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RESEARCH

Six-month maintenance under monochromatic LED light modulates the productivity of caffeic acid derivatives in *Cynara cardunculus* var. altilis DC calli

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Abstract

Artichoke is valued as a food crop and as a source of bioactive phenolic compounds. Its pharmacological properties are attributed to caffeic acid derivatives (CAD) because of their anti-inflammatory and antioxidant properties. The successful cultivation of artichokes with high CAD levels requires specific climatic conditions. Cell culture represents a stable and efficient approach for obtaining highly productive artichoke biomass. One prospective, effective and nature-like approach to activate the biosynthesis of phytochemicals in plant cell culture is the use of a modulated light-emitting diode (LED). Typically, variations in light intensity and spectrum have been investigated. However, the effect of prolonged exposure remains unclear. In the present study, the effects of a monochromatic LED (red, blue, and green) with a 100 µmol $m^{-2}s^{-1}$ intensity during six repeated subcultures of artichoke (*Cynara cardunculus* var. altilis DC) calli were examined for the first time. A stable, dark-grown artichoke callus culture was used for six-month repeated subcultures under white, red, blue, and green light. CAD biosynthesis, growth, morphology, and IAA homeostasis were investigated over the course of a six-month exposure period. Two and three repeated subcultures under blue light are the most effective approaches for optimizing the total CAD productivity in artichoke calli, which is more than threefold that of dark-grown calli (135 vs. 45 mg L^{-1}). Under the tested conditions, prolonged red light exposure was associated with no effect on biomass accumulation, whereas biomass accumulation decreased markedly in the sixth subculture under white, blue, and green light.

Keywords Caffeoylquinic acids · Callus culture · *Cynara cardunculus* · Light-emitting diode (LED) lighting · Monochromatic LED light · Repeated subculturing

Introduction

Artichoke, a Mediterranean agricultural crop, is currently being actively studied from both nutritional and medical perspectives (Shanker et al. 2007). Its pharmacological and cosmetic properties are attributed to its high content of caffeic acid derivatives (CAD), mainly because of its anti-inflammatory and antioxidant properties (Jan et al. 2021; Koç and Karayiğit 2023; Shanker et al. 2007). However, the successful cultivation of artichokes with high CAD levels requires specific climatic conditions (Abbas et al. 2022; Pandino et al. 2020). Consequently, indoor or in vitro cultivation leads to a reduction in CAD content (Pandino et al. 2010). Caffeoyl-quinic acid or chlorogenic acid and its derivatives accumulate in plants as end products of

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secondary metabolism and perform protective functions (Alcázar Magaña et al. 2021). The hepatoprotective, antiviral, antioxidative, peroxynitrite-scavenging, and antidiabetic effects of artichoke are largely ensured by the activity of caffeoylquinic acids (Kim et al. 2020; Alcázar Magaña et al. 2021; Park 2010; Rodrigues et al. 2023).

Over the past few decades, plant biotechnology has advanced significantly, and many uses of callus and suspension cell cultures in pharmacology, pharmaceuticals, horticulture, and agriculture have been reported (Efferth 2019; Kutschera and Ray 2022). The most important of these methods is tissue culture (Ozyigi et al., 2023). Therefore, finding effective, environmentally friendly, and natural ways to stimulate secondary metabolism in plant cell cultures is crucial. Artificial lighting is currently being actively explored as a noninvasive and eco-friendly method for modulating physiological and biochemical processes in plant cells. The results of numerous studies indicate that variations in monochromatic lighting alter the morphology and synthesis of phytochemicals in plants (Taulavuori et al. 2017; Veremeichik et al. 2023, 2024a). However, the effects of long-term monochromatic light treatment on callus cultures during repeated subculturing have not yet been studied.

Cell culture represents a more stable and efficient approach for obtaining highly productive artichoke biomass. Using modulated light-emitting diode (LED) lighting to cultivate plant cell cultures is a contemporary method that satisfies these needs. In recent years, the effects of artificial light on plant callus cultures have been actively studied. However, this research focused primarily on morphogenetic processes (Dehestani-Ardakani et al. 2025). Data on the role of different lighting options on secondary metabolism in cell cultures are much less extensive and often contradictory. Thus, more biomass accumulates in callus cultures when they are grown in the dark and under red light, whereas growth is inhibited by blue light treatment (Sobhani Najafabadi et al. 2019; Veremeichik et al. 2024b, 2025b; Ullah et al. 2019). In other works, the growth of calli was not affected under blue light (Bajwa et al. 2023). Compared with dark-grown calli, *V. corymbosum* cultivated under red light presented the highest anthocyanin concentration (Abou El-Dis et al. 2021). In *Gynura procumbens* calli, blue LED (50 µmol $m^{-2}s^{-1}$) treatment led to increases in phenolic content, total flavonoid content, and antioxidant activity (Lian et al. 2019). Compared with white light, blue light significantly increased capsaicin and dihydrocapsaicin contents in *Capsicum annuum* calli (Arya et al. 2025). Blue and red/blue light stimulates the accumulation of verbascoside and isoverbascoside in *Verbena officinalis* L. calli (Kubica et al. 2020). Thus, despite the availability of a large body of experimental data, it is not possible to discern general trends regarding the effects of monochromatic light on the physiology of plant callus tissue. The primary challenge lies in the inconsistency of experimental conditions. Furthermore, the "strength" of the light treatment—defined here as a combination of factors such as intensity and duration of exposure—is often insufficient to reveal the true effect of a specific light treatment.

In this study, we investigated the long-term sequential cultivation (or repeated subculturing) of callus tissue under monochromatic illumination. On the basis of the data obtained, we suggest that light spectra play important roles in regulating the distribution of cellular resources, growth and biosynthesis in undifferentiated plant callus tissue. In the present work, we investigated the long-term (six subcultures) sequential maintenance of a callus culture of cultivated artichoke or *Cynara cardunculus* var. altilis DC (Asteraceae) under monochromatic LED light. We used a cell culture obtained previously, in 2012, from stem explants that were continuously cultivated for more than 150 passages in the dark (Vereshchagina et al. 2014). We previously showed that a single exposure to monochromatic light during a 30-day subcultivation of a callus culture significantly affects the biosynthesis of phytochemicals. Treatment with green light (510–520 nm) at 300 µmol m^{-2} s^{-1} led to a 3.5-fold increase in rosmarinic content (Veremeichik et al. 2024b) in the calli of *Mertensia maritima* L. (Boraginaceae). Low-intensity red light can increase the productivity of shikonin in *Lithospermum erythrorhizon* Sieb. et Zucc calli. Blue light blocked the growth of the *M. maritima* culture and the biosynthesis of shikonin in the *L. erythrorhizon* culture (Veremeichik et al. 2025a). However, for artichoke cell culture, blue light at low intensities does not block growth or activate CAD biosynthesis during a single cultivation. Moreover, blue light with an intensity of 50 µmol $m^{-2}s^{-1}$ was the most cost-effective treatment for improving CAD productivity in *C. cardunculus* calli. However, an increase in blue light intensity to 300 µmol $m^{-2}s^{-1}$ led to growth inhibition. Red light with a wide range of intensities did not affect the growth of *C. cardunculus* calli (Veremeichik et al. 2025b). Thus, the goal of the present work was to determine the effects of prolonged exposure to monochromatic light (red, blue, and green) on the growth, morphogenesis, and secondary metabolism of *C. cardunculus* calli. For the experiments, we chose a medium intensity of exposure (100 µmol $m^{-2}s^{-1}$) at which we found no negative effect on growth after a single exposure. *C. cardunculus* calli grown in the dark served as a control. With respect to the dynamics of subcultivation over six months (6 subcultures for 30 days), indicators such as growth, morphogenesis, IAA content and CAD content were analysed.

