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ORIGINAL ARTICLE

# Artificial monochromatic red and green light induces the biosynthesis of rosmarinic acid in long-term cultured calli of *Mertensia maritima* (L.)

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## Abstract

Rosmarinic acid is a metabolite that improves cognitive function and may prevent the development of Alzheimer's disease. The long-term continuous cultured calli of the extreme halophytic plant *Mertensia maritima* L. (Boraginaceae) constitute a source of rosmarinic acid. However, the biosynthetic activity of this calli culture gradually decreased over 12 years of continuous cultivation. In this work, we have shown that this process can be overcome by using monochromatic light, especially green light, in the wavelength range of 510–520 nm. In recent years, LEDs (light-emitting diodes) have been the subject of research within the fields of plant growth, development, and phytochemical biosynthesis. As determined by HPLC–DAD-ESI-HRMS, when this culture was exposed to normal (100 µmol $m^{-2}$ $s^{-1}$) or moderate (300 µmol $m^{-2}$ $s^{-1}$) green light, the content of rosmarinic acid increased by 2 and 3.5 times, respectively. A green light-induced increase in the content of rosmarinic acid was accompanied by the inhibition of the biosynthesis of glucosides of coumaric and ferulic acid, which are also produced in long-term cultured calli of *M. maritima*. Thus, monochromatic light can be used to increase the biosynthetic capacity of callus cultures during long-term cultivation.

## Key message

Exposure of the long-term cultivated callus culture of *M. maritima* under green light with moderate (300 µmol $m^{-2}$ $s^{-1}$) intensities provided the highest productivity of 12-year-old *M. maritima*, equal to the productivity of the initial calli.

**Keywords** Artificial light · LED · Rosmarinic acid · Long-term cultured calli · *Mertensia maritima* L

## Introduction

Plants are the main source of phytochemicals for medicinal and cosmetic purposes (Jamshidi-Kia et al. 2018). However, the production and accumulation of secondary metabolites occurs in plants under various stresses. In addition, some plants that produce important phytochemicals are rare, endemic, or protected species. Some of these issues can be resolved by using plant cell culture technology, which offers alternative sources of specialised metabolites (Fehér, 2019; Krasteva et al. 2021; Kutschera and Ray 2022; Pantchev et al. 2018). As shown previously (Fedoreyev et al. 2012), the initial callus culture of *Mertensia maritima* L. (Boraginaceae) is a producer of polyphenolic compounds, the main component of which is rosmarinic acid. Rosmarinic acid was first isolated from *Rosmarinus officinalis* L. (Lamiaceae). Later, rosmarinic acid was described as a phenolic antioxidant in thirty-nine plant families (Petersen et al. 2009). Rosmarinic acid is an ester of caffeic acid and lactic acid. Caffeic acid is a structural unit of various types of secondary metabolites, including ferulic acid, isoferulic acid, and chlorogenic acid (Petersen et al. 2009). Rosmarinic acid is well known for its antioxidant properties as well as its protective function against infections and herbivores (Khojasteh et al. 2020).

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Rosmarinic acid and its derivatives possess promising biological activities, such as improved cognitive performance, prevention of the development of Alzheimer's disease, cardioprotective effects, reduction in the severity of kidney diseases, and cancer chemoprevention (Bulgakov et al. 2018).

It is known that long-term continuous cultivation negatively affects the ability of calli to produce secondary metabolites (Liu et al. 2009). An important task in the cultivation of cell cultures is the stabilisation of their productivity and, in this context, the search for the most effective approaches to the activation of secondary metabolism. In recent years, light-emitting diodes (LEDs) have become the subject of research in the fields of plant growth, development, and the biosynthesis of phytochemicals (Nhut et al. 2015). Compared with traditional lighting systems, the use of LEDs can reduce energy costs by 50–75% (Vu et al. 2020). In the present work, we explored LED treatment as an approach for activating secondary metabolism in long-term continuous cultivated callus culture. The natural light intensity at 30% solar surface illumination (common conditions) ranges from 195 to 369 µmol $m^{-2}$ $s^{-1}$ depending on the time of day (Eskandarzade et al. 2023). According to Rahman et al. (2021), a LED light intensity of 100–200 µmol $m^{-2}$ $s^{-1}$ is commonly used for growing both plants and cell cultures. An intensity above 100 µmol $m^{-2}$ $s^{-1}$ can be called "normal," and that above 600 µmol $m^{-2}$ $s^{-1}$ is "high." Therefore, intensities in the range of 300–600 µmol $m^{-2}$ $s^{-1}$ can be considered "moderate" (Rahman et al. 2021). Many studies have investigated the use of LEDs in micropropagation techniques. Although the role of monochromatic light in plant morphogenesis in vitro has been well studied, much less is known about the effects of light quality on secondary metabolism (Batista et al. 2018). The effects of low-intensity LEDs (approximately 50 µmol $m^{-2}$ $s^{-1}$) on secondary metabolite biosynthesis have been studied, but higher-intensity light has received less attention (Taulavuori et al. 2017). The aim of the present work was to investigate the effects of increasing the intensity of monochromatic light on the growth and secondary metabolism of long-term continuously cultivated *M. maritima* calli.

In this work, we tested for the first time how long-term cultivated callus cultures respond to artificial monochromatic light. We compared the growth and biosynthetic characteristics of *M. maritima* callus culture obtained in 2012 with those of the same culture after 12 years of continuous cultivation. The biosynthetic characteristics of the culture decreased over time. However, the present study revealed that monochromatic light treatment at an intensity of 100 µmol $m^{-2}$ $s^{-1}$ or 300 µmol $m^{-2}$ $s^{-1}$ can overcome this problem.

## Materials and methods

### Plant materials, growth conditions, and experimental design

Previously, in 2012, a callus culture of *M. maritima* (L.) S.F. Gray (designated the Mm line) was obtained from stem explants using modified Murashige–Skoog media (ammonium nitrate content was reduced to 400 mg/l). This medium was supplemented with the following components (mg/l): thiamine-HCl (0.2), nicotinic acid (0.5), pyridoxine–HCl (0.5), meso-inositol (100), peptone (100), sucrose (25,000), agar (6000), 6-benzyladenine (BA, 0.5) and α-naphthaleneacetic acid (NAA, 2.0). Calli were cultivated in the same medium in the dark at 24 °C for 12 years and subcultured once per month. For the experiments, 12-year-old continuously cultivated calli of *M. maritima* line Mm growing in the dark were used. After inoculation (2 g of inoculant per 50 mL of the same medium in Erlenmeyer flasks), 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 for further chemical analysis. Ten jars with 2 g of inoculant were treated with every variant of light as a technical replicate. Three independent experiments were performed as biological replicates.

