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ORIGINAL ARTICLE

Differential regulation of caffeoylquinic acid production in *Cynara cardunculus* var. altilis DC calli by modulating qualitative and quantitative LED-lighting parameters

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Abstract

Numerous bioactive secondary metabolites (SMs) of plants are used in a variety of industries. Plant biotechnology has made significant advances and applications of callus cultures in pharmacology, pharmaceuticals, horticulture, and agriculture have emerged over the past few decades. The search for effective, environmentally friendly, and nature-like approaches to activate the biosynthesis of SMs in plant cell cultures is an urgent task. A modern approach that meets these requirements is the use of modulated light-emitting diode (LED) lighting. In the present work, we investigated the ability of modulated LEDs to increase the productivity of callus cultures of artichoke (*Cynara cardunculus* var. altilis DC) cultivated continuously for more than 150 passages. The Mediterranean crop artichoke is useful in pharmaceuticals because of its caffeic acid derivatives (CADs). In the present study, we investigated the effects of warm white and monochromatic lights with a wide range of intensities (50–600 µmol m^{-2} s^{-1}) on the growth and biosynthetic capacity of *C. cardunculus* calli. Red light had the greatest growth-stimulating effect regardless of intensity. Cultivation under red light at an intensity of 100 µmol m^{-2} s^{-1} improved the growth of calli more than 1.7-fold. We observed changes in the contents of the two most abundant defined caffeoylquinic acids in *C. cardunculus* calli, monocaffeoylquinic acid (mCQ) and dicaffeoylquinic acid (dCQ). Considering growth and content, low-intensity (50 µmol m^{-2} s^{-1}) blue light, which was more than 10 times greater (71 mg/L) than the dark-grown control, was the most effective treatment for increasing caffeoylquinic acid productivity in *C. cardunculus* calli. The effect of red light on the productivity of caffeoylquinic acids was not as strong but was stable and did not depend on intensity.

Keywords Artificial light · Caffeoylquinic acids · Callus culture · *Cynara cardunculus* · LED · Long-term cultured calli

Introduction

Numerous bioactive plant-derived secondary metabolites (SMs) are used in food, cosmetics, agriculture, pharmaceuticals and other industries. Plant biotechnology has made significant advances, and numerous applications of callus and suspension cell cultures have emerged in pharmacology, pharmaceuticals, horticulture and agriculture over the past few decades (Efferth 2019). As a result of the high demand, SMs must be acquired in large quantities. In addition to production via traditional methods, biotechnology approaches can be used to attain a large output. There are various ways that plant biotechnology can be used for this purpose. Among these techniques, tissue culture and gene transfer are the most crucial. In addition to being sources of numerous secondary metabolites with therapeutic value,

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genetically modified plants are more productive and efficient in the marketplace, making them useful instruments for both industrial and medical applications (Ozyigit et al. 2023). However, in most countries, the use of GMOs is against the law. Thus, the search for effective, environmentally friendly and nature-like approaches to activate secondary metabolism in plant cell cultures is an urgent task. A modern approach that meets these requirements is the use of modulated light-emitting diode (LED) lighting for the cultivation of plant cell cultures.

In the present work, we investigated the possibility of increasing the productivity of the callus culture of cultivated artichoke or *Cynara cardunculus* var. altilis DC (Asteraceae) via modulated LED light. We used a cell culture obtained previously, in 2012, from stem explants that were continuously cultivated for more than 150 passages. Control untransformed *C. cardunculus* calli were not investigated because they were used as source material for genetic transformation (Vereshchagina et al. 2014). In 2014, two transgenic cell cultures were obtained from control untransformed *C. cardunculus* calli: empty vector-transformed calli and calli transformed with the *Agrobacterium* oncogene *rolC*. The caffeoylquinic acid content of empty vector-transformed calli could be extrapolated to those of untransformed *C. cardunculus* calli. Thus, the contents of mCQ and dCQ in vector-transformed calli were approximately 1.1 and 5.4 mg/g DW, respectively (Vereshchagina et al. 2014). Transformation of this culture with the agrobacterial oncogene *rolC* led to a threefold increase in the productivity of CAD (Vereshchagina et al. 2014). The Mediterranean crop artichoke is useful in the pharmaceutical, nutraceutical, and green chemistry industries because of its high biomass and secondary metabolite production as well as its superior climate change adaptation (Abbas et al. 2022). The pharmaceutical properties of artichoke are due to its high content of caffeic acid derivatives (CADs). Caffeoylquinic acids (CQs) exhibit hepatoprotective, antiviral, antioxidative, peroxynitrite-scavenging, antiobesity, and antidiabetic properties (Park 2010). As observed in Alzheimer's disease, diCQs have a strong ability to scavenge free radicals and protect the brain from long-term inflammatory stress (Alcázar Magaña et al. 2021). Chlorogenic acids and caffeine from coffee byproducts are effective for skin care (Rodrigues et al. 2023). 5-Caffeoylquinic acid inhibits melanogenesis by chelating copper cations from the copper–tyrosinase complex. Thus, 5-caffeoylquinic acid may be useful in cosmetics as a skin whitening agent (Kim et al. 2020).

Plant growth and metabolism are influenced by both the quantity and quality of light, including the photoperiod and light intensity (Cavallaro and Muleo 2022). Because of their greater luminous efficiency and lower power consumption than traditional fluorescent lights do, light-emitting diode (LED) lights are widely used in the growth of many plant species, particularly horticultural plants. LED light affects postharvest quality, plant defence, cell wall construction, gene expression, and enzyme function (Sena et al. 2024). A range of bioactive plant secondary metabolites, including alkaloids (such as vinblastine), phenolics, flavonoids, and terpenoids, are produced as a result of stress caused by various light sources, including UV light but also excessive light. A variety of light sources, including UV, fluorescent, and light-emitting diodes (LEDs), can elicit secondary metabolites (Hashim et al. 2021; Zhang et al. 2021; Veremeichik et al. 2023a, b).

