Cent. Eur. J. Biol. • 3(2) • 2008 • 161–167
DOI: 10.2478/s11535-008-0006-9

VERSITA

Central European Journal of **Biology**

In vitro culture of *Chrysanthemum* plantlets using light-emitting diodes

Research Article

Anželika Kurilčik[1,2*], Renata Miklušytė-Čanova[2], Stasė Dapkūnienė[2], Silva Žilinskaitė[2], Genadij Kurilčik[3], Gintautas Tamulaitis[3], Pavelas Duchovskis[1,4], Artūras Žukauskas[3,4]

[1] *Lithuanian Institute of Horticulture, LT-54333 Babtai, Lithuania*

[2] *Botanical Gardens of Vilnius University, LT-10239 Vilnius, Lithuania*

[3] *Institute of Materials Science and Applied Research, Vilnius University, LT-10222 Vilnius, Lithuania*

[4] *UAB Hortiled, LT-44403 Kaunas, Lithuania*

Received 25 September 2007; Accepted 15 January 2008

Abstract: Effects of illumination spectrum on the morphogenesis of chrysanthemum plantlets (*Chrysanthemum morifolium* Ramat. 'Ellen') grown *in vitro* were studied using an illumination system consisting of four groups of light-emitting diodes (LEDs) in the following spectral regions: blue (450nm), red (640nm), red (660nm), and far-red (735nm). Taking into account all differences in shoot height, root length, and fresh and dry weight (FW and DW, respectively), observed while changing the total photon flux density (PFD), the optimal total PFD for growth of chrysanthemum plantlets *in vitro* was estimated. For 16 h photoperiod and typical fractions of the spectral components (14%, 50%, 28%, and 8%, respectively), the optimal total PFD was found to be 40 μmol m^{-2} s^{-1}. Our study shows that the blue component in the illumination spectrum inhibits the plantlet extension and formation of roots and simultaneously increases the DW to FW ratio and content of photosynthetic pigments. We demonstrate photomorphogenetic effects in the blue region and its interaction with the fractional PFD of the far-red spectral component. Under constant fractional PFD of the blue component, the root number, length of roots and stems, and fresh weight of the plantlets have a correlated nonmonotonous dependence on the fractional PFD of the far-red component.

Keywords: *Blue-light • In vitro plant cultivation • Far-red light • Red light • Solid-state lighting • Morphogenesis*

1. Introduction

Light is the key factor influencing the growth of green plants. Plants react to light mainly *via* photosynthetic, photomorphogenetic, and phototropic response. These responses depend on the photon flux density (PFD), light quality, and photoperiod [1]. In addition to considerable interest for general plant photophysiology, these responses are of commercial importance for plant cultivation both in greenhouses and *in vitro*.

Up to now, fluorescent lamps were the main light source commonly used for *in vitro* cultivation of plants. Fluorescent lamps have fixed emission spectra composed of many bands in the wavelength range from 320 to 800 nm without the possibility of varying illumination parameters (spectrum and time characteristics). Due to the development of a new generation of high-power LEDs with a wide diversity of emission wavelengths, LED-based illuminators have become versatile and commercially attractive light sources for different applications in plant cultivation. Compared to conventional fluorescent lamps, LED-based illuminators have improved features, including

* *E-mail: a.kurilcik@lsdi.lt*

smaller mass and volume, longer lifetime, and tailored spectrum [2,3]. Thus, LED-based illuminators provide an alternative to fluorescent lamps as a light source with controllable spectrum that can be used for plant cultivation [4,5].