### Light treatments and growth chamber construction

The callus culture was grown in four-section chambers (100 × 50 × 50 cm, Fig. 5a) with light sources designed and manufactured at the IACP FEB RAS. The matrices of light sources were composed of three-watt (CHANZON, China) LEDs of different colours in the amount of 24 LEDs in each matrix, which created one integrated light source. To diffuse light, the chambers were covered inside with reflective aluminum foil. The temperature (24 °C) was maintained using an FFB1212SH 12025 exhaust fan (power: 14.8 W, speed: 3700 rpm, air volume: 140.16 cfm, China). The air humidity was maintained at 70%, with a photoperiod of 16–8 h (light/dark). Inside the chamber, the same spectrum was created with different levels of intensity in each separated section. Chambers of different spectra were distant from each other to minimise the mutual influence of light of different spectra on each other.

In this study, 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 characteristics, including normal, commonly used (100 µmol $m^{-2}$ $s^{-1}$) and moderate (300 µmol $m^{-2}$ $s^{-1}$) light intensities. Each section contained 1–10 light-emitting matrices, which provided the required level of photosynthetic photon flux density (PPFD). The intensity of the light in each section of the chamber was regulated by changing the supply current for each matrix. The spectra were measured using a PG200N spectrophotometer (UPRtek, Taiwan). A UT61A digital multimeter (Uni-T, China) controlled the driver supply currents. The normalised spectra of the light sources are shown in Fig. 5b.

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

### Chemicals

An analytical standard of *p*-coumaric acid was obtained from Serva (Germany). Research-grade standard samples of rosmarinic acid were obtained as described previously (Fedoreyev et al. 2005). Ultrapure water (Millipore, Bedford, MA, USA) was used for the preparation of all the extraction solutions and eluents. All solvents were of analytical grade.

### Sample preparation

Sample preparation for HPLC–UV-MS was performed according to our previously reported protocol (Veremeichik et al. 2023a). In brief, dried and powdered callus tissue (50 mg) was sonicated in 1 ml of 80% v/v methanol for 20 min and vortexed (800 rpm) overnight at 45 °C. The supernatant was filtered (0.45-µm membrane, Millipore, Bedford, MA, USA) and used for analysis.

### Analytical chromatography and mass spectrometry

The reversed-phase high-performance liquid chromatography method was applied for polyphenol determination. An Infinity 1260 analytical HPLC instrument (Agilent Technologies, Santa Clara, California, USA) was used for analysis. An analytical Zorbax C18 column (150 mm, 2.1 mm i.d., 3.5 µm, Agilent Technologies, USA) was used for separation at 40 °C. The mobile phase for gradient elution with a flow rate of 0.2 ml/min consisted of 0.1% aqueous formic acid (A) and acetonitrile (B). A linear gradient profile from 0 to 40% B for 35 min was used. UV spectra were recorded with a DAD in the range of 200–500 nm, and chromatograms were obtained at a wavelength of 320 nm. The MS studies were performed using an ion trap mass spectrometer Bruker HCT ultra PTM Discovery System (Bruker Daltonik GmbH, Bremen, Germany) interfaced with an HPLC system. The mass spectra were collected by applying ESI conditions for negative ion detection. The following settings were used: the range of *m/z* detection was 100–1,000, the drying gas ($N_2$) flow rate was 8.0 l/min, the nebuliser gas ($N_2$) pressure was 25 psi, 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 high-resolution MS data were acquired using a Shimadzu LCMS-IT-TOF instrument (Shimadzu, Japan) including a tandem ion trap/time-of-flight mass spectrometer with a resolution of 12,000. The following settings were used: the drying gas ($N_2$) pressure was 100 kPa, the nebuliser gas flow rate was 1.5 l/min, the ion source potential was varied from −3.8 to 4.5 kV, and the interface temperature was 200 °C. The external standard method was used for quantification of the identified compounds.

The production of rosmarinic acid was calculated as follows:

Productivity (mg/l) = Content × DW, where "Content" denotes the content of rosmarinic acid (mg/g DW) and DW denotes the dry weight (g) of the callus biomass per liter of medium (g/l).

## 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

## Growth and biosynthetic parameters of a 12-year-old M. maritima callus culture

It is believed that with long-term, multiyear cultivation, the ability of calli to regenerate and produce a high synthesis of secondary metabolites decreases (Liu et al. 2009). In the present work, we analysed the growth and biosynthetic characteristics of the *M. maritima* callus culture obtained in 2012 after 12 years of continuous cultivation (more than 150 passages).

Previous $^1$H and $^{13}$C NMR, UV, ECD, and HPLC−MS analyses of newly obtained *M. maritima* calli have shown that the calli contain rosmarinic acid (Fedoreyev et al. 2012). We analysed an extract from a 12-year-old callus culture of *M. maritima*. The compounds found in the extract of the 12-year-old *M. maritima* callus culture were identified on the basis of their chromatographic behavior, and UV and mass spectral data were obtained using HPLC–DAD-ESI-HRMS (MS2) and compared with standards (rosmarinic acid) and published data (Figs. 1, 2, 3**,** Table 1). A reversed-phase HPLC technique with UV and MS detection was used to determine phenolic compounds in *M. maritima* callus tissue. High-resolution mass spectra were obtained usingvia a time-of-flight mass spectrometer, and molecular formulas were obtained for the detected compounds with a mass error of less than 3 mDa. Compounds 1 and 2 (with molecular formulas $C_{15}H_{18}O_8$ and $C_{16}H_{20}O_9$, respectively) were characterised as monosaccharide derivatives of coumaric and ferulic acids (Figs. 2, Table 1). Their identification was based on $MS^2$ experiments and comparisons of the results with those of previously published studies (Hong et al. 2021). The well-observed fragment ions (at *m/z* 163 and 193 for compounds 1 and 2, respectively) were produced by elimination of the hexose moiety (162 Da). The chromatographic and mass–spectral data for the studied compounds are summarised in Table 1. Compound 3 was identified as rosmarinic acid because of its full coincidence with the standard sample (Figs. 1, 2, 3, Table 1).