We previously demonstrated that the ability of long-term-grown calli of *Mertensia maritima* L. (Boraginaceae) to produce rosmarinic acid declined over 12 years of growth. This process can be overcome using monochromatic light. Treatment with green light (510–520 nm) at normal (100 µmol m^{-2} s^{-1}) or moderate (300 µmol m^{-2} s^{-1}) intensities led to 2- and 3.5-fold increases in rosmarinic content, respectively (Veremeichik et al. 2024b). Thus, in the present study, we investigated the effects of warm white and monochromatic light with a wide range of intensities (50–600 µmol m^{-2} s^{-1}) on the growth and biosynthetic capacity of *C. cardunculus* callus cultures during long-term cultivation.

Materials and methods

Plant materials, growth conditions, and experimental design

The callus line *C. cardunculus* was obtained previously, in 2012, from stem explants (Vereshchagina et al. 2014). The modified Murashige–Skoog media (ammonium nitrate content was reduced to 400 mg/L) was supplemented with the following components (mg/L): nicotinic acid (0.5), thiamine-HCl (0.2), meso-inositol (100), peptone (100), pyridoxine-HCl (0.5), sucrose (25,000), agar (6000), 6-benzyladenine (BA, 0.5) and α-naphthaleneacetic acid (NAA, 2.0). *C. cardunculus* calli were continuously cultivated in the same medium in the dark at 24 °C for 12 years and subcultured once every 30 days. For the experiments, we used 12-year-old continuously cultivated *C. cardunculus* calli. 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). Calli growing in the dark were used as a control. 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. Three separate experiments were conducted as biological replicates. As a technical replication, ten jars were exposed to each type of

light for each experiment. 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 growth chamber construction

The calli were cultivated in four-section chambers (100 × 50 × 50 cm, Supplementary Fig. S2) with light sources designed and manufactured at the IACP FEB RAS. The light source matrices were made up of 24 three-watt (CHANZON, China) LEDs of various colors, creating one integrated light source as described previously (Veremeichik et al. 2024b). The chambers were covered with reflective aluminum foil to diffuse light, and an FFB1212SH 12025 exhaust fan (power: 14.8 W, speed: 3700 rpm, air volume: 140.16 cfm, China) was used to maintain the temperature (24 °C). 70% air humidity was maintained, and the photoperiod (light/dark) lasted 16–8 h. The same spectrum, with varying intensities in each divided section, was produced inside the chamber. Chambers of different wavelengths were distant from one another to reduce the mutual influence of light of different spectra on each other.

In this study, in addition to warm white light (designated "W"), the following varieties of monochromatic light were used: red (660 nm, designated "R"), blue (440 nm, designated "B"), and green (520 nm, designated "G"). Each section of the chamber was equipped with LED lamps with different light intensities: 50, 100, 300, and 600 µmol m^{-2} s^{-1}. Each segment had 1–10 light-emitting matrices, which produced the requisite level of photosynthetic photon flux density (PPFD). The minimum intensity was provided by a matrix with a minimum number of LEDs. An increase in the PPFD was achieved by increasing the current strength and the number of matrices. The intensity of the light in each chamber was adjusted by altering the supply current for each matrix. The spectra and actual PPFD were measured with a PG200N spectrophotometer (UPRtek, Taiwan). Currents in the driver supply system were managed by a UT61A digital multimeter (Uni-T, China). The normalized spectra of the light sources are shown in Supplementary Fig. S2. The main characteristics of the light are listed in Supplementary Table S1.

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

Chemicals

Analytical standards of CQAs (chlorogenic acid and cynarin) were obtained from Sigma−Aldrich (St. Louis, MO, USA). All the eluents and extraction solutions were prepared with ultrapure water (Millipore, Bedford, MA, USA). All solvents were of analytical grade.

Sample preparation for analytical chromatography

Polyphenol extraction was performed according to a previously published protocol (Veremeichik et al. 2024b). Briefly, ultrasonic extraction of dried and powdered callus material in 80% aqueous methanol was performed. The extracts were purified via a 0.45-µm membrane (Millipore, Bedford, MA, USA) before HPLC analysis.

Analytical chromatography and mass spectrometry

The polyphenol extracts were studied at the Instrumental Centre of Biotechnology and Gene Engineering of IBSS FEB RAS using an 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) for polyphenol separation was applied at 40 °C. The mobile phase consisted of a gradient elution of ultrapure water (A) and acetonitrile (B) with 0.1% formic acid added in both cases. The following linear gradient at a flow rate of 0.2 mL/min was used: 0 min, 5% B; 20 min, 30% B; and 30 min, 100% B. A photodiode array detector was employed in the range between 200 and 600 nm to obtain UV−Vis spectra. Chromatograms for quantification were recorded at a wavelength of 325 nm. The MS instrument was operated in electrospray ionization (ESI) mode, and negative ions were detected. The following settings were used: the range of *m/z* detection was 100–650, the drying gas (N_2) flow rate was 8.0 L/min, the nebulizer gas (N_2) pressure was 25 psi, the ion source potential was −3.8 kV, and the drying gas temperature was 325 °C. Tandem mass spectra were acquired in Auto-MS^2 mode (smart fragmentation) by increasing the collision energy. The fragmentation amplitude was set to 1 V.

