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Adventitious regeneration of blackberry and raspberry shoots and the assessment of the LED-lighting impact

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Abstract

Blackberry (*Rubus fruticosus* L.) and raspberry (*Rubus idaeus* L.) are well known throughout the world due to their nutritional and medicinal importance. Obtaining regenerants is an important stage for the application of cell technologies in plant growing, plant breeding, and genetic engineering. The objective of this study was to determine the best regeneration pathways for understudied blackberry and raspberry cultivars. Adventitious shoot regeneration of thornless blackberry cultivars 'Smoothstem', 'Triple Crown' and 'Karaka Black', and raspberry cultivars 'Glen Ample', 'Gusar' and 'Maria' has been studied depending on the cultivar, type explant, hormonal composition of nutritional medium, and LED lighting. To induce adventitious shoot bud formation, three types of explants: leaves, internodes, and roots obtained from *in vitro* plants, were cultured in a growth chamber on MS medium supplemented with different concentrations of plant growth regulators. In most cases, regeneration became visible after three weeks of cultivation. Blackberry cultivar 'Smoothstem' showed the best results and regenerated at a maximum rate of 73.3% on leaf blades and stem segments on MS supplemented with 2.0 mg L^{-1} TDZ. The best shoot proliferation rates of raspberries (65.5%) were observed in the 'Glen Ample' on root segments at MS medium supplemented with 0.5 mg L^{-1} ZEA. The blackberry and raspberry explants were exposed under LED lighting with a ratio of quanta red (R) to blue (B) light at 1:1, 1:2, 2:1, 1:4 and 4:1, monochrome red (650–670 nm) and monochrome blue (440–460 nm), and recorded regeneration. Efficient regeneration percentage (>80%) was obtained by the incubation of raspberry and blackberry explants under illumination with a ratio of red to blue spectrum: R2:B1 and R1:B1. Monochrome blue and red light inhibited shoot growth.
The obtained results indicate that certain combinations of spectra LED lighting enhances plant morphogenesis. The regeneration system described here will be useful for developing a gene transfer system and can be efficient for selected raspberries and blackberries cultivars.

Keywords: *Rubus fruticosus*, *Rubus idaeus*, LED-light dependent, thidiazuron, zeatin.

Introduction

Blackberry (*Rubus fruticosus* L.) and raspberry (*Rubus idaeus* L.) (Rosaceae) are widespread in most countries in the Northern Hemisphere as a popular and economically important berry crops (Tridge, 2021). The berries are used for food, and the leaves are used in herbal medicine as an antioxidant, antimicrobial, antitumor, antidiarrheal, and antidiabetic agent due to the content of alkaloids, flavonoids, glycosides, terpenoids, trace elements, vitamins, pectin, sugars, and other components (Zia-Ul-Haq et al., 2014; Skrovankova et al., 2015; Teng et al., 2017; Krzepiłko et al., 2021). The demand for the berries of these crops and their production has been steadily increasing, especially in Europe (CBI Ministry of Foreign Affairs, 2018). However, having valuable nutritional, technological and pharmacological properties, these crops are susceptible to fungal (anthracosis, septoria) and bacterial (root cancer) diseases and viral infections: Black raspberry necrosis virus (BRNV), Rubus yellow net virus (RYNV), Raspberry mottle virus (RMoV), etc. Genetic engineering and biotechnology enable the expression of novel traits such as pest resistance, disease resistance and traits leading to quality improvement (Ouyang et al., 2017).

The *in vitro* plant regeneration system is a prerequisite for genetic transformation and most often depends on the genotype (Georgieva et al., 2004). Several studies have examined the effect of various conditions on *in vitro* shoot regeneration in *Rubus* species. It was reported that thidiazuron (TDZ) at a level of 1.0 mg L^{-1} in combination with 0.02 mg L^{-1} indolyl-3-butyric acid (IBA) was effective for regeneration of the blackberry after transformation by *Agrobacterium tumefaciens* (Súkeníková et al., 2015). A high regeneration rate was observed for three-month-old blackberry cultivar 'Boysenberry' leaf explants cultured at 0.05 mg L^{-1} TDZ. The addition of 1.5 mg L^{-1} kinetin and 0.1 mg L^{-1} NAA (α-naphthylacetic acid) was the optimal medium for proliferation. The best rooting medium was 1/2 MS with 0.03 mg L^{-1} NAA (Wang et al., 2012). Testing of the influence of different 6-benzylaminopurine (BA) concentrations on shoot formation showed that the genotype and BA concentration significantly influenced the ability of shoot proliferation in *Rubus* cultivars (Hunková et al., 2016). The blackberry cultivar 'Chester Thornless' optimally regenerated on a medium with 2.0 mg L^{-1} BA and 0.2 mg L^{-1} IBA (Kefayety et al., 2018).