The growth parameters were analysed, and the data collected before and after 12 years of regular subculture revealed that the growth of Mm callus culture was unchanged (Fig. 4a, Fedoreev et al., 2012). Previous quantitative HPLC analysis of *M. maritima* calli (Mm'2012) revealed that the calli produced 0.74% rosmarinic acid on a dry weight basis (Fedoreev et al., 2012). After 12 years of cultivation, the content of rosmarinic acid decreased more than 3.5-fold, from 7.4 to 2.5 mg/g DW (Fig. 4b). With similar growth but a decrease in the content of rosmarinic acid in

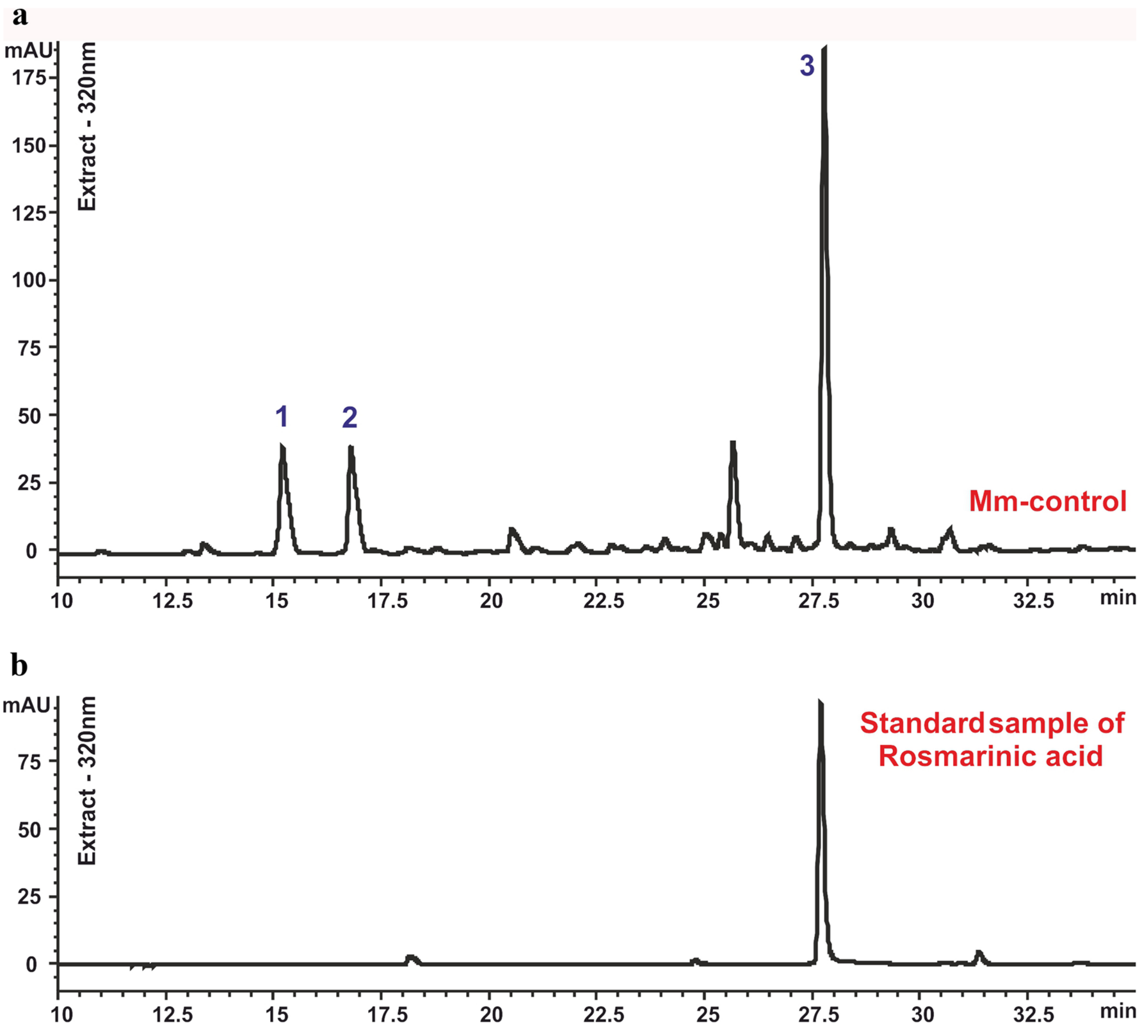

**Fig. 1** HPLC–UV profiling of phenolic compounds identified in crude extracts of *M. maritima* callus tissue. Chromatograms were recorded at 320 nm. The peak abbreviations correspond to the components listed in Table 1. HPLC analysis of the phenolic compounds in *M. maritima* calli. HPLC separation (**a**) of the phenolic compounds in Mm in a 12-year-old callus culture of *M. maritima* obtained in 2012 compared with a standard sample of rosmarinic acid (**b**)