Materials and methods

Plant materials

The callus line *C. cardunculus* was obtained previously, in 2012, from stem explants (Vereshchagina et al. 2014). Modified Murashige and Skoog medium supplemented with 6-benzyladenine (BA, 0.5 mg L^{-1}) and α-naphthaleneacetic acid (NAA, 2.0 mg L^{-1}) (Veremeichik et al. 2025c) was used for *C. cardunculus* callus cultivation. The *C. cardunculus* calli used in the experiments were continuously cultivated in the same medium in the dark at 24 °C for 12 years and subcultured once every 30 days. The study employed specimens (strains) deposited in the Bioresource Collection of the Federal Scientific Centre of East Asia Terrestrial Biodiversity of the Far East Branch of the Russian Academy of Sciences (reg. number 2797657).

Light treatments and experimental design

The IACP FEB RAS designed and produced the light sources used to cultivate the *C. cardunculus* calli in four-section chambers (100 × 50 × 50 cm, Supplementary Figure S1, Supplementary Table S1) as previously described (Veremeichik et al. 2024b). The following types of monochromatic light were employed in this investigation in addition to warm white light (called "W"): red (660 nm, designated "R"), blue (440 nm, designated "B"), and green (520 nm, designated "G") with a 24 h photoperiod. LED lights with light intensities of 100 µmol $m^{-2}s^{-1}$ were installed in every area of the chamber. The photosynthetic photon flux density (PPFD) was determined for each segment's 1–10 light-emitting matrices. Each matrix's supply current was changed to change the amount of light in each chamber. A PG200N spectrophotometer (UPRtek, Taiwan) was used to measure the spectra and real PPFD at the actual culture level. A UT61A digital multimeter (Uni-T, China) controlled the currents in the driver supply system. The primary light attributes are listed in Supplementary Table S1. The temperature was controlled by an exhaust fan FFB1212SH 12025 (power: 14.8 W; speed: 3700 rpm; air volume: 140.16 cfm, China). The front of each chamber was covered by a lid lined on the inside with reflective aluminum foil. Chambers with different emission spectra were positioned at some distance from one another to minimize any mutual interference between the various light spectra.

After inoculation (2 g of inoculant per 50 mL of the same solid medium in 250-mL *Erlenmeyer* flasks; Simax, Czech Republic), the cells were immediately transferred under different light conditions for one passage (30 days). After 30 days, 2 g of the treated calli (uniform cell aggregates) was transferred to fresh medium and grown under the same light conditions. Sequential subculturing was performed six times for each light treatment. Twelve replications of each treatment were obtained for further analysis: six flasks with a callus biomass were grown for each subculturing round under different light treatments: two for subculturing and four for analysis. The experiment was conducted in three independent runs. Following 3 successive subcultures from the first experiment, a second pool derived from the original culture was subjected to experimentation. Upon completion of the first experimental run, a third pool was placed under the same conditions. Calli growing in the dark were used as a control. The dark-grown control was measured for every subculture, as were the light-treated calli. After 30 days of cultivation under different light conditions, the calli were harvested, weighed, photographed, and dried (45 °C for 12–16 h) for further chemical analysis.

Phenolic acid extraction and HPLC-DAD-ESI-MS/MS conditions

Chemicals

Chlorogenic acid and cynarin analytical standards were acquired from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water (Millipore, Bedford, MA, USA) was used to prepare the eluents and extraction solutions. All the solvents were of analytical grade.

Sample preparation for analytical chromatography

A previously reported procedure was followed for the extraction of polyphenols (Veremeichik et al. 2025b). In short, dried and powdered callus material was extracted by ultrasonication in 80% aqueous methanol. Prior to HPLC analysis, the extracts were purified using a 0.45-µm membrane (Millipore, Bedford, MA, USA). For each biological replicate, two technical replicates were prepared and analysed; their mean values were then used for further analysis.

Analytical chromatography and mass spectrometry

Analytical chromatography and mass spectrometry were performed at the Instrumental Centre of Biotechnology and Gene Engineering of IBSS FEB RAS via a 1260 Infinity analytical HPLC system (Agilent Technologies, Santa Clara, California, USA) interfaced with an ion trap mass spectrometer (Bruker HCT ultra PTM Discovery System, Bruker Daltonik GmbH, Bremen, Germany). An analytical Zorbax C18 column (150 mm, 2.1 mm i.d., 3.5 µm; Agilent Technologies, USA) was used for the separation of ultrapure water (A) and acetonitrile (B) with 0.1% formic acid added to each instance to make up the mobile phase. The

following linear gradient was applied at a flow rate of 0.2 mL/min: 0 min, 5% B; 20 min, 30% B; and 30 min, 100% B. UV spectra were obtained using a photodiode array detector operating in the 200–600 nm range. A wavelength of 325 nm was used to record chromatograms for quantification. MS data were obtained in negative electrospray ionization (ESI) mode. The ion source potential was −3.8 kV, the drying gas temperature was 325 °C, the nebulizer gas (N2) pressure was 25 psi, the drying gas flow rate was 8.0 L min^{-1}, and the m/z detection range was 100–650. Tandem mass spectra were obtained in auto-MS2 mode (smart fragmentation).

The productivity of CAD was calculated as follows:

$$Productivity(mg/L) = Content \times DW$$

where Content denotes the content of CAD (mg g^{-1} DW) and DW denotes the dry weight (g) of the callus biomass per liter of medium (g L^{-1}).

The cumulative values were calculated for each replicate using the mean dry weight.

IAA ELISA

Chemicals

ELISA was performed *via* an ELISA kit for indole-3-acetic acid (IAA) (Cloud-Clone Corp., China). All the extraction solutions were prepared with ultrapure water (Millipore, Bedford, MA, USA). All the solvents were chemically pure grade.

Sample preparation for ELISA

To compare the IAA content in untreated and light-treated *C. cardunculus* calli by ELISA, sample preparation was performed according to a previously described method (Veselov et al. 1992). Samples of *C. cardunculus* calluses were powdered with liquid nitrogen and immediately weighed to 300 mg. Extraction was performed in 1 ml of 80% ethanol for 16 h at 4 °C with continuous shaking. After incubation, the extracts were centrifuged, and the supernatant was dried (Concentrator Plus, Eppendorf). The dried pellet was dissolved in 1 ml of distilled water acidified with HCl (pH 2–3). IAA was subsequently concentrated twice with diethyl ether. The ether phase was dried; the pellet was dissolved in 50 µl of 80% ethanol and used immediately for the ELISA.

IAA content analysis

ELISA was performed *via* an ELISA kit for indole-3-acetic acid (IAA) (Cloud-Clone Corp., China) according to the manufacturer's instructions. The samples were analysed in a 96-well plate using a spectrophotometer (Benchmark Plus Microplate Reader, Bio-Rad, USA) at a wavelength of 450 nm. The results were obtained from a calibration curve constructed according to the optical density of a standard (320, 1600, 8000, and 40000 ng ml^{-1}). The analysis of IAA content was repeated three times, with two biological replicates and four technical replications.

Light microscopy

For histological analysis, fragments of *C. cardunculus* callus tissue from the experiment (0.5–2 cm^3) were fixed with 70% ethanol, 4% formaldehyde, and glacial acetic acid (SAF fixative; 36:7:7 v/v) by vacuum infiltration, dehydrated, and embedded in paraffin. Afterward, 12 µm thick sections were prepared using an HM 340E rotary microtome (Microm, Thermo Scientific, United Kingdom). The sections were deparaffinized and stained with hematoxylin and alcian blue according to the methods of Zhinkina and Voronova (2000). After histochemical staining, the preparations were examined using an Axio Imager A1 light microscope (Carl Zeiss, Germany) with an AxioCam HRc video camera (Zeiss, Germany) and AxioVision 4.8 software. For analysis, two biological replicates (separate experiments) with four technical replicates were used. When the samples were prepared for microscopy, a field of 1 cm^2 was defined, within which the total number of cells ranged from 300 to 500. For each replicate, the percentage of cells in the four size groups was calculated on the basis of the total number of cells in the sample.

