

IN VITRO CULTIVATION OF GRAPE CULTURE UNDER SOLID-STATE LIGHTING

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We report on the influence of illumination spectrum on grape morphogenesis. The plantlets were cultivated *in vitro* using an illumination system based on light-emitting diodes (LEDs). Four groups of LEDs emitting in red (at the wavelengths of 660 nm and 640 nm), blue (450 nm), and far-red (735 nm) were exploited and the ratios of the photon flux densities (PFDs) of these spectral components were maintained at selected values. The plantlets were simultaneously cultivated in five growth modules, which were identical except of a preset illumination spectrum. Culture of grape *Vitis vinifera* L. 'Gailiūnė' has been cultivated.

Morphological and biometric parameters and concentration of photosynthetic pigments in the plants were measured after 28 or 30 days of the treatment. The total PFD of 40–55 $\mu\text{mol m}^{-2}\text{s}^{-1}$ was found to be optimal for growth of the grape plantlets. Our study was focused on elucidation of the morphogenetic influence of illumination in the blue and far-red regions on the development of grape explants.

We demonstrate that spectrally selective solid-state lighting is an effective tool for cultivation of grape culture *in vitro* and discuss the prospective for using this technique on a commercial scale.

Key words: *in vitro* plant cultivation, light-emitting diodes, morphogenesis, *Vitis vinifera*.

Introduction. Light is the energy source for photosynthesis and the main factor of plant development. Lighting conditions are very important for growth and development of plantlets in culture vessels of many *in vitro* grown species, including grapevines. The control and optimization of illumination is essential for a successful acclimatization, increase of the survival rate, and decrease of the acclimatization period (Amâncio et al., 1999). The common sources of light currently used for *in vitro* plant cultivation are fluorescent lamps. Metal halide, high-pressure sodium, and incandescent lamps are being also applied. However, these sources emit a large

portion of light in spectral regions that are unnecessary for efficient growth of plants. Recently, solid-state lighting sources based on light-emitting diodes (LEDs) with narrow-band emission have been introduced for more efficient *in vitro* plant growth (Heo et al., 2006; Jao et al., 2005; Kim et al., 2004).

In comparison with conventional fluorescent lamps, LED illuminators have several advantages, such as longer lifetime, smaller mass and volume, lower thermal emission, and selective monochromic spectrum (Bula et al., 1991; Brown et al., 1995; Tanaka et al., 1998). LED-based illuminators provide an alternative to fluorescent lamps, as a light source with tailored spectrum that can meet specific needs of plants (Žukauskas et al., 2002). Recently, computer-controlled LED illuminators were successfully introduced into greenhouse plant cultivation (Bliznikas et al., 2004; Tamulaitis et al., 2005).

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops cultivated worldwide (Das et al., 2002). Harvest of the grapevine is used in wine industry and for the production of grapes and raisins. Grapevine is propagated by hardwood cuttings or via somatic embryogenesis *in vitro* (Martins et al., 2003; Croce et al., 2005). Grapevine tissue and organ culture have important practical applications in viticulture. The basic purpose of *in vitro* propagation is the elimination of grapevine diseases via meristem culture. In breeding programs, *in vitro* propagation can be successfully used as an alternative for grapevine rapid multiplication (Slavtcheva and Dimitrova, 1999).

The influence of illumination spectrum on *in vitro* growth of various plants has been studied by using solid-state illumination systems (Lian et al., 2002; Nhut et al., 2003; Jao et al., 2005). However, few reports are published on the micropropagation of grapevine by applying LED-based illumination. Heo et al. (2006) described the effect of light quality of a LED-based lamp on the growth of grapevine cultivated *in vitro*. However, the effects of illumination on morphogenesis of the *in vitro* cultured grapevine explants have not been carried out up to now.

The objective of the present study was to analyze the growth and morphogenesis of the grapevine plantlets cultured *in vitro* under various illumination spectra and total PFDs.