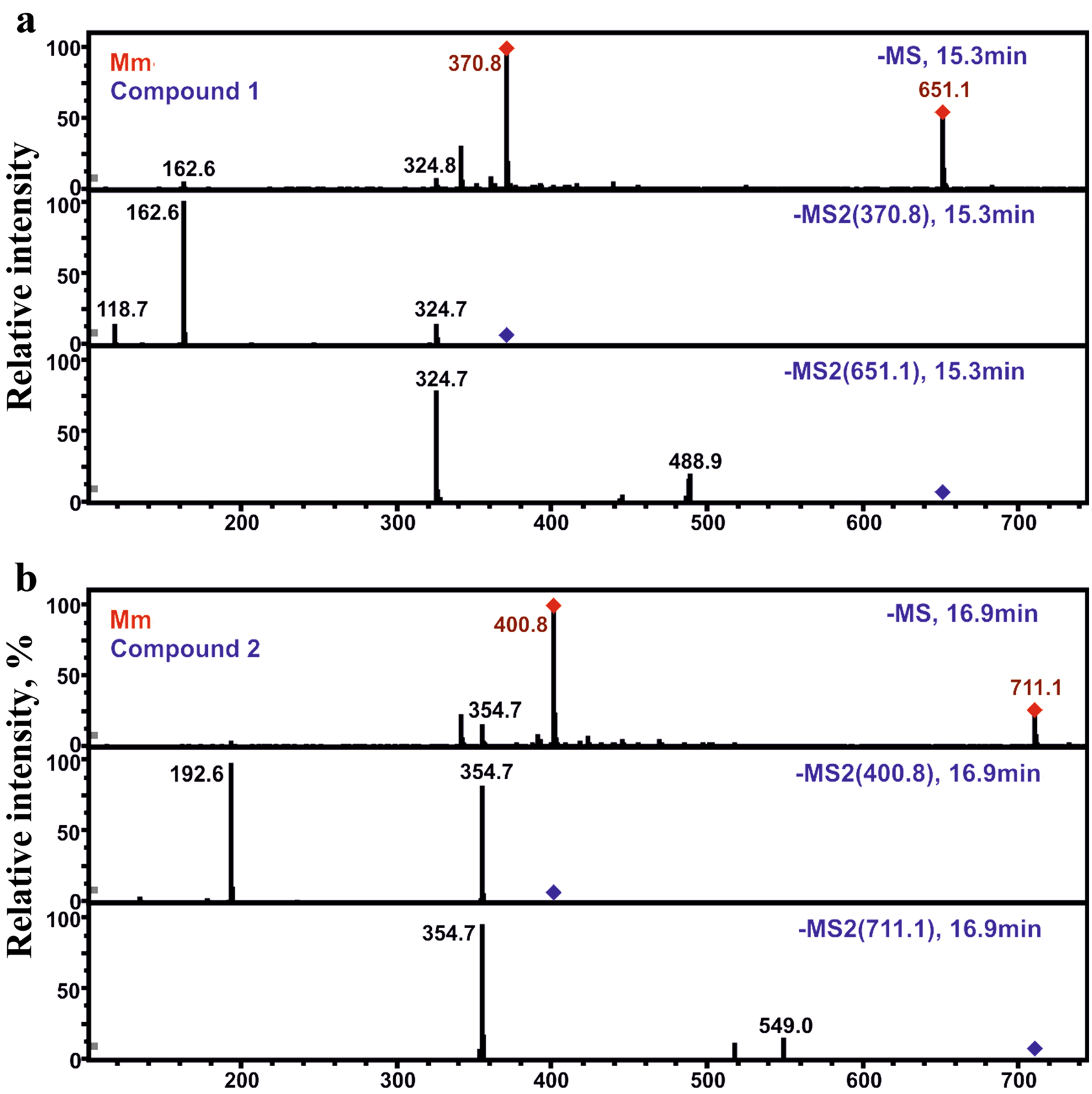

**Fig. 2** MS/MS$^2$ spectra of phenolic compounds (1, **a** and 2, **b**) identified in the crude extracts of Mm, a 12-year-old *M. maritima* callus culture, were acquired from the peak tops (Fig. 1). Mass spectra were obtained at low resolution using an ion trap mass spectrometer (Bruker HCT ultra PTM Discovery System) operated in ESI mode. All identification data are listed in Table 1

calli, the production of rosmarinic acid decreased by more than 3.5 times (Fig. 4c).

## Effects of artificial light on the growth of 12-year-old M. maritima callus cultures

It is currently known that LED light quality and intensity can improve plant secondary metabolism, and this effect is species-specific (Taulavuori et al. 2017; Veremeichik et al. 2023a). We hypothesised that exposure to light of varying intensities and spectra could induce rosmarinic acid biosynthesis in long-term cultivated *M. maritima* calli. Different light intensities can either reduce or stimulate plant growth (Parrine et al. 2021; Yavari et al. 2021). The following variants of monochrome lighting were used (Fig. 5a): red (R), blue (B), and green (R), with different characteristics (Fig. 5b). On the basis of our previous experiments (Veremeichik et al. 2023a), the light intensities chosen were normal (100 µmol m$^{-2}$ s$^{-1}$) and moderate (300 µmol m$^{-2}$ s$^{-1}$).

As shown in Fig. 5c**,** the growth of the 12-year-old *M. maritima* callus culture during a single passage under conditions of normal (100 µmol m$^{-2}$ s$^{-1}$) and moderate (300 µmol m$^{-2}$ s$^{-1}$) R or G light did not differ from that under the control dark conditions. In contrast, blue light treatment completely inhibited callus growth (Fig. 5c). The morphology of R and G light-treated calli was not significantly different from that of calli grown under dark conditions. However, the color of the light-treated calli