Statistical analysis

Statistical analysis was conducted using the STATISTICA software tool (StatSoft, Inc., USA). For the analysis of growth data, a minimum of 12 biological replicates (as the experimental unit) were used. For the statistical analysis of data on individual and total CAD content, the mean values from two technical replicates (as the experimental unit) of the HPLC analysis—obtained for at least 12 biological replicates—were used. The mean ± standard error (SE) for growth data and total CAD content and productivity were obtained from descriptive statistics, whereas robust mean ± standard error for mCQ and dCQ were obtained from a robust tool of descriptive statistics and confirmed *via* Tukey's biweight (Statistica Visual Basic SVB) for differing values. Datasets of the content and productivity of the individual compounds (mCQ and dCQ) were compared using Tukey's HSD (honestly significant difference). Datasets of growth and total CAD content and productivity were compared using Fisher's least significant difference (LSD) post hoc test (ANOVA). The statistical significance threshold was set at $p < 0.05$. Comparisons were conducted among all treatment-by-subculture combinations.

Results and discussion

The growth of *C. cardunculus* calli during six months of maintenance under different light treatments

The dynamics of the growth parameters of *C. cardunculus* calli were studied. The morphology of the light-treated calli significantly changed after the third subculture. As shown in Fig. 1a, the control dark-grown calli were loose and uniform in consistency and had a light creamy hue. After the three subcultures were subjected to W, B, and G light treatments, the consistent culture lost its homogeneity, and the culture acquired a green tint. Further cultivation under these conditions resulted in growth inhibition and darkening of the cell biomass on the sixth subculture to a greater extent for blue and white and to a lesser extent for green variants of light exposure. Moreover, subculturing under red light had no noticeable effect on the appearance of the culture throughout the entire experimental period. The color and consistency of the cell culture remained unchanged compared with those of the dark-grown control calli (Fig. 1, a).

Compared with those of the dark-grown control cultures, the growth dynamics of *C. cardunculus* calli grown under different artificial light conditions were measured and are represented as percentages (Fig. 1, b, c**);** the absolute values are given in Supplementary Table S2. As shown in Fig. 1, compared with the control conditions, long-term

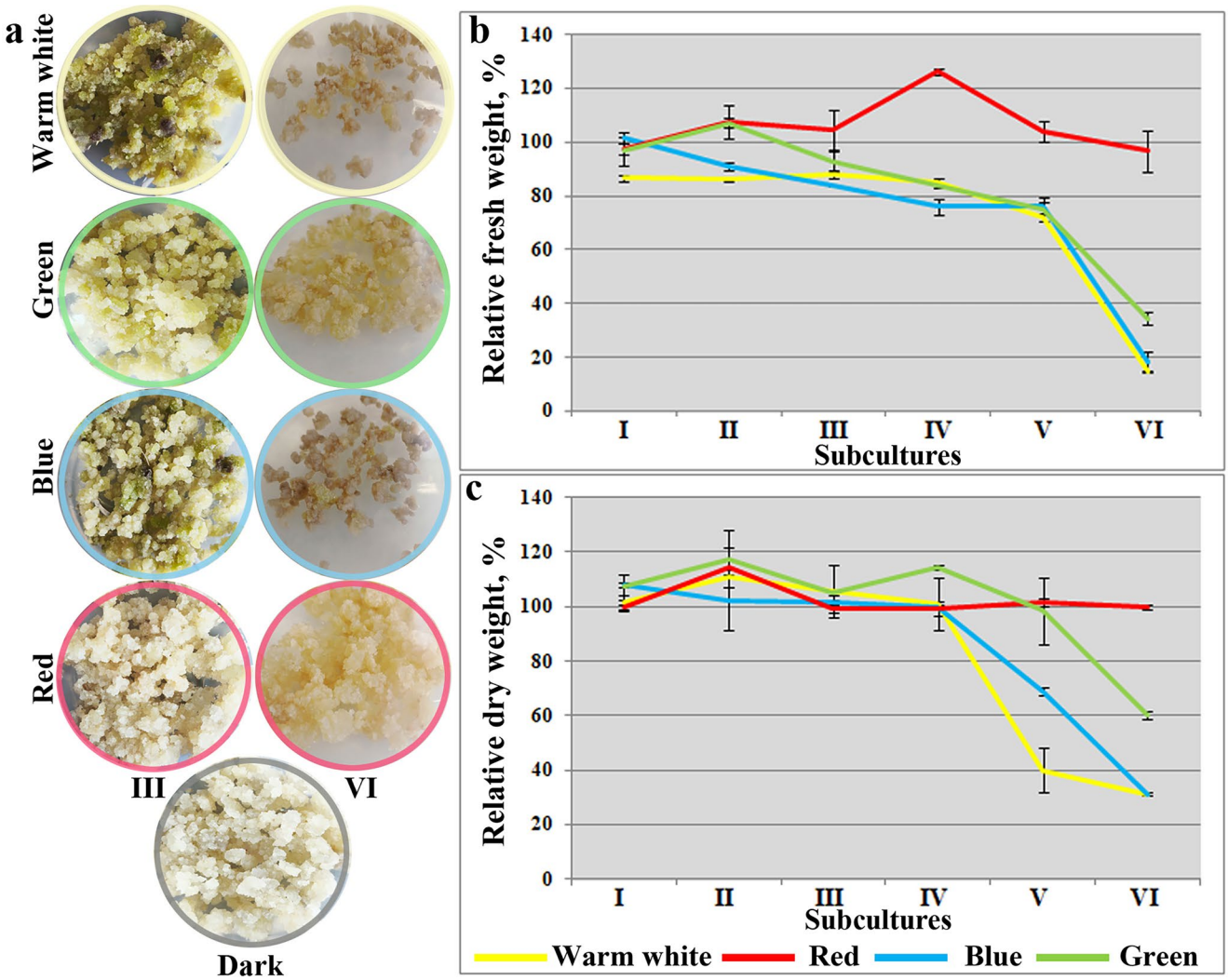


Fig. 1 Morphology and weight dynamics of *C. cardunculus* callus cultures under different light conditions during six subcultures. Morphology (**a**) of *C. cardunculus* callus cultures cultured under warm white, green, blue, and red light with intensities of 100 µmol $m^{-2}s^{-1}$ and in darkness (shown for passages III and VI). Dynamics of biomass accumulation (**b**, fresh weight; **c**, dry weight) during continuous maintenance (six 30-day-long subcultures, I–VI) for callus cultures exposed to different light conditions. The data are presented as a percentage of the dark-grown control. The absolute values of the mean ± standard error of the mean are presented in Supplementary Table S2. Statistical analysis of the data from the twelve replicates is presented in Supplementary Tables S4 and S5, and the red color of the table cell indicates a statistically significant difference (ANOVA, Fisher's LSD, $p < 0.05$)

subcultivation of *C. cardunculus* calli under different light conditions significantly affected the growth of the callus despite the use of monochromatic red light treatment. Compared with those in the control dark-grown calli, the growth values in the III subcultures were stable at 100–110% (Fig. 1, b; Supplementary Table S2). Warm white, blue, and green light treatments had similar effects on the biomass accumulation of *C. cardunculus* calli. During the first three passages, the culture growth did not differ from that of the dark-grown control calli (Fig. 1, b; Supplementary Table S2). However, after the 3rd subculture, under these light treatments, the growth of the treated calli gradually decreased, and by the 6th subculture, growth markedly decreased. As shown in Fig. 1, b, c, both dry and fresh weight data indicate a negative effect of warm white, blue, and green light treatments on callus growth during long-term cultivation, in contrast to exposure to red light (Fig. 1, b; Supplementary Table S4 and S5).