Materials and methods. **Plant materials and culture condition.** Grapevine plantlets (*Vitis vinifera* L. 'Gailiūnė') were grown *in vitro* in Murashige & Skoog (1962) modified nutrient medium (MS + IAA 0.2 mg/l + BAP 0.05 mg/l, $\frac{1}{2}$ NH_4NO_3 , $\frac{1}{2}$ KNO_3 , without vitamins, mio-inositol, and glycine) at 26/22°C (day/night) temperatures maintained within 1°C. Five millilitres 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. Explants in our experiments were taken from grapevine regenerants, which were grown up *in vitro* under white luminescent lamps OSRAM L 36 W/20. In sterile conditions, the top part of a shoot with one axillary bud was cut off and used as an explant. The length and fresh weight of the grapevine explants were 1 ± 0.1 cm and 0.0105 ± 0.0012 g, respectively.

Light treatments. The cultures of *in vitro* plantlets were illuminated using red (at the wavelengths 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 light for a 16 h photoperiod.

Table 1. Photon flux densities (in $\mu\text{mol m}^{-2} \text{s}^{-1}$) of illumination spectral components in growth treatments of the first experiment

1 lentelė. Apšvietimo spektro komponentių fotonų srauto tankiai ($\mu\text{mol m}^{-2} \text{s}^{-1}$) skirtinguose pirmojo eksperimento variantuose

Treatment Variantas	Photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$ and %) Fotonų srauto tankis, $\mu\text{mol m}^{-2} \text{s}^{-1}$ ir %				
	Total Bendras (100%)	450 nm (14%)	640 nm (50%)	660 nm (27%)	735 nm (9%)
A1	25	3.5	12.5	6.8	2.2
A2	40	5.6	20.0	10.8	3.6
A3	55	7.7	27.5	14.8	5.0
A4	70	9.8	35.0	18.9	6.3
A5	85	11.9	42.5	23.0	7.6

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). The fractional PFDs of the light components were maintained constant in all treatments. The fractions were fixed at 14% for 450-nm, 50% for 640-nm, 27% for 660-nm, and 9% for 735-nm components, respectively. The total PFD in different treatments was changed from 25 ± 5 in treatment A1 to $85 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ in treatment A5 (Table 1).

The second experiment was aimed at the study of explant 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 $43 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$. The experiment was focused on the revealing of the influence of the 450-nm and 735-nm light components on the morphogenesis of the grapevine. 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 ($22 \mu\text{mol m}^{-2} \text{s}^{-1}$).

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

The influence of the blue component at a fixed fractional PFD in the far-red region can be traced by comparison of the results of treatment pairs B1 versus B3 (no far-red illumination is applied) and B2 versus B4 (the PFD of the far-red component is maintained at $4 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Table 2. Photon flux densities (in $\mu\text{mol m}^{-2} \text{s}^{-1}$) of illumination spectral components in growth treatments of the second experiment

2 lentelė. Apšvietimo spektro komponentių fotonų srauto tankiai ($\mu\text{mol m}^{-2} \text{s}^{-1}$) skirtinguose antrojo eksperimento variantuose

Treatment Variantas	Photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$ and %) Fotonų srauto tankis, $\mu\text{mol m}^{-2} \text{s}^{-1}$ ir %				
	Total Bendras	450 nm	640 nm	660 nm	735 nm
B1	43 (100%)	0	21 (49%)	22 (51%)	0
B2	43 (100%)	0	17 (40%)	22 (51%)	4 (9%)
B3	43 (100%)	12 (28%)	9 (21%)	22 (51%)	0
B4	43 (100%)	12 (28%)	5 (12 %)	22 (51%)	4 (9%)
B5	43 (100%)	12 (28%)	0	22 (51%)	9 (21%)

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 grapevine plantlets were studied after 30 and 28 days of cultivation in the first and second experiment, respectively. 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 rest 15 replicates were used to measure the concentrations of the photosynthetic pigments. After extraction with 100% acetone according to the Wettstein (Gavrilenko et al., 2003) 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).

Results. Optimization of the total PFD. The biometric parameters of the grapevine plantlets grown *in vitro* for 30 days under different photon flux densities are shown in Fig. 1. The parameters do not show a significant dependence on total PDF, which was varied from 25 to 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The length of the shoots show a slight tendency to increase with increasing total PFD from 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in treatment A1 to 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in treatment A5 (Fig. 1A). The length and number of roots followed the same trend (Fig. 1A-B). Meanwhile, no significant differences in leaf number were observed in grapevine plantlets grown under different total PFDs (Fig. 1B).

