

## Effect of the photoperiod duration on the growth of *Chrysanthemum* plantlets *in vitro*

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We report on the influence of the photoperiod duration on chrysanthemum growth that was studied using light-emitting diode (LED)-based illuminator. After transplantation, culture of chrysanthemum (*Chrysanthemum morifolium* Ramat. 'Ellen') was grown *in vitro* in Murashige & Skoog modified nutrient medium in a phytotron for 42 days at 26/22 °C day/night temperature. Five groups of plants were simultaneously grown under independently set different photoperiod regimes: 8 h, 12 h, 16 h, 20 h and 24 h, respectively. All treatments were illuminated using an illumination system consisting of four groups of LEDs emitting in the blue (450 nm), red (640 and 660 nm), and far-red (735 nm) spectral regions. The intensity ratio of the light components was fixed at 14 % for the 450 nm, 36 % for 640 nm, 36 % for 660 nm, and 14 % for 735 nm components, respectively. The total photon flux density (PFD) in all treatments was maintained at the same level ( $56 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Morphological and biometric parameters and concentration of photosynthetic pigments in the plantlets were measured after the experiment. With an increase of photoperiod duration from 8 h to 24 h, the dry and fresh weight (DW and FW, respectively) as well the number of leaves and DW to FW ratio continually increases. The highest values of the length of shoots and roots, and number of roots were observed in plantlets grown at 16 h photoperiod. Meanwhile, differences in concentration of photosynthesis pigments were not significant.

**Key words:** *Chrysanthemum morifolium*, *in vitro* plant cultivation, light-emitting diodes, photoperiod.

**Introduction.** Photoperiod, light intensity, and light quality influence plant growth and development from seed germination to flowering. Photoperiod has an effect on the development of some plant species, and no effect on growth of other plants. The influence of the photoperiod duration on flowering and rooting of ornamental plants *in vivo* received a lot of attention (Runkle and Heins, 2006; Cameron et al., 2005). However, few results were published on the growth under *in vitro* conditions. In particular, the influence of 8 h and 16 h photoperiod on the microtuberization and growth of potato plantlets *in vitro* (Seabrook et al., 1993; Kozai et al., 1995) as well

as on subsequent yield of greenhouse-grown potato tubers (Seabrook et al., 1995) was described. Some research on the influence of photoperiod on *in vitro* growth and floral initiation of *Nicotiana tabacum* and chicory (Altamura et al., 1991; Demeulemeester et al., 1995), on stem elongation and growth of *Mentha rotundifolia* (Jeong et al., 1996), and on the bulb formation of garlic and *Hyacinthoides paivae* (Takagi and Qu, 1995; Iglesias et al., 1999) was also conducted. In the majority of papers, only two photoperiod regimes *in vitro* were checked: short day (SD) at 8 h and long day (LD) at 16 h, respectively. A few works are devoted to the investigation of other photoperiod regimes (Lu et al., 2004; Vaz et al., 2004).

From an application standpoint, plant morphogenesis can be influenced by an appropriate choice of lighting, which may affect photoreceptors of the plants. The common sources of light currently used for *in vitro* plant cultivation are fluorescent lamps. However, they have no possibility to vary illumination parameters (spectrum and time characteristics). LED-based illuminators provide an alternative to fluorescent lamps, as a light source with a tailored spectrum, which can meet specific needs of plants (Bula et al., 1991; Brown et al., 1995; Žukauskas et al., 2002; Bliznikas et al., 2004; Tamulaitis et al., 2005). Investigation of the effect of illumination intensity and spectrum on plant growth *in vitro* has been carried out by applying LED illumination to a few species of plants (Tanaka et al., 1998; Lian et al., 2002; Nhut et al., 2003; Jao et al., 2005; Heo et al., 2006).

