



Impact of photon flux density and light spectral quality on biomass production and arbutin compound accumulation in *Origanum majorana* L. plantlets

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Abstract

Light is one of the factors that influence the growth, development and synthesis of secondary metabolites of different plant species in vitro. *Origanum majorana* is an aromatic plant belonging to a group of plants that produce arbutin, a substance of importance in the cosmetic industry. The objective of this study was to verify the effect of light intensity and wavelength on the growth and accumulation of arbutin in vitro in plantlets. Nodal segments were grown in MS culture medium under photon flux densities of 26, 51, 69, 94 and 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In the light spectrum, nodal segments were grown under different light-emitting diodes LED: red; blue; a combination of blue and red light (30% B:70% R; 50% B:50% R; 70% B:30% R); and white, yellow and green. After 40 days, the growth, photosynthetic pigments and arbutin accumulation were evaluated by HPLC-DAD. The lowest light intensity (26 $\mu\text{mol m}^{-2} \text{s}^{-1}$) provided a higher content of photosynthetic pigments, and the intensity of 94 $\mu\text{mol m}^{-2} \text{s}^{-1}$ increased dry biomass accumulation. Greater accumulation of arbutin was observed under a higher light intensity of 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Regarding the quality of light, different wavelengths did not significantly affect the number of shoots or shoot length. The white light spectrum stimulated a greater accumulation of dry biomass. The green light provided a greater accumulation of photosynthetic pigments. The amount of arbutin was influenced by the different light spectra. The red spectrum promoted the highest arbutin content, and the yellow inhibited.

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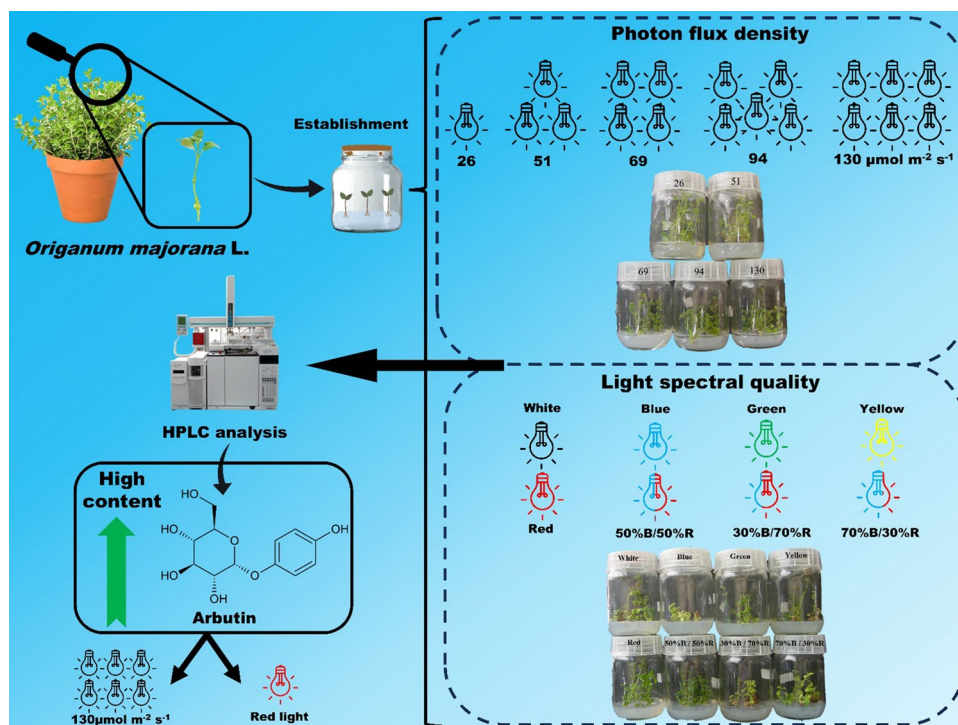
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Graphical abstract



Key message

The intensity of 94 $\mu\text{mol m}^{-2} \text{s}^{-1}$ increased dry biomass and 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$ increased arbutin accumulation. The red spectrum promoted the highest arbutin content, and the yellow inhibited.

Keywords Light-emitting diodes · HPLC · In vitro · Marjoram

Introduction

Origanum majorana L. is a species capable of synthesizing arbutin, a polyphenol widely used in the cosmetics industry for the treatment of hyperpigmentation disorders without causing melanocytotoxicity; thus, it is an option for replacing the use of hydroquinone and corticosteroids (Cala et al. 2021; Lukas et al. 2010; Zhu and Gao 2008). Arbutin is a hydroquinone glycoside and is one of the main constituents of the *Arctostaphylos uva-ursi* plant and is found in significant amounts in *Origanum majorana* (Gallo et al. 2015; Saeedi et al. 2021). This compound is widely used to lighten the skin in cosmetic products due to its property of suppressing melanin biosynthesis in human skin (Boo 2021; Saeedi et al. 2021). Arbutin is obtained synthetically, but natural arbutin and its derivatives have increasingly attracted interest for exhibiting a range of biological properties, such as antioxidants, antimicrobials, antimalarials and cytotoxics (Xu et al. 2015). The industrial production of arbutin occurs through chemical synthesis (Wang et al. 2006), plant

extraction (Migas and Krauze-Baranowska 2015) and enzymatic biotransformation (Nishimura et al. 1994, Jin et al. 2020). Recently, Skrzypczak-Pietraszek et al. (2017) pointed out the cultivation of marjoram shoots as another alternative source. However, the data provided by these authors are still insufficient to attest to the culture of *O. majorana* shoots as an alternative source for obtaining this bioactive constituent. Considering the intriguing structures and promising biological activities, the natural derivatives of arbutin have garnered growing attention (Xu et al. 2015). Obtaining arbutin in tissue culture offers several advantages. In the context of arbutin, tissue culture can be a valuable approach for several reasons: controlled production, sustainability and resource conservation, rapid production scale-up, and reduced dependence on climatic conditions. Obtaining arbutin through tissue culture provides a more controlled, sustainable and efficient approach compared to traditional extraction methods, opening the door to advances in the production of this compound with diverse applications.

Several processes for the production of arbutin in different species, including *Origanum vulgare* (Rychlinska and Nowak 2012), *O. majorana* (Skrzypczak-Pietraszek et al. 2017), *Fragaria spp.*, *Vaccinium corymbosum* (Kim et al. 2018), and *Bergenia crassifolia* (Nikulin et al. 2019), through tissue culture have been described in the literature. Skrzypczak-Pietraszek et al. (2017) introduced the species *O. majorana* as an alternative source of arbutin. Based on a biotransformation study, the authors demonstrated that in vitro shoot culture of *O. majorana* converted hydroquinone into arbutin.

Several factors contribute to the growth and development of the explant in vitro and metabolism, such as the culture medium, growth regulator, light intensity, wavelength and sugar concentration. However, light is directly involved in plant growth and development, both in primary and secondary metabolism, and drives the immense multiplicity of biochemical processes that constitute living matter (Sáez et al. 2013). A microclimate is created inside the flask or test tube that affects photosynthetic pigments, photomorphogenesis and plant metabolism. In addition, it can cause physiological and morphological disorders, such as malformation of the root system and chloroplast and high hyperhydricity. Therefore, intensity and wavelength are important abiotic factors to develop plants with quality and thus enable better acclimatization. The response of the plant to the production of biomass and secondary compounds in relation to intensity and spectral quality depends on the genotype. The growth of *Achillea millefolium* in vitro developed better under a low light intensity of $27 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Alvarenga et al. 2015), whereas the species *Lippia gracilis* showed better growth under $94 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Lazzarini et al. 2018). The quality of light in combination with different wavelengths or monochromatic light also affects growth and bioactive compounds in vitro. The species *Lippia rotundifolia* had the best growth in the combination of 70% red and 30% blue spectrum (Hsie et al. 2019), whereas the species *L. gracilis* had the best growth in monochromatic red light (Lazzarini et al. 2018).