The results of this study revealed a negative effect of warm white, blue, and green light treatments on callus growth during long-term cultivation, in contrast to exposure to red light. The information gathered in this study is consistent with earlier findings for both callus cultures and plants: compared with that of the control plants, the biomass of the callus cultures of *Hypericum perforatum* increased when the plants were grown in the dark and under red light, whereas growth was inhibited by blue light treatment (Sobhani Najafabadi et al. 2019). Interestingly, artichoke seedlings grown in red light grew 60–100% faster than those grown in a greenhouse did, but blue light had the opposite effect (Rabara et al. 2017). It has been repeatedly demonstrated that blue light inhibits the growth of callus tissue (Veremeichik et al. 2024b, 2025b; Ullah et al. 2019). Five-week blue light treatment inhibited the accumulation of root biomass in *Hypericum perforatum* (Sobhani Najafabadi et al. 2019). Moreover, in our previous work, we showed that while low blue light had a slight positive effect on the growth of *C. cardunculus* calli, increasing the blue light intensity (up to 100, 300, and 600 µmol $m^{-2}s^{-1}$) led to growth inhibition (Veremeichik et al. 2025b). Increasing the intensity to 300 µmol $m^{-2}s^{-1}$ resulted in a 3-fold reduction in growth, whereas at 600 µmol $m^{-2}s^{-1}$, complete growth inhibition occurred. In the experiment described, we subjected the calli to light exposure for one subculture (30 days). Thus, we suggest that excess blue light (in terms of both intensity and exposure time) inhibited the growth of *C. cardunculus* calli. On the basis of the present and previously obtained (Veremeichik et al. 2025b) data, red light, in contrast, does not inhibit the growth of callus tissue either with increasing intensity or with prolonged exposure. In our view, determining whether this observation is specific to the culture used in this study or whether it represents a universal effect of blue light is highly interesting. It is possible that the calli of different plant species possess varying thresholds of sensitivity to blue light; therefore, to confirm the growth-inhibitory effect of blue light on calli from various plants, more extensive research—incorporating variations in both light intensity and duration—is needed.

LED light-dependent alterations in cell morphology and IAA content in *C. cardunculus* callus cultures

To hypothesize how light exposure during long-term cultivation modulates cell growth in callus tissue, we performed a histological analysis. Histological analysis of *C. cardunculus* calli cultivated under different light conditions was performed after two subcultures via hematoxylin/alcian blue staining. Histology (Fig. 2, a–e) of the *C. cardunculus* calli cultured under different light treatments and in the dark as a control revealed that the calli samples contained parenchymal (80–95%) and vascular (10–20%) cells (Fig. 2, a–e, arrows). No structural or tissue differentiation in the light-treated cells was observed over time. We identified four groups of cells: small (2–10 µm) with high division potential, medium (11–50 µm), actively dividing, large (51–100 µm), and giant artifact cells (> 100 µm). The last two categories have low division potential and serve a storage function. The analysis revealed that the control dark-grown culture was dominated by medium-sized cells (approximately 60%). The remaining 3 groups are represented in equal proportions of 15–20%. During the second subculture, a statistically significant change was observed for cells grown under the blue and red light treatments (Fig. 2, g). Both light treatments led to a decrease in the number of small cells to 5%. However, at the same time, the number of large cells stored in blue light-treated cells increased, whereas the number of actively dividing middle cells increased in red light-treated cells. By the sixth subculture, only red-light-treated cells were viable. Analysis revealed that long-term maintenance under red light leads to an equal redistribution of cell sizes. With an equal ratio of actively dividing and storing cells, the number of small cells dramatically decreased.

As we noted above, the control dark-grown *C. cardunculus* callus tissue is predominantly composed of parenchyma cells. However, as shown in the figure, after just the second subculture under green and blue light, the number of vascular cells increased significantly and more than it doubled for blue light (Fig. 3, a). Red light did not cause an increase in the

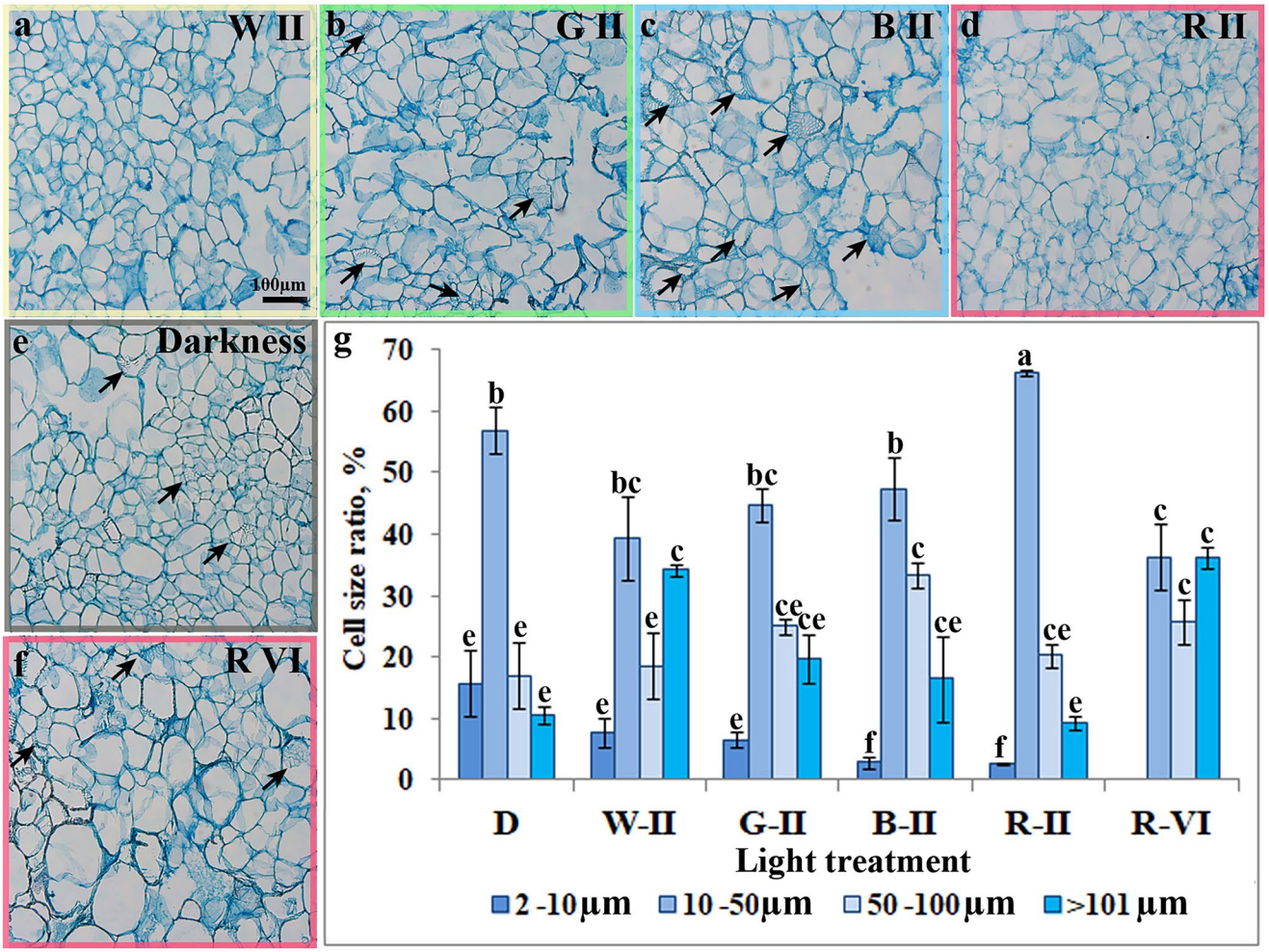


Fig. 2 Histological analysis of the cell size of *C. cardunculus* callus cultures cultivated under different light conditions. Histology (**a**–**g**) of the cell cultures (hematoxylin/alcian blue stained) of *C. cardunculus* cultured during two subcultures under warm white (**a**, W-II), green (**b**, G-II), blue (**c**, B-II), and red (**d**, R-II) light with intensities of 100 µmol $m^{-2}s^{-1}$, in darkness (**e**), and under red light (**f**, R-VI) during six subcultures. The arrows indicate the vascular cells. The cell size ratio (%, **g**) was calculated from three independent measurements in two independent experiments for callus cultures exposed to different light conditions. The data are presented as the mean of three replicates ± standard error of the mean. Different letters above the bars indicate statistically significant differences (ANOVA, Fisher's LSD test, $p < 0.05$)