Investigation of the effect of illumination PFD and spectrum on plant growth *in vitro* has been carried out by applying LED-based illumination to few species of plants. Tanaka *et al*. [6] reported on enhanced growth of *Cymbidium* plantlets cultured *in vitro* with sugar-free medium under super-bright red and blue LED light source. Lian *et al*. [7] analyzed morphogenesis and growth of bulblets from *in vitro* cultured scale segments of *Lilium* under separate blue and red and joint blue and red LED-based illumination. Nhut *et al*. [8] showed that the best growth of strawberry plantlets cultured *in vitro* was observed under LED-based illumination with 70% red and 30% blue spectral components, respectively, while the optimal total PFD was found to be 60 µmol m^{-2} s^{-1}. Jao and Fang studied *in vitro* growth of potato plantlets using LEDs [9,10]. The effects of red and blue light on *in vitro* growth and tuber formation of *Zantedeschia* plantlets were reported by Jao *et al*. [11]. Recently, the influence of LED-based sources with various spectra on growth and carbohydrate accumulation of grape rootstock was reported by Heo *et al*. [12].

The *Chrysanthemum* is the second economically most important floricultural (cut-flower) crop following the Rose [13]. Micropropagation of chrysanthemum shoots grown using LEDs were previously reported. Kim *et al.* [14] showed that shoot growth, stem and internode elongation, net photosynthetic rate, and stomatal characteristics of chrysanthemum plantlets are affected by several components of the illumination spectrum. However, the effect of illumination spectrum composed of simultaneously operated blue, red and far-red light components on morphogenesis of *in vitro* cultured chrysanthemum explants has not been carried out so far.

The present study was aimed at the analysis of morphogenesis of chrysanthemum plantlets, which were cultured *in vitro* under illumination with various spectra and photon flux densities using LEDs. Special attention has been paid to the influence of blue and far-red light. An LED-based illumination system containing four groups of LEDs emitting in blue, red and far-red regions was employed.

2. Experimental Procedures

2.1. Plant materials and culture conditions

Chrysanthemum plantlets (*Chrysanthemum morifolium* Ramat. 'Ellen') were grown *in vitro* in Murashige & Skoog [15] modified nutrient medium (MS + IAA 0.2 mg/l + BAP 0.05 mg/l, ½ NH_4NO_3, ½ KNO_3, without vitamins, mio-inositol and glycine) at 26/22°C (day/night) temperatures maintained within 1°C. Five milliliters of medium were dispensed in 16 x 150 mm tubes covered with PVC caps with air exchange. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 min. One explant per tube was planted and 36 tubes per treatment were prepared.

2.2. Light treatments

The cultures of *in vitro* plantlets were illuminated using red (at the wavelenghts of 660 nm and 640 nm), blue (450 nm), and far-red (735 nm) LEDs powered by a self-designed driver. The plantlets were exposed to a 16 h photoperiod.

The first experiment was aimed at the optimization of the total PFD. The fractional PFDs of the spectral components are specified in Table 1. Before the experiment, the PFDs were measured at the level of plantlets using a radiometer-photometer (model *RF-100 G.PAR-100*, Sonopan, Poland). An optimal spectral power distribution for photosynthesis and most photomorphogenic processes should be 10-15% of the energy in the blue region of the spectrum, preferably at about 440 to 460 nanometers, and 85-90% of the energy in the red (between 600 and 700 nm) and far-red (700 to 800 nm) region of the spectrum [16]. The fractional PFDs of the light components were maintained constant in all treatments of the experiment. The fractions were fixed at 14% for 455-nm, 50% for 640-nm, 28% for 660-nm, and 8% for 735-nm components, respectively. The total PFD in different treatments was varied from 25±5 in treatment A1 to 85±5 µmol m^{-2} s^{-1} in treatment A5 (Table 1).

Treatments	Photon flux density, μmol m^{-2} s^{-1} and %				
	Total (100%)	455 nm (14%)	640 nm (50%)	660 nm (28%)	735 nm (8%)
A1	25	3.5	12.5	7.0	2.0
A2	40	5.6	20.0	11.2	3.2
A3	55	7.7	27.5	15.4	4.4
A4	70	9.8	35.0	19.6	5.6
A5	85	11.9	42.5	23.8	6.8

Table 1. Spectral components applied in Exp. 1, using a constant fractional PFD of the light components.