Therefore, studies on the effects of light intensity and spectral quality on the primary and secondary metabolism of plants have reported different responses. For instance, the intensity $69 \mu\text{mol m}^{-2} \text{s}^{-1}$ and monochromatic blue increased the carvacrol content in *Plectranthus amboinicus* L. Spreng. (Silva et al. 2017). Also, *Hyptis suaveolens* (L.) Poit cultivated under 47 and $69 \mu\text{mol m}^{-2} \text{s}^{-1}$ increased sabinene, β -pinene, β -phellandrene and bicyclogermacrene compounds and lower light intensity of 13 and $28 \mu\text{mol m}^{-2} \text{s}^{-1}$ increased *E*-caryophyllene and germacrene D. (Andrade et al. 2017). Lazzarini et al. (2018) reported that low intensity ($26 \mu\text{mol m}^{-2} \text{s}^{-1}$) increased γ -terpinene content and concomitantly decreased carvacrol and blue light showed higher production of carvacrol of *L. gracilis* Schauer. Hsie et al. (2019) described in *L. rotundifolia* that

the monochromatic blue spectrum increased the myrcene and limonene content but decreased the myrcenone content, which was increased by red light. The in vitro cultivation of *Aeollanthus suaveolens* under white LEDs increased linalool and linalool acetate, while fluorescent light increased α -santalene and (Z)- β -farnesene contents. And green and yellow monochromatic light led to greater accumulation of massoia lactone (Araújo et al. 2021). Coelho et al. (2021) working with *Urtica dioica* L. under $130 \mu\text{mol m}^{-2} \text{s}^{-1}$ increased phenolic compound and flavonoid production. Also, showed that plantlets grown under 30% red/70% blue produced 5.53 times more phenolic compounds and 8.63 times more flavonoids than plants under fluorescent lights. *Lippia dulcis* cultivated under $139 \mu\text{mol m}^{-2} \text{s}^{-1}$ reach greater production of 6-methyl-5-hepten-2-one and 3-methyl-2-cyclohexen-1-one and the blue light showed greater accumulation of total phenolic compounds (Rocha et al. 2022). These different responses in the secondary metabolism of plantlets under intensity and wavelength are highly dependent on the genotype.

The production of secondary metabolites is related to numerous light-related factors: the type of spectrum, light intensity, photoperiod and the type of lamp used in the growth room. Although the literature cites studies on the use of light in medicinal and spice plants, more studies are needed to elucidate the importance of light in the growth and accumulation of bioactive molecules. Although several reports underline the benefits of light on the growth and secondary metabolism of plants, there is no information available about the effect of photon flux density and wavelength on arbutin accumulation in *O. majorana*. The present study is the first report of the effect of the *O. majorana* response to light on arbutin accumulation. With this information, the present study aimed to evaluate the impact of different photon flux densities and spectral qualities of light on biomass production and arbutin accumulation in *O. majorana* plantlets. The hypothesis that intensity and wavelength may influence plant development and secondary metabolism of plants in vitro.

Materials and methods

Establishment of the in vitro explant

O. majorana L. exsiccate was deposited in the PAMG Herbarium of the Agricultural Research Company of Minas Gerais (EPAMIG) under registration number 58898. The mother plants were cultivated in the Medicinal Garden of Federal University of Lavras (UFLA). For in vitro establishment, nodal segments (1 cm in length) of mother plants of *O. majorana* L. were used as explants. The explants were washed with detergent and kept in running water (30 min).

The explants were then disinfected with bleach (1.25% active chlorine) for 15 min and then washed three times with sterile deionized water. Subsequently, the leaves were removed, and the nodal segments were inoculated into test tubes for explant establishment containing 15 mL of MS medium (Murashige and Skoog 1962) without growth regulator and supplemented with 30 g L⁻¹ sucrose, 6.0 g L⁻¹ of agar, and the pH was adjusted to 5.7 ± 0.1 before autoclaving (for 20 min at 121 °C).

Light intensity

Nodal segments (1 cm in length) were obtained from plantlets grown in vitro and inoculated under aseptic laminar flow in flasks (200 mL) containing 50 mL of MS medium supplemented with 30 g L⁻¹ sucrose and 6 g L⁻¹ agar (Himedia®, type I), and the pH was adjusted to 5.7 ± 0.1. After inoculation, the explants were cultivated under five light intensities: 26, 51, 69, 94, and 130 μmol m⁻² s⁻¹, obtained with light emitting diodes (LEDs), under a 16 h photoperiod and temperature of 26 ± 1 °C. The experimental design was completely randomized with 5 treatments and 4 replicates—3 flasks per replicate, with 5 explants per flask, totaling 60 flasks and 300 explants. The light intensities were measured by a QSO-S Procheck + Sensor-PAR Photon Flux device (Decagon Devices-Pullman-Washington-USA).

Wavelength

Nodal segments (1 cm in length) were obtained from plantlets grown in vitro and inoculated under aseptic laminar flow in flasks (200 mL) containing 50 mL of MS medium supplemented with 30 g L⁻¹ sucrose and 6 g L⁻¹ agar (Himedia®, type I), and the pH was adjusted to 5.7 ± 0.1. After inoculation, the explants were cultivated under different light-emitting diode LEDs (TECNA® Piracicaba, Brazil): red (R); blue (B); a combination of blue and red light (30% B:70% R; 50% B:50% R; 70% B:30% R); and white LED (W), yellow (Y) and green (G). The experimental design was completely randomized with 8 treatments and 3 replicates—2 flasks per replicate, with 5 explants per flask, totaling 48 flasks and 240 explants. The intensity of the diodes was 42 μmol m⁻² s⁻¹. The light spectra were measured using a manual SPECTRA PEN Z850 spectrometer (Qubit Systems- Kingston, Ontario-USA).

Growth analysis

At 40 days, the plantlets were evaluated for shoot length (SL), shoot number (SN), leaf dry weight (LDW), stem dry weight (SDW), and aerial part dry weight (ShDW). To

determine the dry weight of the leaves and stems, the respective parts were placed in Kraft paper bags and placed in a forced air oven at 40 °C until reaching a constant weight (approximately 72 h). Subsequently, the dehydrated material was weighed on a precision scale.

Analysis of photosynthetic pigments

The extraction and measurement of photosynthetic pigments followed the method developed by Hiscox and Israelstam (1979). For analysis of photosynthetic pigments, 50 mg of fresh leaf matter of each treatment was collected in a dark room lit only with green light. Only the second pair of leaves from each plantlet was used. Remove the central vein of the leaves and the pieces were placed in Falcon tubes and wrapped in aluminum foil, and then 10 mL of dimethyl sulfoxide (DMSO) was added. The tubes were then placed in an oven at 65 °C for 24 h. After this period, 3 mL aliquots of the extract were collected and transferred to quartz cuvettes, and the absorbances were read at wavelengths of 480 nm (carotenoids), 649 nm (chlorophyll *a*) and 665 nm (chlorophyll *b*). The TECAN INFINITY M200 PRO spectrophotometer, operated with the I-control® data processing system (version 3.37), was used to read the optical density. The chlorophyll and carotenoid concentrations were expressed as milligrams of pigment per gram of fresh leaf tissue (mg g⁻¹), calculated according to the following equations, according to the methodology of Wellburn (1994):

$$\text{Chlorophyll } a \text{ (Ca)} = [(12.47 \times A_{665.1}) - (3.62 \times A_{649.1})] \quad (1)$$

$$\text{Chlorophyll } b \text{ (Cb)} = [(25.05 \times A_{649}) - (6.5 \times A_{665})] \quad (2)$$

$$\text{Carotenoids} = \frac{(1000 \times A_{480} - 1.29 \times \text{Ca} - 53.78 \times \text{Cb})}{220} \quad (3)$$

Sample preparation and qualitative and quantitative analyses of arbutin

Sample preparation was based on the method described in Rychlinska and Nowak (2012). Fifty-milligram aliquots of dry leaves of *O. majorana* from the light experiments were pulverized in a mortar. Next, the plant material was extracted by sonication (3 cycles × 15 min) using 3 mL of methanol MeOH and H₂O (50% H₂O + 50% MeOH) in each cycle. The extracts were combined and concentrated on a rotary evaporator at 40 °C under reduced pressure. The residue was resuspended in 1 mL of ultrapure water, transferred to microtubes and subjected to sonication for 2 min, followed by centrifugation at 10,000 rpm for 10 min. The supernatant was automatically injected into the chromatograph.