The *Chrysanthemum* is the second economically most important floricultural (cut-flower) crop following the Rose (Teixeira da Silva, 2004). Micropropagation of chrysanthemum shoots grown using LEDs were reported. Kim et al. (2004) showed that shoot growth, stem and internode elongation, the net photosynthetic rate, and stomatal characteristics of chrysanthemum plantlets are affected by light quality. Shimizu and Ma (2006) showed that blue light from LEDs inhibits stem elongation of chrysanthemum *in vivo*. However, the effect of photoperiod on growth and morphogenesis of *in vitro* cultured chrysanthemum explants under LEDs has not been carried out so far.

The present study was aimed at the analysis of the growth of chrysanthemum plantlets that were cultured *in vitro* under illumination at photoperiods of 8 h : 16 h, 12 h : 12 h, 16 h : 8 h, 20 h : 4 h, and 24 h light : 0 h darkness, respectively. An LED-based illumination system containing four groups of LEDs emitting in blue, red and far-red regions was used.

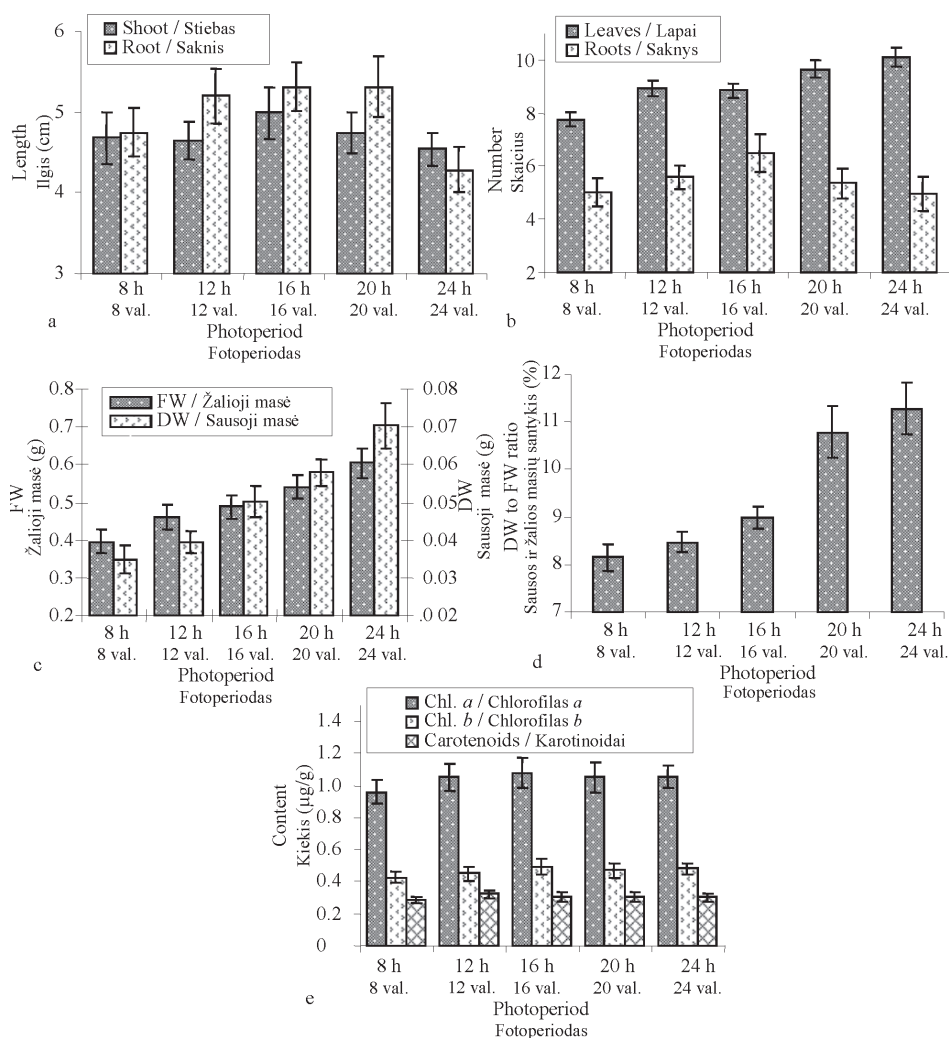
**Object, methods and conditions.** Plant materials and culture condition. Chrysanthemum plantlets (*Chrysanthemum morifolium* Ramat. 'Ellen') were grown *in vitro* in Murashige and Skoog (1962) modified nutrient medium (MS + IAA 0.2 mg/l + BAP 0.05 mg/l, S NH<sub>4</sub>NO<sub>3</sub>, S KNO<sub>3</sub>, 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 × 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.