number of vascular cells, even in the sixth subculture. Undifferentiated callus tissue consists mainly of parenchyma cells, and vascular formation is a sign of morphogenetic transformation. Moreover, the growth of vascular tissue is accompanied by an imbalance of endogenous auxins (Fehér 2023). Therefore, we further determined the level of endogenous IAA in *C. cardunculus* calli after primary light exposure and after secondary subcultivation under different light treatments (Fig. 3, b). Compared with that in unaffected polychromatic W-treated calli, the IAA content in *C. cardunculus* calli was dramatically affected. The second subcultivation under light treatment led to the stabilization of the IAA content in green light-treated cells; stable increases and stable decreases in the IAA content were induced by blue and red light treatment, respectively (Fig. 3, b).

Undifferentiated callus tissue consists mainly of parenchyma cells, and vascular formation is a sign of morphogenetic transformation. Moreover, the growth of vascular tissue is accompanied by an imbalance of endogenous auxins (Fehér 2023; Pasternak and Steinmacher 2024; Shimomura and Fujihara 1978) through AUXIN RESPONSE FACTOR 5 (Fehér 2023, 2026). However, we found no published studies on the effect of monochromatic lighting on endogenous auxin balance. It can be hypothesized that blue light destabilizes the hormonal balance even in stable cultures, leading to the transformation of parenchymal tissue into vascular tissue, which, in turn, results in growth inhibition. However, red light helps to stabilize the auxin balance, ensuring stable crop growth regardless of the intensity and duration of exposure to red light. Therefore,

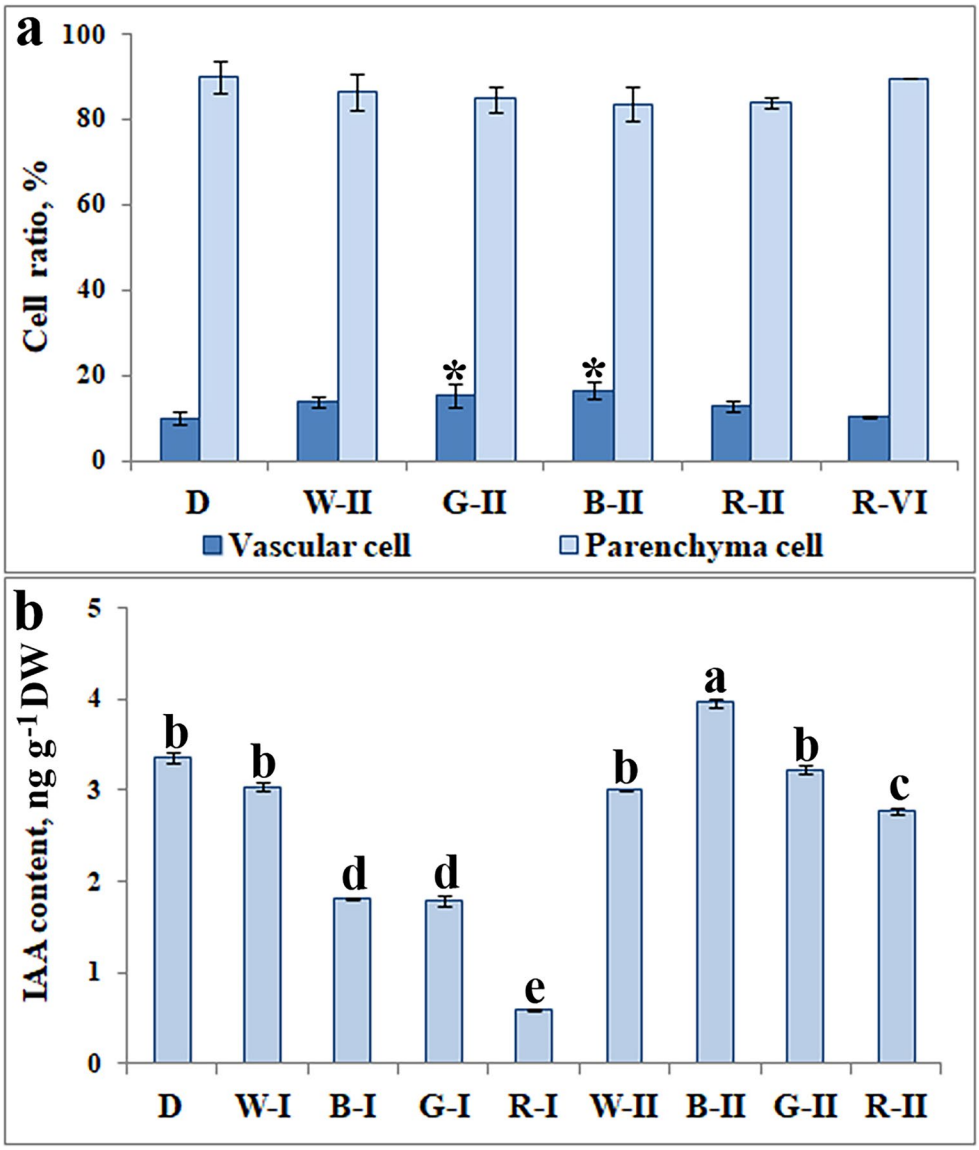


Fig. 3 Abundance of the vascular cells and IAA content of *C. cardunculus* calli cultivated under different light conditions. Vascular and parenchyma cell ratio (%, **a**) of *C. cardunculus* calli cultured under warm white (W), green (G), blue (B), and red (R) light with intensities of 100 µmol $m^{-2}s^{-1}$ for two subcultures (II) and red light for six subcultures (VI) and in darkness. IAA contents (**b**) in *C. cardunculus* callus cultures cultured under warm white (W), green (G), blue (B), and red (R) light with intensities of 100 µmol $m^{-2}s^{-1}$ for one (I) two subcultures (II) and in darkness were measured using ELISA and are presented as ng g^{-1} FW. The data are presented as the mean ± standard error of the mean of three replicates. Asterisks (**a**) and different letters (**b**) above the bars indicate statistically significant differences (ANOVA, Fisher's LSD test, $p < 0.05$)

regardless of intensity or duration, red light has the strongest growth-stimulating effect. Growth is not adversely affected by green light. There was a substantial ($p < 0.05$) unfavourable effect when the blue light intensity was increased. Recent years have seen an increase in the significance and relevance of research on illumination settings that might increase PFAL sustainability and profitability (Orsini et al. 2020). However, the ability of *C. cardunculus* calli to withstand blue light may be species specific, and this treatment may be disastrous for other callus cultures. On the basis of textual evidence, it can also be inferred that this pattern is typical of cell cultures in general. There may be species-specific resistance to blue light intensity. It can be speculated that blue light tolerance may depend on species- or tissue-specific IAA levels.