The second experiment was aimed at the study of explants' morphogenesis. The fractional PFDs of the spectral components for five treatments are specified in Table 2. The total PFD was maintained constant in all treatments. The value of the total PFD was fixed at 43±5 μmol m^{-2} s^{-1} that is close to the value obtained as an optimum in the first experiment. The experiment was focused on revealing the influence of the 455-nm and 735-nm light components on the morphogenesis of the chrysanthemum. Therefore, the PFDs of these two components were varied, while the red 660-nm component, which is the main component contributing to photosynthesis, was fixed at a constant level (22 μmol m^{-2} s^{-1}).

Treatments	Photon flux density, μmol m^{-2} s^{-1}				
	Total	455 nm	640 nm	660 nm	735 nm
B1	43	0	21	22	0
B2	43	0	17	22	4
B3	43	12	9	22	0
B4	43	12	5	22	4
B5	43	12	0	22	9

Table 2. Spectral components applied in Exp. 2, using a constant total PFD.

Treatments B1 and B2 were arranged to reveal the influence of the far-red component without the blue component. The blue 455-nm component was switched off and the far-red 735-nm component was maintained at 0 and 4 μmol m^{-2} s^{-1} in treatments B1 and B2, respectively. The blue component was fixed at 12 μmol m^{-2} s^{-1} in treatments B3, B4, and B5. These three treatments were arranged to show the influence of the increasing PFD of the far-red component on the plantlet growth. The PFD of the far-red 735 nm component was maintained at 0, 4, and 9 μmol m^{-2} s^{-1} in treatments B3, B4, and B5, respectively.

2.3. Data collection

The fresh and dry weight (FW and DW, respectively), stem and root length, number of leaves and roots, and amount of photosynthesis pigments of chrysanthemum plantlets were studied after 42 days of cultivation. In some treatments one explant did not survive, therefore 20 replicates were randomly selected for biometric analysis. To determine the DW, plantlets were oven-dried at 105°C until a constant mass was reached. The other 15 replicates were used for measurement of the photosynthetic pigment concentrations. After extraction with 100% acetone according to the Wettstein [17] method, the total chlorophyll *a* and *b* and carotenoid content in leaf tissues per one gram of green foliage mass was analysed by a double-array spectrophotometer (model *Genesys 6*, Thermospectronic, USA).

3. Results

3.1. Optimization of the total PFD

The biometric parameters of the chrysanthemum plantlets grown *in vitro* for 6 weeks under different photon flux densities are shown in Figure 1. The length of shoots of the plantlets increased with increasing total PFD from 25 μmol m^{-2} s^{-1} to 40 μmol m^{-2} s^{-1}. Further increase of the PFD caused a decrease in shoot length. The root length followed the same trend but the differences in root length were less significant (Figure 1a).

The highest value of the FW and DW was also observed in plantlets grown at 40 μmol m^{-2} s^{-1} (Figure 1b). The further increase of PFD from 40 μmol m^{-2} s^{-1} to 85 μmol m^{-2} s^{-1} resulted in no significant influence on the DW, while the FW continually decreased. The influence of PFD on accumulation of DW is illustrated by the DW to FW ratio (Figure 1c). The dry matter content of the plantlets increased with increasing of the total photon flux from 25 μmol m^{-2} s^{-1} to 85 μmol m^{-2} s^{-1} (Figure 1c).

Meanwhile, differences of leaf and root number observed in plantlets grown under different total PFDs were not significant (Figure 1d).

Thus, the optimum PFD of chrysanthemum plantlets grown *in vitro* in our study was achieved at 40 μmol m^{-2} s^{-1} (treatment A2). This PFD was maintained in the second experiment (morphogenesis analysis).

3.2. Study of the influence of blue and far-red spectral components on plantlet morphogenesis

The influence of the blue component was revealed by comparison of the results in two pairs of treatments B1 *versus* B3 and B2 *versus* B4, respectively. The plantlets grown without the blue component showed a two-fold increase in height and number of roots and a 20-70% larger rooting rate but a significantly lower DW to FW ratio and lower concentrations of all photosynthetic pigments under study (see Figure 2). This trend is more obvious in treatments B1, B3, where the illumination spectrum contained no far-red component.