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 total photon flux density (PFD) in all treatments was maintained at the same level ( $56 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The intensity ratio of the light components was fixed at 14 % for the 450 nm, 36 % for 640 nm, 36 % for 660 nm, and 14 % for 735 nm components, respectively. Selection of the range of the PFDs used in the experiments was based on our previous studies (Kurilčik et al., 2008). The photoperiod duration in different treatments was maintained at 8 h, 12 h, 16 h, 20 h, and 24 h, respectively

**Data collection and statistical analysis.** The fresh and dry weight (FW and DW, respectively), stem and root length, number of leaves and roots, and amount of photosynthetic pigments of the chrysanthemum plantlets were studied after 42 days of cultivation. 35–36 replicates were used for the biometrical analysis. Among those, 18 replicates were randomly selected for the dry weight measurement. To determine the dry weight, the plantlets were oven-dried at 105 °C until a constant mass was reached. The other 17–18 replicates were used for the measurement of the photosynthetic pigment concentrations. After extraction with 100 % acetone according to the Wettstein method (Гавриленко, Жыгало́ва, 2003), 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). Organogenesis stages of chrysanthemum plantlets were also determined (Куперман, 1982). After 42 days, the regenerants were at the II<sup>nd</sup> organogenesis stage. All the data were evaluated for significance by the analysis of variance (ANOVA).

**Results.** The biometric parameters of the chrysanthemum plantlets grown *in vitro* under different photoperiod regimes are shown in Fig. The estimated parameters exhibit the dependence on the photoperiod duration that was varied from 8 h to 24 h per day. The length of the shoots shows a tendency to increase with increasing photoperiod from 8 h to 16 h (Fig. a). The further increase of the photoperiod duration to 24 h showed a tendency for a decrease of the length of shoots. The length and number of roots followed the same trend (Fig. a–b). Meanwhile, the leaf number, fresh and dry weight, and DW/FW ratio continually increased with the increase of the photoperiod from 8 h to 24 h (Fig. b–d). At round-the-clock irradiation, the regenerants of chrysanthemum had by two leaves more, than those grown at 8 h photoperiod. They also have accumulated up to one and a half times more fresh weight and twice as more dry weight. Meanwhile, the variation of the amount of photosynthetic pigments in treatments with different photoperiods was not significant (Fig. e).



**Fig.** Parameters of chrysanthemum regenerants grown under different photoperiod regimes: shoot and roots length (a), number of leaves and roots (b), fresh and dry weight (c), DW/FW ratio (d), contents of photosynthetic pigments in leaves (e)

**Pav.** Chrizantemų regenerantų parametrai skirtingo fotoperiodo sąlygomis: stiebo ir šaknų ilgis (a), lapų ir šaknų skaičius (b), žalioji ir sausoji masė (c), sausosios ir žaliosios masių santykis (d), fotosintezės pigmentų kiekis lapuose (e)

**Discussion.** This experiment was aimed at the determination of the optimal photoperiod for the growth and development of the chrysanthemum plantlets *in vitro* under LEDs. Our research has shown that a change of the photoperiod influences the estimated parameters in different ways. For the plantlets height and for the development of roots, the optimum photoperiod of 16 h was established (Fig. a–b). Note that Kozai et al. (1995) also showed suppressed root growth of potato plantlets under conditions of 8 h photoperiod in comparison to 16 h photoperiod; however, other photoperiod

treatments have not been investigated by these authors.

