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Effects of monochromatic red and blue light-emitting diodes and phenyl acetic acid on *in vitro* mass production of *Pyrus communis* 'Arbi'

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ABSTRACT

Purpose: In Tunisia, *Pyrus communis* 'Arbi' is broadly imperiled by *Erwinia amylovora*. The breeding of resistant rootstocks is an effectual control strategy for disease management. Therefore, a sound protocol for micropropagation and for the extensive production of high-quality plantlets was developed. **Research method:** Three groups of LED treatments were carried out: (1) 100% blue (B) LED, (2) 100% red (R) LED and (3) 50% B + 50% R (=BR) LED. Stock plants were micropropagtaed on modified Murashige and Skoog (MS) medium with half concentration of NH_4NO_3 and KNO_3. **Findings:** Throughout the propagation stage, red LED displayed important advantages: it produced optimal shoot height and leaf surface. The least leaf area was obtained with fluorescent light. The blue / red combination yielded considerable amelioration. Shoot weight/callus weight was maximal, along with shoot number and shoot length. The root formation of *in vitro* grown pear plantlets was greatly influenced by the various light types and by the incorporation of a new root-promoting substance, phenyl acetic acid (PAA). When combining red light and PAA, 89 % of rooting was observed in pear plantlets. The acclimatized pear vitroplants achieved rapid growth without morphological anomalies. **Research limitations:** In order to improve the survival rates of the acclimatized vitroplants, the acclimatization stage needs to be further studied. **Originality/Value:** The study compared the impact of different combinations of monochromatic blue and red LED lights and phenyl acetic acid against that of fluorescent light during the micropropagation, rooting and acclimation of a resistant pear (*Pyrus communis* L., cv. Arbi).

INTRODUCTION

Light is a crucial element influencing morphogenesis *in vitro*. With its wide frequency spectrum (between 350 and 750 nm) (Gupta & Jatothu, 2013), fluorescent light (Flu) is generally applied in plant tissue culture rooms (Hung et al., 2016). However, this light is of poor quality for promoting plant growth (Morrow, 2008). Highly energy consuming, Flu generates heat (Yeh & Chung, 2009) and must be placed away from plants to avoid the effects of photo-stress (Gupta & Agarwal, 2017). It is, therefore, necessary to have an energy-efficient light that could cut costs and increase the production and quality of tissue culture plants (Miler et al., 2019).

Light-emitting diodes (LEDs) have been lately suggested as a promising light source for plant growth *in vitro* (Yeh & Chung, 2009) since they offer several advantages. The LED lamps' low energy consumption makes plant propagation *in vivo* and *in vitro* more profitable (Larraburu et al., 2018). LEDs can indeed have a relatively long lifespan that ranges between 25.000 and 100.000 hours, as contrasted to fluorescent tubes that last between 10.000 and 15.000 hours (Yeh & Chung, 2009; Gupta & Jatothu, 2013). In addition, most of the LED energy is transformed into luminosity that can take one of several chosen shapes while its heat generation remains minimal, which optimizes photosynthetic and photomorphogenic responses (Bello-Bello et al., 2017). All these qualities make of LEDs a perfect source of light for plant tissue culture.

The use of LED lighting for the rapid and large-scale micropropagation of plants has recently aroused considerable interest. Previous research proven the positive impact of LED lamps on the *in vitro* development of species such as *Vaccinium corymbosum* (Hung et al., 2016), *Populus euroamericana* (Kwon et al., 2015) and *Saccharum* spp. (Ferreira et al., 2017) and have shown that plant propagation is determined by the light frequency to which the plantlets are subjected and that it differs depending on the plant genus (Dewir et al., 2006).