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Regeneration from the somatic tissue of the red raspberry cultivar 'Biryulevskaya' increased on MS medium containing a triple dose of iron chelate Fe-EDTA and 1.0 mg L^{-1} BA (Khlebova et al., 2019). Seven-day-old leaf plates increased the morphogenesis of raspberry cultivars 'Joan J' and 'Polana' to 70% and 82.2% after incubation on woody plant medium with 2.5 μm BA + 1.0 μm TDZ and with 2.5 μm BA + 0.1 μm TDZ, respectively (Kim, Dai, 2020).

In addition, it has been shown that light quality is one of the main factors of light signalling that activates or deactivates physiological responses and controls growth and development (Dou et al., 2017; Gupta, 2017). Red light is important for photosynthesis, chloroplast function, growth, and the reproductive system, while blue light controls phototropism, leaf outgrowth, photomorphogenesis, stomatal opening processes, accumulation of pigments, and secondary metabolites (Liscum et al., 2014). It has been proven that the combination of LED spectra promotes better plant growth, improved morphogenic response and increased biosynthesis of secondary metabolites (Naznin et al., 2019).

Light-emitting diodes (LEDs) allow for combining the spectra and provide an illumination source for *in vitro* photobiological research. They are environmentally friendly, have a low mass, cool surfaces, and long functional life (Dutta Gupta, Jatothu, 2013), but the physiological response of the plant triggered by the quality of light varies depending on the species and/or cultivars (Lykins et al., 2021).

A good regeneration protocol is essential for enhanced percentage of the transformants. The success regeneration response has been found to be medium composition, the type of explant, and the incubation conditions (Farhadi et al., 2017). Therefore, regeneration protocols must be adapted to specific cultivars for efficient use and the best result. However, there are no reports of multiple shoot induction and explant regeneration for the important but insufficiently studied blackberry cultivars 'Triple Crown', 'Smoothstem', and 'Karaka Black', and raspberry cultivars 'Glen Ample', 'Maria', and 'Gusar'. The ability of LEDs of different spectra to increase the efficiency of adventive shoots regeneration has not been previously evaluated for the genus *Rubus*.

Given the above, in this communication, an improved and reproducible protocol for the regeneration of adventive seedlings in selected cultivars of blackberries and raspberries depending on the type of explant, hormonal medium composition, and LED lighting spectrum is reported. This protocol will be useful for developing a gene transfer system and can be efficiently applied for selected raspberries and blackberries cultivars.

Materials and methods

The experiment was carried out at the Department of Genetic Engineering, Institute of Cell Biology and Genetic Engineering, Ukraine, in 2019.

Plant material and culture conditions. In vitro cultures of thornless blackberry (*Rubus fruticosus* L.) cultivars 'Smoothstem' (American selection), 'Triple Crown' (American selection), and 'Karaka Black' (New Zealand selection) and raspberry (*Rubus idaeus* L.) cultivars 'Glen Ample' (Scottish selection), 'Gusar' (Russian selection), and 'Maria' (Ukrainian selection) were introduced into *in vitro* culture from a stock bush using earlier described our method (Medvedeva et al., 2007). For this, the apical and lateral stem buds and etiolated root buds from two-year-old plants were collected. In a cabinet under a laminar flow, explants were dipped in 70% ethanol; their surface was then sterilized with 0.1% $HgCl_2$ (mercury chloride) for 5–6 min followed by four rinses with sterile distilled water. For shoot formation, sterilized explants were cultured on semi-saline Murashige and Skoog (MS) medium supplemented with 0.5 mg L^{-1} 6-benzylaminopurine (BA). Subsequently, the plants of all cultivars were subcultivated every 4–5 weeks in complete MS medium supplemented with 2% sucrose and 0.7% agar. To reduce chlorosis, the raspberries were grown on a medium replacing Fe-EDTA with the highly effective chelated form of Fe-EDDHA. For blackberry micropropagation, the medium was supplemented with 1.0 mg L^{-1} BA in combination with 0.1 mg L^{-1} IBA (indolyl-3-butyric acid) and 0.02 mg L^{-1} NAA (α-naphthylacetic acid), and for raspberry – 0.5 mg L^{-1} BA. Control explants were incubated on hormone-free MS medium.