In our study, the development of leaves and the accumulation of fresh and dry weight were faster at 24 h photoperiod (Fig. b–c). This is in line with the observations of Adams and Langton (2005). These authors revealed that long-day (LD = 16 h) treatments usually promote an increase in dry weight of various plants that otherwise grow in short days (SD = 8 h).

In this study the largest DW/FW ratio was also observed at 24 h photoperiod (Fig. d). Meanwhile, Kozai et al. (1995) showed that 16 h photoperiod in comparison to 8 h photoperiod led to an increase in the fresh and dry weights of potato plantlets but maintained similar percentage of dry matter (DW/FW ratio). We suppose that in our study the dry matter content depends on the duration of dark period. A decrease of the dark period resulted in an increase of the DW to FW ratio.

In addition, our study shows that the concentration of photosynthetic pigments per 1 g of leaves FW does not depend on photoperiod duration (Fig. e). Meanwhile, Adams and Langton (2005) showed that chlorophyll amount per unit leaf area is occasionally increased by LD (16 h) treatment, which may increase photosynthesis and constitute a second mechanism that increases dry weight. Lu et al. (2004) also recorded significantly negative correlations between chlorophyll content in leaves of potato plantlets and darkness length.

The stage of the development of chrysanthemum plantlets after the experiment was similar for various photoperiods (data not shown). After 42 days, the regenerants were at the II<sup>nd</sup> organogenesis stage (Куперман, 1982) and no distinctions have been established for different photoperiods. Our results are in line with the review of Carvalho and Heuvelink (2001), where data on the influence of various factors on flower formation were summarized. According to these data, at the temperature of 27 °C that was maintained in our study, the development of flowers lasts about 120 days. Therefore, at the 42<sup>nd</sup> day, no distinctions between the treatments could be expected. Meanwhile the same review infers that at 18 °C, the development of flowers lasts about 60 days. Therefore we assume that in our study the morphogenesis of flowers was hindered by a higher temperature and was less sensitive to the duration of photoperiod. An additional study including variation of temperature is necessary for a deeper understanding of this phenomenon.

**Conclusions.** Taking into account all differences observed while changing the photoperiod duration, the optimal photoperiod for growth of shoots and roots of chrysanthemum plantlets *in vitro* was estimated to be 16 h. However, morphogenesis of new leaves and accumulation of DW and FW was most prominent at 24 h photoperiod. This data may be helpful for improving of the efficiency of micropropagation of chrysanthemum.