According to the literature, the impact of LED light on pear has been rarely examined, neither when LED light is used on its own nor when in combination with other colors. Lotfi et al. (2019) found that the treatment of *Pyrus communis* with BR (Blue Red) LEDs increased shoot number, compared to fluorescent white light. Comparable results have been recorded in *Phoenix dactylifera* (Al-Mayahi, 2016) and *Malus domestica* 'JM7' (Huang et al., 2018). Dewir et al. (2005) and Waman et al. (2016) equally recorded that the cultivation of *Spathiphyllum cannifolium* and *Musa* spp under R (Red) LED yielded a higher shoot multiplication rate than under B (Blue) or BR LEDs. For their part, Werbrouck et al. (2011) revealed the adverse impact of BR LED on the proliferation of *Ficus benjamina* and revealed that, compared to other light treatments, B LED caused callus formation at the base of the vitroplants.

It is also noteworthy that, under R LED, plant height was maximal in *Malus domestica* 'Budagovsky 9' and 'Geneva 30' (Geng et al., 2015), *Vitis* sp (Poudel et al., 2008), *Carica papaya* (da Silva, 2014) and *Gerbera jamesonii* (Pawłowska et al., 2018). However, Kostadinova et al. (2021) recorded that shoot length in *Pyrus communis* 'OHF333' was more important under white and B LED.

As reported by Lotfi et al. (2019), the biggest leaf size in *Pyrus communis* was observed under R LED. Contrariwise, leaf size in *Pyrus communis* 'OHF333' under white LED was larger than under R, B and BR-Far red LED (Kostadinova et al., 2021). Plantlet fresh weight was greater under BR LEDs; it was in equal proportions in *Lilium* x *hybridum* (Lian et al., 2002), *Musa* sp (Nhut et al., 2001), *Fragaria* sp (Nhut et al., 2003) and *Myrtus communis* (Cioć et al., 2018). Conversely, no improvement in fresh weight was observed in *Vitis* sp under B LED light exposure (Heo et al., 2006).

As for the impact of different light spectra on pear, two related studies can be cited. Bertazza et al. (1995) assessed the effect of R, B, far-red and Flu on *in vitro* rooting of *Pyrus communis* using spectral filters.

Another report comes from Lotfi et al. (2019), who showed that the adventitious root development of *in vitro*-cultivated pear was considerably impacted by the use of various light frequencies and by the addition of *trans*-cinnamic acid. Phenyl Acetic acid (PAA) is a natural auxin (Cook, 2019); it is used to induce lateral roots, root hairs and adventitious rooting in *Cicer arietinum* (Ghanti et al., 2009). It has also been shown that PAA stimulates auxin response in the same manner as IAA in Arabidopsis (Sugawara et al., 2015).

In order to develop an efficient system for high-quality large-scale micropropagation of pear, the effects of B, R and BR LED were examined against those of Flu throughout the shoot multiplication and rooting stage. Our earlier work revealed that pear reacts to auxins by the formation of friable callus at the root base (Lotfi et al., 2020). In an experiment to develop a substitute rooting agent, the influence of PAA was tested in combination with that of monochromatic LED light quality.

MATERIALS AND METHODS

Stock cultures

The research was performed on *Pyrus communis* 'Arbi', a superior pear rootstock that is distinguished by its high compatibility with other pear cultivars (Mars et al., 1994) and by its tolerance to both biotic stress and abiotic stress (Lotfi et al., 2020). Stock plants were micropropagated for 5 subcultures on modified Murashige and Skoog (1962) medium (MS) with 50% content of NH_4NO_3 and KNO_3, 30 g/L sucrose, 5 µM *meta*-topolin (*m*T) and 6 g/L agar-agar. The medium (pH 5.8) was autoclaved at 121°C for 20 min. Thirty *in vitro* 1 cm-long shoots were used as explants per light treatment. Six glass jars of 380 mL, with 100 mL medium each, were kept per light source. After two months, the number of shoots per explant, shoot length, shoot weight and callus weight were measured. The real leaf area was obtained by using ImageJ® software.

In vitro rooting

In order to remedy the problem of poor rooting and to overcome acclimatization problems, a series of combination treatments based on LED light and Phenyl Acetic Acid (PAA) were performed. To assess the impact of these treatments on *in vitro* rooting, thirty unrooted offshoots, each 3 cm long, were used as explants. For each treatment, 6 boxes contained 120 mL of ½ MS medium, enriched with 5 µM PAA. The plants were placed under different light spectra during the multiplication phase. Rooting rate and root number and root length were measured after 21 days of culture.