Segmented leaf blades, internodes, and roots were isolated from four-week cultures *in vitro* (after micropropagation) and cultured so that the surface was in contact with the shoot bud induction medium (MS with plant growth regulators (Tables 1 and 2). For each treatment, 12–15 explants of leaf segments (~0.5 cm^2) or pieces of internodes or roots (~0.5 cm long) or five of each explant were cultured on five Petri dishes containing 25 ml MS medium. The medium pH was adjusted to 5.6 prior to autoclaving at 121°C temperature and 103 kPa for 20 minutes.

Light conditions. Specialized LED lighting systems with specific spectral characteristics were used, and seven LED plant lighting options were organized. The design of the systems made it possible to provide illumination with a ratio of quanta red (R) to blue (B) light at 1:1, 1:2, 2:1, 1:4, and 4:1, and monochrome red (650–670 nm) and monochrome blue (440–460 nm). Cultures were maintained in light at an intensity photosynthetic photon flux density (PPFD) at least 320 μmol m^{-2} s^{-1} in the growth point. The applied photosynthetic photon flux densities were measured using a quantum meter (Nanbei, China). The light system was designed and made by the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine. The effect of leaf pre-cultivation on shoot induction was also investigated. *In vitro* plants were illuminated with selected spectra for two weeks. The resulting explants were cultured on MS medium supplemented with 1.0 mg L^{-1} TDZ (thidiazuron). The explants were cultivated under fluorescent lamps TL-D 36W/54, cap G13 (Philips) and 3 mm round with domed top LED lamps (Wurth Elektronik, Germany) of various spectral composition. As control, plants grown under fluorescent white lighting were used.

The explants of all cultivars were cultured in controlled conditions at a 20 ± 2°C temperature and a 16/8 light/dark photoperiod. The experiment was carried out with three replications. The regeneration frequency was calculated as a percentage of regenerated explants of the total number of explants, and the regeneration efficiency was calculated as the number of shoots per explant averaged over the total number of explants.

Statistical processing and analysis were conducted in *Excel* application using the standard *Microsoft Office XP* (Microsoft, USA) and *SPSS*, version 17.0 (IBM, USA). To compare normally distributed samples, the variances of which did not differ conclusively, Student's *t*-test was used. Two hypotheses were preliminarily tested: the correspondence of sample distributions to the normal law according to the Kolmogorov-Smirnov consistency criterion and the homogeneity of the variance of values in the samples based on the Leuven test. The samples, whose distribution deviated from the normal or whose variance values were statistically significantly different, were compared using the nonparametric Mann-Whitney U-test. All the data presented are the mean values of three independent sets of experiment ± standard deviation (SD) or standard error.

Results and discussion

The morphogenic response was initiated differently in blackberry and raspberry. The primary reaction in all blackberry cultivars was the formation of the callus tissue at leaf plates and stem segments

on all cultural medium (Figure 1a). After three weeks, shoots were formed on the green callus of the internodes (Figure 1b). After 30 days, adventive shoots were formed on the callus tissues of the leaf explants (Figure 1c). Solid green callus with expressed meristematic focus was capable of the morphogenic response. Shoots appeared both separately and in the form of clusters (from three to ten). Solid green callus with expressed meristematic focus had the ability to undergo morphogenesis. Shoots appeared both separately and in the form of clusters (from three to ten). The root segments formed a light, loose, and non-morphogenic callus (Figure 1d).

An individual reaction of cultivars to various combinations of hormones was observed. The explants cultured on MS medium (control) did not show any regeneration response. The data about the effect of plant growth regulators (PGR) on blackberry adventitious morphogenesis are shown in Table 1.