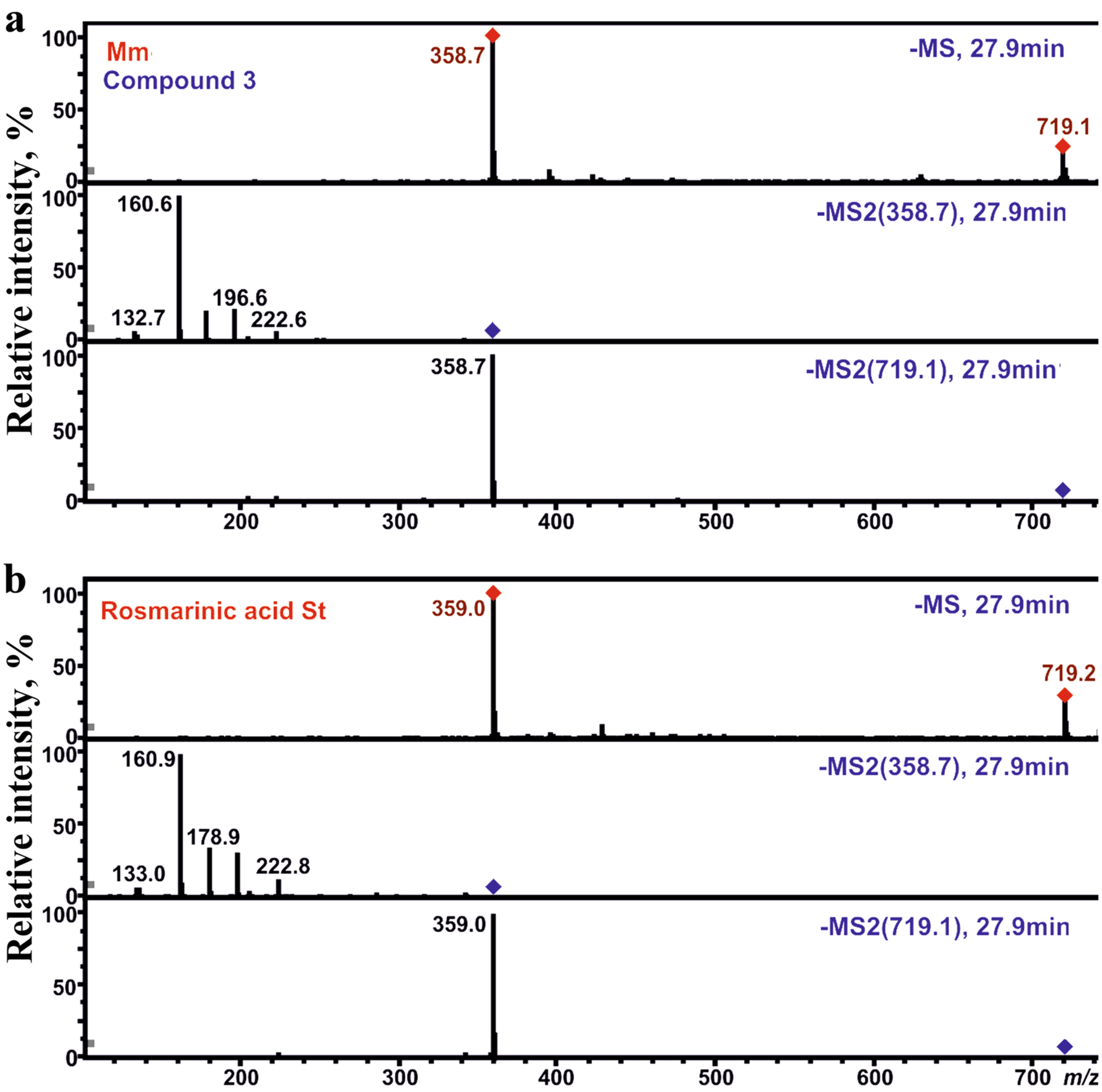

**Fig. 3** MS/MS$^2$ spectra of rosmarinic acid (compound 3) identified in the crude extracts of Mm, 12-year-old *M. maritima* callus tissue (**a**) and a standard sample of rosmarinic acid (**b**) were acquired from the peak tops (Fig. 2). Mass spectra were obtained at low resolution using an ion trap mass spectrometer (Bruker HCT ultra PTM Discovery System) operated in ESI mode. All identification data are listed in Table 1

**Table 1** List of phenolic compounds identified in the crude extracts of *M. maritima* callus tissue by HPLC–UV-ESI–MS (MS$^2$)

| № | Rt, min | Compound assignment | UV max, nm | Molecular Formula | ESI–MS(MS$^2$) data | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Ion composition | *m/z* values | Error, mDa | MS$^2$, *m/z* |
| 1 | 15.2 | Coumaric acid glucoside | 295 | $C_{15}H_{18}O_8$ | $[2\,M\text{-}H]^-$ | 651.1919 | 1.2 | 489, 325 |
| | | | | | $[M\text{-}H+Fa]^-$ | 371.0959 | 2.5 | 325, 163, 119 |
| | | | | | $[M\text{-}H]^-$ | 325.0903 | 2.6 | nd |
| | | | | | $[M\text{-}H\text{-}Hex]^-$ | 163.0420 | 1.9 | nd |
| 2 | 16.8 | Ferulic acid glucoside | 293, 314 | $C_{16}H_{20}O_9$ | $[2\,M\text{-}H]^-$ | 711.2143 | 0.1 | 549, 517, 355 |
| | | | | | $[M\text{-}H+Fa]^-$ | 401.1083 | 0.6 | 355, 193 |
| | | | | | $[M\text{-}H]^-$ | 355.1017 | 1.8 | nd |
| | | | | | $[M\text{-}H\text{-}Hex]^-$ | 193.0519 | 1.3 | nd |
| 3 | 27.8 | Rosmarinic acid | 293 sh | $C_{18}H_{16}O_8$ | $[2\,M\text{-}H]^-$ | 719.1611 | 0.92 | 359 |
| | | | 327 | | $[M\text{-}H]^-$ | 359.0764 | 0.8 | 223, 197, 179, 161, 133 |

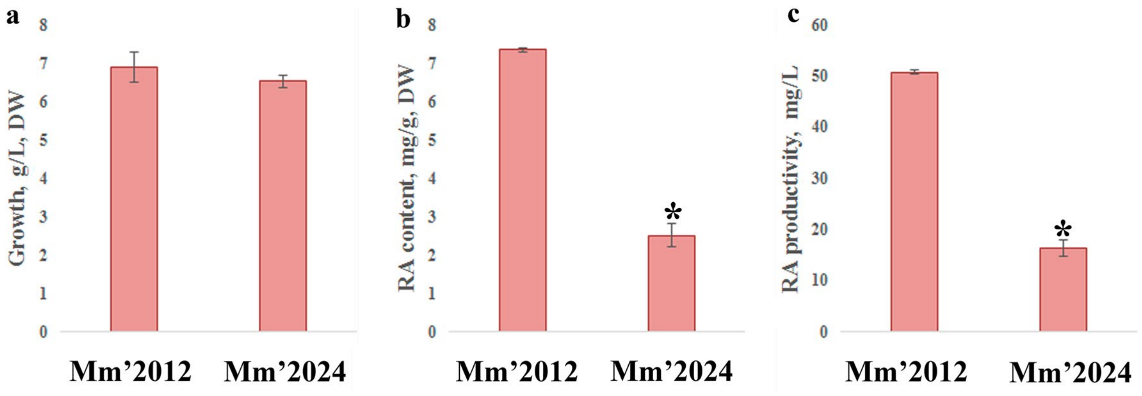

**Fig. 4** Impact of long-term cultivation on the growth and RA biosynthesis of *M. maritima* calli. **a**, biomass accumulation (g/L) of 30-day-old calli (0.2 g inoculants/50 mL of solid medium); **b**, content (mg/g DW) of RA; **c**, RA productivity (mg/L). Mm' 2012, initial callus culture obtained in 2012; Mm'2024, subcultured continuously for 12 years in the dark. The data obtained from three independent experiments with ten biological replicates are presented as the mean ± standard error of the mean, and the asterisks above the error bars indicate statistically significant differences (*t*- test, $p < 0.05$)

was more intense. Calli grown under B light with normal (100 µmol $m^{-2}$ $s^{-1}$) or moderate (300 µmol $m^{-2}$ $s^{-1}$) intensities were completely unviable (Fig. 6).

### Contents of phenolic compounds in M. maritima calli grown under different artificial lighting conditions

We analysed the effects of different types of artificial lighting on the contents of rosmarinic, coumaric, and ferulic acids in 12-year-old *M. maritima* callus cultures. Calli were grown one passage under normal (100 µmol $m^{-2}$ $s^{-1}$) and moderate (300 µmol $m^{-2}$ $s^{-1}$) intensities of R or G light (Fig. 7). The reversed-phase HPLC results revealed that treatment with R or G monochromatic light led to a significant doze-dependent increase in rosmarinic acid content (Fig. 7a). An increase in the content of rosmarinic acid was accompanied by a decrease in the contents of coumaric and ferulic acid glucosides. In addition, the increase in rosmarinic acid biosynthesis was accompanied by a decrease in the content of coumaric and ferulic acid glucosides (Fig. 7b).