Cultivation conditions and light treatments

The shoots were incubated in the growth room at 25 ± 1°C under a photoperiod (light: dark) 16:8. In each step of micropropagation (multiplication and rooting), three groups of LED treatments were performed: (1) 100% blue (B) LED (maximum wavelength: 454 nm), (2) 100% red (R) LED (maximum wavelength: 660 nm) and (3) 50% B + 50% R (=BR) LED. The control light treatment was cool-white fluorescent lamps (Flu) (wavelength between 350-750 nm), supplied by PHILIPS master TLD 36 W 830 Reflex ECO tubes (40 µmol m^{-2} s^{-1} PAR).

Acclimatization

At the end of the 21 days of in vitro culture, rooted pear plants of 3.5 cm height from different light treatments were transplanted into vinyl pots filled with a mix of coco peat and perlite as substrate with the Osmocote® fertilizer and then were relocated to a greenhouse to be acclimatized at 28 ± 1°C, 95% relative humidity, 16-h photoperiod, light frequency of 85 µmol m^{-2} s^{-1} and with a fog system. Four weeks later, the plants were transplanted to pots including the same soil mixture and were kept in the greenhouse at 20 ± 2°C, 85% RH and 16-h photoperiod.

Statistical analyses

A completely randomized design was adopted for all experiments which were reduplicated three times. Results were submitted to a one-way analysis of variance (ANOVA) using the statistical program SPSS (Version 24 for Windows Inc., Chicago, IL, USA). In the present study, Duncan's multiple range test t ($p \leq 0.05$) was used.

RESULTS

The different light sources significantly influenced the *in vitro* shoot formation and growth habit of *Pyrus communis* 'Arbi' (Table 1, Fig. 1). Under R LED, maximal leaf area (189.5±0.98 mm^2) was recorded, but shoot production was minimal (1.3±0.34). Conversely, blue LED yielded comparatively small leaves and a mean of 4.3±0.9 shoots per explant.

Interestingly, under BR LEDs, the plantlets joined the largest shoot number (6.2±0.49) with the uppermost shoot length (3.8±0.45) and a middling leaf area. Maximal shoot weight/callus weight was recorded under blue red LED. The smallest shoot size (2.2±0.34 cm) and leaf area (19±0.61 mm^2) were detected under fluorescent light.

The adventitious root formation of *in vitro* pear plantlets was highly affected by the different light spectra and PAA presence. Rooting ratio, root number and root length are listed in Table 2. In the presence of PAA, maximal rooting percentage was obtained under red LED (89%), blue LED (76%) and blue red LEDs (63%). When PAA was used, the total number of primary and secondary roots per plants was superior under BR (4±0.58), and root length was highest under R LED (6.4±0.79 cm) and B LED (5.2±0.57 cm) (Fig. 2).

Table 1. Effects of light type on shoot growth and quality of *Pyrus communis* 'Arbi' after two months of *in vitro* cultivation.

Light types	No. of shoots/ explants	Shoot length (cm)	Leaf area (mm^2)	Shoot /callus weight
Blue	4.3±0.9 b	2.8±0.41 c	102±0.37 c	6.21±0.22 d
Red	1.3±0.34 d	3.1±0.53 b	189.5±0.98 a	13.05±0.41 b
Blue + Red	6.2±0.49 a	3.8±0.45 a	121±0.42 b	18.98±0.72 a
Fluorescent light	2.7±0.81 c	2.2±0.34 d	19±0.61 d	08.15±0.11 c

Values labeled by different letters designate significant statistical disparities (Duncan, $p \leq 0.05$).

Table 2. Impact of various light types and PAA on rooting ratio and root number and length of *Pyrus communis* 'Arbi' plantlets by the end of 21 days of *in vitro* cultivation.