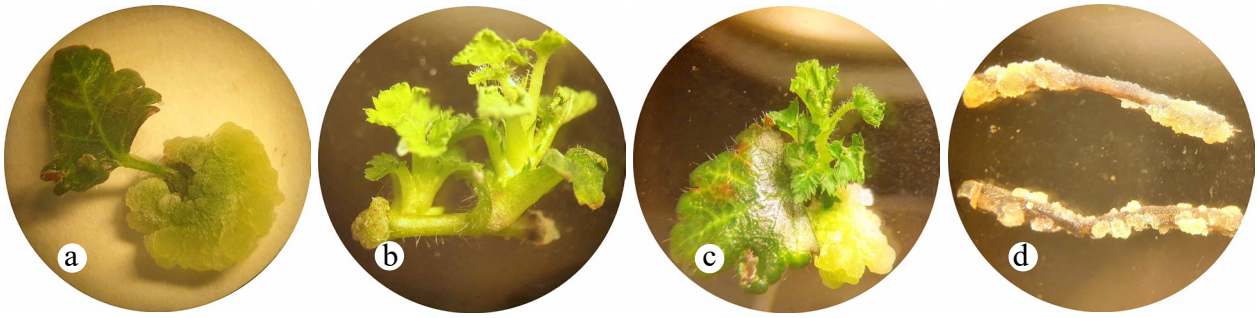


Figure 1. Blackberry regeneration stages: callus tissue on leaf plates and stem (a), shoots on green callus on internodes (b), adventive shoots on leaf explants (c), and light, loose, and non-morphogenic callus on root segments (d)

Table 1. Composition of various plant growth regulators (PGR) used for regeneration experiment of blackberry cultivars

PGR mg L^{-1}			Explants showing bud formation %			Number of shoots per explant		
TDZ	BA	IBA	'Smooth-steem'	'Triple Crown'	'Karaka Black'	'Smooth-steem'	'Triple Crown'	'Karaka Black'
0.5			24.1	19.2	13.1	1.41 ± 0.62*	0.41 ± 0.05	0.33 ± 0.04
0.5		0.1	22.4	25.1	19.4	3.34 ± 0.11*	0.23 ± 0.08	0.13 ± 0.1
0.5		1.0	29.1	28.1	20.9	1.95 ± 0.52*	0.62 ± 0.12	0.71 ± 0.12
1.0			53.6	43.3	68.8	9.93 ± 0.74**	8.16 ± 0.69**	7.96 ± 0.41**
1.0		0.1	45.4	44.9	35.2	6.36 ± 0.89**	6.24 ± 1.38**	3.14 ± 1.01*
1.0		1.0	37.5	35.1	33.4	3.68 ± 0.77*	2.27 ± 0.64*	1.11 ± 0.61*
1.0	1.0		46.7	69.0	53.7	8.21 ± 1.02**	6.83 ± 0.67**	5.53 ± 0.32**
1.0	2.0		15.4	14.7	19.8	3.01 ± 0.96*	3.92 ± 0.99*	3.87 ± 0.44*
2.0			73.3	46.6	62.1	9.73 ± 1.1**	8.53 ± 1.3**	8.13 ± 1.1**
2.0		0.1	31.8	36.7	23.3	4.36 ± 0.94**	3.67 ± 0.96*	4.71 ± 0.56**
2.0		1.0	37.6	33.2	31.5	3.31 ± 0.91*	1.55 ± 0.95*	2.02 ± 0.44*
2.0	1.0		28.2	20.8	22.2	4.46 ± 0.16**	2.91 ± 0.12*	2.12 ± 0.24*
2.0	2.0		12.5	15.3	10.8	3.34 ± 0.08*	5.18 ± 0.54**	4.77 ± 0.53**
	1.0	0.1	9.0	10.1	7.0	1.79 ± 0.5*	1.19 ± 0.34*	1.34 ± 0.41*
	1.0	1.0	1.0	3.9	2.1	0.2 ± 0.09	0.14 ± 0.06	0.05 ± 0.06
	2.0	0.1	18.4	5.6	9.4	0.84 ± 0.22	0.33 ± 0.09	0.13 ± 0.09
	2.0	1.0	6.8	6.4	4.4	0.76 ± 0.31	0.73 ± 0.1	1.41 ± 0.1*

Note. TDZ – thidiazuron, BA – 6-benzylaminopurine, IBA – indolyl-3-butyric acid; concentration means of three replications ± SD; significantly different from the control value at * – $p < 0.05$ or ** – $p < 0.01$, respectively.

The highest percentage of shoot-forming explants was obtained on MS medium supplemented with 2.0 mg L^{-1} TDZ for the 'Smoothstem' (73.3%) and 1.0 mg L^{-1} TDZ in combination with 1.0 mg L^{-1} BA for the 'Triple Crown' (69.0%); an amount of 1.0 mg L^{-1} TDZ was optimal for the 'Karak Black' (68.8%). Supplementing the medium with BA or combining BA with IBA did not increase the percentage of shoot formation in any of the studied cultivars. For effective shoot formation, auxin (IBA) was not required in most cases – regenerating samples produced 3–4 shoots on average. The greatest shoot number per explant (up to ten) was observed with the addition of 2.0 and 1.0 mg L^{-1} TDZ for the 'Smoothstem'. The forming shoots were actively developing and were intensely green (Figure 2a). Leaf explants and internodes were not efficiently regenerated. In most cases, raspberry shoots were obtained on root explants mainly without callus formation (Figure 2b).