Contents of caffeoylquinic acids in *C. cardunculus* calli grown under different artificial lights during six subcultures

Long-cultivated *C. cardunculus* callus culture has been studied previously (Vereshchagina et al. 2014) and, to date, has successfully produced caffeoylquinic acids (Veremeichik et al. 2025b). In the present work, we observed changes in the contents of two of the most abundant defined caffeoylquinic acid derivatives (CAD) in *C. cardunculus* calli grown under different light treatments (Fig. 4), monocaffeoylquinic acid (mCQ, peak with RT = 12.1 min, identified as chlorogenic acid) and dicaffeoylquinic acid (dCQ, peak with RT = 20.9 min, identified as 1,5-dicaffeoylquinic acid). The CQAs were quantified by HPLC-UV at the wavelength of

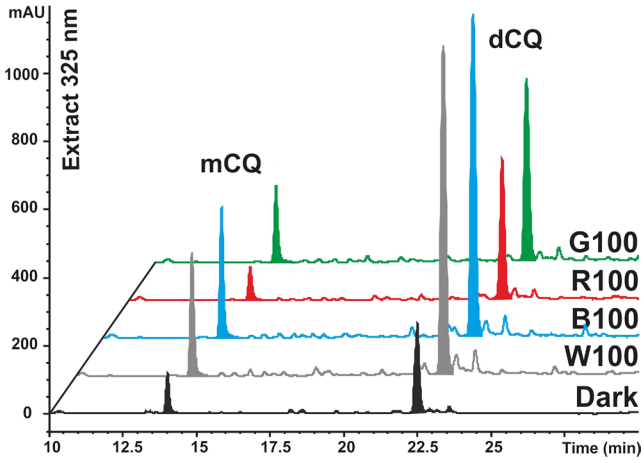


Fig. 4 HPLC–UV profiling of mCQ and dCQ identified in crude extracts of *C. cardunculus* calli recorded at 325 nm. Chromatograms are overlaid at the same scale for comparison of the samples obtained from the experiments: the warm white, blue, red, and green light treatments are designated W, B, R, and G, respectively, with intensities of 100 µmol $m^{-2}s^{-1}$. The peak at RT = 12.1 min (mCQ) was identified as chlorogenic acid. The peak at RT = 20.9 min (dCQ) was identified as 1,5-dicaffeoylquinic acid

the maximum UV–Vis absorbance by the external standard method using the available standards of chlorogenic acid and cynarin.

Compared with the dark-grown control, the third subculture under blue light led to increases in the contents of mCQ and dCQ by 4.5–5 and 3 times, respectively. However, after IV subculture, the mCQ and dCQ contents decreased gradually. Compared with the dark-grown control, the third subculture under warm white light led to a moderate increase of up to 250% for mCQ and dCQ, with a gradual decrease thereafter. The subsequent maintenance of *C. cardunculus* calli under green light led to a twofold increase in mCQ and dCQ contents during the five subcultures. Compared with those in the dark-grown control, the contents of mCQ and dCQ in the sixth subculture decreased more than twofold. Compared with the dark-grown control, the subsequent maintenance of *C. cardunculus* calli under red light did not significantly affect the contents of mCQ and dCQ (Fig. 5, Supplementary Table S3).

These findings reveal both parallels and inconsistencies with previously acquired data. The optimal conditions for the economic and energy-sustaining production of *E. sativa* plants with high aboveground FW accumulation and high flavonol accumulation were blue light with an intensity of 100 µmol $m^{-2}s^{-1}$ (Veremeichik et al. 2023). Furthermore, the amount of caffeic acid derivatives in the roots of *O. basilicum* plants is unaffected by red or green light (Veremeichik et al. 2024a). Red light increased the contents of lycopene, β-carotene, total phenolic compounds, and total flavonoids in both the outer and inner sections of the tomatoes (Sena et al. 2024). Compared with dark-grown calli, *V. corymbosum* cultivated under red light presented the highest anthocyanin concentration (Abou El-Dis et al. 2021). In *Gynura procumbens* calli, blue LED (50 µmol $m^{-2}s^{-1}$) treatment led to increases in phenolic content, total flavonoid content, and antioxidant activity (Lian et al. 2019). In comparison with the corresponding controls treated with white LEDs, the callus cultures of *R. chalepensis* and the field-grown plants showed the greatest accumulation of polyphenols under blue LED treatment (Juneja et al. 2022). Compared with white light, blue light significantly increased capsaicin and dihydrocapsaicin contents by 41.43-fold and 4.36-fold, respectively, in *Capsicum annuum* calli (Arya et al. 2025). Blue and red/blue light stimulates the accumulation of verbascoside and isoverbascoside in *Verbena officinalis* L. calli (Kubica et al. 2020). The highest atropine content was obtained under blue LED conditions in *Hyoscyamus niger* L. calli (Giglou et al. 2025). The same results were obtained for coumarins in *Ruta chalepensis* calli (Juneja et al. 2022).

Productivity of caffeoylquinic acids in *C. cardunculus* calli grown under different artificial lights during six subcultures

Thus, to adequately interpret the level of the stimulatory effect of light exposure on CAD biosynthesis, the productivity of light-treated calluses was calculated. To determine which light conditions are most effective, we compared the individual and total CAD productivity in light-treated *C. cardunculus* calli with that in dark-grown control calli (Figs. 6 and 7; Supplementary Table S3). Considering the influence of illumination on growth characteristics and CAD content, the most effective approach for the optimization of mCQ production in *C. cardunculus* calli involves three sequential subcultures under blue light exposure (Fig. 6, a; Supplementary Table S3). Under these conditions, the productivity of mCQ in *C. cardunculus* calli was four times greater than that in the dark-grown control calli and reached 50 mg L^{-1} of mCQ. However, the productivity of dCQ was the same after three sequential subcultures under blue and warm white light exposure. Under these conditions, the productivity of the dCQ in *C. cardunculus* calli was three times greater than that in the dark-grown control calli and reached 8 mg L^{-1} dCQ (Fig. 6, a; Supplementary Table S3). Sequential cultivation under green light had a weak activating effect on CAD productivity in *C. cardunculus* calli, with a more than twofold decrease by the sixth subculture. Cultivation under red light had no effect on CAD productivity in *C. cardunculus* calli (Fig. 6, a; Supplementary Table S3).