Light treatments	Rooting ratio (%)	Total root number	Root length (cm)
Blue	76 b	1.5±1.86 d	5.2±0.57 b
Red	89 a	3.5±0.30 b	6.4±0.79 a
Blue + Red	63 c	4±0.58 a	3.2±0.64 d
Fluorescent light	52 d	3±0.74 c	3.6±0.71 c

Values labeled by different letters indicate significant statistical disparities (Duncan, $p \leq 0.05$).

Fig. 1. Effect of light quality on shoot proliferation of *Pyrus communis* 'Arbi' after 60 days of *in vitro* culture (Bar = 1cm). (a) Blue LED; (b) Red LED; (c) Blue and Red LEDs; (d) Fluorescent Lamp.

DISCUSSION

In our research, growing pear under BR LED revealed particular advantages: optimal shoot number and shoot height were reached. Our results agree with Kwon et al. (2015), who demonstrated that a combination of BR LED yielded an optimal rate of *in vitro* regeneration in *Populus euramericana*, compared to blue LED, red LED or fluorescent light. Al-Mayahi (2016) also found that treating *Phoenix dactylifera* 'Alshakr' with BR LED occasioned a notable rise in shoot number, as opposed to fluorescent white light. Similar findings have been reported in other horticultural plants such as *Anthurium andreanum* (Budiarto, 2010), *Dendranthema grandiflorum* (Kim et al., 2004), *Pyrus communis* (Lotfi et al., 2019) and *Vaccinium corymbosum* (Hung et al., 2016). It has often been demonstrated that, under blue red LED, *in vitro*-raised plantlets display considerable amelioration in growth and morphogenesis, compared to single LEDs (Gupta & Jatothu, 2013).

Fig. 2. Effects of different light sources and PAA on rooting of *Pyrus communis* 'Arbi' after 21 days of *in vitro* culture (Bar = 1cm). (a) Blue LED; (b) Red LED; (c) Blue and Red LEDs; (d) Fluorescent Lamp.

In addition, our study showed that R LED inhibited the multiplication of pear shoots, compared to other light spectra. Our observations do not correlate with those of Dewir et al. (2006) and Waman et al. (2016), who noted that the culture of *Spathiphyllum cannifolium* and of *Musa* spp under R LED produced an increase in shoot multiplication, compared to the results obtained under B or BR LEDs. Geng et al. (2015) also noted that R LED increased the shoot number of *Malus domestica* 'Budagovsky 9' and 'Geneva 30' rootstocks, compared to white or B LED, while the growth of *in vitro* plants of 'Geneva 41' showed no great effect between these three light sources.

Long and robust pear shoots were produced under BR, R and B LEDs. Our findings were in accordance with proposed by Bello-Bello et al. (2016), who demonstrated that B and BR LEDs stimulate shoot elongation in *Vanilla planifolia*. Other authors have observed that the best shoot elongation was obtained with R LED in *Vitis riparia* × *Vitis vinifera* (Poudel et al.,

2008), *Musa* spp (Waman et al., 2016), *Dendranthema grandiflorum* (Kim et al., 2004) and *Petunia hybrida* (Witomska & Ładyżyńska, 2001). *Gerbera jamesonii* plants grown under R LED were longer than the ones cultivated under B LED or fluorescent light (Wang et al., 2011). In addition, Gabryszewska and Rudnicki (1994) reported the positive influence of R light on shoot elongation of *Ficus benjamina* grown on a medium devoid of growth regulators and a medium enriched with 0.5 mg l^{-1} IAA. In contrast, B LED inhibited the shoot elongation of *Dendranthema grandiflora* (Kurilčik et al., 2008) and of *Ficus benjamina* 'Golden King' (Gabryszewska & Rudnicki, 1994).

Our study shows that the leaf area of pear plants highly increased under the R and blue red LED treatments, compared to the rest of the light spectra. Our observations are thus in agreement with Shin et al. (2008), who recorded that the largest leaf surface was detected in *Doritaenopsis* plantlets developed under BR and R LED light, while B LED inhibited leaf expansion. On the other hand, Li et al. (2010) noted that the largest leaf area was observed in *Gossypium hirsutum* plants developed under B LED.