Chromatographic conditions of HPLC-DAD analysis

Analyses by high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD) were performed in an Agilent 1200 liquid chromatography system (Agilent Technologies®, Waldbronn, Germany) equipped with a quaternary pump (G1311A) with a degassing system (G13222A), an ALS autosampler (G1322A) and a TCC heater set (G1316A). A variable-wavelength ultraviolet detector (G1315D) was used to obtain chromatograms at 220 nm. The equipment was controlled by OpenLAB software, version A07.04, build 04.07.28.

The separations were performed on a C18 reversed-phase analytical column (Eclipse XDB-C18, Agilent Technologies®, USA) with silica-based packing (150 mm × 4.6 mm di, 5 µm) and a precolumn (12.5 mm × 4.6 mm ID, 5 µm). A volume of 10 µL (50 mg mL⁻¹ LDW) of the samples was injected into the column with the temperature maintained at 25 °C and a constant flow rate of 1.0 mL min⁻¹. Ultrapure water (A) and methanol (B) were used as eluents.

The elution program was as follows: 10% B in isocratic conditions for 0–5 min, followed by a linear gradient until 95% B for 5 min, remaining in isocratic conditions for another 5 min, returning to the initial condition in a linear gradient reversed for 5 min, totaling 20 min of analysis. Each run was followed by an equilibration period of 10 min.

Characterization of the presence of arbutin in the samples

Characterization of the presence of arbutin (Sigma-Aldrich ≥ 98%) in the samples was performed by TLC and HPLC-DAD chromatographic profiles. The TLC analyses used 60G silica gel plates, 10 × 10 cm², prepared manually. Aliquots of 10 µL of the sample and of the arbutin reference solution (1 mg mL⁻¹) were applied to the plates, which were eluted with a mixture of ethyl acetate, methanol and water (70:20:10). After drying, the plate was sprayed with a solution of sulfuric anise aldehyde, followed by heating. The characterization of the presence of arbutin was performed by comparing the retention times of the sample and reference substance analyzed under the same conditions, coinjection with the reference substance and by the spectral homogeneity of the arbutin peak in the sample compared to that of the reference substance at 220 nm.

Quantification of arbutin

Arbutin quantification was performed using the external standardization method (Ribani et al. 2004). To construct the analytical curve of arbutin, aiming to evaluate the linearity of the method, it was necessary to initially estimate the concentration range of this substance in the extract of dry

leaves of *Origanum majorana*. Estimates were based on the relative areas of the peak corresponding to arbutin in the samples and from a reference solution with a concentration of 1 mg mL⁻¹. The samples analyzed came from plantlets grown in vitro in preliminary assays. The linearity of the method was determined from two analytical curves using five different concentrations. Each point of the analytical curves was obtained by injecting, in triplicate, different aliquots of the reference solutions (2 mg L⁻¹ and 1 mg mL⁻¹) in the range of 2 to 10 µL. The analytical curves were determined in relation to the injected mass of arbutin. The data obtained for each calibration curve were subjected to linear regression analysis by the method of least squares, and the corresponding coefficients of determination (R²) were calculated. The curves obtained on the two consecutive days were statistically compared by analysis of covariance ($p < 0.05$) in *GraphPad Prism 6 software*. Arbutin levels are expressed as mg per gram of plant (mg g⁻¹).

Statistical analyses

The data were subjected to analysis of variance, and the means were compared by the Scott–Knott test at 5% probability using SISVAR statistical software (Ferreira 2019). Statistica® version 13.5 software (StatSoft; Tulsa, OK, USA) was used to perform principal component analysis (PCA).

Results and discussion

Analysis of growth under light intensity

The in vitro growth of *O. majorana* was affected by different light intensities. There was very little root induction in *O. majorana* plantlets without the addition of a growth regulator. Etiolation was observed in plantlets at 26 µmol m⁻² s⁻¹ with thin stems (Fig. 1). On average, the highest number of shoots (2.6) and shoot length (6 cm) were observed under cultivation at 51 and 94 µmol m⁻² s⁻¹, respectively. The in vitro cultivation of *O. majorana* under increased light intensity tended to reduce the number of regenerated shoots (Fig. 2a). In *H. suaveolens* cultivation, increased light intensity (13 to 69 µmol m⁻² s⁻¹) increased the number of shoots and shoot length, with the best growth being observed under light intensities of 47 and 69 µmol m⁻² s⁻¹ (Andrade et al. 2017). In the in vitro culture of *Withania somnifera*, the plantlets had higher growth under cultivation at 30 µmol m⁻² s⁻¹ (Lee et al., 2007). Higher light intensities were favorable for leaf and stem expansion of *O. majorana*, probably because light intensity regulates the size of leaves and stems, as well as their morphogenesis. Therefore, controlling light intensity, wavelength and photoperiod allows the production of plants with the best desired characteristics (Cavallaro



Fig. 1 *Origanum majorana* plantlets from nodal segments cultivated in vitro under different intensities 26, 51, 69, 94 and 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at 40 days. Replicates $n=4$

et al. 2022). Many abiotic factors, such as light, can affect plant growth in vitro, thus making it difficult to choose a more effective light intensity and spectrum for many species. This shows that each genotype may respond differently according to the light regime subjected.

Different light intensities significantly influenced ($p < 0.05$) the production of leaf dry weight (LDW), stem dry weight (SDW) and aerial part dry weight (ShDW). A greater gain of stem and leaf dry weight was observed from 69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 2). The intensity of 94 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was higher than all other treatments regarding leaf dry weight (48.8 mg^{-1}), stem (34.7 mg^{-1}) and aerial part dry matter (83.5 mg^{-1}). The plantlets of *U. dioica* (Coelho et al. 2021) and *gracilis* (Lazzarini et al. 2018) cultivated under 94 $\mu\text{mol m}^{-2} \text{s}^{-1}$ also obtained greater dry weight gain and growth.

This study and others confirm the importance of light intensity in growth and dry weight accumulation in plants grown in vitro. The results of this study and others showed that an intensity above 94 $\mu\text{mol m}^{-2} \text{s}^{-1}$ affected the dry weight accumulation in the plantlets. The *O. majorana* plantlets at the highest intensity of 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$ reduced dry weight accumulation, which may have occurred due to the reduced efficiency of photosynthesis and damage to the reaction center (Fan et al. 2013; Hsie et al. 2019). When plantlets are under prolonged excessive irradiation, photoinhibition can occur, leading to impaired growth and development (Kitao et al. 2000). Other studies also reported a reduction in the total dry weight gain of plantlets grown in vitro under high light intensities, such as those of *U. dioica* (Coelho et al. 2021), *L. rotundifolia* (Hsie et al. 2019) and *amboinicus* (Silva et al. 2017).

The dry weight gain of plant organs varies with the species. The cultivation of *Gerbera jamesonii* with increasing

light intensities from 40 to 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ did not affect dry weight production or the number of shoots (Cioć et al. 2019). The in vitro cultivation of *H. suaveolens* under different light intensities showed an increase in growth variables with increasing light intensity from 13 to 69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Andrade et al. 2017). Other species respond to different intensities, for example, *A. suaveolens* (Araújo et al. 2021) and *L. dulcis* (Rocha et al. 2022) at light intensities of 139 $\mu\text{mol m}^{-2} \text{s}^{-1}$, *P. amboinicus* 69 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and *L. rotundifolia* at low intensities of 20 to 54 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Hsie et al. 2019).