The productivity of CAD was calculated as follows:

$$\text{Productivity (mg/L)} = \text{Content} \times \text{DW},$$

where Content denotes the content of CAD (mg/g DW) and DW denotes the dry weight (g) of the callus biomass per liter of medium (g/L).

Energy efficiency was calculated for each container. The total lamp power was divided into 20 jars (1 L of culture medium) for each experimental light treatment and was reduced to a total value per kilowatt hour. The energy efficiency for growth was calculated as follows:

$$\text{Energy costs } \left(\text{g h}^{-1}\text{l}^{-1}\text{kW}^{-1}\right) = \text{P}/(1000/\text{Power})/\text{Total time},$$

where P denotes the productivity of a compound of *C. cardunculus* calli per liter of culture medium (mg/L), power denotes the total lamp power, and total time denotes the total hours of light (480 h for each experiment).

Statistical analysis

The STATISTICA software package (StatSoft, Inc., USA) was used for the statistical analysis. All values are presented as the mean±standard error (SE). Student's t test was employed for the statistical assessment to compare two independent groups. Analysis of variance (ANOVA) was used, together with a multiple comparison approach, to compare several datasets. The cut-off point for statistical significance was fixed at $p < 0.05$.

Results

The growth of *C. cardunculus* calli cultivated under different light treatments

We investigated the growth parameters of *C. cardunculus* calli cultivated under different light conditions for 30 days. The callus cultures were grown in four-section chambers. Warm white (W) and the following monochromatic lighting options were used: red (R), blue (B), and green (R) with different characteristics, as described in the "Materials and Methods" section. The light intensities chosen for the experiments were low (50 and 100 µmol m^{-2} s^{-1}), moderate (300 µmol m^{-2} s^{-1}) and high (300 µmol m^{-2} s^{-1}). Callus cultures grown in the dark were used as controls.

As shown in Fig. 1, compared with the control dark conditions, light treatments with low intensities (50 and 100 µmol m^{-2} s^{-1}) did not affect the growth of the *C. cardunculus* callus culture during a single passage. The exception was red light with low intensities (50 and 100 µmol m^{-2} s^{-1}), which led to increases in growth of 1.5 and 1.7 times, respectively. Increasing the light intensity to 300 µmol m^{-2} s^{-1} led to a significant decrease (more than 2.5 times) in blue light and had no effect on the other light variants. Moreover, *C. cardunculus* calli were much darker (Fig. 1a; Supplementary Table S2) than those of the control or other light variants at an intensity of 300 µmol m^{-2} s^{-1}. A further increase in intensity to 600 µmol m^{-2} s^{-1} resulted in the darkening of calli growing under white and green light, accompanied by decreases in growth of 1.6 and 1.3 times, respectively, compared with the control. In addition, red light with an intensity of 600 µmol m^{-2} s^{-1} had no effect on the growth of the calli, whereas blue light with an intensity of 600 µmol m^{-2} s^{-1} led to total growth inhibition compared with the control (Fig. 1b; Supplementary Table S2).

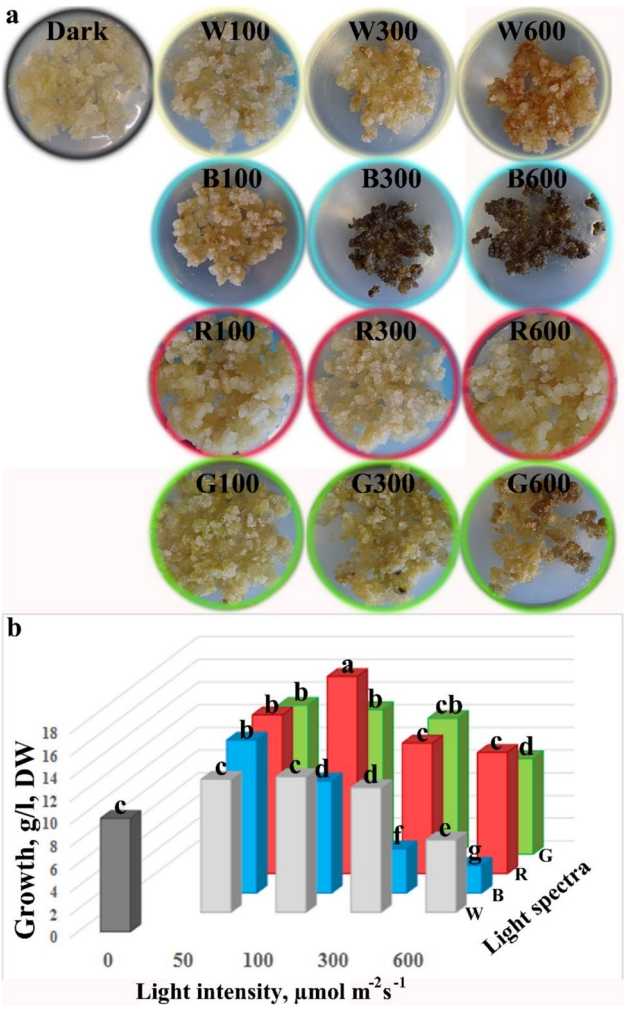

Fig. 1 The growth of *C. cardunculus* callus cultures cultivated under different light treatments. Morphology (**a**) and biomass accumulation (**b**, g/L) of *C. cardunculus* 30-day-old calli (2 g FW inoculants per 50 ml of solid medium) grown for 30 days under different light treatments: D, darkness; warm white, blue, red, and green light treatments are designated W, B, R, and G, respectively. Listed light variants were used with intensities of 50, 100, 300 and 600 µmol m^{-2} s^{-1}. The data obtained from three independent experiments with ten biological replicates are presented as the means, and the different letters above the bars indicate statistically significant differences (ANOVA, $p < 0.05$). The absolute values of the mean±standard error of the mean are presented in Supplementary Table S2

