



The use of different LEDs wavelength and light intensities for in vitro proliferation of cherry rootstock: influence on photosynthesis and photomorphogenesis

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Abstract

Plant growth and development are strongly influenced by light quality conditions in their environment. In this study, in vitro shoot proliferation, photosynthetic pigments, leaf anatomy and photosystem II photochemistry of Gisela 6 cherry rootstock (*Prunus cerasus* x *Prunus canescens*) were investigated. The culture medium used was the MS (Murashige and Skoog) containing 4.4 μM 6-benzyladenine (BA), 0.05 μM α -naphthalene acetic acid (NAA) and 0.3 μM gibberellic acid (GA_3) (30 days, 16 h photoperiod, 22 ± 2 °C). Initially, two different light wavelength absorption spectra: 400–700 nm provided by white fluorescent light lamps (mainly blue–green, BG at equal ratio 1:1; WFL-40) and, 430–690 nm provided by two Light Emitting Diodes (LED) types, differing in the spectral ratio of multiple light emitting wavelengths [LED2-40 (blue–red, BR) and LED3-40 (blue–green–yellow–orange–red, BGYOR)] were studied, under 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light intensity. Following that, three LED intensities-20 (LED1-20), 40 (LED2-40), and 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (LED4-120) were investigated under BR spectrum. WFL-40 exhibited non-significant differences with LED2-40 (shoot number 4.25, proliferation rate 4.30, shoot height 0.91 cm). LED2-40 displayed higher fresh and dry shoot biomass, better PSII functionality, increased Chl a/b, Chl b and carotenoids content as well as decreased number of stressed explants. Leaf anatomy under LED2-40 and WFL-40 was similar. LED4-120 presented the highest rate of stressed explants, with their leaf anatomy being severely affected, as leaves had a reduced mesophyll cell density. In LED4-120, the proportion of the absorbed energy used in photochemistry (Φ_{PSII}) was decreased whereas the increased quantum yield of non-regulated energy loss in PSII (Φ_{NO}) could not be compensated by the increased photoprotective energy dissipation (Φ_{NPQ}). Therefore, LED2-40 is proposed as the most beneficial for growing cherry rootstock Gisela 6 plantlets in vitro.

Key Message

Higher proliferation rate, shoot biomass, photosystem II functionality, Chl a/b and carotenoids under LED2-40 (BR) [430–690 nm, mainly blue-red at a 1:3 ratio, 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$] whereas high light stress [LED4-120 (BR)] (120 $\mu\text{mol m}^{-2} \text{s}^{-1}$) adversely affected leaf anatomy.

Keywords Gisela 6 · Light-emitting diodes · Chlorophyll content · Chlorophyll fluorescence parameters · Light microscopy · Micropropagation

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Introduction

The *Prunus cerasus* × *Prunus canescens* hybrid (Gisela 6) is a semi-dwarf rootstock, compatible with most of the sweet cherry varieties, commonly used in cherry tree nurseries, because it grows in a broad range of soils, mainly the heavy ones (Andersen et al. 1999). Large-scale production of Gisela 6 through cuttings and layering—to meet the growing internal demand for high quality, disease-free and uniform planting material, seems to be difficult (Aka-Kacar et al. 2010). However, the use of micropropagation could assist to overcome these encountered problems.

Light, being simultaneously both a signal and an energy source, is an environmental factor absolutely essential in commercial micropropagation laboratories, for establishing optimal plant growth and development. When in vitro cultures are being illuminated with fluorescence lamps (FLs), power consumption used for lighting represents the 65% of the total energy used (Bornwaver and Tantau 2012). During the recent years, LEDs as a lighting source has attracted considerable interest. LEDs show numerous advantages, such as a customizable spectra, a relative decreased mass and volume, adaptable light intensity, high energy conversion effectiveness, favoring reduced production expenses (Gupta and Jatothu 2013), i.e. 10 to 100 fold lower energy consumption than conventional lighting e.g. FLs (Dutta Gupta 2017).