G light of normal and moderate intensity was found to be more effective at activating rosmarinic acid biosynthesis than R light. Red light of normal (100 µmol $m^{-2}$ $s^{-1}$) or moderate (300 µmol $m^{-2}$ $s^{-1}$) intensity increased the content of rosmarinic acid by 1.2 and 2 times, respectively, whereas green light of normal (100 µmol $m^{-2}$ $s^{-1}$) or moderate (300 µmol $m^{-2}$ $s^{-1}$) intensity increased the rosmarinic acid content by 2 and 2.9 times, respectively (Fig. 7c). Since rosmarinic acid has beneficial effects on humans (Bulgakov et al. 2018), we calculated the total productivity of calli, an important biotechnological characteristic, under different light conditions (Fig. 7d). As expected, the productivity of the 12-year-old *M. maritima* callus culture increased under monochromatic light conditions, with a maximum yield of 54 mg/L compared with 18 mg/L in the dark (i.e., three times greater). Thus, we concluded that moderate-intensity green light (300 µmol $m^{-2}$ $s^{-1}$) is an effective inducer of rosmarinic acid production in long-term cultivated callus cultures of *M. maritima*.

## Discussion

The production of secondary metabolites, including pharmacologically active compounds, in native plants is low (usually less than 1% of dry weight) and mainly depends on the physiological state and developmental stage of the plant (Wu et al. 2021). In vitro plant cell culture technology has become widespread and has found its niche both in fundamental biology and in the applied field. The stability of cell cultures is an important issue. In many cases, the ability of calli to produce high levels of secondary metabolites decreases with prolonged cultivation (Liu et al. 2009). By long-term cultivation, we refer to 10–20 years of continuous subculture. In the present work, we compared the growth and biosynthetic characteristics of *M. maritima* callus cultures obtained in 2012 and the characteristics of the same culture after 12 years of continuous subcultivation. We showed that after 12 years of continuous subcultivation, the content of the main polyphenolic compound, rosmarinic acid, decreased by more than three times. Moreover, the growth characteristics did not change. For polyphenolic compounds such as RA, long-term cultivation is critically important. Therefore, the following question arises: how can the biosynthesis of secondary metabolites be activated in long-term cultured plant cells? It is difficult to find experimental data or review articles that compare the biosynthetic parameters

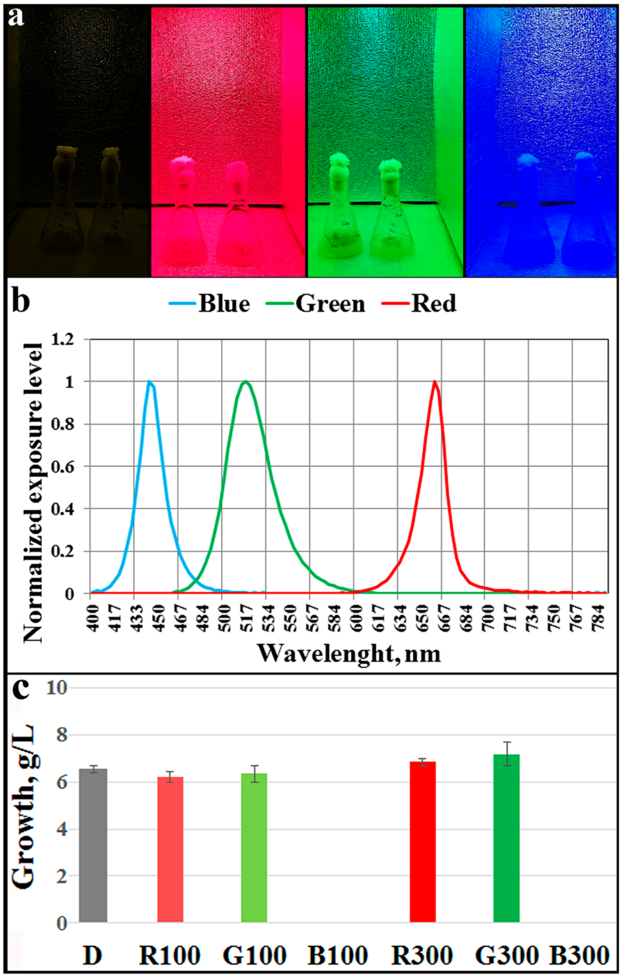

**Fig. 5** Growth chambers, characteristics of light, and growth parameters of light-treated 12-year-old *M. maritima* calli. Growing chambers (**a**) with artificial lighting variations (left to right): darkness and monochromatic sources such as red (R), blue (B) and green (G). Normalised spectral characteristics of light emission intensities as a function of wavelength (nm) for the light varieties are shown for monochromatic light sources (**b**). Impact of artificial lighting on the growth biomass accumulation (g/L) of *M. maritima* calli (2 g inoculants per 50 mL of solid medium): D, darkness; R100 and R300, red light with intensities of 100 and 300 µmol m$^{-2}$ s$^{-1}$, respectively; G100 and G300, green light with intensities of 100 and 300 µmol m$^{-2}$ s$^{-1}$, respectively; B100 and B300, blue light with intensities of 100 and 300 µmol m$^{-2}$ s.$^{-1}$, respectively. The data obtained from three independent experiments with ten biological replicates are presented as the mean ± standard error of the mean (ANOVA, $p < 0.05$)

of newly obtained and long-term cultivated callus cultures; in most studies, long-term cultivation means 6–36 months (Kosturkova et al. 2017). Freshly obtained cell cultures from various plants are often referred to as alternative sources of RA. For example, cultures of *Origanum dictamnus* produce rosmarinic acid at up to 6.6% dry weight (Sarropoulou et al. 2023), *Salvia miltiorrhiza* at 1.27% dry weight (Wu et al. 2016), and *Eritrichium sericeum* at 4.6% dry weight (Inyushkina et al. 2007). However, little is known about the biosynthetic stability of rosmarinic acid-producing plant cells during long-term culture. A study from our group revealed that a callus culture of *Lithospermum erythrorhizon* produced rosmarinic acid (1.1% dry weight) after a 15-year period of cultivation (Fedoreyev et al. 2005). Numerous studies have identified an effective approach for activating rosmarinic acid biosynthesis in cell cultures. The addition of sucrose, hormones, and yeast extract to culture media can increase the rosmarinic acid content to 8.5% dry weight, but this increase is accompanied by growth inhibition (Dalal and Dantu 2023). Several examples of long-term cultivation and high productivity of plant cell cultures have been reported. The most prominent examples are isoflavones in soybean suspension culture (25 years; Federici et al. 2003), naphthoquinones and rosmarinic acid (15 years; Fedoreyev et al. 2005), anthraquinones (14 years; Veremeichik et al. 2019, 2023b), and caffeoylquinic acid metabolites (21 years; Grishchenko et al. 2022). However, the lack of dynamic comparisons with initial callus cultures makes it impossible to determine whether these cultures are stable.