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

The long-cultivated *C. cardunculus* callus culture has been studied previously (Vereshchagina et al. 2014) and, to date, has successfully produced caffeoylquinic acids. In the present work, we observed changes in the contents of two of the most abundant defined caffeoylquinic acids in *C. cardunculus* calli grown under light treatment (Fig. 2, Supplementary Fig. S2), monocaffeoylquinic acid (mCQ,

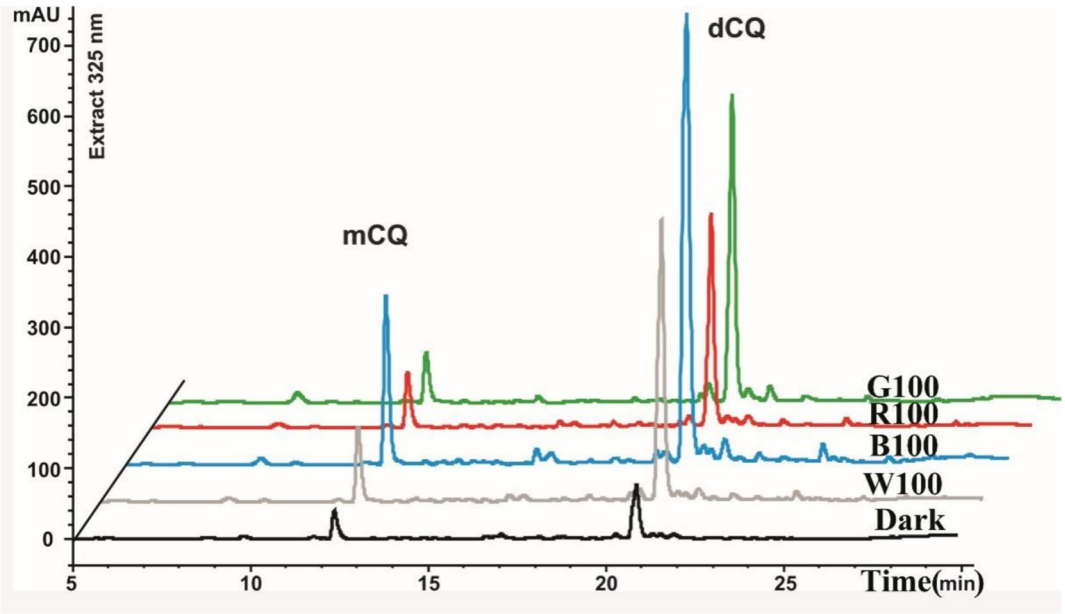

Fig. 2 HPLC–UV profiling of CQs identified in crude extracts of *C. cardunculus* calli recorded at 325 nm. Chromatograms are overlaid at the same scale for comparison of the samples obtained from the experiments: D, darkness; warm white, blue, red, and green light treatments are designated W, B, R, and 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 (dCQA) was identified as 1,5-dicaffeoylquinic acid

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). Mass spectrometric studies were carried out to confirm the previous identification. The UV and MS data of these compounds correlated well with the data obtained earlier (Vereshchagina et al. 2014). The CQAs were quantified by HPLC-UV at the wavelength of the maximum UV−Vis absorbance by the external standard method using the available standards of chlorogenic acid and cynarin. After 13 years of continuous cultivation, the contents of mCQ and dCQ in the *C. cardunculus* calli grown in the dark were 0.28 and 0.7 mg/g DW, respectively, which are almost 8 times lower than those reported for the vector culture 13 years prior (Vereshchagina et al. 2014).

Different light treatments had similar effects on the biosynthesis of both mCQ and dCQ (Fig. 3, Supplementary Table S2). Warm white light led to increases in mCQ and dCQ contents in a dose-dependent manner. With increasing W light intensity from 50 to 100 and 300 µmol m^{-2} s^{-1}, the contents of mCQ and dCQ increased by 4, 5, and 8 times, respectively, compared with those of the dark-grown control. However, increasing the W light intensity to 600 µmol m^{-2} s^{-1} led to a decrease in the CAD content up to the control level. The blue light treatment had another effect on CAD biosynthesis. Compared with the control, blue light treatment at low intensities (50 and 100 µmol m^{-2} s^{-1}) led to an approximately eightfold increase in the contents of both mCQ and dCQ, whereas high intensities (300 and 600 µmol m^{-2} s^{-1}) led to full inhibition of CAD biosynthesis. Red light treatment led to a threefold increase in the content of both mCQ and dCQ regardless of intensity. The same effect was shown for the green light treatment. The CAD content was more than 7 times greater in the green light-treated calli than in the control calli, regardless of light intensity (Fig. 3, Supplementary Table S2).