The morphogenic response was less expressed in raspberry cultivars 'Glen Ample', 'Maria', and 'Gusar' (Table 2).

A significant difference in the proliferative response was observed among the selected cultivars: 'Glen Ample' and 'Gusar' proved to be more regenerative than 'Maria'. In 'Glen Ample', morphogenesis was stimulated with 2.0 mg L^{-1} TDZ on root and leaf/steam explants with a frequency of 46.6% and 3.7%, respectively. The

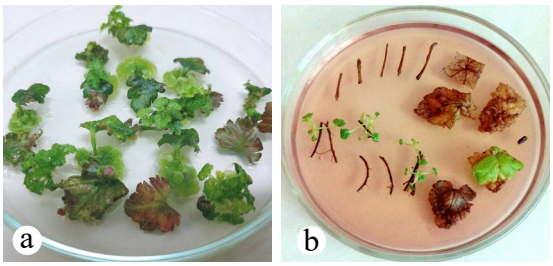


Figure 2. Plant regeneration: blackberry shoots on leaf and stem explants (a) and raspberry shoots on root explants (b)

addition of 1.0 mg L^{-1} TDZ initiated proliferation in 33.3% root and in 5.3% leaf and stem explants. 'Gusar' mainly formed callus on a medium supplemented with 0.5 mg L^{-1} TDZ. Shoots and roots in all plants were misshapen and etiolated at a 3.0 mg L^{-1} TDZ.

In combination with IBA or GA, ZEA stimulated shoot formation on the root sections of 'Glen Ample' (frequency: up to 65.5% with 0.5 mg L^{-1} ZEA), and the average number of regenerants per explant was up to 3.5. Regeneration was not obtained on leaf plates and stem in any of the eight variants of the medium. The most favourable regeneration medium for 'Gusar' was also MS supplemented with 0.5 mg L^{-1} ZEA. Shoots were formed

Table 2. Composition of various plant growth regulators (PGR) used for regeneration experiment of raspberry cultivars

PGR mg L^{-1}					Explants showing bud formation %						Number of shoots per explant		
					'Glen Ample'		'Maria'		'Gusar'				
TDZ	ZEA	BA	IAA	GA	a	b	a	b	a	b	'Glen Ample'	'Maria'	'Gusar'
		2.0	0.5		9.2	–	8.3	–	22.4	–	2.2 ± 0.6**	2.1 ± 0.8**	1.8 ± 0.8*
		3.0	0.5		3.8	–	4.2	–	24.6	–	1.8 ± 0.6**	1.5 ± 0.6*	1.3 ± 0.5*
		4.0	0.1		–	–	–	–	21.5	–	–	–	0.9 ± 0.3*
0.5					15.0	–	13.3	–	14.5	23.4	1.5 ± 0.5**	1.2 ± 0.4*	2.1 ± 0.6*
0.5				0.5	12.1	–	22.2	–	9.6	21.5	0.6 ± 0.2	0.5 ± 0.3	0.6 ± 0.3
1.0					8.3	33.3	5.3	6.7	2.5	5.9	2.3 ± 0.4**	1.6 ± 0.6**	0.7 ± 0.4
1.0				0.5	5.8	9.1	3.3	–	3.8	8.4	0.4 ± 0.1	1.0 ± 0.5*	1.2 ± 0.5*
1.5					9.7	27.8	13.3	2.8	–	4.8	2.4 ± 0.6**	1.2 ± 0.4*	1.0 ± 0.2*
1.5				0.5	2.4	8.8	1.5	7.1	–	–	0.6 ± 0.2	0.3 ± 0.1	–
2.0					3.7	46.6	3.7	–	–	–	2.3 ± 0.6**	0.8 ± 0.3	–
	0.5				–	65.5	–	–	21.2	57.6	3.5 ± 0.4**	–	3.2 ± 1.2**
	1.0				–	13.3	–	–	–	35.8	1.1 ± 0.3*	–	2.1 ± 0.8*
	0.5		0.1		–	26.7	–	–	2.4	42.6	0.6 ± 0.1	–	1.8 ± 0.6*
	1.0		0.1		–	13.3	–	–	3.8	24.5	1.1 ± 0.4*	–	1.0 ± 0.4*
	0.5			0.1	–	33.3	–	–	6.2	36.4	1.5 ± 0.5**	–	2.6 ± 1.1*
	0.5			0.5	–	26.7	–	–	5.8	29.6	1.2 ± 0.3*	–	1.2 ± 0.4*
	1.0			0.1	–	58.0	–	–	–	11.6	0.7 ± 0.3	–	0.6 ± 0.2
	1.0			0.5	–	13.3	–	–	–	9.4	1.1 ± 0.3*	–	0.5 ± 0.1