The use of LED light is becoming a new way to grow plants and activate the biosynthesis of secondary metabolites. Green light is rarely used for plant cultivation. However, it has been shown that wide-spectrum LEDs composed of white, red, and blue LEDs are more suitable for plant cultivation than red and blue LEDs only (i.e., light lacking a green region), with an average proportion of 65:20:15 red:green:blue LEDs at moderate (300 µmol m$^{-2}$ s$^{-1}$) light intensity (Darko et al. 2022). Green light is able to reverse the positive effect of blue light on anthocyanin accumulation but increases the accumulation of carotenoids in plants (Thoma et al. 2020). Flavonoid content is affected mainly by the absolute amount of blue light, but especially in the case of flavones, red light is also important. The influence of the green region of the spectrum on flavonoids has a moderate effect (Darko et al. 2022). Moreover, a powerful stimulating effect of green monochromatic light on the biosynthesis of secondary metabolites was observed in actinomycetes, where green light stimulated the biosynthesis of benzisochromanequinone metabolites (Kanchanabanca et al. 2023). Orsini et al. (2020) noted that the development of optimal light conditions for cost-effective plant growth is the main scientific direction in this area. In the present work, we investigated the effects of monochromatic artificial light of normal and moderate intensity (100–300 µmol m$^{-2}$ s$^{-1}$) on the biosynthesis of RA in a long-term cultured cell line of *M. maritima*. Importantly, in our study, we examined not only the activation of secondary metabolism but also the possibility of overcoming the blockage of secondary metabolism, which apparently occurs in calli during long-term cultivation. Our previous observations

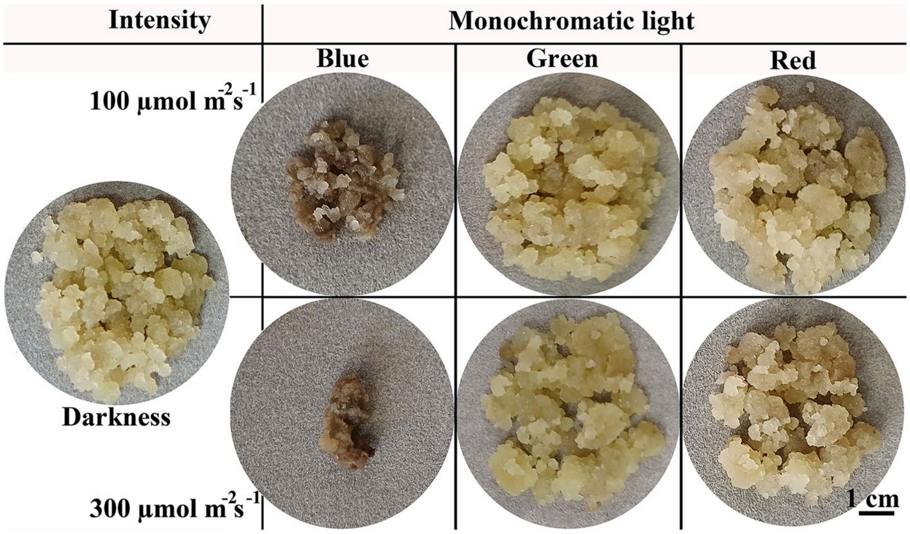

**Fig. 6** Morphology of a 12-year-old *M. maritima* callus culture grown under different light treatments. Impact of artificial lighting on the growth of 30-day-old *M. maritima* calli: D, darkness; blue, red, and green monochromatic light with intensities of 100 and 300 μmol m$^{-2}$ s.$^{-1}$