Toning LED wavelengths suited to plant photoreceptors could present a solution for the best possible crop production (Massa et al. 2008). The two absorption peaks of chlorophyll in the sunlights spectrum (300–2600 nm) are at wavelengths from 430–450 nm and 640–660 nm (Long et al. 2018) so, the wavelengths which directly influence plant photosynthesis are between 400 and 700 nm, in which blue (B) light (420–500 nm) and red (R) light (620–700 nm) play important roles, while yellow, orange and green are minimally absorbed (Pan 2008). Since, the relation between light quality and quantity with the endogenous hormonal levels influences plant growth (Ouyang et al. 2015), it has been noted that B and R LEDs, stimulated the growth of several horticultural plants (Naznin et al. 2019), while studies also revealed that different LED lighting combinations caused a biomass increase (Idrees et al., 2018), affected shoot formation and ex vitro survival rate (Dutta Gupta and Sahoo 2015).

Leaves grown under FLs (a light source traditionally used in in vitro cultures) show different anatomical features, than those of plants grown in vitro under LED lighting (Gonçalves et al. 2008). This indicates that LEDs do not alter leaf anatomy like traditional light conditions do, being at the same time energy efficient. However, the internal leaf structure could be affected by different

light qualities (Slattery et al. 2018), so leaf specific anatomical traits could appear altered by differential LED illumination.

Highly increased light intensities hamper normal photomorphogenesis, and lead to capital forfeit due to augmented power use (Dobránszki and Mendler-Drienyovszki 2014). Researchers so far, have studied the relation between light and growth, photosynthetic efficiency features, carbon and nitrogen consumption, volatile secretion and leaf anatomy in many plants of increased agricultural/horticultural value (Ma et al. 2021). An approach to understand the optimal light intensity is to estimate light energy use efficiency (Montgomery and Givnish 2008). In this context, chlorophyll fluorescence is a sensitive method that could be applied to calculate and study any alteration in photosynthetic efficiency, detect how numerous external factors affect plant growth (Ouzounis et al. 2015), and generally monitor plant health status (Moustakas et al. 2020). As already established, in vitro phytomorphogenesis, is influenced by the use of monochromatic (alone or in combination) red and blue LEDs (Gupta and Jatothu 2013).

The aim of this study was to determinate the optimal proportions of blue, green, yellow, orange and red LED lights suitable for microshoot in vitro grown of Gisela 6, as well as the appropriate LED light intensity on shoot proliferation, photosynthetic pigments, photosystem II photochemistry and leaf anatomy, evaluating the optima qualitative and quantitative parameters of LED lighting which could substitute WFLs.

Materials and methods

Plant material

Shoot tips explants of Gisela 6 sub-cultured every 4 weeks on MS (Murashige and Skoog 1962) medium fortified with 4.4 μM BA, 0.05 μM NAA, 0.3 μM GA₃, 20 g L⁻¹ sucrose (Duchefa, The Netherlands), (pH: 5.8) and 6 g L⁻¹ Plant Agar (Duchefa, The Netherlands) as the solidifying agent, were used as the stock plant material for further experimentation. The culture medium was autoclaved at 121 °C for 20 min. The explants were then transferred into Magenta vessels (62 mm × 95 mm, 200 mL, Sigma-Aldrich), each containing 35 mL of medium. Following that, the explants were incubated in a growth chamber under controlled conditions of temperature (22 ± 2 °C), photoperiod (16 h light/8 h darkness), and light intensity (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$) provided by cool white FLs (WFLs).

Different light absorption wavelength spectra and intensities on in vitro shoot proliferation

The experimental materials used were shoot node explants 1–1.5 cm long with 2–3 buds obtained from the previous stock in vitro cultures. The environmental conditions related to temperature, photoperiod, light intensity and illumination type (WFLs) were similar as previously stated.