Although a slight decrease of the fresh weight and leave weight (FW and LW, respectively) of the grapevine plantlets by increasing of the total PFD from 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in treatment A1 to 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in treatment A5 was observed, the differences between treatments were not significant (data not shown). The length of the largest leaf of the plantlets somewhat increased with the increase of the total PFD from 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in treatment A1 to 55 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in treatment A3 (Fig. 1C). The further increase of the PFD to 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in treatment A5 showed a tendency for a decrease of the length of the largest leaf. Meanwhile, no significant differences in leaf width were observed in grapevine plantlets grown under the total PFD in the range 25-70 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Only in treatment A5 (under 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$), this parameter was significantly lower (Fig. 1C).

The variations of the amount of photosynthetic pigments between treatments

with different PFDs were not significant (Fig. 1D), except of a small decrease of all pigments by 15% in treatment A3 (55 $\mu\text{mol m}^{-2}\text{s}^{-1}$). The ratio of chlorophyll *a* and *b* concentrations slightly increased with the increase of the total PFDs from 25 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in treatment A1 to 85 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in treatment A5 (Fig. 1E). Meanwhile, the ratio of the total chlorophyll concentration (*a* + *b*) to the concentration of carotenoids was similar in all treatments (data not shown).

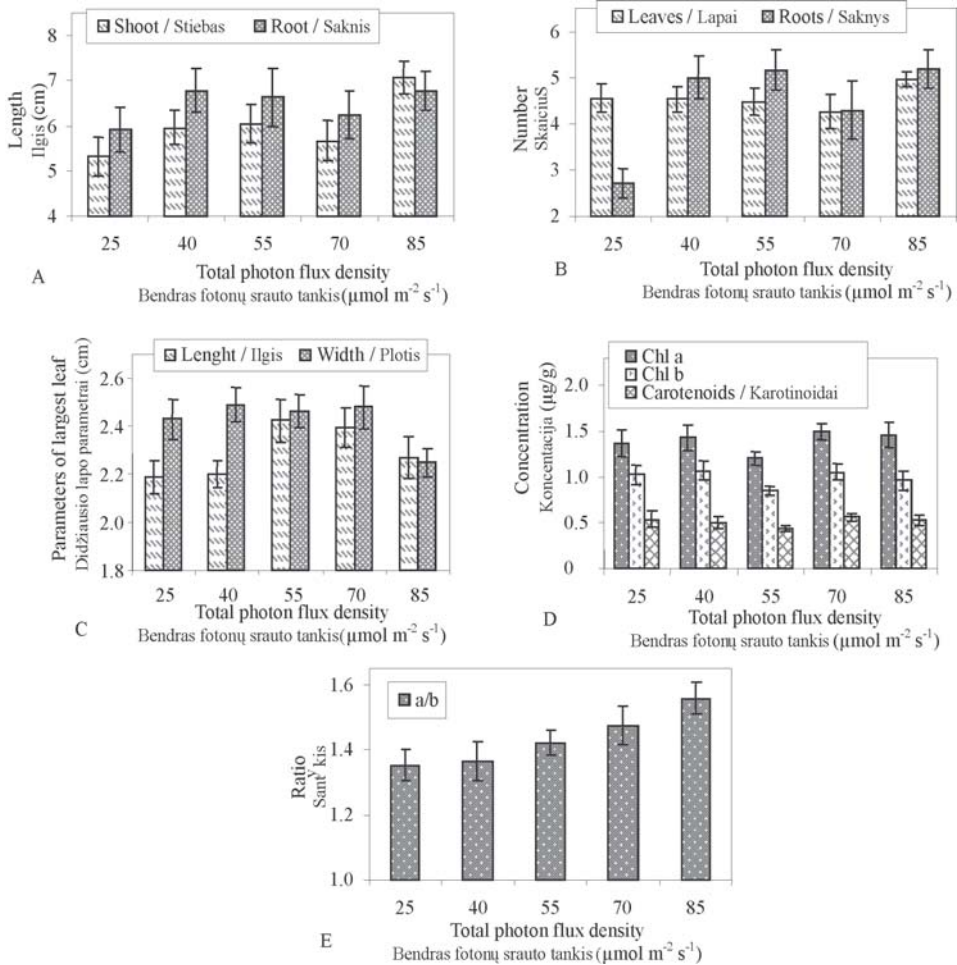


Fig. 1. Biometric parameters of grape explants grown *in vitro* under illumination at different total flux densities as specified in Table 1: shoot and root length (A), number of leaves and roots (B), parameters of the largest leaf (C), content of photosynthetic pigments in leaves (D), ratio of the concentrations of chlorophylls *a* and *b* (E).