Shoot/callus weight ratio was maximal under blue LED; it has tripled compared to the ratio obtained under BR LED. Our results corroborate Werbrouck et al. (2011) on *Ficus benjamina* and Lotfi et al. (2019) on *Pyrus communis*, showing that, when under B LED, shoot weight/callus weight doubled, compared to Flu light.

Previous studies have demonstrated that without auxins, this pear cultivar is extremely difficult to root *in vitro* when using fluorescent light. Adding 5 µM of IAA or IBA to the rooting medium culture, we observed that the adventitious roots developed on the calluses prevent a great vascular communication between the roots and the shoots (Lotfi et al., 2019). However, under Flu, adding 5 µM of PAA stimulated rooting rate (52%) without callus formation. According to Ayele et al. (2017), PAA effectively stimulates rooting and the initiation of lateral roots in *Vanilla planifolia* plantlets. Lateral root induction was also detected in our experiments. In *Capsicum annuum*, adding 14.7 µM of PAA was more effective at stimulating root growth than when adding similar concentrations of IAA (Mythili et al., 2017). These results suggest that PAA may serve a predominant role in root induction and root growth as well as in maintaining normal cellular growth (Cook, 2019).

In combination with PAA, 89% rooting and maximal root length and number were recorded under R. Red light is beneficial for rooting according to many authors, such as Poudel et al. (2008) in *Vitis* sp, Gabryszewska and Rudnicki (1994) in *Ficus benjamina*, Baque et al. (2010) in *Morinda citrifolia*, Hung et al. (2016) in *Vaccinium corymbosum*, Kurilčik et al. (2008) in *Chrysanthemum morifolium* and Lotfi et al. (2019) in *Pyrus communis*. Similar results were reported for cherry 'Colt' rootstock (Iacona & Muleo, 2010). In pear, B and BR were also effective when in combination with PAA. Bertazza et al. (1995) also observed a high rooting efficiency in *Pyrus communis* 'conference', with secondary root formation under R and B light.

The plants transferred to the greenhouse survived with a frequency of 60%. No variations in growth or morphological characteristics were detected.

CONCLUSION

High quality shoots and effective root growth of a resistant pear rootstock were achieved. During the multiplication stage, where *m*T was employed as cytokinin, a mixture of 50% blue and 50% red LEDs gave a maximum number of superior quality shoots. During the rooting stage, combining R with PAA enhanced root induction up to 89%. These results revealed that LED light could be more efficient than fluorescent light for rapid propagation with good root induction ability.

Conflict of interest

The author declares that there is no conflict of interest.

REFERENCES

Al-Mayahi, A. M. W. (2016). Effect of red and blue light emitting diodes "CRB-LED" on *in vitro* organogenesis of date palm (*Phoenix dactylifera* L.) cv. Alshakr. *World Journal of Microbiology and Biotechnology*, *32*(10), 1-8. https://doi.org/10.1007/s11274-016-2120-6

Ayele, Y. B., Tefera, W., & Bantte, K. (2017). Enhanced Protocol Development for *in vitro* Multiplication and Rooting of Vanilla (*Vanilla planifolia* Andr.) Clone (Van. 2/05). *Biotechnology Journal International*, 1-11. https://doi.org/10.9734/BJI/2017/33726

Baque, M. A., Hahn, E. J., & Paek, K. Y. (2010). Induction mechanism of adventitious root from leaf explants of *Morinda citrifolia* as affected by auxin and light quality. *In vitro Cellular & Developmental Biology-Plan*t, *46*(1), 71-80. https://doi.org/10.1007/s11627-009-9261-3

Bello-Bello, J. J., Perez-Sato, J. A., Cruz-Cruz, C. A., & Martinez-Estrada, E. (2017). Light-emitting diodes: *Progress in Plant Micropropagation*. *InTech*, *6*, 93-103.