The *O. majorana* plantlets at the lowest intensity of 26 $\mu\text{mol m}^{-2} \text{s}^{-1}$ reduced dry weight accumulation (Fig. 2). In general, plants grown in low light environments have lower biomass values due to the low production of photoassimilates, which is the result of inefficient photosynthesis. The opposite result was found with *A. millefolium* where the highest values of shoot dry weight was observed under the lowest light intensity of 27 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Alvarenga et al. 2015).

Analysis of photosynthetic pigments under light intensity

The different light intensities significantly ($p < 0.05$) affected the concentration of photosynthetic pigments of *O. majorana* plantlets grown in vitro. The highest values for chlorophyll *a* and *b* and carotenoids were observed in cultivation under a light intensity of 26 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The species *L. rotundifolia* also obtained higher values of photosynthetic pigments under a low light intensity of 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Hsie et al. 2019). In this study, the lowest values of chlorophyll and

Table 1 Concentration of photosynthetic pigments in *Origanum majorana* plantlets cultivated in vitro under different light intensities, at 40 days

Intensity $\mu\text{mol m}^{-2} \text{ s}^{-1}$	Chlorophyll			Carotenoids
	<i>a</i>	<i>b</i>	Total	
	(mg g ⁻¹ FM)			
26	0.59a	0.14a	0.73a	0.15a
51	0.36c	0.11b	0.47c	0.10c
69	0.46b	0.11b	0.57b	0.12b
94	0.29d	0.07d	0.36d	0.08d
130	0.28e	0.08c	0.37d	0.10c
CV (%)	11.76	13.39	11.23	9.30

Means followed by the same letter in the column do not differ from each other, according to the Scott Knott test, at the 5% probability level

Replicates $n=4$

FM fresh matter

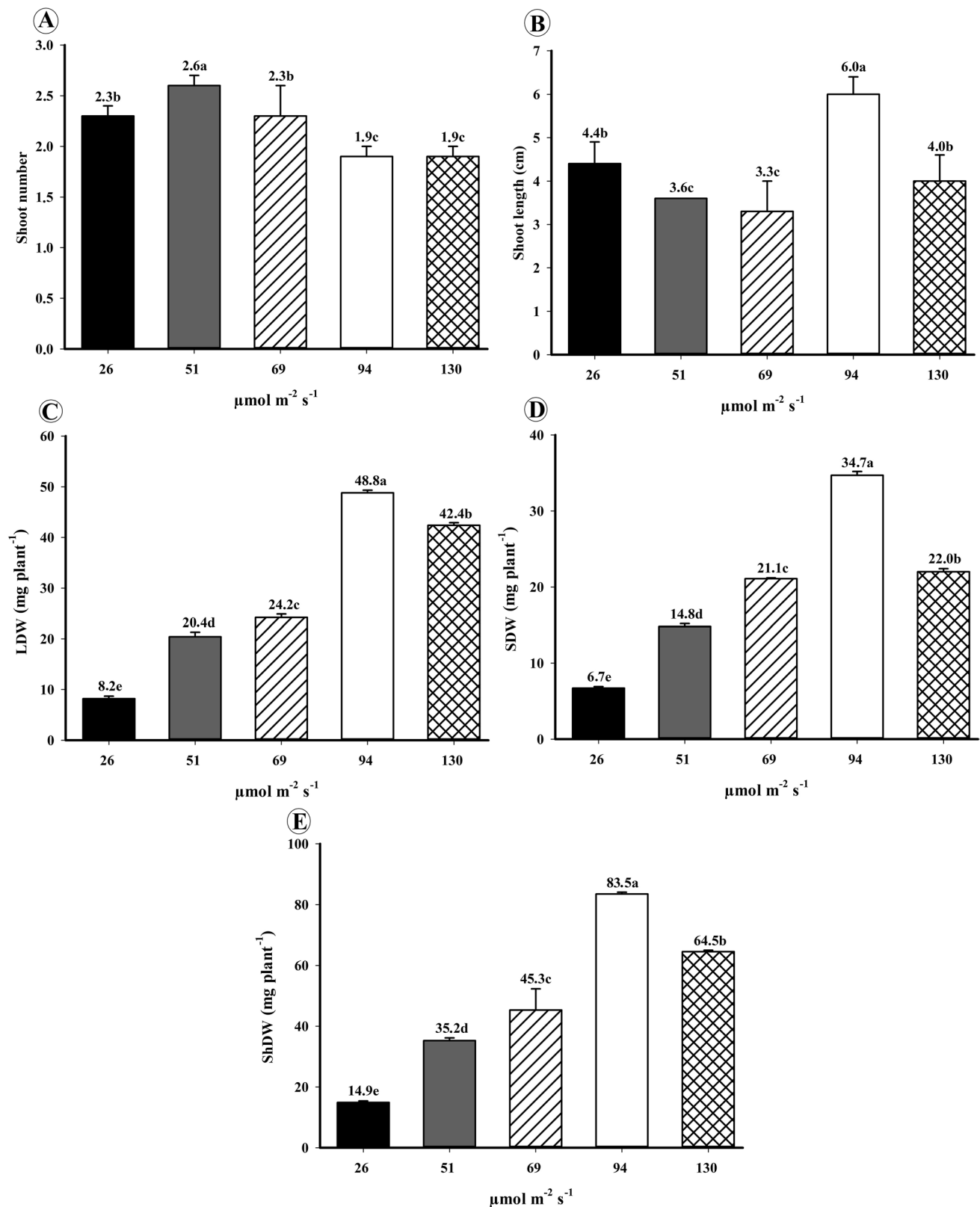


Fig. 2 a Shoot number (SN), b shoot length (SL), c leaf dry weight (LDW), d stem (SDW), e shoot dry weight (ShDW), of plantlets of *Origanum majorana* from nodal segments cultivated in vitro in the

intensities 26, 51, 69, 94 and 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at 40 days. Means followed by the same letter do not differ from each other, in the Scott Knott test, at the 5% probability level. Replicates $n = 4$

carotenoids were observed in cultivation under intensities of 94 and 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 1).

Light intensity is one of the key factors in the regulation of genes responsible for chlorophyll and carotenoid biosynthesis. The results obtained in the present study indicate that the values of chlorophyll and carotenoids decrease in *O. majorana* with increasing light intensity, thus suggesting a correlation between greater light intensity and reduced concentration of photosynthetic pigments due to photodegradation (Cavallaro et al. 2022; Silva et al. 2017). The plantlets are grown in flasks where there is high humidity, resulting in less epicuticular wax (EW) on the leaves. EW has been identified as an important physical adaptation to a wide range of environmental factors, such as droughts, high irradiance and herbivory. Thus, in vitro plantlets with lower wax accumulation become susceptible to high light conditions and prone to photoinhibition due to damage to the photosynthetic apparatus and photosynthetic pigment synthesis (Silva et al. 2017; Singh and Patel 2014). Nevertheless, in this study, dry weight accumulation was not affected by high radiation in *O. majorana* (Fig. 2).

However, not all species exhibit this behavior in vitro. In the cultivation of *A. millefolium*, when evaluating different light intensities (13 to 69 $\mu\text{mol m}^{-2} \text{s}^{-1}$), the authors observed a higher content of photosynthetic pigments at 69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Alvarenga et al. 2015). Similar results were described in the in vitro cultivation of *G. jamesonii* under different light intensities (40 to 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$), where the photosynthetic pigment content increased with increasing intensity, with an intensity of 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ accumulating higher pigment contents (Cioć et al. 2019). Thus, it is clear that different species in vitro need different light conditions, adapting morphologically and physiologically to different light intensities in a unique way.

Quantification of arbutin under different light intensities

Light is an abiotic factor that affects the secondary metabolite compounds of plants. In this study, different light intensities significantly affected arbutin accumulation in *Origanum majorana* plantlets grown in vitro (Figs. 3 and 4). The increase in light intensity stimulates the production of phenolic compounds in plants, with the function of protecting the photosynthetic apparatus, because light affects the production of secondary metabolites such as phenolic compounds and flavonoids, however, its action in increasing or decreasing the content of compounds is specific to the species and the constituent of interest (Jing et al. 2018; Taulavuori et al. 2018).