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## References

1. Adams S. R., Langton F. A. 2005. Photoperiod and plant growth: a review. *Journal of Horticultural Science and Biotechnology*. 80(1) 2–10.
2. Altamura M. M., Pasqua G., Monacelli B., Tomassi M., Archilletti T., Falasca G. 1991. Flower formation *in vitro* in a quantitative short-day tobacco: interrelation between photoperiod and infructescence development. *Physiologia Plantarum*. 82(3): 333–338.
3. Bliznikas Z., Breivė K., Tamulaitis G., Kurilčik G., Novičkovas A., Žukauskas A., Duchovskis P., Ulinskaitė R., Brazaitytė A., Šikšnianienė J. B. 2004. Puslaidininkinė lempa augalų fotofiziologiniams procesams tirti ir valdyti. *Elektronika ir elektrotechnika*. 7(56): 74–79.
4. Brown C. S., Schuerger A. C., and Sager J. C. 1995. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. *Journal of the American Society for Horticultural Science*. 120(5): 808–813.
5. Bula R. J., Morrow R. C., Tibbitts T. W., Barta D. J., Ignatius R. W., and Martin T. S. 1991. Light-emitting diodes as a radiation source for plants. *HortScience*. 26(2): 203–205.
6. Cameron R. W. F., Harrison-Murray R. S., Judd H. L., Marks T. R., Ford Y. Y., Bates C. H. A. 2005. The effects of photoperiod and light spectrum on stock plant growth and rooting of cuttings of *Cotinus coggygia* ‘Royal Purple’. *Journal of Horticultural Science & Biotechnology*. 80(2): 245–253.
7. Carvalho S. M. P. and Heuvelink E. 2001. Influence of greenhouse climate and plant density on external quality of chrysanthemum (*Dendranthema grandiflorum* (Ramat.) *Kitamura*): First steps towards a quality model. *The Journal of Horticultural Science and Biotechnology*. 76(3): 249–258.
8. Demeulemeester M. A. C., Voet A., Van de Mierop and De Proft M. P. 1995. Stem elongation and floral initiation on *in vitro* chicory root explants: influence of photoperiod. *Plant Growth Regulation*. 16(3): 233–238.
9. Heo J. W., Shin K. S., Kim S. K. and Paek K. Y. 2006. Light quality affects *in vitro* growth of grape ‘Teleki 5BB7’. *Journal of Plant Biology*. 49(4): 276–280.
10. Iglesias I., Feijyo M. C., and Fernández S. F. 1999. Temperature and photoperiod influences on *in vitro* bulb formation of *Hyacinthoides paivae*. *Acta Horticulturae*. 486: 339–342.
11. Jao R.-C., Lai C.-C., Fang W., and Chang S.-F. 2005. Effects of red light on the growth of *Zantedeschia* plantlets *in vitro* and tuber formation using light-emitting diodes. *HortScience*. 40(2): 436–438.
12. Jeong B. R., Kozai T. and Watanabe K. 1996. Stem elongation and growth of *Mentha rotundifolia* *in vitro* as influenced by photoperiod, photosynthetic photon flux, and difference between day and night temperatures. *Acta Horticulturae*. 440: 539–544.
13. Kim S. J., Hahn E. J., Heo J. W., and Paek K. Y. 2004. Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets *in vitro*. *Scientia Horticulturae*. 101: 143–151.

14. Kozai T., Watanabe K. and Jeong B. R. 1995. Stem elongation and growth of *Solanum tuberosum* L. *in vitro* in response to photosynthetic photon flux, photoperiod and difference in photoperiod and dark period temperatures. *Scientia Horticulturae*. 64(1–2): 1–9.
15. Kurilčik A., Miklušytė-Čanova R., Dapkūnienė S., Žilinskaitė S., Kurilčik G., Tamulaitis G., Duchovskis P., Žukauskas A. 2008. *In vitro* culture of *Chrysanthemum* plantlets using light-emitting diodes. *Central European Journal of Biology*. 3(2): 161–167.
16. Lian M. L., Murthy H. H., and 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: 365–370.
17. Lu C. W., Wang J. C., Tang D. B., Gai Q. H., Lu S. M. 2004. Effects of photoperiod on inducing initiation of potato microtuber *in vitro* (Abstract). *Chinese Potato Journal*. 23(2): 68–72.
18. Murashige T., and Shoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*. 15: 473–494.
19. Nhut D. T., Takamura T., Watanabe H., Okamoto K., and 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: 43–52.
20. Runkle E. S. and Heins R. D. 2006. Manipulating the light environment to control flowering and morphogenesis of herbaceous plants. *Acta Horticulturae*. 711: 51–60.
21. Seabrook J. E. A., Coleman S. and Levy D. 1993. Effect of photoperiod on *in vitro* tuberization of potato (*Solanum tuberosum* L.). *Plant Cell Tissue and Organ Culture*. 34(1): 43–51.
22. Seabrook J. E. A., Percy J. E., Douglas L. K., Tai G. C. C. 1995. Photoperiod *in vitro* affects subsequent yield of greenhouse-grown potato tubers. *American potato journal*. 72(6): 365–373.
23. Shimizu H. and Ma Z. 2006. Blue light inhibits stem elongation of chrysanthemum. *Acta Horticulturae*. 711: 363–367.
24. Takagi H. and Qu Y. 1995. Effects of light quality, photoperiod and cold treatment on *in vitro* bulbing of garlic soot tip. *Acta Horticulturae*. 393: 181–188.
25. Tamulaitis G., Duchovskis P., Bliznikas Z., Breivė K., Ulinskaitė R., Brazaitytė A., Novičkovas A., and Žukauskas A. 2005. High-power light-emitting diode based facility for plant cultivation. *Journal of Physics D: Applied Physics*. 38: 3 182–3 187.
26. Tanaka M., Takamura T., Watanabe H., Endo M., Yanagi T., and Okamoto K. 1998. *In vitro* growth of *Cymbidium* plantlets cultured under superbright red and blue light-emitting diodes. *Journal of Horticultural Science & Biotechnology*. 73(1): 39–44.
27. Teixeira da Silva J. A. 2004. Ornamental chrysanthemums: improvement by biotechnology – Review of Plant Biotechnology and Applied Genetics. *Plant Cell Tissue and Organ Culture*. 79: 1–18.