Bertazza, G., Baraldi, R., & Predieri, S. (1995). Light effects on *in vitro* rooting of pear cultivars of different rhizogenic ability. *Plant Cell, Tissue and Organ Culture*, *41*(2), 139-143. https://doi.org/10.1007/BF00051582

Budiarto, K. (2010). Spectral quality affects morphogenesis on Anthurium plantlet during *in vitro* culture. *AGRIVITA, Journal of Agricultural Science*, *32*(3), 234-240. http://doi.org/10.17503/agrivita.v32i3.20

Cioć, M., Szewczyk, A., Żupnik, M., Kalisz, A., & Pawłowska, B. (2018). LED lighting affects plant growth, morphogenesis and phytochemical contents of *Myrtus communis* L. *in vitro*. *Plant Cell, Tissue and Organ Culture* (PCTOC), 132(3), 433-447. https://doi.org/10.1007/s11240-017-1340-2

Cook, S. D. (2019). An historical review of phenylacetic acid. *Plant and Cell Physiology*, *60*(2), 243-254. https://doi.org/10.1093/pcp/pcz004

da Silva, J. A. T. (2014). Photoauto-, Photohetero-and Photomixotrophic *in vitro* propagation of papaya (*Carica papaya* L.) and response of seed and seedlings to light-emitting diodes. *Science & Technology Asia*, 57-71.

Dewir, Y. H., Chakrabarty, D., Hahn, E. J., & Paek, K. Y. (2006). The effects of paclobutrazol, light emitting diodes (LEDs) and sucrose on flowering of *Euphorbia millii* plantlets *in vitro*. *European Journal of Horticultural Science*, *71*(6), 240.

Dewir, Y. H., Chakrabarty, D., Kim, S. J., Hahn, E. J., & Paek, K. Y. (2005). Effect of light-Emitting Diode on Growth and Shoot Proliferation of *Euphorbia millii* and *Spathiphyllum cannifolium*. *Horticulture Environment and Biotechnology*, *46*(6), 375-379.

Ferreira, L. T., de Araújo Silva, M. M., Ulisses, C., Camara, T. R., & 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, Tissue and Organ Culture (PCTOC)*, *128*(1), 211-221. https://doi.org/10.1007/s11240-016-1101-7

Gabryszewska, E., & Rudnicki, R. M. (1994, January). The effects of light quality on the growth and development of shoots and roots of *Ficus benjamina in vitro*. *In III International Symposium on Artificial Lighting in Horticulture* 418. (pp. 163-168).

Geng, F., Moran, R., Day, M., Halteman, W., & Zhang, D. (2015). *In vitro* shoot proliferation of apple rootstocks 'B9','G30', and 'G41'grown under red and blue light. *HortScience*, *50*(3), 430-433. https://doi.org/10.21273/HORTSCI.50.3.430

Ghanti, S. K., Sujata, K. G., & Rao, M. S. (2009). The effect of phenyl acetic acid on shoot bud induction, elongation and rooting of chickpea. *Biologia Plantarum*, *53*(4), 779-783. https://doi.org/10.1007/s10535-009-0143-7

Gupta, S. D., & Agarwal, A. (2017). Light emitting diodes for agriculture. *In LED Supplementary Lighting*, 27-36.

Gupta, S. D., & Jatothu, B. (2013). Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. *Plant Biotechnology Reports*, *7*(3), 211-220.