The increase in light intensity promoted greater accumulation of arbutin in the leaves of *O. majorana* in vitro. From the light intensity of 51 $\mu\text{mol m}^{-2} \text{s}^{-1}$, arbutin accumulated

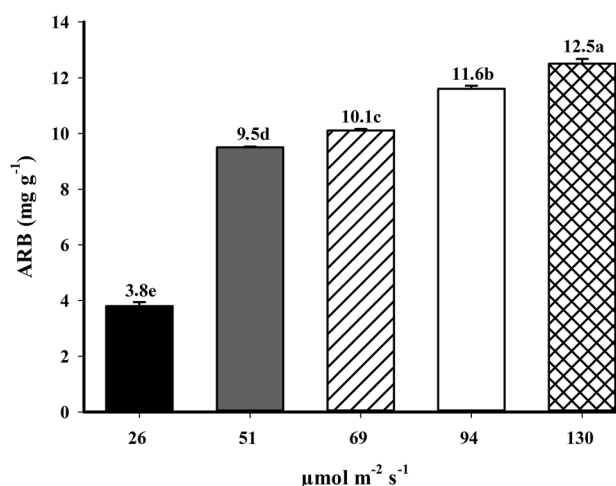


Fig. 3 Quantification of ARB (mg g⁻¹) in leaves produced in in vitro cultivation of *Origanum majorana* under different light intensities. Means followed by the same letter do not differ from each other, according to the Scott Knott test, at the 5% probability level. Replicates n=3

more than twice compared to the control (26 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The increase in light intensity leads to a greater production of photoassimilates to a level where harmful reactions to the plant begin to occur, caused by light stress (Taiz et al. 2017). Light stress did not favor the accumulation of photosynthetic pigments, as discussed above, but favored the accumulation of arbutin (12.5 mg g⁻¹) as a possible response to light stress in plantlets grown under 139 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Quantitative analysis of *Arbutus andrachne* L. leaves in in vivo and in vitro cultivation using high-performance liquid chromatography (HPLC) showed that the in vivo leaves contained a higher arbutin content (0.3–0.81% w/w) than in vitro leaves (0.09% w/w) (Mostafa et al. 2010). In this study, in vitro cultivation of *O. majorana* using HPLC revealed that the leaves contained an arbutin content of 1.25% under the highest light intensity.

Similar results with the secondary metabolites regarding light irradiance were observed in the in vitro culture of *Digitalis mariana* under different light intensities (20 to 139 $\mu\text{mol m}^{-2} \text{s}^{-1}$), where the increase in light intensity led to an increase in the cardenolide content, justifying this increased stress due to exposure to high light intensity (Silva et al. 2022). Another study with in vitro cultivation under different light intensities changed the profile of volatile compounds and increased the content of carvacrol in *P. amboinicus* (Silva et al. 2017). In contrast to the in vitro cultivation of *L. gracilis* under different light intensities, researchers observed an increase in the γ -terpinene content and a decrease in the carvacrol content under cultivation at 26 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Lazzarini et al. 2018).

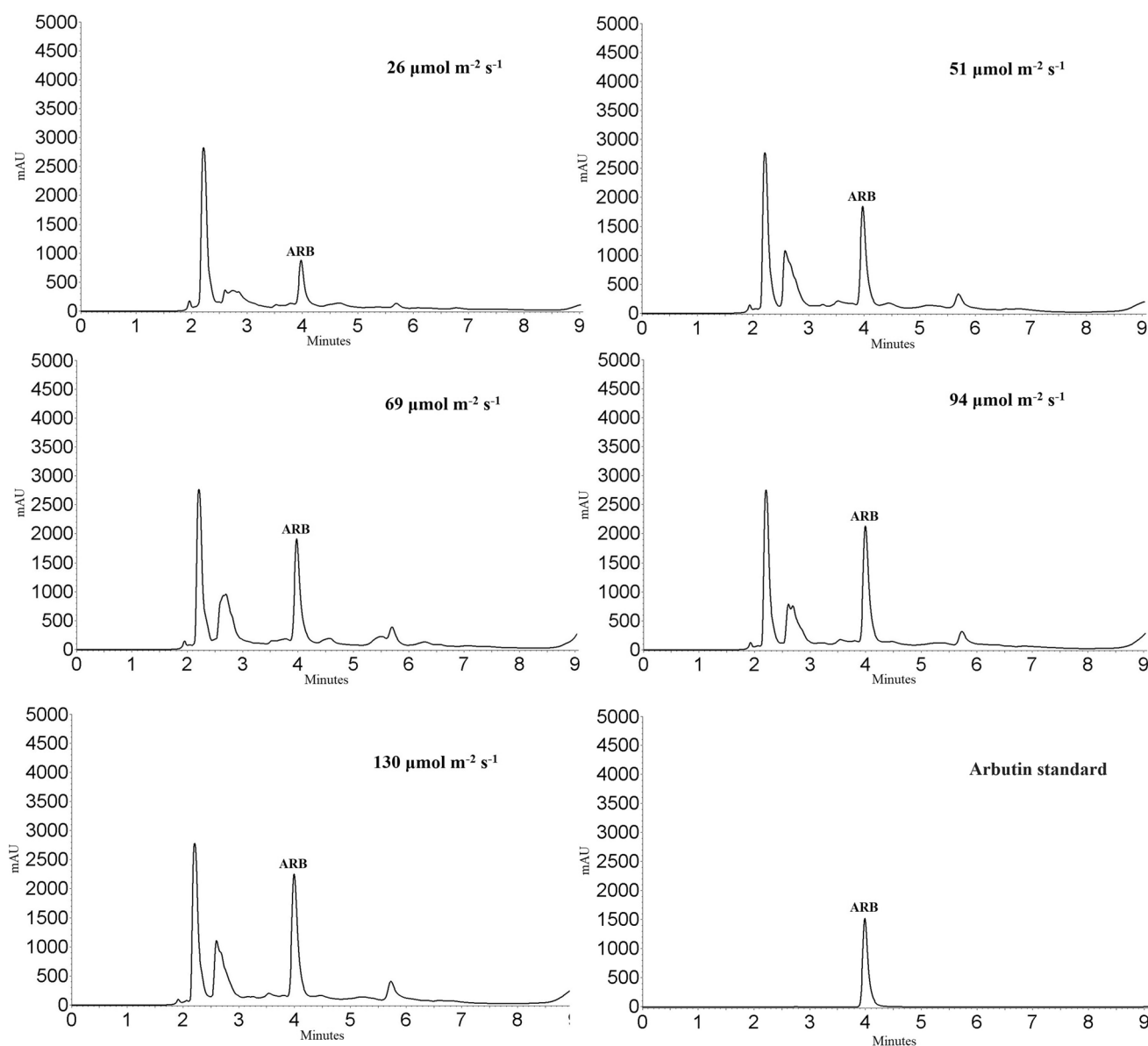


Fig. 4 Chromatographic profiles obtained by HPLC-DAD (quantification of arbutin in dry leaves, wavelength 220 nm). RT ARB=4.2 min. produced in the in vitro cultivation of *Origanum*

majorana under different light intensities: $26 \mu\text{mol m}^{-2} \text{s}^{-1}$, $51 \mu\text{mol m}^{-2} \text{s}^{-1}$, $69 \mu\text{mol m}^{-2} \text{s}^{-1}$, $94 \mu\text{mol m}^{-2} \text{s}^{-1}$, $130 \mu\text{mol m}^{-2} \text{s}^{-1}$

Growth analysis under different wavelengths

The wavelength can affect secondary metabolism, shoot multiplication and the development and growth characteristics of the plantlets in vitro, i.e., shoot length, dry weight and photosynthetic pigments. The growth of *O. majorana* in vitro was significantly influenced by the spectral quality of light (Figs. 5 and 6). The number of shoots regenerated under white, green, yellow, red and 70%B:30%R light spectra had no significant effect (Fig. 6A). Plantlets grown under green and yellow light were etiolated and had small leaves. Higher values of shoot length were observed under

cultivation under the white light spectrum (7.3 cm). This result can be explained because white light has a broad spectrum, which is different from the specificity of monochromatic lights (Cavallaro et al. 2022). However, monochromatic blue light spectra and combinations of blue and red light had an inhibitory effect on shoot length (Fig. 6B). Different aspects of plant growth and development are regulated by various blue light receptors. The wavelength region of light from UV-A to blue (B, 320–500 nm) is perceived by three photoreceptors that act in plants. The three photoreceptors act as chromophores called cryptochromes (CRY1, CRY2, and CRY3). At high blue light irradiation, CRY1



Fig. 5 *Origanum majorana* plantlets from nodal segments cultivated in vitro under different light spectra: white, blue (B), green, yellow, red (R), 50%B/50%R, 30%B/70%R, 70%B/30%R at 40 days. (Colour figure online)

inhibits stem growth and reduces internode elongation, and CRY2 inhibits it at low blue light irradiation (Cavallaro et al. 2022; Lin 2000).