1 pav. Vynmedžių eksplantų biometriniai parametrai, esant skirtingam suminiam fotonų srauto tankiui (žr. lentelėje 1): stiebo ir šaknų ilgis (A), lapų ir šaknų skaičius (B), didžiausio lapo parametrai (C), fotosintezės pigmentų kiekis lapuose (D), chlorofilų *a* ir *b* koncentracijų santykis (E).

Taking into account all small differences observed while changing the total PFD, the optimal total PFD for growth of grapevine plantlets *in vitro* was estimated to be between 40 and 55 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (treatments A2-A3). The value of the PFD was selected from this range for the second experiment.

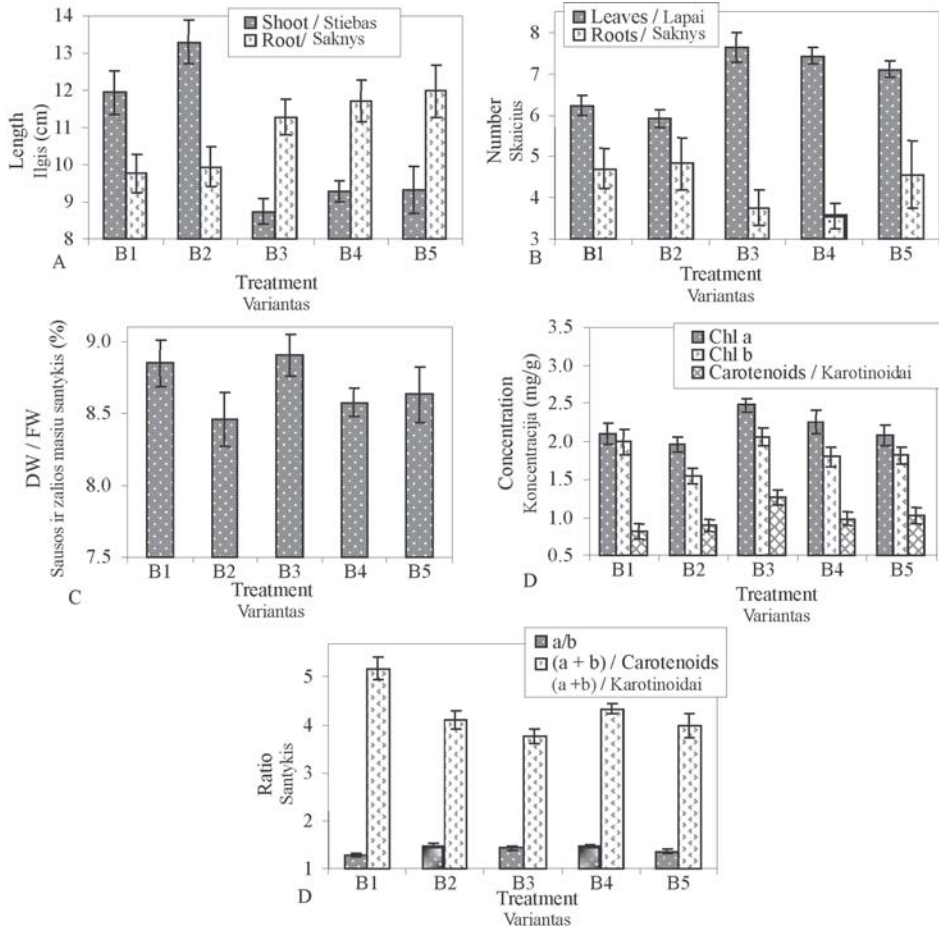


Fig. 2. Parameters of grape explants grown under different illumination regimes as specified in Table 2: shoot and roots length (A), number of leaves and roots (B), DW/FW ratio (C), contents of photosynthetic pigments in leaves (D), ratio of the concentration of chlorophylls *a* and *b*, ratio of the total content of chlorophylls to the content of carotenoids (E).