Under the PFD of the blue component fixed at 12 μmol m^{-2} s^{-1}, the increase of the PFD of the far-red component from 0 to 4 μmol m^{-2} s^{-1} (in treatments B3 and B4, respectively) resulted in increased shoot and root lengths, and also increased FW, DW and number of roots (Figure 2a,b,d), while the DW to FW ratio (Figure 2c) and concentrations of all photosynthetic pigments (Figure 3a) under study decreased. Further increase of the far-red component fractional PFD up to 9 μmol m^{-2} s^{-1} in treatment B5 caused opposite changes of both biometric and phytochemical parameters.

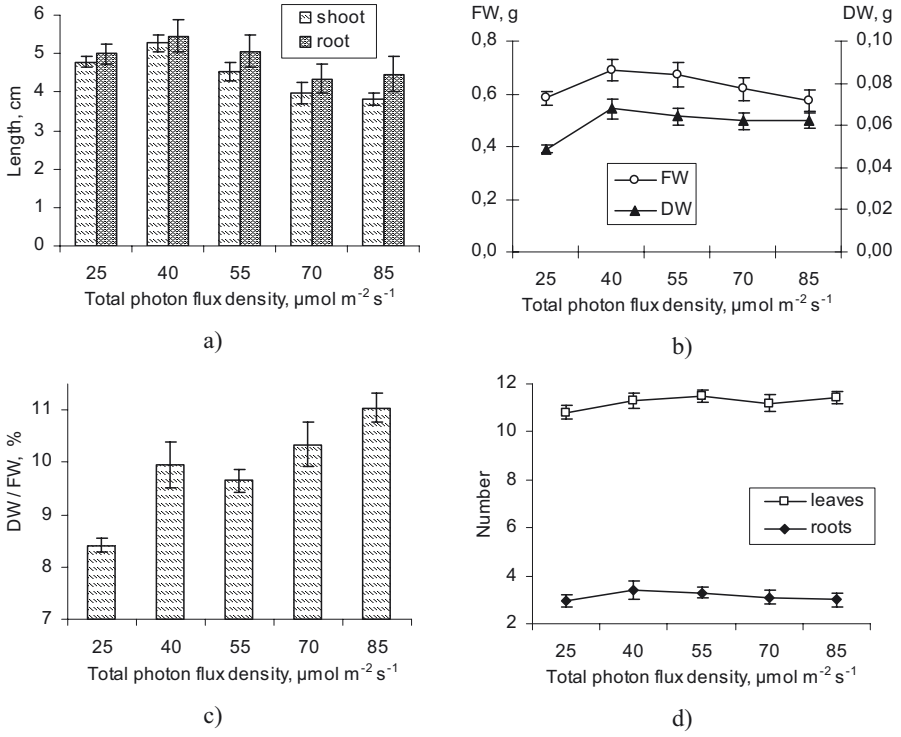

Figure 1. Biometric parameters of *Chrysanthemum* explants grown *in vitro* under illumination at different total flux densities as specified in Table 1: shoot and root length (a), fresh and dry weight (b), dry matter content (c), number of leaves and roots (d). Results are expressed in means ± SEM, n=20.