The leaf dry weight (LDW) of plantlets grown under white LED light accumulated the highest value (28.1 mg), followed by plantlets grown under a combination of blue and red light (70% B:30% R) (Fig. 6C). Plantlets grown under monochromatic blue, green and yellow light accumulated less dry weight (Fig. 6C, D, E). White- and red-light spectra were more efficient in the production of SDW, with blue and green monochromatic lights, and 50% B:50% R showed lower accumulation of SDW. In general, higher aerial part dry weight (ShDW) production was favored by cultivation in the white- and red-light spectra and a combination of blue and red (70% B:30% R and 30% B:70% R). Similar to the present study, Sandhya et al. (2021) reported that *O. majorana* plantlets maintained under white fluorescent light intensity of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ had good growth.

Different species responded to dry weight accumulation unique way. The cultivation of *U. dioica* under the combination of 70% R:30% B resulted in higher dry weight accumulation than that under white light (Coelho et al. 2021). The species *L. rotundifolia* also obtained the best biometric data under the combination of 70% R:30% B (Hsie et al. 2019). The species *A. suaveolens* obtained the highest dry weight gain under white and fluorescent light (Araújo et al. 2021). For the light spectra, *L. dulcis* plantlets grown under combinations of 30% B:70% R and 50% B:50% R provided greater light accumulation of dry weight (Rocha et al. 2022). Monochromatic red led to a greater gain of dry weight in the species *P. amboinicus* (Silva et al. 2017) and in *L. gracilis* (Lazzarini et al. 2018).

All these results indicate that the responses of plants to LED lighting are species and/or cultivar dependent.

Analysis of photosynthetic pigments under different wavelengths

The accumulation of photosynthetic pigments was significantly influenced by the light spectrum (Table 2). The leaves of *O. majorana* plantlets accumulated higher levels of chlorophyll *a*, total chlorophyll and carotenoids under cultivation under green light. There was a significant increase in chlorophyll *a* content under green light ($0.68 \text{ mg g}^{-1} \text{ FW}$), followed by the red-light spectrum ($0.46 \text{ mg g}^{-1} \text{ FW}$). High values of photosynthetic pigments observed under green light cultivation are indicative of plant stress, and this increase is a way to compensate for the lack of photosynthetically active light (Cavallaro et al. 2022). This compensation is explained by the penetration of green light into the plants, which favors plant growth, increasing photosynthesis in the leaves more efficiently than blue and red light (Alvarenga et al. 2015). Similar results were observed in the cultivation of *A. millefolium* under the green light spectrum, which showed higher values of chlorophyll *a* and *b* and carotenoids (Alvarenga et al. 2015).

Lower accumulation of chlorophyll *a* and *b* was observed under the yellow (0.16 and $0.04 \text{ mg g}^{-1} \text{ FW}$, respectively) and blue (0.23 and $0.06 \text{ mg g}^{-1} \text{ FW}$) spectra (Table 2). Weremczuk-Jeżyna et al. (2021) observed contrary results, where the highest chlorophyll content in the cultivation of *Dracocephalum forrestii* was observed under the blue light spectrum. Likewise, in the cultivation of *Saccharum officinarum*, the combination of blue- and red-light spectra (70% B:30% R and 50% B:50% R) induced the production of total and chlorophyll *a*, the authors justify these results by the fact that blue light plays an important role in the synthesis of chlorophyll, inducing its production and chloroplasts (Silva et al. 2014). In the in vitro culture of *P. amboinicus*, they observed an increase in the production of photosynthetic pigments under the spectra of the combinations of 50% B:50% R and 30% B:70% R (Silva et al. 2017). Therefore, according to data in the literature compared to the results of the present study, it was noted that the increase in photosynthetic pigments was dependent on species and light quality.

The spectral quality of light significantly influences the biological response of the aerial part. Since plant photoreceptors responsible for plant development and photosynthesis are known to be stimulated mainly and most significantly by the red and blue regions of the light spectrum, most studies have evaluated the impact of monochromatic red (660 nm), blue (460 nm) and combined blue (440–480 nm) with red lights (630–665 nm). Little information is available