2 pav. Vynmedžių eksplantų parametrai skirtingo apšvietimo sąlygomis (žr. 2 lentelę): stiebo ir šaknų ilgis (A), lapų ir šaknų skaičius (B), sausas ir šviežios masių santykis (C), fotosintezės pigmentų kiekis lapuose (D), chlorofilų *a* ir *b* koncentracijų santykis, suminės chlorofilų koncentracijos ir karotinoidų koncentracijos santykis (E).

Study of the influence of blue and far-red spectral components on plantlet morphogenesis. The impact of the blue component was revealed by comparison of the results obtained in two pairs of treatments: B1 versus B3 and B2 versus B4. The

plantlets grown without the blue component had larger height and number of roots but lower length of roots, number of leaves and concentrations of all photosynthetic pigments (see Fig. 2A, B, D).

Under the PFD of the blue component fixed at $12 \mu\text{mol m}^{-2}\text{s}^{-1}$, the increase of the partial PFD of the far-red component from 0 to $9 \mu\text{mol m}^{-2}\text{s}^{-1}$ (treatments B3-B5) resulted in a tendency for an increase of the shoot and root lengths and number of roots, while the number of leaves and concentrations of all photosynthetic pigments showed a tendency for a decrease (Fig. 2A, B, D).

The FW and DW were similar within experimental error in all treatments (data not shown), whereas the DW/FW ratio showed a decrease by the increasing of the far-red component PFD from $0 \mu\text{mol m}^{-2}\text{s}^{-1}$ to $4 \mu\text{mol m}^{-2}\text{s}^{-1}$, both with and without the blue component (see treatments B1 versus B2 and treatments B3 versus B4 in Fig. 2C).

The plantlets grown in treatment B1 without both the blue component and the far-red component had a maximum value of the concentration ratio of chlorophyll *a* and *b*, but a minimum value of the ratio of the total chlorophyll concentration (*a* + *b*) to the concentration of carotenoids (Fig. 2E).

Discussion. The first experiment was aimed at the determination of the optimal total PFD for the growth and development of the grapevine plantlets. Selection of the range of the PFDs used in our experiments was based on the published data (Kim et al., 2004; Jao et al., 2005). In spite of the weak dependence of the plantlet parameters on the total PFD, the range $40\text{-}55 \mu\text{mol m}^{-2}\text{s}^{-1}$ can be pointed out as the optimal total PFD for the grapevine plantlets. In comparison with regenerants of grapevines grown under fluorescent lamps (Dapkūnienė et al., 2004), plants grown under solid-state irradiation exhibited smaller height, reduced numbers of leaves and roots, as well as lower amounts of chlorophylls *a* and *b*. Meanwhile the length of roots and fresh weight were found larger for plants grown under LED treatments.

It is worth noting that no abnormalities imposed to the grapevine plantlets by intense illumination at the maximal PFD used in our experiments were observed. This is in contrast to our results of similar study of chrysanthemum plantlets (to be published). This difference is possibly an indication that the grapevine plantlets need higher total PFDs than the chrysanthemum plantlets do.

Our second experiment shows that the presence of the 450-nm component in the illumination spectrum inhibits the plantlet elongation and simultaneously enhances concentrations of all photosynthetic pigments. Such effects were observed also by Nhut et al. (2003) in strawberry, Kim et al. (2004) in *Chrysanthemum*, and Jao et al. (2005) in *Zantedeschia* grown *in vitro*. Our study revealed a different influence of the blue component on the growth and development of roots and leaves. The plantlets grown under the treatments with the blue component had a lower height and number of roots but a larger length of roots and larger number of leaves per plantlet (see treatments pairs B1 versus B3, and B2 versus B4 on Fig. 2A, B).

This study did not show any significant differences in accumulation of fresh and dry weight, which have been demonstrated by Nhut et al. (2003) and observed in our study of chrysanthemum. Meanwhile, the influence of the far-red light PFD on the DW to FW ratio was established. The increase in PFD of the far-red component

resulted in a decrease of the DW/FW ratio, both with and without the blue component (see treatment B1 versus B2 and treatments B3-B5 in Fig. 2C). A stronger impact was observed in treatments without the blue light. Thus both blue and far-red light components participate in this process via phytochrome and cryptochrome systems.