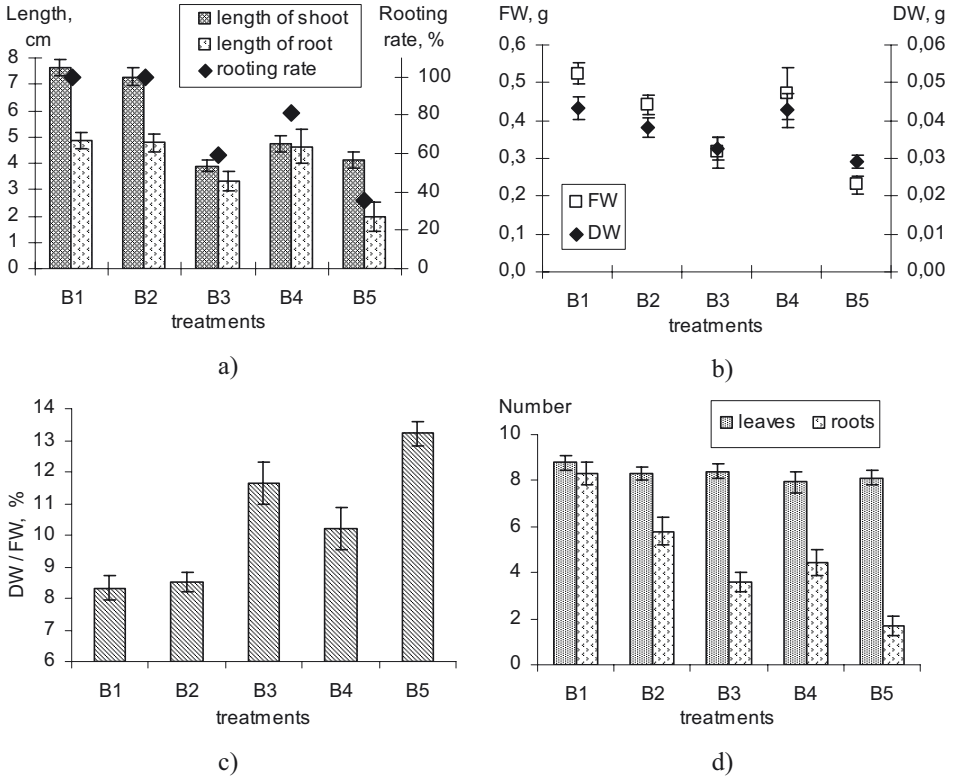

Figure 2. Measured parameters of *Chrysanthemum* explants grown *in vitro* under different illumination regimes as specified in Table 2: shoot and root length and rooting rate (a), fresh and dry weight (b), dry matter content (c), number of leaves and roots (d). Results are expressed in means ± SEM, n=20.

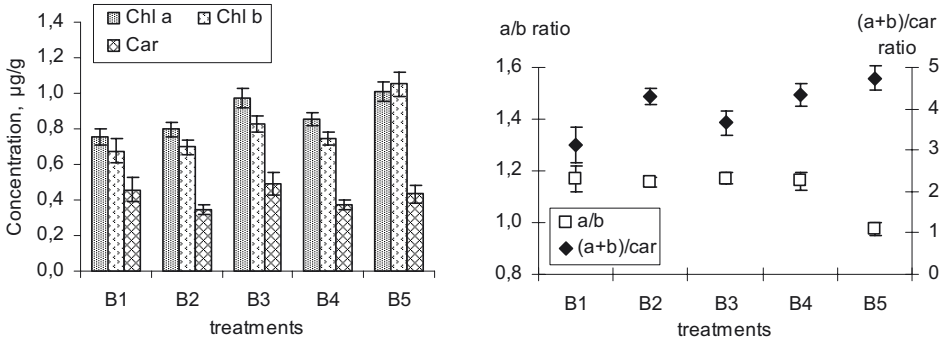

Figure 3. Photosynthetic parameters of *Chrysanthemum* explants grown *in vitro* under different illumination regimes as specified in Table 2: photosynthetic pigments content in leaves (a), chlorophylls a/b ratio, sum of chlorophylls, and carotenoids (a+b)/car ratio (b). Results are expressed in means ± SEM, n=15.

The leaf number was not significantly influenced by the treatments (Figure 2d). The ratio of chlorophyll *a* and *b* concentrations was also similar in all treatments, except in treatment B5. Meanwhile, no significant differences in the ratio of the total chlorophyll (*a* + *b*) concentration to the concentration of carotenoids was observed (Figure 3b).

4. Discussion

The first experiment enabled a quantification of the optimal total PFD for growth and development of chrysanthemum plantlets. The range of the PFDs used in our experiments was selected by taking into account the published data on PFDs necessary for healthy growth of plants *in vitro* [11,14]. We noticed that the maximal PFD (85 µmol m^{-2} s^{-1}) used in our experiment induced slight abnormalities of the leaf surface (roughness and formation of necrotic spots on top of the leaves). We suspect this might be due to water droplets condensed on leaves that focused light and caused burning.