Note. TDZ – thidiazuron, ZEA – zeatin, BA – 6-benzylaminopurine, IAA – indole-3-acetic acid, GA – gibberellic acid; a – leaf and stem explants, b – root explants; concentration means of three replications ± SD; significantly different from the control value at * – $p < 0.05$ or ** – $p < 0.01$, respectively.

on root explants twice as often as on leaf ones (57.6% and 21.2%, respectively) with the average number of regenerants per explant up to 3.2. Adding ZEA and TDZ did not stimulate morphogenesis effectively in 'Maria' on any type of explants; this shows the low regenerative potential of this cultivar.

The data obtained indicate that in stimulating morphogenesis in both blackberries and raspberries TDZ is the most effective of the used hormones. This hormone is a cytokinin-like substance and is of significantly superior efficiency than purine cytokinin. Due to the combination of cytokinin and auxin activity, TDZ can directly or indirectly modify endogenous growth regulators and cause reactions in the cells that are necessary for their division and regeneration (Guo et al., 2011). Results of our experiment confirm the data that higher (from 3.0 mg L^{-1}) TDZ concentrations cause morphological abnormalities and thus are not suitable for either regeneration or micropropagation (Qu et al., 2000).

Using information about the stimulating role of red and blue LEDs in various combinations for shoot regeneration and subsequent growth in different plants such as orchids (Teixeira da Silva, 2014), stevia (Ramírez-Mosqueda et al., 2017), potatoes (Edesi et al., 2014), strawberries (Hung et al., 2015) and others, the light effect was studied. Lighting was used with ratios of light quanta red to blue in the following combinations: 1R:1B, 2R:1B, 1R:2B, 4R:1B, and 1R:4B, also, monochrome red and monochrome blue. Two options were considered: *in vitro* plants grown under white fluorescent lighting and plants pre-incubated under the same LED lighting options.

Morphological differences in regenerating explants under LED lighting. With an equal R:B ratio, the leaf plates remained dark green with reddening at the site of cuts. The callus and shoots were intensely green (Figure 3a). The increase in the red proportion caused the formation of a dense globular morphogenic callus and the shoot colour became dark green with burgundy veins (Figure 3b). A fourfold increase in blue stimulated the appearance of a loose light green callus and reduced the shoots number (Figure 3c).

Under the lighting ratios R1:B1 and R2:B1, a larger percentage of regenerants formed on nodular callus. Monochrome lighting resulted in the minimum number of shoots. Monochrome red caused the elongation of individual induced shoots due to the elongation of internodes. This weakened them and hindered their further development. Monochrome blue slowed down the growth of shoots: their leaves were small and dense. Cultures exposed under LEDs in the ratios R1:B1 and R2:B1 gave a higher percentage of shoot regeneration than the white lighting (control) variant: 69.8% and 79.4% *versus* 63.1% (Figure 4a). A twofold increase in blue and a fourfold increase in red reduced the frequency of regeneration to control values. Incubation under monochrome (both red and blue) light reduced these values by more than 10% in relation to the control. The shoots number per explant was also higher under R2:B1 lighting (Figure 4b). Lighting R1:B1 and R2:B1 resulted in more shoots than the control; other options were not effective.