Productivity and energy efficiency of the different artificial lights of CAD in *C. cardunculus* calli

Compared with the dark-grown control, different light treatments had different effects on the growth and biosynthesis of individual phytochemical compounds in *C. cardunculus* calli. To evaluate which of the light treatments are most effective, we analyzed the productivity of both individual compounds (Fig. 4, Supplementary Table S2) and their sum in the *C. cardunculus* callus culture. In terms of growth and content, low-intensity (50 µmol m^{-2} s^{-1}) blue light was the most effective treatment for increasing mCQ and dCQ productivity in *C. cardunculus* calli compared with that of the dark-grown control, which was more than 10 and 8 times greater (23 and 48 mg/L), respectively (Fig. 4, Supplementary Table S2). Warm white light with an intensity of 300 µmol m^{-2} s^{-1} had a similar effect but to a lesser extent. The effect of red light on the productivity of mCQ and dCQ was not as strong but was stable and did not depend on intensity. Green light had a stable, intensity-independent

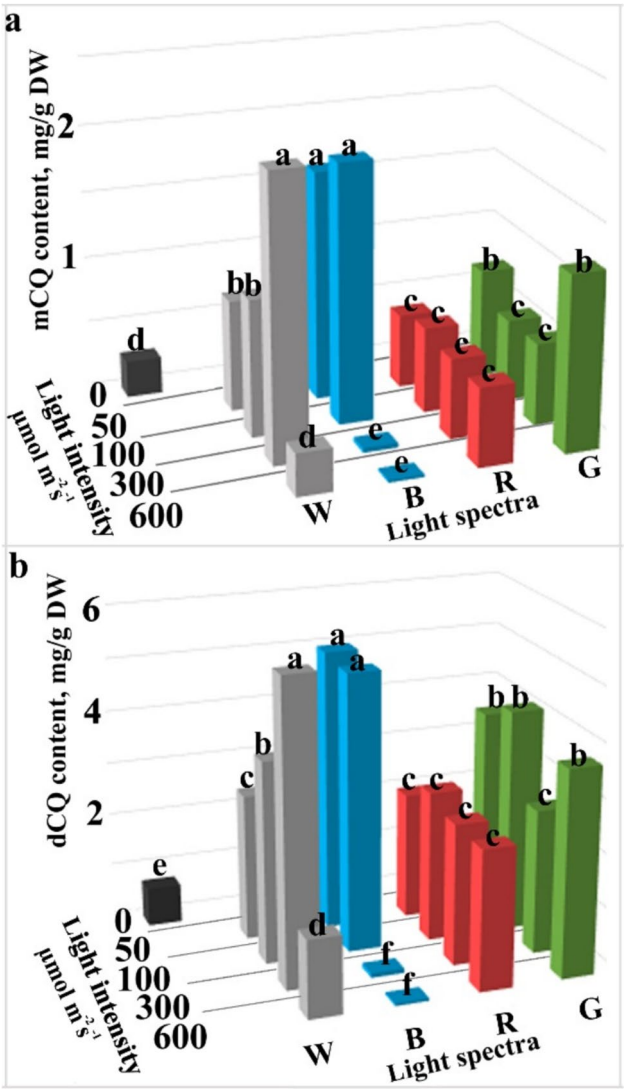

Fig. 3 Contents of CQ acids in *C. cardunculus* calli grown under different lighting conditions. Contents of mCQ (**a**) and dCQ (**b**) acids in *C. cardunculus* calli (mg/g DW) grown for 30 days under different light treatments: D, darkness; warm white, blue, red, and green light treatments are designated W, B, R, and G, respectively. Listed light variants were used with intensities of 50, 100, 300 and 600 µmol m^{-2} s^{-1}. The data obtained from three independent experiments with ten biological replicates are presented as the means, and the different letters above the bars indicate statistically significant differences (ANOVA, $p < 0.05$). The absolute values of the mean±standard error of the mean are presented in Supplementary Table S2

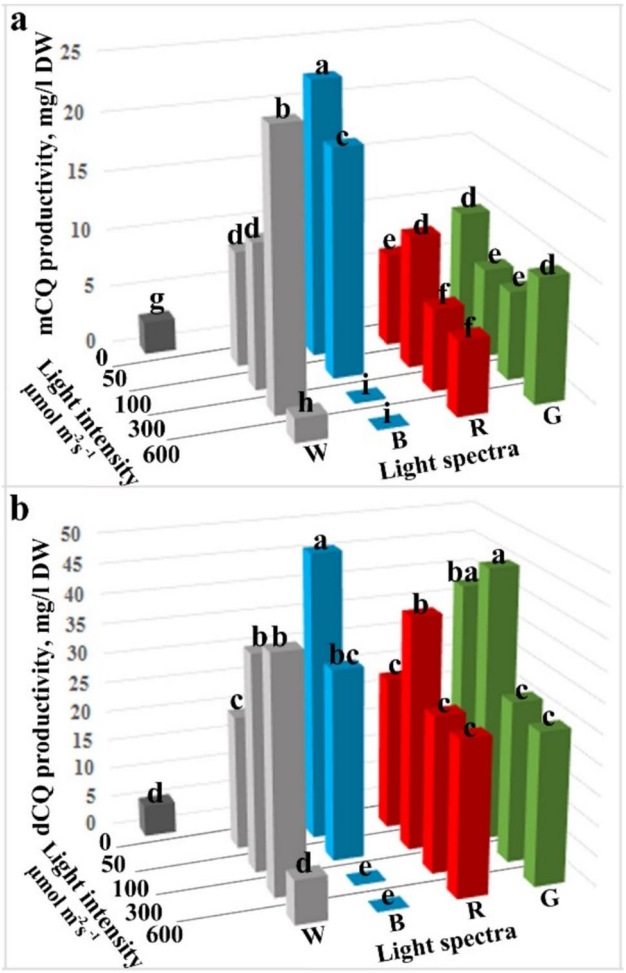

Fig. 4 Production of CQ acids in *C. cardunculus* calli grown under different lighting conditions. Production of mCQ (**a**) and dCQ (**b**) acids in *C. cardunculus* calli (mg/L) grown for 30 days under different light treatments: D, darkness; warm white, blue, red, and green light treatments are designated W, B, R, and G, respectively. Listed light variants were used with intensities of 50, 100, 300 and 600 µmol m^{-2} s^{-1}. The data obtained from three independent experiments with ten biological replicates are presented as the means, and the different letters above the bars indicate statistically significant differences (ANOVA, $p < 0.05$). The absolute values of the mean±standard error of the mean are presented in Supplementary Table S2

effect on the productivity of mCQ, while the productivity of dCQ significantly decreased with increasing light intensity (Fig. 4, Supplementary Table S2).