The optimal value of the PFD for plantlets depends on the species of plant in question and its particular ecological adaptation. It can be also cultivar specific. For example for banana and strawberries it constitutes 60 µmol m^{-2} s^{-1} [8,18]. In comparison with field conditions, the optimal value of this parameter for *in vitro* culture is relatively low. In our study the optimal value of the PFD for the plantlets of chrysanthemum was found to be approximately 40 µmol m^{-2} s^{-1}. Such optimization is important for a better development of plantlets grown *in vitro* for their further transplantation *ex vitro*. Optimization of the total PFD corresponding to a healthy plant growth is also important for a proper choice of reference conditions in more detailed experiments on plant illumination.

Our second experiment shows that the blue component in the illumination spectrum inhibits plantlet extension and increases both the dry matter content and the content of photosynthetic pigments (Figure 2a,c and Figure 3a). This is in line with the observations by Nhut *et al*. in strawberry [8], Kim *et al*. in *Chrysanthemum* [14], and Jao *et al*. in *Zantedeschia* [11] grown *in vitro*.

In addition to known data, our study shows that the blue component also inhibits the formation of roots (Figure 2a,d). The inhibiting influence of the blue light on root formation for *in vitro* grown plants has previously been reported by Tanaka *et al*. for *Cymbidium* [6] and Fuernkranz for *Prunus serotina* [19]. We observed that the percentage of explants with roots (rooting rate) and the number of roots per rooted plantlet also depends on the fractional PFD of the far-red component. The root number and rooting rate initially increases with increasing fractional PFD of the far-red component (treatments B3 to B4); however, further increase in the far-red component (treatment B5) causes a decrease of these parameters. Note that in comparison with leaf number, root number is much more sensitive to the fractional PFDs of the blue and far-red components (Figure 2a,d).

The complex influence of the simultaneous illumination in blue and red/far-red regions might be interpreted by synergistic interactions between the photoreceptors of blue and red light (cryptochromes and phytochromes, respectively). These photomorphogenic pigments might be responsible for photoperception and triggering of the rhizogenesis process. Phytochromes possibly govern the rhizogenesis process *via* phytohormone systems. However, the cryptochromes can also influence the root development. Using fluorescent lamps, Teixeira da Silva [20] showed that the presence or absence of light significantly affected the rhizogenic response of chrysanthemum from stem transverse thin cell layers.

Our study revealed that blue (450 nm) and far-red (735 nm) spectral components have a strong influence on root growth, FW and DW and the development of photosynthetic pigments. Thus, a fine tailoring of the illumination spectrum, which is feasible using the LED technology, is of commercial importance for growing *Chrysanthemum* plantlets *in vitro*. According to Chory *et al.* [21], many processes of plant development are controlled by light *via* the systems of phytochromes and phytohormones. Therefore, a further study of interaction between the illumination spectrum and endogenous growth regulators on plantlets rooting would be interesting.

In conclusion, the optimal value of the total PFD constituting blue (450-nm, 14%), red (640-nm and 660-nm, 50% and 28% respectively) and far-red (735-nm, 8%) spectral components for *in vitro* grown chrysanthemum plantlets was found to be approximately 40 μmol m^{-2} s^{-1}. An increase in the blue component of the PFD has an inhibitory influence on root formation. Moreover, the influence of the blue component depends on the PFD of the far-red component. However, the shoot length and weight show a considerable sensitivity to the PFD in these spectral regions. Again, with a constant PFD blue component, the length and FW have nonmonotonous dependence on the far-red component. These peculiarities have to be taken into account while tailoring an optimal illumination spectrum for *in vitro* cultivation of chrysanthemum plantlets as well as within a broader context, for plant cultivation under artificial illumination.

Acknowledgements

This study was partially supported by the Lithuanian State Science and Studies Foundation.