28. Vaz A. P. A, Figueiredo-Ribeiro R. C. L. and Kerbauy G. B. 2004. Photoperiod and temperature effects on *in vitro* growth and flowering of *P. pusilla*, an epiphytic orchid. *Plant Physiology and Biochemistry*. 42(5): 411–415.
29. Žukauskas A., Shur M. S., and Gaska R. 2002. *Introduction to Solid State Lighting*. New York, Willey. 220.
30. Гавриленко В. Ф., Жигалова Т. В. 2003. *Большой практикум по фотосинтезу*. Москва, Академия. 256.
31. Куперман Ф. М., Ржанова Е. И., Мурашев В. В., Львова И. Н., Седова Е. А., Ахундова В. А., Щербина И. П. 1982. *Биология развития культурных растений*. Москва, Высшая школа. 343.

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### **Fotoperiodo trukmės poveikis chrizantemų eksplantų augimui *in vitro***

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*Santrauka*

Aptariamas fotoperiodo trukmės poveikis chrizantemų eksplantų augimui ir vystymuisi. Tyrimo objektas – chrizantemų veislė ‘Ellen’ (*Chrysanthemum morifolium* Ramat. ‘Ellen’) auginama *in vitro* 16 × 150 mm mėgintuvėliuose su 5 ml maitinamosios terpės. Augalai 42 paras buvo auginami kietakūnio apšvietimo šviestuvuose. Kiekviename iš jų atskirai programiškai valdomi 450, 640, 660 ir 735 nm spektrinių komponenčių fotonų srautai, ir tomis pačiomis sąlygomis auginami 36 augalai. Bendras fotonų srauto tankis palaikomas  $56 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$  ribose, temperatūra 26 °C/22 °C. Dienos/nakties fotoperiodo trukmė penkiuose apšvietimo deriniuose atitinkamai buvo 8/16 val., 12/12 val., 16/8 val., 20/4 val. ir 24/0 val. Eksperimento pabaigoje buvo vertinami kiekvieno augalo augimo parametrai bei nustatomas fotosintezės pigmentų kiekis.

Nustatyta, kad ilgėjant fotoperiodui nuo 8 val. iki 24 val. per para, chrizantemų eksplantų sausoji ir žaliąji masės, o taip pat vidutinis lapų kiekis bei sausos ir žalios masių santykis nuosekliai didėja. Stiebų ir šaknų ilgis bei šaknų skaičius buvo didžiausi 16/8 val. fotoperiodo sąlygomis. Fotoperiodo trukmės pokyčiai neturėjo reikšmingos įtakos fotosintezės pigmentų kiekiui lapuose.

**Reikšminiai žodžiai:** augalų kultivavimas *in vitro*, *Chrysanthemum morifolium*, fotoperiodas, šviestukai.