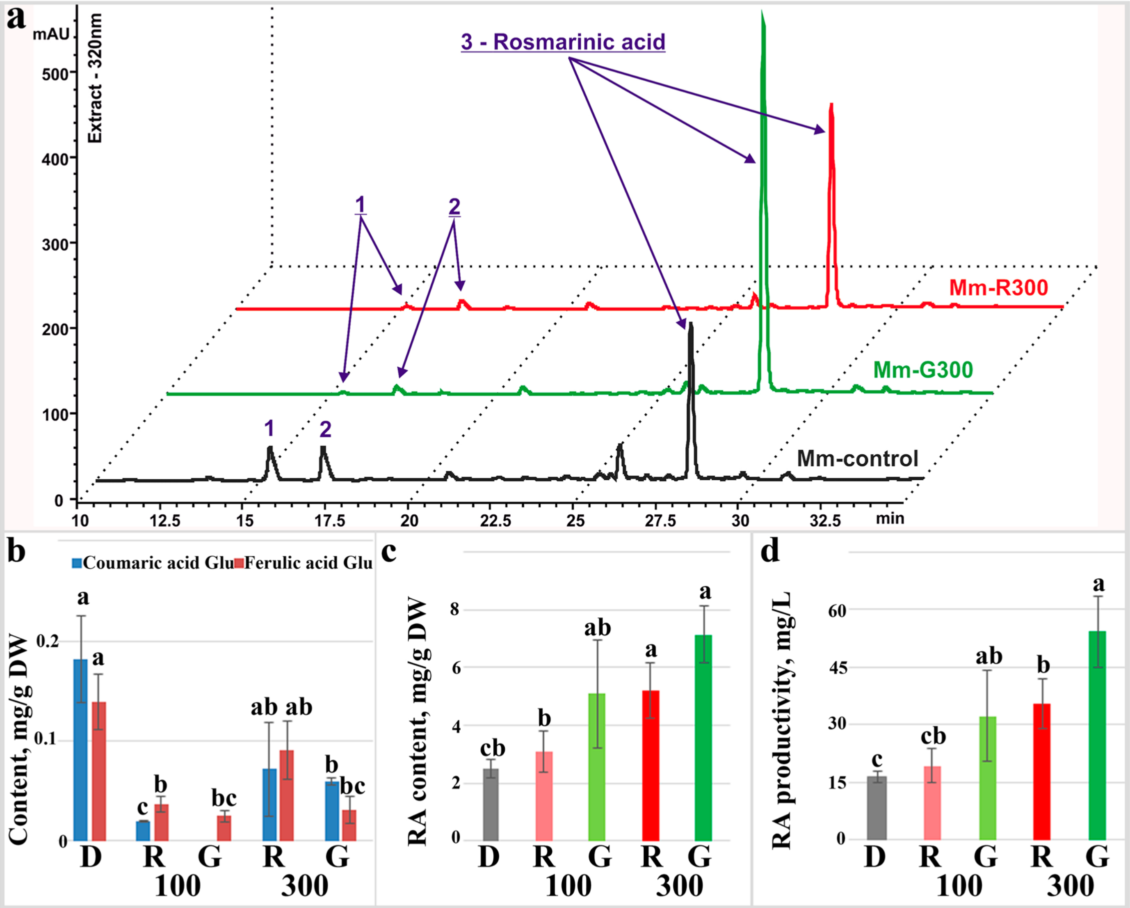

**Fig. 7** Contents of phenolic compounds in *M. maritima* calli grown under different lighting conditions. HPLC separation of phenolic compounds: **a**, Comparison of the UV absorption profiles of samples Mm (calli growing in the dark), Mm R300, and Mm G300 recorded at 520 nm. **b**, Contents of coumaric acid glucoside, ferulic acid glucoside (**b**), and rosmarinic acid (**c**) in *M. maritima* calli (mg/g DW). Production of rosmarinic acid in 12-year-old *M. maritima* callus cultures grown for 30 days under different lighting conditions: red (R) and green (G) light intensities of 100 and 300 μmol m$^{-2}$ s.$^{-1}$. Data from three independent experiments with ten biological replicates and three technical replicates are presented as the mean ± standard error of the mean, and asterisks above the error bars indicate the presence of statistical significance (ANOVA, $p < 0.05$)

revealed that a significant increase in flavonoid content can be achieved using moderate (300 µmol $m^{-2}$ $s^{-1}$) or high-intensity (1000 µmol $m^{-2}$ $s^{-1}$) monochromatic light (Veremeichik et al. 2023a), although there are examples of using low-intensity light. 25 µmol $m^{-2}$ $s^{-1}$ of various combinations of blue and red LED light had no effect on the callus tissue of *Panax vietnamensis* (Nhut et al. 2015). Blue light (50 µmol $m^{-2}$ $s^{-1}$) blocked the initiation of calli, but red light increased the content of secondary metabolites by twofold in a callus culture of *Withania somnifera* (Adil et al. 2019). Another study revealed that 40–50 µmol $m^{-2}$ $s^{-1}$ blue light slightly increased the rosmarinic acid content in the initial callus culture of *Ocimum basilicum* (Nadeem et al., 2018). Red light with 40–50 µmol $m^{-2}$ $s^{-1}$ intensity had no effect on growth or polyphenol composition in *Eclipta alba* cell culture (Khurshid et al. 2020).

Most studies on the effects of monochromatic LEDs on secondary metabolism in plant cell cultures have revealed that low light intensities (less than 50 µmol $m^{-2}$ $s^{-1}$) have no significant effect (Nhut et al. 2015; Adil et al. 2019; Nadeem et al. 2019; Khurshid et al. 2020). However, when used in certain devices, such as LEDitSHAKE (multiplexed customised illumination within a single shaking incubator), low intensities induce a powerful effect on specific groups of compounds, such as anthocyanins, in *Vitis vinifera* suspension cultures (Beuel et al. 2021). Overall, it can be concluded that low-intensity monochromatic light (20–50 µmol $m^{-2}$ $s^{-1}$) has little effect on the growth and productivity of plant cell cultures. These results are consistent with data obtained in plants; for example, low-intensity monochromatic light (30 µmol $m^{-2}$ $s^{-1}$) did not affect the growth or flavonoid content of *Anoectochilus roxburghii* plants (Wang et al. 2018). In the present work, we showed that increasing the monochromatic light intensity to 100 µmol $m^{-2}$ $s^{-1}$had a significant effect on both growth and secondary metabolism. A normal intensity (100 µmol $m^{-2}$ $s^{-1}$) of blue light completely blocked callus growth, whereas red and green light supported normal growth and activated the biosynthesis of rosmarinic acid in the long-term cultivated callus culture of *M. maritima*. Increasing the intensity to 300 µmol $m^{-2}$ $s^{-1}$ further increased the rosmarinic acid content more than two- and threefold for red and green light, respectively.

Thus, we conclude that moderate-intensity green light effectively activates the biosynthesis of rosmarinic acid. This approach may be applicable to other long-term cultivated callus cultures, as it allows the productivity of calli to return to the original level. This area of research needs further investigation. It is necessary to determine what kind of light and what intensity will be effective for different groups of compounds.

## Conclusion

In this study, the impact of artificial monochromatic light at normal (100 µmol $m^{-2}$ $s^{-1}$) and moderate (300 µmol $m^{-2}$ $s^{-1}$) intensities on the growth and biosynthesis of long-term cultivated callus cultures of *M. maritima* was investigated for the first time. In general, the following conclusions can be drawn: i) In the long-term cultivated callus culture of *M. maritima*, the content of phenolic compounds decreased after 12 years of cultivation. The content of the main polyphenolic compound, rosmarinic acid, decreased by more than three times. ii) Blue light with an intensity of 100 µmol $m^{-2}$ $s^{-1}$ completely inhibited callus growth. Moreover, normal (100 µmol $m^{-2}$ $s^{-1}$) and moderate (300 µmol $m^{-2}$ $s^{-1}$) intensities of red or green light had no negative effect on the growth of calli. iii) Treatment with normal (100 µmol $m^{-2}$ $s^{-1}$) or moderate (300 µmol $m^{-2}$ $s^{-1}$) intensities of red or green light strongly activated rosmarinic acid biosynthesis. iv) Cultivation under moderate (300 µmol $m^{-2}$ $s^{-1}$) green light intensity resulted in the highest productivity of 12-year-old *M. maritima* calli, equal to the productivity of the original calli.

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

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare no competing interests.

**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.

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