In addition, the results of treatments B1 versus B3 and B2 versus B4 demonstrate that the blue component also enhances the synthesis of the photosynthetic pigments. Meanwhile, the far-red component inhibits this process, especially in the presence of the blue light (see treatments B1 versus B2 and B3-B5 on Fig. 2D). Our study showed that the blue and far-red components interfere in the control of the photosynthetic pigments development. This conclusion is supported by the decrease in the ratio of the total concentration of chlorophylls and the concentration of carotenoids in absence of blue light due to far-red component (treatments B1 and B2 in Fig. 2E) and by a low sensitivity of these parameters to far-red component in the presence of blue light (treatments B3-B5).

Our results imply that the blue (450 nm) and far-red (735 nm) spectral components are involved in the plantlets growth and control of photosynthetic pigment development by synergetic interactions between blue/red light photoreceptors, cryptochromes and phytochromes. We suggest that the response of plants to variation of illumination spectrum depends on plant species. Some features of this response are common for all plants (e.g., inhibition of shoot elongation by blue light), whereas other features are individual. Thus, a fine tailoring of the illumination spectrum for commercial growth of the grapevine plantlets *in vitro* using the LED-based lighting technology is feasible, but requires further optimization.

Conclusions. The optimal value of the total PFD for *in vitro* growth of grapevine plantlets using a four-component LED lamp was found to be in the range 40–55 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The presence of the blue component in the illumination spectrum has an inhibiting influence on the plantlet elongation. Simultaneously, blue light enhances leave formation and increases the concentrations of all photosynthetic pigments. An increase in PFD of the far-red light has a suppressive influence on the processes of DW/FW accumulation and synthesis of photosynthetic pigments. An additional study including analysis of endogenous hormones is necessary for a deeper understanding of the effect of the illumination spectrum on grapevine growth and development.

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VYNMEDŽIŲ KULTIVAVIMAS *IN VITRO* KIETAKŪNIO APŠVIETIMO SĄLYGOMIS

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Santrauka

Aptariamas apšvietimo spektro poveikis vynmedžio eksplantų morfogenezei. Tyrimo objektas – vynmedžio veislė ‘Gailiūnė’ (*Vitis vinifera* L. ‘Gailiūnė’) auginama *in vitro* 16 x 150 mm dydžio mėgintuvėliuose su 5 ml maitinamosios terpės. Augalai 30 arba 28 paras buvo apšviečiami šviestuvuose, pagamintuose pagal HORTILED projektą. Šviestuvo kompleksą sudaro 5 moduliai, kiekviename iš jų atskirai programiškai valdomi 450, 640, 660 ir 735 nm spektrinių komponentų fotonų srautai, ir tomis pačiomis sąlygomis auginami 36 augalai. Dienos/nakties fotoperiodo trukmė – 16/8 val., temperatūra 26°C/22°C. Eksperimentų pabaigoje buvo vertinami kiekvieno augalo augimo parametrai bei nustatomas fotosintezės pigmentų kiekis.

Nustatyta, kad vynmedžių eksplantų, auginamų *in vitro*, optimaliausios augimo sąlygos yra apie 40–55 μmol m⁻²s⁻¹ suminio fotonų srauto tankio ribose. Nustatytas mėlynosios šviesos inhibuojantis poveikis eksplantų stiebo tismui, kartu didėjant lapų

skaičiui ir fotosintezės pigmentų koncentracijai. Įvertintas tolimosios raudonosios šviesos dalyvavimas sausos ir žalios masių akumuliacijos procesuose bei fotosintezės pigmentų sintezėje.

Pademonstruota, kad derinamo spektro šviestuvai, pagaminti panaudojant puslaidininkinius šviestukus, yra universali ir lanksti priemonė augalų kultivavimui *in vitro* ir leidžia optimizuoti vynuodžių eksplantų fotofiziologinius procesus.

Reikšminiai žodžiai: augalų kultivavimas *in vitro*, morfogenezė, šviestukai, *Vitis vinifera*.