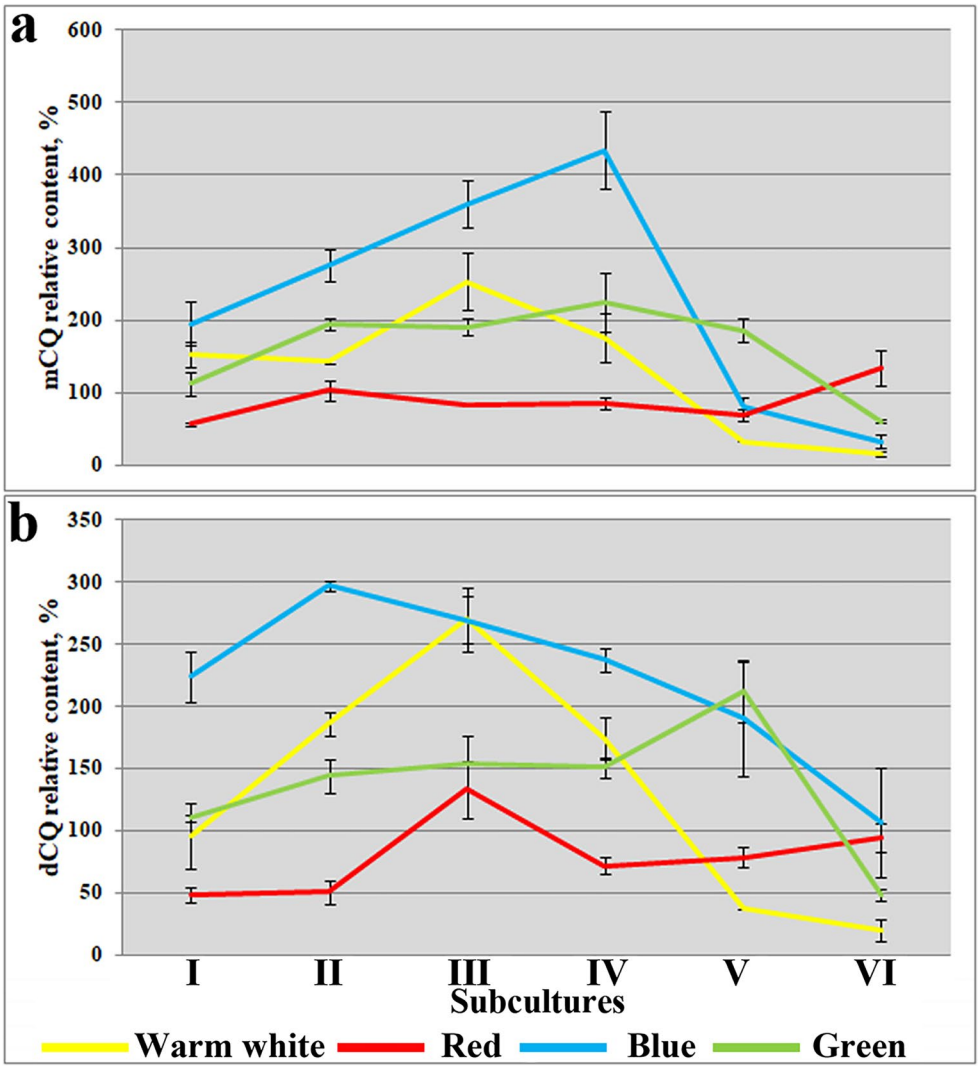


Fig. 5 Dynamics of the relative mCQ (**a**) and dCQ (**b**) contents of *C. cardunculus* callus cultures during continuous cultivation (six subcultures of 30 days each) under warm white, green, blue, and red light with an intensity of 100 µmol m^{-2} s^{-1}. The data are presented as a percentage of the dark-grown control. The absolute values of the mean ± standard error of the mean are presented in Supplementary Table S3. Statistical analysis of the data from the twelve replicates is presented in Supplementary Tables S6 and S7, and the red color of the table cell indicates a statistically significant difference (Tukey's biweight and Tukey's HSD, $p < 0.05$)

To determine the total productivity of the light-treated *C. cardunculus* calli grown under different artificial light conditions during subsequent continuous maintenance compared with the dark-grown control culture, the total content of CAD (CADSum) was calculated and represented as well as total CAD productivity in percentages (Fig. 7); absolute values are given in Supplementary Table S3. Considering the effects of light treatments on growth characteristics and total CAD content, the most effective approach for optimizing total CAD productivity in *C. cardunculus* calli involves two and three sequential subcultures under blue light and exposure (Fig. 7, b; Supplementary Table S3). Under blue light, CADsum productivity reached 135.41 and 134.84 mg L^{-1} after two and three subcultures, respectively, approximately three times greater than that of the dark-grown control (45.42 mg L^{-1}). Compared with dark-grown control calli, *C. cardunculus* calli cultivated under red light did not affect the productivity of CADsum (Fig. 7, b; Supplementary Table S3).

As seen from the data on growth and CAD content, when growth was inhibited in the 5th–6th subcultures under white and blue light, biosynthetic processes within the cells were likewise suppressed. Previously, it was shown that a single exposure (for one 30-day-long subculture) to blue light with an intensity of 50 µmol $m^{-2}s^{-1}$ increased the productivity of the CAD in *C. cardunculus* calli up to 8 times greater than that in the dark-grown control (Veremeichik et al. 2025b). In the present work, we investigated the long-term (six sequential subcultures) effect of light exposure on various parameters of callus tissue. We demonstrated that blue light with an intensity of 100 µmol $m^{-2}s^{-1}$ is more effective for second and third subcultures than for single-exposure subcultures.

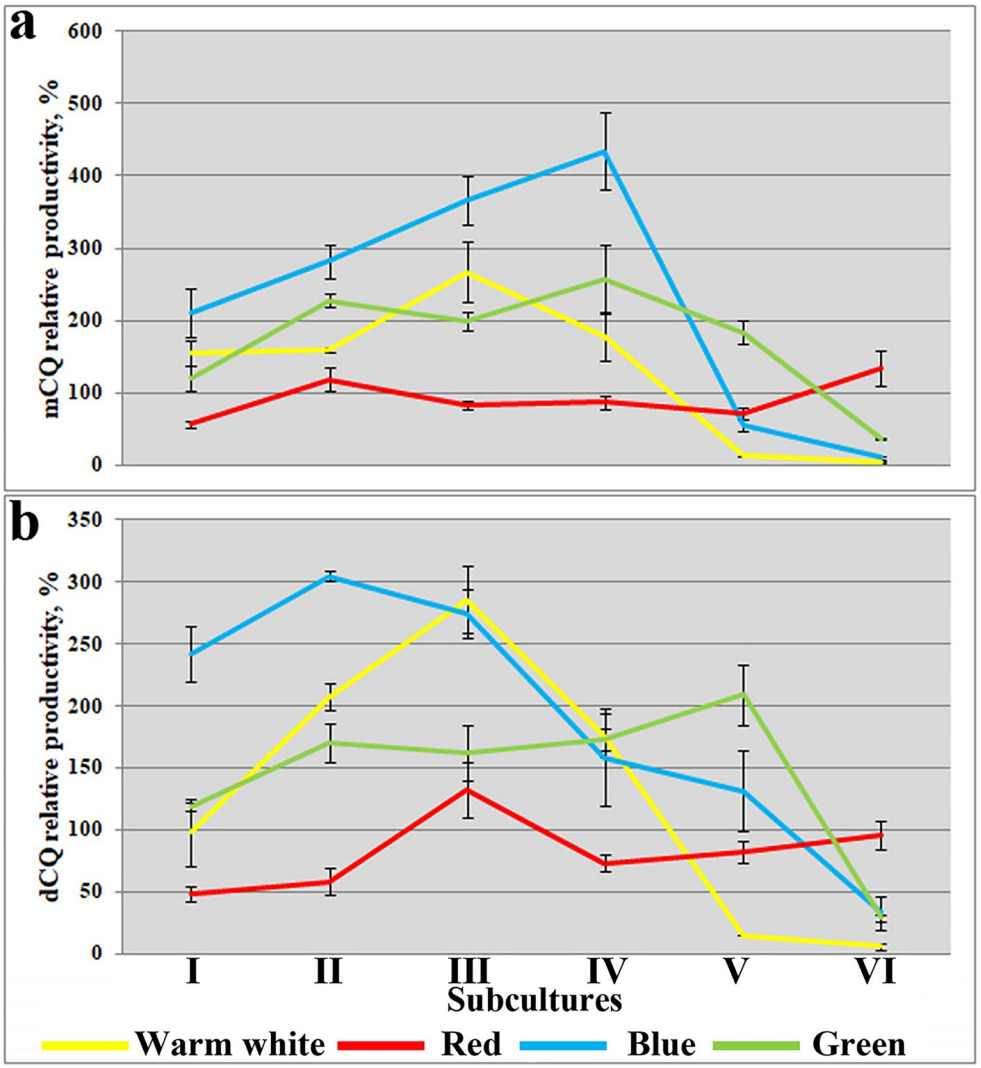


Fig. 6 Dynamics of the relative productivity of mCQ (**a**) and dCQ (**b**) of *C. cardunculus* callus cultures during continuous cultivation (six subcultures of 30 days each) under warm white, green, blue, and red light with an intensity of 100 µmol m^{-2} s^{-1}. The data are presented as a percentage of the dark-grown control. The absolute values of the mean ± standard error of the mean are presented in Supplementary Table S3. Statistical analysis of the data from the twelve replicates is presented in Supplementary Tables S8 and S9, and the red color of the table cell indicates a statistically significant difference (Tukey's biweight and Tukey's HSD, $p < 0.05$)