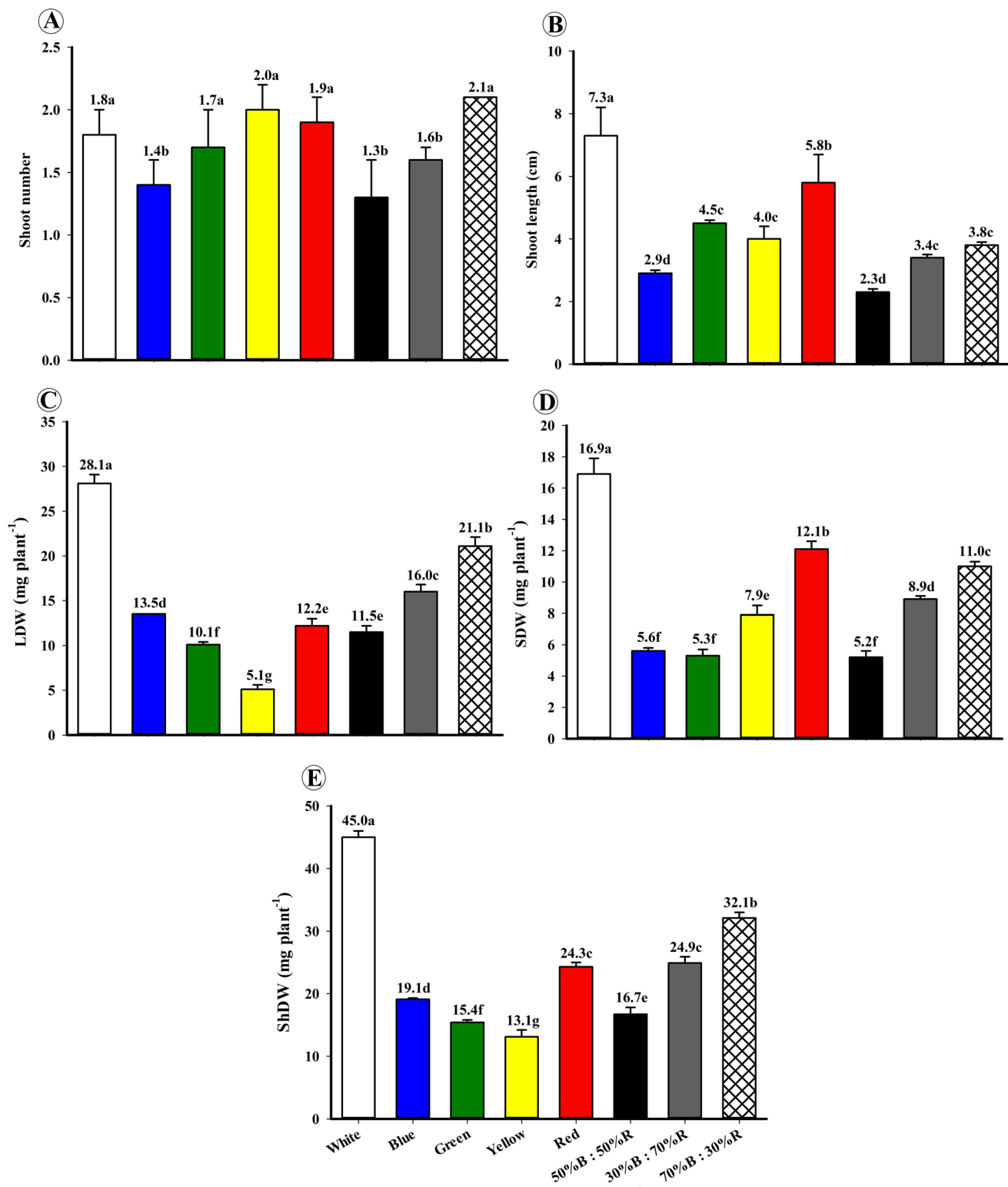


Fig. 6 *Origanum majorana* plantlets from nodal segments cultivated in vitro under different light spectra, at 40 days. **A** Shoot number (SN), **B** Shoot length (SL), **C** leaf dry weight (LDW), **D** stem (SDW) and **E** Shoot dry weight (ShDW). Replicates $n=3$

Table 2 Concentration of photosynthetic pigments in *Origanum majorana* plantlets cultivated in vitro under different light spectra, at 40 days

Spectra	Chlorophyll			Carotenoids
	<i>a</i>	<i>b</i>	Total	
	(mg g ⁻¹ FM)			
White	0.41c	0.16b	0.57b	0.12c
Blue (B)	0.23f	0.06d	0.29e	0.07f
Green	0.68a	0.16b	0.80a	0.17a
Yellow	0.16 g	0.04e	0.20f	0.05d
Red (R)	0.46b	0.11c	0.57b	0.13b
50%B:50%R	0.33d	0.11c	0.44d	0.11d
30%B:70%R	0.29e	0.22a	0.50c	0.10e
70%B:30%R	0.45b	0.12c	0.57b	0.13b

Means followed by the same letter in the column do not differ from each other, according to the Scott Knott test, at the 5% probability level

Replicates n = 3

FM fresh matter

on the effects of the far red, green and yellow regions of the spectrum (Cavallaro et al. 2022).

Quantification of arbutin at different wavelengths

The arbutin levels in *O. majorana* plantlets were affected by the spectral quality of light (Fig. 7). The chromatographic profile of the plantlets grown under the different light spectra can be seen in Fig. 8. Higher values.

of arbutin production were observed under cultivation under red monochromatic light (13 mg g⁻¹), 35% higher than under white light (9.6 mg g⁻¹) and 2.8 times higher than under monochromatic yellow light. The study shows that the plantlets that were cultivated with the highest proportion of red in relation to the blue spectrum (30% B:70% R) had the highest arbutin accumulation (10.3 mg g⁻¹). The red monochromatic light accumulated 94% more than the blue monochromatic light (6.7 mg g⁻¹). The use of monochromatic or composite LED lights with different spectral qualities may be a tool for the induction of bioactive metabolites and pigments of interest in in vitro culture. Lower values of arbutin were observed under blue (6.7 mg g⁻¹), green (6.5 mg g⁻¹) and yellow (4.6 mg g⁻¹) light culture. These results can be explained by the fact that plants respond to the environment differently, allocating their photoassimilates for the biosynthesis of elements necessary for their survival and adaptation to the environmental conditions offered by different light sources.

The reports that the variation in secondary compounds under cultivation at different wavelengths is species dependent. The in vitro culture of *D. mariana* cultivated under

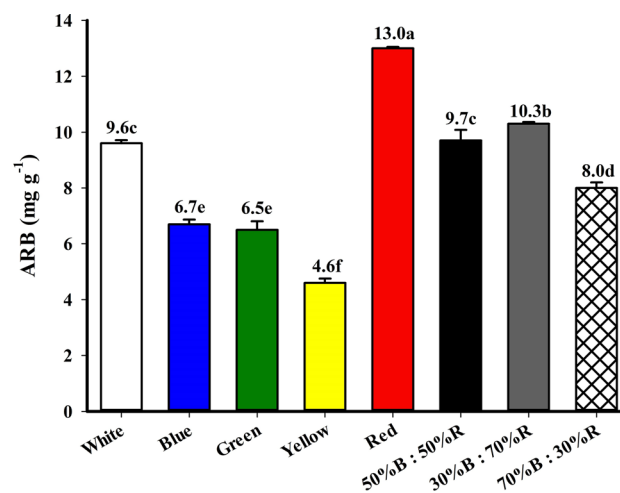


Fig. 7 Quantification of arbutin (ARB-mg g⁻¹) in leaves produced in in vitro cultivation of *Origanum majorana* under different light spectra. Replicates n = 3

combined red and blue light (30% R:70% B and 50% R:50% B) showed higher cardenolide content (Silva et al. 2022). Red and blue light are mostly responsible for the biosynthesis of secondary metabolites due to their participation in photosynthesis. Silva et al. (2017) reported a higher accumulation of secondary metabolites in the in vitro culture of *P. amboinicus* under the blue light spectrum, where they obtained a higher carvacrol content. The monochromatic blue spectrum increased the content of myrcene and limonene compounds and the highest content of pentadecane under the white spectrum and combinations of red and blue in the essential oil of *L. rotundifolia* (Hsie et al. 2019). The volatile compounds in the species *A. suaveolens* increased the linalool content under white LED light (Araújo et al. 2021). The wavelength of 30% R:70% B obtained a 5.53-fold increase in the concentration of phenolic compounds and an 8.63-fold increase in the concentration of flavonoids compared to the fluorescent lamp (Coelho et al. 2021). Several studies emphasize that the production of phenolic compounds depends on the species, cultivar, and exposure time and that each phenolic compound has a specific response to light quality (Loi et al. 2021). Since arbutin is a phenol, its production in *O. majorana* under different light spectra is expected to be species specific.

Principal component analysis (PCA)

PCA (scores and loadings) was used to evaluate the correlations between dry weights, arbutin content and photosynthetic pigments of *O. majorana* treated under different wavelengths and light intensities. The PCA of light intensity was based on 8 parameters (growth and chemical compounds) that accounted for 96.51%, and for light quality, it