Analyzing the energy efficiency of the various artificial light variations for CAD productivity in *C. cardunculus* calli was the last phase of the study. The content of the sum of CAD was calculated for calli grown under different light treatments compared with the control dark-grown calli. The maximum CAD sum content was shown for *C. cardunculus* calli grown under white light with an intensity of 300 µmol m^{-2} s^{-1} and blue light with intensities of 50 and 100 µmol m^{-2} s^{-1}, up to 5.7 mg/g DW, which was 7 times greater than that of the dark-grown control calli (Fig. 5a, Supplementary Table S2). An increase in the intensity of both white and blue light led to a decrease in the CAD content. Red light treatment had a stable, intensity-independent effect on the CAD content. The increase in the CAD content under red light was no more than 3.5 times greater than that in the dark-grown calli. The green light treatment had

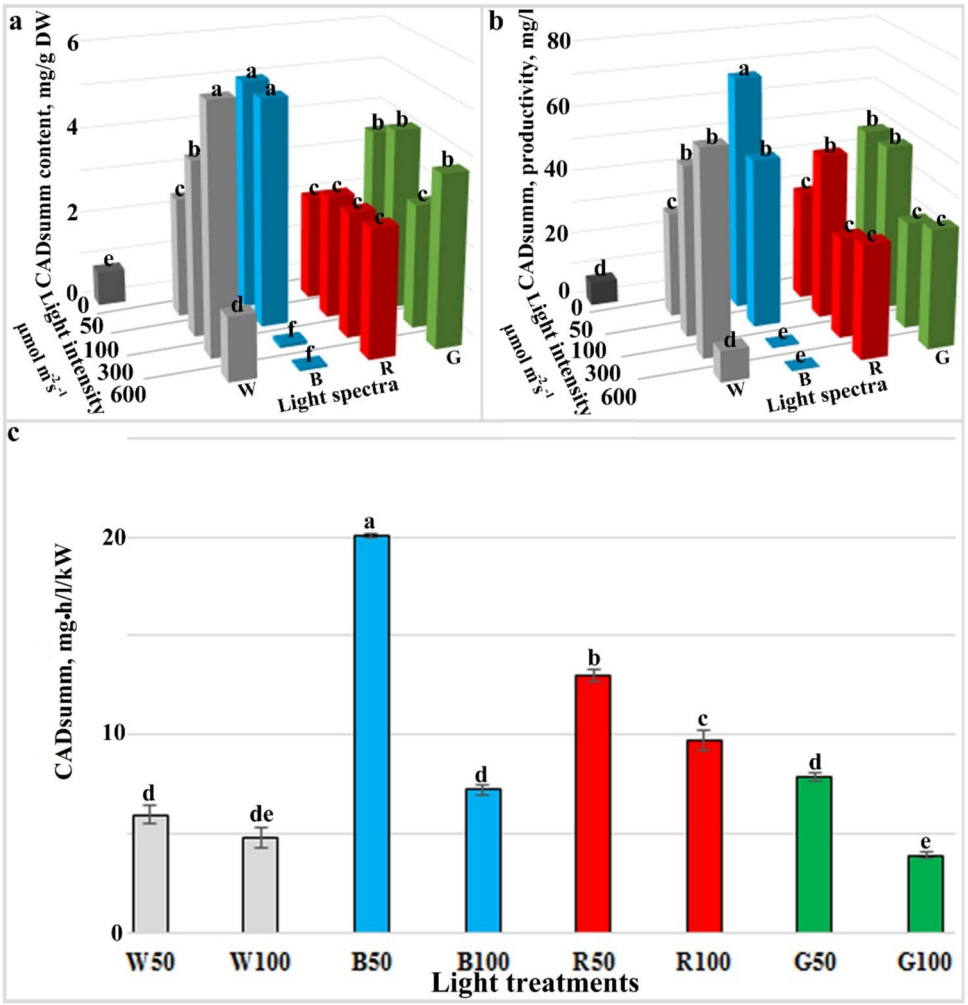

Fig. 5 Content, production, and energy efficiency of the sum of CQ acids in *C. cardunculus* calli grown under different lighting conditions. Content (**a**, mg/g DW) and production (**b**, mg/L) of sum of CQ acids (CADsumm) in *C. cardunculus* calli grown for 30 days under different light treatments: D, darkness; warm white, blue, red, and green light treatments are designated W, B, R, and G, respectively. Listed light variants were used with intensities of 50, 100, 300 and 600 μmol m^{-2} s^{-1}. The data obtained from three independent experiments with ten biological replicates are presented as the means, and the different letters above the bars indicate statistically significant differences (ANOVA, $p < 0.05$). The absolute values of the mean±standard error of the mean are presented in Supplementary Table S2. Energy efficiency (**c,** mg $h^{-1}l^{-1}kW^{-1}$) of the different artificial light treatments with intensities of 50 and 100 μmol m^{-2} s^{-1} for CADsumm productivity. Data are presented as the mean±standard error of the mean, and different letters above the error bars indicate the presence of statistical significance (ANOVA, $p < 0.05$)

a similar effect. The effect of light exposure more clearly shows the productivity of the CAD sum (Fig. 5b, Supplementary Table S2). Thus, the most effective treatment for CAD production is blue light treatment at an intensity of 50 μmol m^{-2} s^{-1}, which results in a yield rate of 70 mg/L, which is tenfold greater than that of the dark-grown control. For the warm white light treatments, the greatest effect was shown for the intensity of 300 μmol m^{-2} s^{-1}, up to 60 mg/L, which was eightfold greater than that of the dark-grown control. An increase in CAD productivity of up to 50 mg/L, which was sevenfold greater than that in the dark-grown control, was shown for the red light treatment, with an intensity of 100 μmol m^{-2} s^{-1}, and the green light treatment, with intensities of 50 and 100 μmol m^{-2} s^{-1}.