Conclusion

As a noninvasive and environmentally benign way to alter physiological and biochemical processes in plant cells, artificial illumination is currently being extensively investigated. A unique feature of the present study is its prolonged duration. We investigated for the first time the effect of monochromatic illumination during repeated sequential subcultures of callus tissue. We used a culture maintained in darkness for 13 years. The absence of significant deviations in the data obtained (stable growth, a uniform biosynthetic response to the stimulus) is due to the stability and homogeneity of the culture used. This culture can be considered completely stabilized and has lost its ability to undergo morphogenesis. We demonstrated the effects of red, blue, and green monochromatic illumination on growth, morphology, IAA homeostasis, and CAD biosynthesis during six months of sequential subculture. We compared the obtained results not only with those of the control growing in the dark but also with those of long-term cultivation under standard warm white light. The study's extended duration is one of its distinctive features. The impact of monochromatic illumination during repeated consecutive subcultures of *C. cardunculus* callus tissue was examined for the first time. We employed a culture that was kept in the dark for thirteen years. During a six-month repeated subculture, we showed how red, blue, and green monochromatic illumination affected growth, morphology, IAA homeostasis, and CAD biosynthesis. We compared the outcomes of both long-term culture under typical warm white light and control growth in the dark. First, too much blue light, both in terms of intensity (according to the previously obtained data) and duration of exposure, led to statistically significant (Fisher's LSD test, $p < 0.05$) growth inhibition. However, extended exposure to red light had no negative effect on the growth of the callus tissue. Second, it can be hypothesized that blue light upsets the hormonal balance, causing parenchymatous

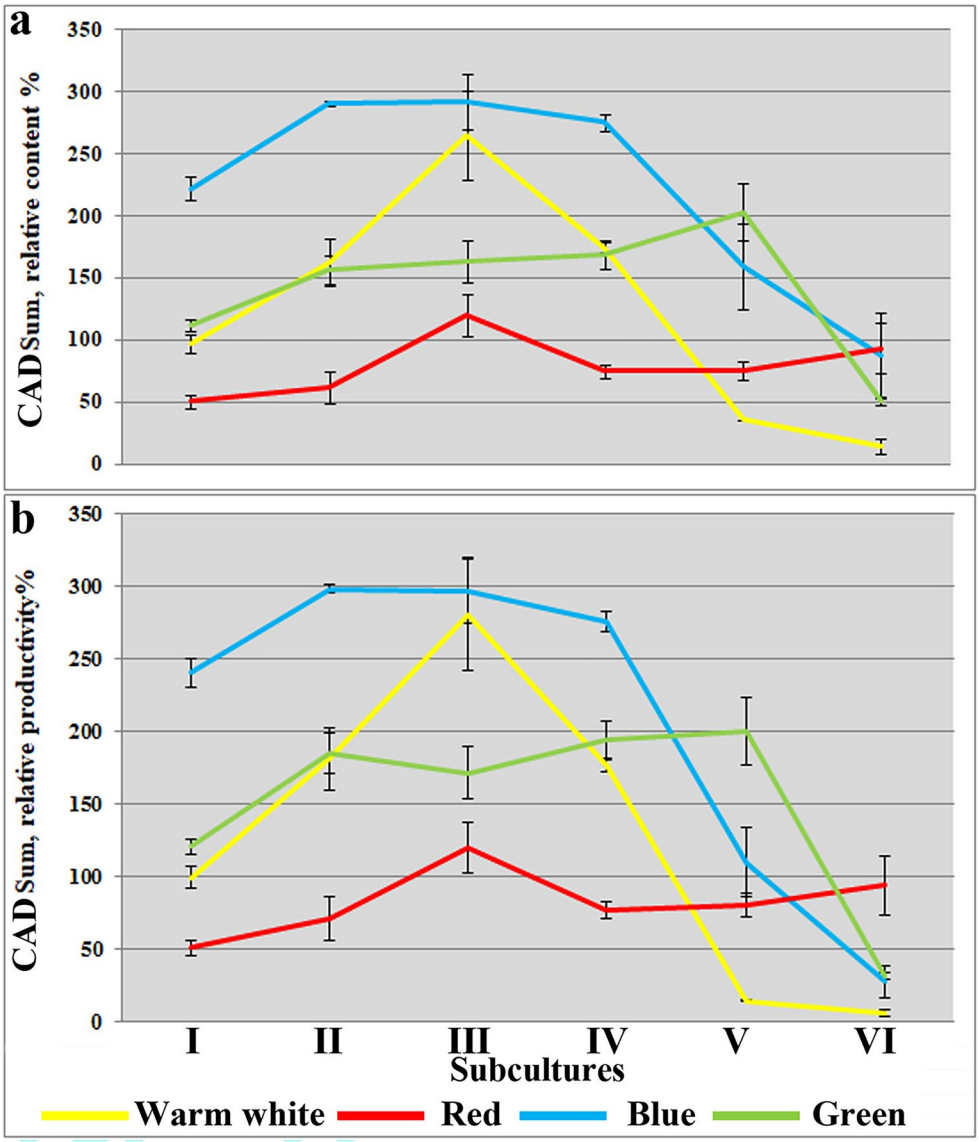


Fig. 7 Dynamics of the relative content (**a**) and productivity (**b**) of CADsum in *C. carduncul*us callus cultures during continuous cultivation (six subcultures of 30 days each) under warm white, green, blue, and red light with an intensity of 100 µmol m^{-2} s^{-1}. The data are presented as a percentage of the dark-grown control. The absolute values of the mean ± standard error of the mean are presented in Supplementary Table S3. Statistical analysis of the data from the twelve replicates is presented in Supplementary Tables S10 and 11, and the red color of the table cell indicates a statistically significant difference (ANOVA, Fisher's LSD, $p < 0.05$)

tissue to degenerate into vascular tissue, which in turn inhibits growth. On the other hand, the absence of a negative effect of red light on cell growth may be linked to the stabilization of the auxin balance. Red light therefore has the greatest growth-stimulating effect. Two and three repeated subcultures under blue light are the most effective approaches for optimizing the total CAD productivity in *C. carduncul*us calli, taking into account the impact of the light treatments on growth parameters and CAD content. Compared with that of the control calli cultivated in darkness, the CAD productivity of *C. carduncul*us calli produced under warm white, blue, and green light significantly decreased after the fifth subculture—more than quadrupling. Compared with that of the dark-grown control calli, the productivity of CADSum in *C. carduncul*us calli was unaffected by cultivation under red light.

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Author contributions G.N. Veremeichik: Conceptualization, Data curation, Project administration, Supervision, Validation, Visualization, Writing – original draft.G.N. Veremeichik; V.P. Grigorchuk; S.A. Silantieva; E.P. Subbotin; E.V. Brodovskaya; O.A. Tikhonova; S.O. Kozhanov, T.Y. Gorpenchenko, V.M. Kazarin,: Investigation, Methodology, Formal Analysis, Writing – original draft.

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Data availability No datasets were generated or analysed during the current study.

Code availability Not applicable.

Declarations

Consent for publication All the authors whose names appeared on the submission approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that the questions related to the accuracy of integrity of any part of the work were appropriately investigated and resolved.

Ethics approval Not applicable.

Consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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