019. Different LED Light Intensities and 6-Benzyladenine concentrations in relation to shoot development, leaf architecture, and photosynthetic pigments of *Gerbera jamesonii* Bolus *in vitro*. Agronomy **9**: 358.

Frank M, Rupp H M, Prinsen E, Motyka V, Van Onckelen H and Schmülling T. 2000. Hormone autotrophic growth and differentiation identifies mutant lines of *Arabidopsis* with altered cytokinin and auxin content or signaling. *Plant Physiol* **122**: 721-29.

He C, Zeng Y, Fu Y, Wu J and Liang Q. 2020. Light quality affects the proliferation of *in vitro* cultured plantlets of *Camellia oleifera* Huajin. *PeerJ8*: e10016.

Ikeuchi M, Momoko, Sugimoto K and Iwase A. 2013. Plant Callus: Mechanisms of Induction and Repression. Plant Cell **25** (9): 3159-73.

Karun A, Siril EA, Radha E and Parthasarathy VA. 2004. Somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants of arecanut (*Areca catechu* L.). *Curr Sci* **86**(12): 1623-28.

Li C, Liu D, Li LL, Hu SX, Xu ZG and Tang CM. 2018. Effects of light-emitting diodes on the growth of peanut plants. *Agron J* **110**: 2369-77.

Mendiburu F D. 2020. agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-3. https://CRAN.R-project.org/package=agricolae

Neema M, Aparna V and Chandran K P. 2022. Contrast analysis recommends flame sterilization for surface depuration in coconut (*Cocos nucifera*) meristem culture. *Current Horticulture* **10** (1): 41-44.

Nhut D T, Huy N P, Tai N T, Nam N B, Luan V Q, Hien V T, Tung H T, Vinh B T and Luan T C. 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.

Pawłowska B, Cioć M and Prokopiuk B. 2018. How LED light rooting *in vitro* affected Gerbera acclimatization efficiency. *Acta Hortic* **1201**: 583-90.

Ramírez-Mosqueda M A, Iglesias-Andreu L G and Luna-Sánchez I J. 2017. Light quality affects growth and development of in vitro plantlet of Vanilla planifolia Jacks. *S Afr J Bot* **109**: 288-93.

Río-Álvarez I, Rodríguez-Herva J J, Martínez P M, González-Melendi P, García-Casado G, Rodríguez-Palenzuela P and López-Solanilla E. 2014. Light regulates motility, attachment, and virulence in the plant pathogen *Pseudomonas syringae*pv tomato DC3000. *Environ Microbiol* **16**(7): 2072-85.

Shengxin C, Chunxia L, Xuyang Y, Song C, Xuelei J, Xiaoying L, Zhigang X and Rongzhan G. 2016. Morphological, Photosynthetic, and Physiological Responses of Rapeseed Leaf to Different Combinations of Red and Blue Lights at the Rosette Stage. *Front Plant Sci* **7**: 1144

Silva M M A, De Oliveira A L B, Oliveira-Filho R A, Gouveia-Neto A S, Camara T J R and Willadino L G. 2014. Effect of blue/red LED light combination on growth and morphogenesis of *Saccharum officinarum* plantlets *in vitro*. In: Proc. SPIE 8947, Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XII, 4th March 2014.

Silva S T, Bertolucci S K V, da Cunha S H B, Lazzarini L E S, Tavares M C and Pinto J E B P. 2017. Effect of light and natural ventilation systems on the growth parameters and carvacrol content in the in vitro cultures of *Plectranthus amboinicus* (Lour.) Spreng. *Plant Cell, Tissue Organ Cult* **129**: 501-10.

Soni J and Swarnkar P L. 1996. Morphogenetic and biochemical variations under different spectral lights in leaf cultures of *Vigna aconitifolia*. *J Phytol Res* **9**(1): 89-93.

Wilms H, De Bièvre D, Longin K, Swennen R, Rhee J and Bart Panis. 2021. Development of the frst axillary *in vitro* shoot multiplication protocol for coconut palms. *Sci Rep* **11**:18367

Xu Y, Yang M, Cheng F, and Liang Y. 2020. Effects of LED photoperiods and light qualities on *in vitro* growth and chlorophyll fluorescence of *Cunninghamia lanceolata*. BMC Plant Biol **20**: 269.

YU Lan, Song C M, Sun L L, Li L L, Xu Z G and Tang C M. 2020. Effects of light-emitting diodes on tissue culture plantlets and seedlings of rice (*Oryza sativa* L.). *J Integr Agric* **19**(7): 1743-54.