Next, we analyzed the energy efficiency for the most effective light variance. On the basis of the indicators of the productivity of CAD sum in light-treated *C. cardunculus* calli, we calculated the energy efficiency for different light intensities of 50 and 100 μmol m^{-2} s^{-1} because these intensities were the most effective for CAD biosynthesis induction without growth inhibition (Fig. 5c). A slightly lower energy efficiency was shown for red light with intensities of 50 and 100 μmol m^{-2} s^{-1}. The energy efficiency of the other lighting options was more than 3 times lower than that of blue light with an intensity of 50 μmol m^{-2} s^{-1} (Fig. 5c).

Discussion

Modulation of LED light quality can regulate plant secondary metabolism and this effect is species-specific (Veremeichik et al. 2023a, b; Veremeichik et al. 2024b; Taulavuori et al. 2017). We hypothesized that light exposure at different intensities and spectra can induce the biosynthesis of caffeoylquinic acids in long-term cultivated *C. cardunculus* callus cultures. Different light intensities can either reduce or stimulate plant growth (Parrine et al. 2021; Yavari et al. 2021). It is believed that with long-term perennial cultivation, the ability of calli not only to regenerate but also to produce secondary metabolites decreases (Liu et al. 2009). In the present work, we analyzed the growth and biosynthetic characteristics of 13-year-old continuously cultivated (more

than 150 passages) *C. cardunculus* callus cultures obtained in 2012. Unfortunately, the control *C. cardunculus* calli were not investigated because they are used as source material for genetic transformation (Vereshchagina et al. 2014). However, we hypothesized that the growth parameters and caffeoylquinic acid content of empty vector-transformed calli could be extrapolated to those of untransformed *C. cardunculus* calli. Thus, the growth of the vector-transformed calli was approximately 19 g/L DW. As we showed in the present work, after 13 years of continuous cultivation, the growth of *C. cardunculus* calli in the dark was less than 10 g/L DW. We suggest that long-term cultivation may be the reason for this significant (approximately twofold) decrease in growth.

Little is known about how the growth of a callus culture changes after more than ten years of continuous cultivation. In our recent work, we showed that a decrease in secondary metabolite biosynthesis in long-term cultured calli was not accompanied by a decrease in growth (Veremeichik et al. 2024b, 2025). We supposed that intensive growth was a feature of the young culture, and with age, it stabilized to a comfortable level. However, cultivation under red light at an intensity of 100 µmol m^{-2} s^{-1} improved the growth of old calli to the juvenile level (Veremeichik et al. 2024b). The data obtained in the present work coincide with the results obtained previously as well as for callus culture and plants: the biomass accumulation of *Hypericum perforatum* callus cultures was significantly ($p < 0.05$) greater when the cultures were grown under dark and red light, whereas blue light treatment had an inhibitory effect on growth (Najafabadi et al. 2019). Notably, the growth of artichoke seedlings under red light was 60–100% greater than that of seedlings grown in a greenhouse, whereas blue light had an inhibitory effect (Rabara et al. 2017). However, in the present work, increasing the intensity of blue light to 300 µmol m^{-2} s^{-1} led to growth inhibition, whereas blue light treatment at intensities of 50 and 100 µmol m^{-2} s^{-1} had no effect on the growth of calli. We previously reported that red and green light had no negative effect on cell culture growth, whereas blue light with an intensity of 100 µmol m^{-2} s^{-1} completely inhibited the growth of *M. maritima* (Veremeichik et al. 2024b).

After 12 years of continuous cultivation, the contents of mCQ and dCQ in the *C. cardunculus* calli grown in the dark were 0.28 and 0.7 mg/g DW, respectively, which are almost 8 times lower than those reported for the vector culture (Vereshchagina et al. 2014). We hypothesized that long-term continuous cultivation could be the reason for this significant decrease in CAD biosynthesis. Thus, monochromatic light treatment can induce the biosynthesis of caffeoylquinic acids in 12-year-old callus cultures of *C. cardunculus* up to 8 times greater than that in the dark-grown control. Modulation of the light spectra or intensity allows for the regulation of growth and biosynthesis separately or together. However, artichoke cell cultures do not always accumulate CAD. Elicitors, such as MeJA, are also used to induce the biosynthesis of mCQ (Abbas et al. 2022). The biosynthetic activity of a *C. cardunculus* cell culture also depends on the variety; in some cases, the CAD content in calli is twofold greater than that in the original plant, up to 5 mg/g DW (Trajtemberg et al. 2006). In a previous study, genetic transformation of artichoke calli with the Agrobacterium *rolC* gene led to a 2–3-fold increase in CAD content (Vereshchagina et al. 2014). Agrobacterium *rol* genes are known universal activators of secondary metabolism (Veremeichik et al. 2023a, b), but in this case, light exposure had a more powerful effect on CQ biosynthesis. Thus, the use of a modulated LED is a promising approach for activating CQ biosynthesis because of its naturalness and high efficiency.