https://doi.org/10.1007/s11816-013-0277-0
Heo, J. W., Shin, K. S., Kim, S. K., & Paek, K. Y. (2006). Light quality affects *in vitro* growth of grape 'Teleki 5BB'. *Journal of Plant Biology*, *49*(4), 276. https://doi.org/10.1007/BF03031155
Huang, W., Yang, G., Li, F., Zheng, L., & Ma, J. (2018). Effects of different light qualities-LED on apple root-stock JM7 tissue culture seedling. *American Journal of Plant Biology*, *3*(2), 17-20.
Hung, C. D., Hong, C. H., Kim, S. K., Lee, K. H., Park, J. Y., Nam, M. W., ... & Lee, H. I. (2016). LED light for *in vitro* and *ex vitro* efficient growth of economically important highbush blueberry (*Vaccinium corymbosum* L.). *Acta Physiologiae Plantarum*, *38*(6), 1-9. https://doi.org/10.1007/s11738-016-2164-0
Iacona, C., & Muleo, R. (2010). Light quality affects *in vitro* adventitious rooting and *ex vitro* performance of cherry rootstock Colt. *Scientia Horticulturae*, *125*(4), 630-636. https://doi.org/10.1016/j.scienta.2010.05.018
Kim, S. J., Hahn, E. J., Heo, J. W., & Paek, K. Y. (2004). Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets *in vitro*. *Scientia Horticulturae*, *101*(1-2), 143-151. https://doi.org/10.1016/j.scienta.2003.10.003
Kostadinova, S., Mollov, I., Dzhambazov, B., Naimov, S., Vassilev, K., & Georgiev, B. (2021). Preliminary Study on the Effect of LED Light and Cytokinin on the Growth of Pear Plants *In Vitro*. In *5th Balkan Scientific Conference on Biology*. 1-8.
Kurilčik, A., Miklušytė-Čanova, R., Dapkūnienė, S., Žilinskaitė, S., Kurilčik, G., Tamulaitis, G., ... & Žukauskas, A. (2008). *In vitro* culture of Chrysanthemum plantlets using light-emitting diodes. *Central European Journal of Biology*, *3*(2), 161-167. https://doi.org/10.2478/s11535-008-0006-9
Kwon, A. R., Cui, H. Y., Lee, H., Shin, H., Kang, K. S., & Park, S. Y. (2015). Light quality affects shoot regeneration, cell division, and wood formation in elite clones of *Populus euramericana*. *Acta Physiologiae Plantarum*, *37*(3), 65. https://doi.org/10.1007/s11738-015-1812-0
Larraburu, E. E., Correa, G. S., & Llorente, B. E. (2019). *In vitro* development of yellow lapacho (Bignoniaceae) using high-power light emitting diode. *Revista Árvore*, 42.
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, Tissue and Organ Culture (PCTOC)*, *103*(2), 155-163. https://doi.org/10.1007/s11240-010-9763-z
Lian, M. L., Murthy, H. N., & Paek, K. Y. (2002). Effects of light emitting diodes (LEDs) on the *in vitro* induction and growth of bulblets of Lilium oriental hybrid 'Pesaro'. *Scientia Horticulturae*, *94*(3-4), 365-370. https://doi.org/10.1016/S0304-4238(01)00385-5
Lotfi, M., Bayoudh, C., Majdoub, A., & Mars, M. (2020). An optimized protocol for *in vitro* propagation of *Pyrus communis* and *Pyrus syriaca* using apical-bud microcuttings. *Journal of Horticulture and Postharvest Research*, *3*(1), 1-10. https://doi.org/10.22077/JHPR.2019.2420.1055
Lotfi, M., Mars, M., & Werbrouck, S. (2019). Optimizing pear micropropagation and rooting with light emitting diodes and *trans*-cinnamic acid. *Plant Growth Regulation*, *88*(2), 173-180. https://doi.org/10.1007/s10725-019-00498-y
Mars, M., Carraut, A., Marrakchi, M., Gouiaa, M., & Gaaliche, F. (1994). Ressources génétiques fruitières en Tunisie (poirier, oranger, fīguier, grenadier). *Elena Hanes*.
Miler, N., Kulus, D., Woźny, A., Rymarz, D., Hajzer, M., Wierzbowski, K., ... & Szeffs, L. (2019). Application of wide-spectrum light-emitting diodes in micropropagation of popular ornamental plant species: a study on plant quality and cost reduction. *In vitro Cellular & Developmental Biology-Plant*, *55*(1), 99-108. https://doi.org/10.1007/s11627-018-9939-5
Morrow, R. C. (2008). LED lighting in horticulture. *HortScience*, *43*(7), 1947-1950. https://doi.org/10.21273/HORTSCI.43.7.1947
Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Mythili, J. B., Rajeev, P. R., Vinay, G., & Nayeem, A. (2017). Synergistic effect of silver nitrate and coconut water on shoot differentiation and plant regeneration from cultured cotyledons of *Capsicum annuum* L. Indian Journal of Experimental Biology, *55*(3), 184-190.