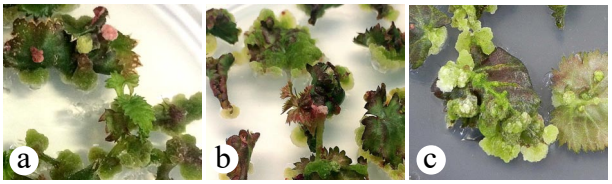
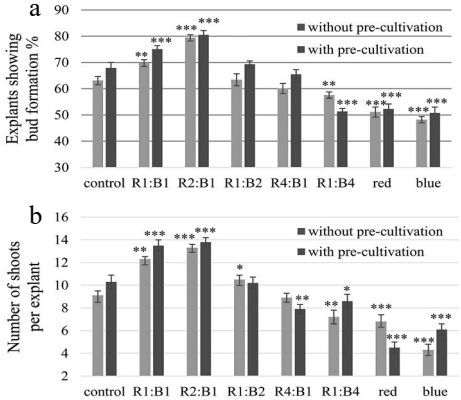


Figure 3. Shoot and callus regeneration under LED lighting of different spectra: red to blue equal ratio 1R:1B (a), twofold red to blue ratio 2R:1B (b), and fourfold blue to red ratio 4B:1R (c)

The use of plants pre-cultured under the same LED spectrum gave some increase in the percentage of regenerative formations and shoots per explant (Figure 4). Enhanced shoot formation on explants with an increased proportion of blue (R1:B4 and monochrome blue) may have a result from the activation of blue light photoreceptors, since it is known that cryptochromes mediate light regulation of gene expression in general (Yang et al., 2017).

Fluorescent lamps are used for growing *in vitro* plants in light boxes, but this type of lamp emits light



Note. Bars represent standard errors; significantly different from the control value at * – $p < 0.05$, ** – $p < 0.01$ or *** – $p < 0.001$, respectively.

Figure 4. LED lighting affects the frequency – explants showing bud formation (a) and efficiency – number of shoots per explant (b) of plant regeneration of the genus *Rubus*

with an insufficient number of red wavelengths for plants. Light-emitting diodes can provide the desired spectral ratio (Dutta Gupta, Jatothu, 2013). The effect of spectral combinations on plant regeneration has been recorded for several plant species grown *in vitro* (Hung et al., 2015; Kwon et al., 2015; Al-Mayahi, 2016; Gupta, 2017).

The results of our experiment have shown an increase in the frequency and efficiency of *Rubus*

regeneration when the R:B LEDs are 1:1 and 2:1. LED lighting 2R:1B and 1R:1B increased the number of regenerated explants by 17.1% and 8.3%, respectively. Shoot formation per explant increased by 4.4% and 3.5%. Irradiation with monochrome red and monochrome blue reduced the regeneration potential by 13%. Pre-cultivation of plants under same-spectrum light increased morphogenesis only by 3–5%.

A recent study shows the effect this LEDs combination has on improving the growth performance of *Rubus* (Nacheva et al., 2021). There are conflicting results for effects of light quality on shoot proliferation in the literature. For *Bacopa monnieri*, longer and shorter shoots were generated under 1R:1B and white LEDs, respectively, but white LED was found to be more effective for regenerating the maximum number of shoots per explant (Karataş et al., 2016). In experiment presented by Poncetta et al. (2017), the mixed LED light yielded less efficient multiplication of red raspberry in comparison to fluorescent lights, but with higher quality shoots. The effect of LED lighting on plant growth, physiology, and secondary metabolism was explained by the fact that light controls shoot regeneration by changing the cytokinins level and/or the response to these hormones (Olle, Viršilė, 2013; Ouzounis et al., 2015). It has been shown that light is important in the biosynthesis and metabolism not only of cytokinins but also auxins, which implies its effect on the growth and development of shoots and roots (Zdarska et al., 2015). The interaction between cryptochromes and phytochromes in *Arabidopsis thaliana* is shown by the example of the activation of protein kinase that regulates seedlings photomorphogenesis at early stages (Malec et al., 2002). A study using flow cytometry showed that combinations of red and blue light increased the shoot regeneration and development of *Populus euramericana* by stimulating cell division, although the response varied among genotypes of the same species (Kwon et al., 2015).

The results of our experiment confirm the available information on the influence of spectral composition of light as one of the factors determining plant morphogenesis. A large number of studies have demonstrated the importance of LEDs lighting in various combinations in shoot regeneration and subsequent plant growth (Gupta, 2017). The differentiated response can be explained by the changing nature of the synergistic interactions of various light-regulated photoreceptors depending on the genetic structure of plants. However, the exact mechanism of the influence of different spectra on plant metabolism at the molecular level is not yet known and requires further study.

Conclusions

1. The dependence of adventitious shoot regeneration of blackberry cultivars 'Smoothstem', 'Triple Crown', and 'Karaka Black' and raspberry cultivars 'Glen Ample', 'Gusar', and 'Maria' on cultivation conditions was established. The optimal cultivars, medium hormonal composition, the explant type, and lighting conditions are proposed.