References

[1] Taiz L., Zeiger E., Plant Physiology, Benjamin/Cummings Publishing Co, New York, 1991

[2] Bula R.J., Morrow R.C., Tibbitts T.W., Barta D.J., Ignatius R.W., Martin T.S., Light-emitting diodes as a radiation source for plants, HortScience, 1991, 26, 203-205

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

[4] Žukauskas A., Shur M.S., Gaska R., Introduction to Solid State Lighting, Willey, New York, 2002

[5] Tamulaitis G., Duchovskis P., Bliznikas Z., Breivė K., Ulinskaitė R., Brazaitytė A., et al., High-power light-emitting diode based facility for plant cultivation, J. Phys. D Appl. Phys., 2005, 38, 3182-3187

[6] Tanaka M., Takamura T., Watanabe H., Endo M., Panagi T., Okamoto K., In vitro growth of Cymbidium plantlets cultured under superbright red and blue light-emitting diodes, J. Hortic. Sci. Biotech., 1998, 73, 39-44

[7] Lian M.L., Murthy H.H., Paek K.Y., Effects of light emitting diodes (LEDs) on the in vitro induction and growth of bulblets of Lilium oriental hybrid 'Pesaro', Sci. Hortic. Amsterdam, 2002, 94, 365-370

[8] Nhut D.T., Takamura T., Watanabe H., Okamoto K., Tanaka M., Responses of strawberry plantlets cultured in vitro under superbright red and blue light-emitting diodes (LEDs), Plant Cell Tiss. Org., 2003, 73, 43-52

[9] Jao R.-C., Fang W., Effects of frequency and duty ratio on the growth of potato plantlets in vitro using light-emitting diodes, HortScience, 2004, 39, 375-379

[10] Jao R.-C., Fang W., Growth of potato plantlets in vitro is different when provided concurrent versus alternating blue and red light photoperiods, HortScience, 2004, 39, 380-382

[11] Jao R.-C., Lai C.-C., Fang W., Chang S.-F., Effects of red light on the growth of Zantedeschia plantlets in vitro and tuber formation using light-emitting diodes, HortScience, 2005, 40, 436-438

[12] Heo J.W., Shin K.S., Kim S.K., Paek K.Y., Light quality affects in vitro growth of grape 'Teleki 5BB7'. J. Plant Biol., 2006, 49, 276-280

[13] Teixeira da Silva J.A., Ornamental chrysanthemums: improvement by biotechnology - Review of Plant Biotechnology and Applied Genetics, Plant Cell Tiss. Org., 2004, 79, 1–18

[14] Kim S.J., Hahn E.J., Heo J.W., Paek K.Y., Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro, Sci. Hortic. Amsterdam, 2004, 101, 143-151

[15] Murashige T., Skoog F., A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plantarum, 1962, 15, 473-494

[16] MacLennan D.A., Turner B.P., Dolan J.T., Ury M.G., Gustafson P., Efficient, full-spectrum, long-lived, non-toxic microwave lamp for plant growth, In: International Lighting in Controlled Environments Workshop, Tibbitts T.W. (Ed), 1994, NASA-CP-95-3309, 243-254

[17] Wettstein D., Formula of chlorophyll determination, Exp. Cell Res., 1957, 12, 427–489
[18] Nhut D.T., Takamura T., Watanabe H., Tanaka M., Efficiency of a novel culture system by using light-emitting diode (LED) on in vitro and subsequent of micropropagated banana, Acta Hort., 2003, 616, 121-128
[19] Fuernkranz H.A., Nowak C.A., Maynard C.A., Light effects on in vitro adventitious root formation in axillary shoots of mature Prunus serotina, Physiol. Plantarum, 1990, 80, 337-341
[20] Teixeira da Silva J.A., Thin cell layer technology for induced response and control of rhizogenesis in chrysanthemum, Plant Growth Regul., 2003, 39, 67–76
[21] Chory J., Chatterjee M., Cook R.K., Elich T., Fankhauser C., Li J., et al., From seed germination to flowering, light controls plant development via pigment phytochrome, Proc. Natl. Acad. Sci. USA, 1996, 93, 12066-12071