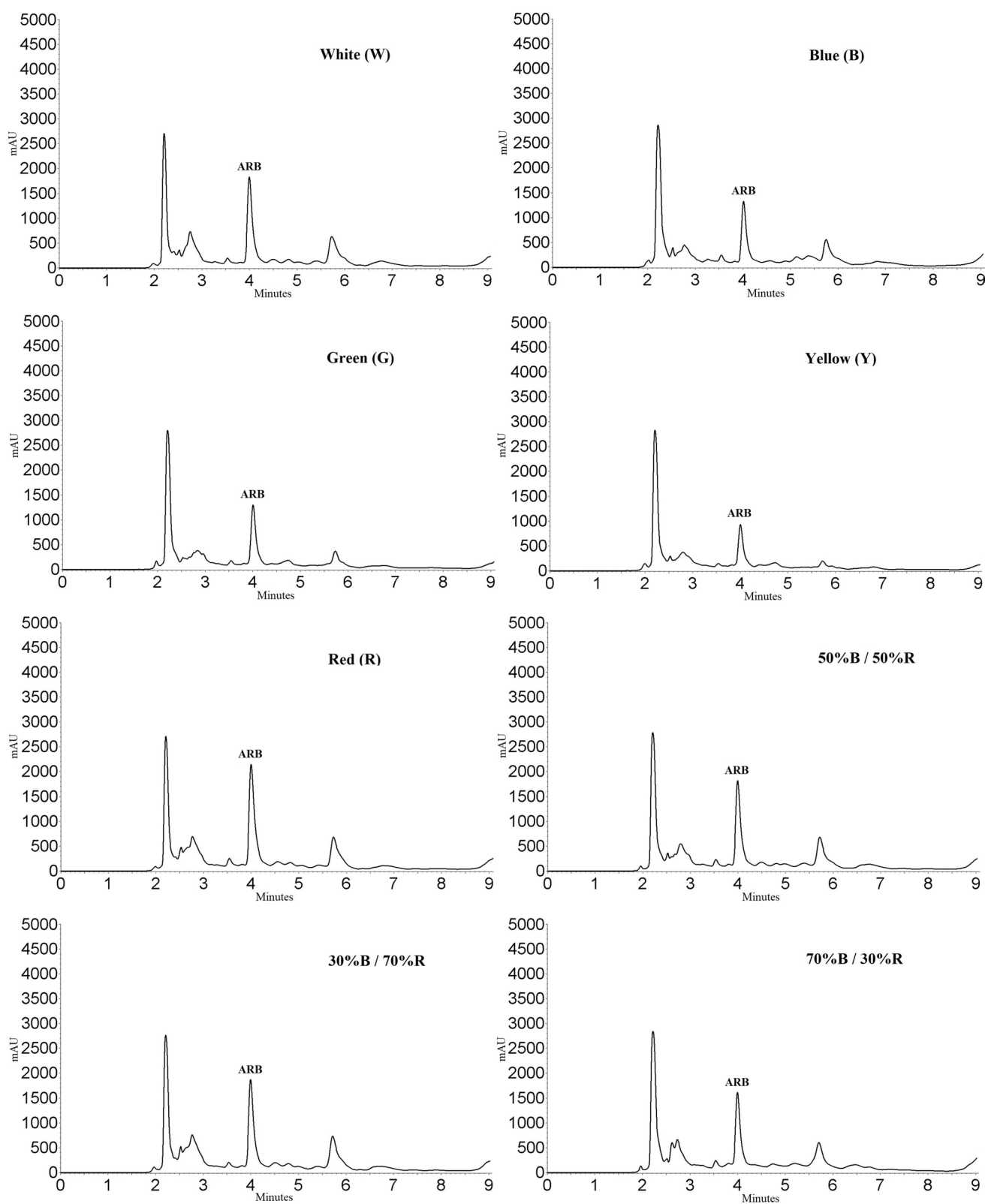
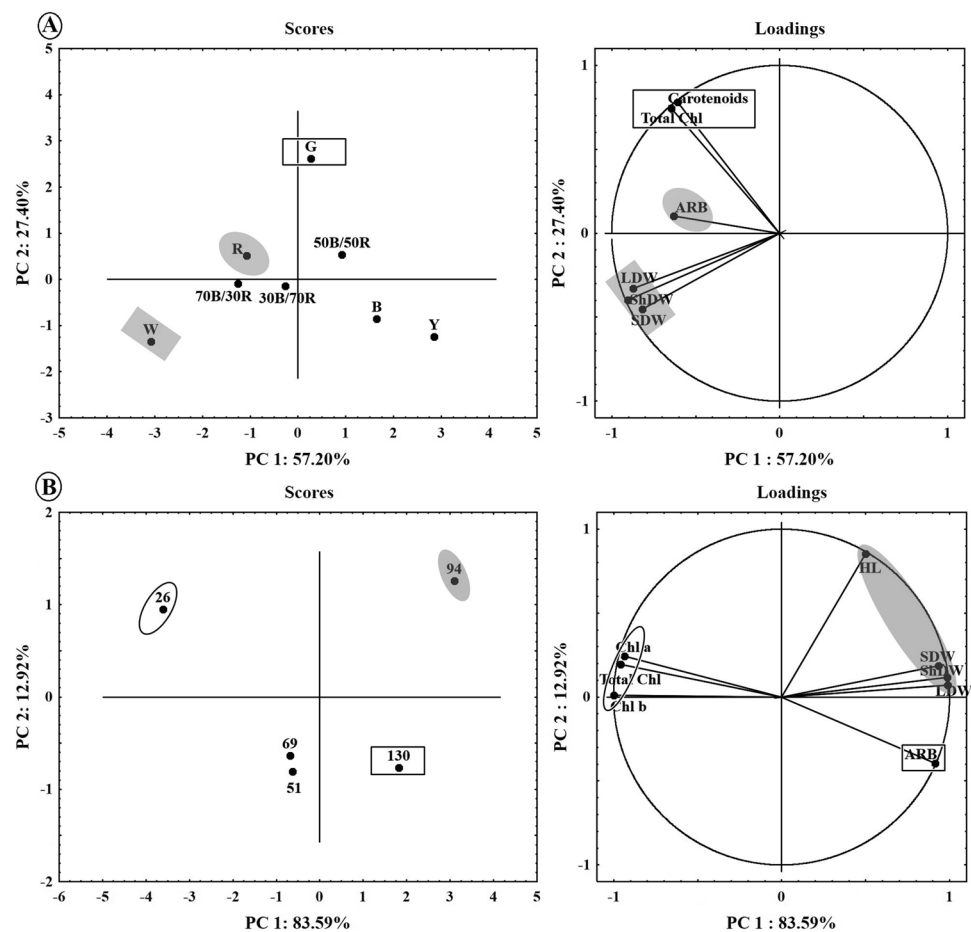


Fig. 8 Chromatographic profiles obtained by HPLC-DAD in the quantification of arbutin in dry leaves, wavelength 220 nm produced in the in vitro cultivation of *Origanum majorana* under different

light spectra: White, Blue, Green, Yellow, Red, 50B:50R, 30R:70B, 70R:30R). (Colour figure online)

Fig. 9 Principal component analysis (PCA) of the constructed matrix correlation using data on photosynthetic pigments, dry weight and arbutin content. **A** light spectral quality, **B** photon flux density



was based on 6 parameters that accounted for 84.6% of the total variation in the experimental procedure. Based on these parameters, it was possible to group the data collected into 3 clusters to evaluate light quality (Fig. 9A) and intensity (Fig. 9B).

Evaluating the correlations of the 6 parameters under the wavelength, PCA showed that plantlets of *O. majorana* under monochromatic red light had higher arbutin content, and plantlets under white light had higher LDW, SDW and ShDW. The plants showed higher levels of carotenoids and total chlorophyll under green light (Fig. 9A). Regarding light intensity, it is possible to conclude that *O. majorana* under higher intensity ($130 \mu\text{mol m}^{-2} \text{s}^{-1}$) had higher arbutin content. Plantlets grown under $94 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity showed higher LDW, SDW, ShDW, and SL, while lower light intensity ($26 \mu\text{mol m}^{-2} \text{s}^{-1}$) accumulated a higher content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and total chlorophyll) (Fig. 9B). The PCA results corroborate and complement the previous analyses and interpretations.

Conclusion

The manipulation of light intensity and quality in the *in vitro* culture of *Origanum majorana* L. influences plantlets growth and the accumulation of photosynthetic pigments and arbutin. Low light intensities (26 and $51 \mu\text{mol m}^{-2} \text{s}^{-1}$) favor the growth and accumulation of photosynthetic pigments. However, for greater accumulation of arbutin, *in vitro* culture under higher light intensity ($130 \mu\text{mol m}^{-2} \text{s}^{-1}$) is necessary. Regarding light quality, different spectra did not significantly affect the number of shoots or shoot length. The combined white and red and blue light spectra stimulated greater accumulation of biomass. The total chlorophyll content spectrum of monochromatic green, white and blue and red light combined provided higher values. The levels of the chemical compound arbutin were influenced by the different light spectra. The red monochromatic spectrum increased the arbutin content, and the yellow monochromatic spectrum decreased the arbutin content.

Author contributions MCVC, JPMR, SKVB and JEBPP designed the research; MCVC, JPMR, RMAA, JJFL and LFT conducted the experiment; MCVC, JPMR, RMAA, JJFL and LFT analyzed the data and wrote the paper; and MCVC, SKVB and JEBPP had primary responsibility for the final content. All authors have read and approved the final manuscript.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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