Nhut, D. T., Takamura, T., Watanabe, H., & Tanaka, M. (2001, September). Efficiency of a novel culture system by using light-emitting diode (LED) on *in vitro* and subsequent growth of micropropagated banana plantlets. *In I International Symposium on Acclimatization and Establishment of Micropropagated Plants* 616. (pp. 121-127).

Nhut, D. T., Takamura, T., Watanabe, H., Okamoto, K., & Tanaka, M. (2003). Responses of strawberry plantlets cultured *in vitro* under superbright red and blue light-emitting diodes (LEDs). *Plant Cell, Tissue and Organ Culture*, *73*(1), 43-52. https://doi.org/10.1023/A:1022638508007

Pawłowska, B., Żupnik, M., Szewczyk-Taranek, B., & Cioć, M. (2018). Impact of LED light sources on morphogenesis and levels of photosynthetic pigments in *Gerbera jamesonii* grown *in vitro*. *Horticulture, Environment, and Biotechnology*, *59*(1), 115-123. https://doi.org/10.1007/s13580-018-0012-4

Poudel, P. R., Kataoka, I., & Mochioka, R. (2008). Effect of red-and blue-light-emitting diodes on growth and morphogenesis of grapes. *Plant Cell, Tissue and Organ Culture*, *92*(2), 147-153. https://doi.org/10.1007/s11240-007-9317-1

Shin, K. S., Murthy, H. N., Heo, J. W., Hahn, E. J., & Paek, K. Y. (2008). The effect of light quality on the growth and development of *in vitro* cultured Doritaenopsis plants. *Acta Physiologiae Plantarum*, *30*(3), 339-343. https://doi.org/10.1007/s11738-007-0128-0

Sugawara, S., Mashiguchi, K., Tanaka, K., Hishiyama, S., Sakai, T., Hanada, K., ... & Kasahara, H. (2015). Distinct characteristics of indole-3-acetic acid and phenylacetic acid, two common auxins in plants. *Plant and Cell Physiology*, *56*(8), 1641-1654. https://doi.org/10.1093/pcp/pcv088

Waman, A. A., Bohra, P., Sathyanarayana, B. N., Umesha, K., Gowda, B., & Ashok, T. H. (2016). *In vitro* shoot multiplication and root induction in Silk banana variety Nanjanagud Rasabale as influenced by monochromatic light spectra. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 86(3), 577-584. https://doi.org/10.1007/s40011-015-0488-y

Wang, Z., Li, G., He, S., da Silva, J. A. T., & Tanaka, M. (2011). Effect of cold cathode fluorescent lamps on growth of *Gerbera jamesonii* plantlets *in vitro*. *Scientia Horticulturae*, *130*(2), 482-484. https://doi.org/10.1016/j.scienta.2011.05.022

Werbrouck, S., Buyle, H., Geelen, D., & Van Labeke, M. C. (2011, September). Effect of red-, far-red-and blue-light-emitting di-odes on *in vitro* growth of *Ficus benjamina*. *In VII International Symposium on In vitro Culture and Horticultural Breeding* 961, (pp. 533-538).

Witomska, M., & Ładyżyńska, K. (2001). Influence of light and auxins on rooting *in vitro* and shoot quality of Ursynia (*Petunia hybrida*). *Zeszyty Naukowe Akademii Rolniczej w Krakowie*, *379*(80), 193-197.

Yeh, N., & Chung, J. P. (2009). High-brightness LEDs—Energy efficient lighting sources and their potential in indoor plant cultivation. *Renewable and Sustainable Energy Reviews*, *13*(8), 2175-2180. https://doi.org/10.1016/j.rser.2009.01.027