2. The regenerative potential of blackberry manifested most actively with supplementing thidiazuron (TDZ) with 1.0–2.0 mg L^{-1} and combining 1.0 mg^{-1} TDZ with 1.0 mg L^{-1} BA (6-benzylaminopurine). Blackberry leaves and internodes regenerated at a frequency of 73%. Blackberry regenerative capacity significantly exceeds that of raspberry and is most effectively realized in the cultivar 'Smoothstem' ($p < 0.05$).

3. In selected raspberry cultivars, 0.5 mg L^{-1} ZEA (zeatin) and 1.0 mg L^{-1} TDZ are the best initiators of adventitious morphogenesis. The addition of auxin (IBA) does not significantly affect the morphogenesis, only raspberry root explants result in a high level of shoot regeneration (up to 65%). Basically, there is direct regeneration from the subepidermal tissue without callus formation stage. The raspberry cultivar 'Glen Ample' had a higher regeneration rate than 'Gusar' and significantly higher than 'Maria' ($p < 0.05$).

4. Cultivation of explants under LED lighting showed that by changing the spectral composition of the incident light, regeneration can be enhanced. The optimum red to blue ratios was R2:B1 and R1:B1. Preliminary plant cultivation under selected spectra increased the efficiency of explant regeneration and the frequency of shoot formation.

5. The obtained data will be used for genetic transformation and production of plants of the genus *Rubus* with altered properties.

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Gervuogių ir aviečių adventyvinių ūglių regeneracija ir LED apšvietimo poveikis

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Santrauka

Raukšlėtoji gervuogė (*Rubus fruticosus* L.) ir paprastoji avietė (*Rubus idaeus* L.) gerai žinomos visame pasaulyje dėl savo mitybinės ir medicininės vertės. Augalininkystėje, augalų selekcijoje ir genų inžinerijoje svarbus ląstelių technologijų taikymo etapas yra regenerantų gavimas. Tyrimo tikslas – nustatyti geriausius nepakankamai ištirtų gervuogės ir avietės veislių regeneracijos būdus. Tirtas bespyglės gervuogės veislių 'Smoothstem', 'Triple Crown' bei 'Karaka Black' ir avietės veislių 'Glen Ample', 'Gusar' bei 'Maria' adventyvinių ūglių regeneravimas priklausomai nuo veislės, eksplantų tipo, hormoninės maitinamosios terpės sudėties ir LED apšvietimo. Siekiant paskatinti adventyvinių ūglių pumpurų formavimąsi, augimo kameroje ant MS terpės, papildytos skirtingomis augalų augimo reguliatorių koncentracijomis, auginti trijų tipų eksplantai: lapai, tarpubambliai ir šaknys, gauti iš *in vitro* augalų. Daugeliu atvejų regeneracija išryškėjo po trijų auginimo savaičių. Geriausius rezultatus parodė gervuogės veislė 'Smoothstem', kurios lapalakščių ir stiebo segmentai MS terpėje, papildytoje 2,0 mg L^{-1} TDZ, regeneravo geriausiai – 73,3 %. Aukščiausias aviečių ūglių dauginimosi rodiklis (65,5 %) nustatytas veislės 'Glen Ample' šaknų segmentuose MS terpėje, papildytoje 0,5 mg L^{-1} ZEA. Gervuogių ir aviečių eksplantai buvo auginami LED šviesoje: raudonos (R) ir mėlynos (B) spalvų santykiui esant 1:1, 1:2, 2:1, 1:4 bei 4:1, vienspalvėje raudonoje ir vienspalvėje mėlynoje šviesoje. Buvo fiksuojama regeneracija. Efektyviausia regeneracija (>80 %) buvo gauta raudonoje ir mėlynoje šviesoje, kai spektro santykiai buvo R2:B1 ir R1:B1. Ūglių augimą slopino vienspalvė mėlyna ir vienspalvė raudona šviesa.
Eksperimento rezultatai rodo, kad tam tikri LED apšvietimo spektrų deriniai stiprina augalų morfogenezę. Aprašyta regeneracijos sistema bus naudinga kuriant genų perkėlimo sistemą ir gali būti veiksmingai taikoma pasirinktoms aviečių ir gervuogių veislėms.

Reikšminiai žodžiai: *Rubus fruticosus*, *Rubus idaeus*, priklausomybė nuo LED apšvietimo, tidiazuronas, zeatinas.