Thus, red light has the greatest growth-stimulating effect regardless of intensity. The effects of green light do not have a negative effect on growth. Increasing the intensity of blue light had a significant ($p < 0.05$) negative effect. Research on illumination settings that can increase the sustainability and profitability of PFALs has recently gained importance and relevance (Orsini et al. 2020). Indicators such as the CO_2 footprint (CF) and energy use efficiency (EUE) are especially important (Yan et al. 2014). The most cost-effective way to produce CAD in *C. cardunculus* calli is blue light treatment with an intensity of 50 µmol m^{-2} s^{-1}. This kind of light treatment has a minimum power and has no inhibitory effect on growth. However, the tolerance of *C. cardunculus* calli to blue light can be a species-specific feature, and for other callus cultures, this treatment can be disastrous. It can also be assumed, on the basis of literary data, that this pattern is characteristic of cell cultures in general. Resistance to blue light intensity may be species specific. Moreover, how cell cultures behave when repeatedly passaged under monochromatic lighting conditions is interesting. It may also be assumed that the blue component of white light has a negative effect on culture growth when the intensity increases.

Thus, the most appropriate approach to increase caffeoylquinic acid productivity in the 12-year-old callus culture of *C. cardunculus* is one-time cultivation for 30 days under blue light with an intensity of 50 µmol m^{-2} s^{-1} compared with the dark-grown control or other light-treated varieties. These data find both coincidences and contradictions with those obtained earlier. Blue light with an intensity of 100 µmol m^{-2} s^{-1} was the best condition for cost-effective and energy-sustainable cultivation of *E. sativa* plants with high aboveground FW biomass accumulation and high flavonol accumulation (Veremeichik et al. 2023a, b). In addition, red and green light had no effect on the content of caffeic acid derivatives in the roots of *O. basilicum* plants (Veremeichik et al. 2024a). However, blue light has been shown repeatedly to have a growth-inhibiting effect. Thus, the growth of the cell culture *M. maritima* was completely inhibited under blue light treatment (Veremeichik et al.

2024b). In both the outer and inner parts of tomatoes, the color development of lycopene, β-carotene, total phenolic, and total flavonoid concentrations was increased by red light. Compared with the other treatments, exposure to 30 min of blue light followed by an 8-min pause resulted in the greatest increase in lycopene, total phenolic compounds, total flavonoids, vitamin C, and soluble sugars (Sena et al. 2024). While the cultures cultivated under red and dark light produced much more biomass, those cultivated under red light produced the most hypericin. One-week blue light treatment of four-week-old roots is an efficient stimulator for increasing total phenolic compounds and hypericins (*Hypericum perforatum*), even though 5-week blue light treatment inhibits the biomass and secondary metabolite synthesis of adventitious roots (Sobhani Najafabadi et al. 2019). The highest anthocyanin content was detected in the callus culture of *V. corymbosum* grown under red light compared with that in the calli grown in the dark, which served as a control (Abou El-Dis et al. 2021). Blue LEDs (50 µmol m^{-2} s^{-1}) elicited the highest antioxidant activity, total flavonoid content, and phenolic content in *Gynura procumbens* calli (Lian et al. 2019). Among the various LED lights, the maximum accumulation of polyphenols was recorded under blue LED treatment in both the callus cultures of *R. chalepensis* and the field-grown plants compared with the respective controls treated with white LEDs (Juneja et al. 2022). However, with the powerful activating effect of blue light on biosynthesis, it appears to have a negative effect on growth with increasing intensity. However, low intensities of blue light that do not inhibit growth may not have a positive effect on secondary metabolism, as is the case for callus cultures of *Lepidium sativum* (Ullah et al. 2019).

In recent years, research on light sources and conditions that can improve the profitability and sustainability of PFALs has been essential and relevant (Orsini et al. 2020). In this study, the impact of artificial monochromatic light at wide ranges of intensities (50, 100, 300, and 600 µmol m^{-2} s^{-1}) on the growth and biosynthesis of caffeic acid derivatives in long-term cultivated *C. carduncul*us callus cultures was investigated for the first time. In general, the following conclusions can be drawn: (i) In long-term cultivated *C. carduncul*us callus cultures, the content of caffeic acid derivatives decreased after 12 years of cultivation. The contents of mCQ and dCQ in the calli were 0.28 and 0.7 mg/g DW, respectively, which are almost 8 times lower than those reported for vector culture 13 years ago. We hypothesized that long-term continuous cultivation could be the reason for this significant decrease in CAD biosynthesis. (ii) Red light has the greatest growth-stimulating effect regardless of intensity. The effects of green light do not have a negative effect on growth. Increasing the intensity of blue light has a significant negative effect. It can also be assumed, on the basis of literary data, that this pattern is characteristic of cell cultures in general. Resistance to blue light intensity may be species specific. Moreover, how cell cultures behave when repeatedly passaged under monochromatic lighting conditions is interesting. It may also be assumed that the blue component of white light has a negative effect on culture growth when the intensity increases. (iii) The most effective treatment for CAD productivity is blue light treatment at an intensity of 50 µmol m^{-2} s^{-1}, up to 70 mg/L, which is tenfold greater than that of the dark-grown control. (iv) The most cost-effective way to produce CAD in *C. carduncul*us calli is to use blue light with an intensity of 50 µmol m^{-2} s^{-1}. This kind of light treatment has a minimum power and has no inhibitory effect on growth. However, the tolerance of *C. carduncul*us calli to blue light can be a species-specific feature, and for other callus cultures, this treatment can be disastrous.

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Declarations

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Ethics approval Not applicable.

Consent to participate Not applicable.

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.

Conflict of interest The authors declare that they have no competing interests.

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