Initially, two different light wavelength absorption spectra: 400–700 nm provided by WFL lamps (mainly blue-green, BG, at equal ratio 1:1) and 430–690 nm provided by two different LED types (Magnus Light-Quality LED Solutions, Biotechnology Company, FOSPAN WORLD-WIDE SL 08912 Badalona-Barcelona, Spain) under light intensity of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ were used. The cool white fluorescent light (WFL) served as the control treatment, thus, three in total treatments were included: WFL-40 (BG, $40 \mu\text{mol m}^{-2} \text{s}^{-1}$), LED2-40 (BR, $40 \mu\text{mol m}^{-2} \text{s}^{-1}$), and LED3-40 (BGYOR, $40 \mu\text{mol m}^{-2} \text{s}^{-1}$). Following that, the effect of three LED light intensities (20, 40 and $120 \mu\text{mol m}^{-2} \text{s}^{-1}$) under 430–690 nm wavelength absorption spectrum (mainly blue-red, 1B:3R) was tested, thus, another three treatments were tested: LED1-20 (BR, $20 \mu\text{mol m}^{-2} \text{s}^{-1}$), LED2-40 (BR, $40 \mu\text{mol m}^{-2} \text{s}^{-1}$), and LED4-120 (BR, $120 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Detailed description of illumination conditions for all treatments tested are presented in Table 1. In addition, absolute spectral value ($\text{W m}^{-2} \text{nm}^{-1}$) distribution and absorption wavelength range (nm) per emitted light wavelength percentages (%) for WFL (Fig. 1a, b), and the two LED types—Magnus Clonekit Pro 120 cm (Fig. 1c, d) and Magnus Clonekit 120 cm (Fig. 1e, f) are provided. The difference between the two LED types was in the spectral ratio of multiple light emitting wavelengths in the same absorption spectrum.

After 30 days of culture, the following data were recorded: number of new shoots ≥ 0.3 cm per explant, shoot length (cm), shoot proliferation rate, fresh weight (FW) and dry weight (DW) of explants, FW/DW ratio, multiple shoot formation percentage (%) (i.e., the number of proliferated explants with formation of new shoots ≥ 0.3 cm/the initial number of explants $\times 100\%$), and percentage (%) of stressed explants (i.e., the number of explants appearing macroscopically stress symptoms/the initial number of explants $\times 100\%$). Mild chlorosis, light brown leaf coloration and bleaching, smaller leaf number and leaf size, less intense leaf green color, and slight twisting inwards of the lower leaves to the base were the observed stress symptoms. Each explant was characterized as stressed when all symptoms as previously mentioned were visible at the same time.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured in 20 min dark-adapted Gisela 6 leaves with the use of an Imaging-PAM fluorometer M-series MINI-version (Walz, Effeltrich, Germany), as described by Moustaka et al. (2018). Five leaves from five different plants were measured from each treatment [LED1-20 (BR), LED2-40 (BR), LED3-40 (BGYOR), LED4-120 (BR) and White Fluorescent Light (WFL-40 (BG)] at the actinic light intensities of $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ for LED1, $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ for LED2, LED3 and WFL and $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ for LED4, similar to their growth light. Via Win software (Heinz Walz GmbH, Effeltrich, Germany), the maximum efficiency of photosystem II (PSII) photochemistry (F_v/F_m), the efficiency of excitation energy capture by open PSII reaction centers (F_v/F_m'), the allocation of absorbed light energy at photosystem II (PSII) that is the effective quantum yield of PSII photochemistry (Φ_{PSII}), the quantum yield of regulated non-photochemical energy loss in PSII (Φ_{NPQ}), the quantum yield of non-regulated energy loss in PSII (Φ_{NO}), the non-photochemical quenching that reflects heat dissipation of excitation energy (NPQ), the photochemical quenching that is the redox state of the plastoquinone pool (q_p) that represents the fraction of open PSII reaction centers and the excess excitation energy (EXC) were assessed (Demmig-Adams et al. 1996).

Photosynthetic pigment analysis

The extraction of the photosynthetic pigments was performed as described by Gao et al. (2020). The optical density (OD) was measured using a PharmaSpec UV-1700 spectrophotometer (Shimadzu, Japan) at 470 nm (OD_{470}) for carotenoids, 663 nm (OD_{663}) for Chl a, and 645 nm (OD_{645}) for Chl b. The content of each pigment in the leaves was calculated using the following formulas (Gao et al. 2020):

$$\text{Chl a} (\text{mg g}^{-1}) = (12.72 \text{OD}_{663} - 2.59 \text{OD}_{645}) V / 1000 W$$

$$\text{Chl b} (\text{mg g}^{-1}) = (22.88 \text{OD}_{645} - 4.67 \text{OD}_{663}) V / 1000 W$$

$$\begin{aligned} \text{Carotenoids} (\text{mg g}^{-1}) \\ = (1000 \text{OD}_{470} - 3.27 \text{Chl a} - 104 \text{Chl b}) V \\ / (229 \times 1000 W), \end{aligned}$$

where V is the total volume of the extract (ml) and W is the FW (g) of the sample.

Table 1 Detailed description of illumination conditions for all treatments tested in this study for in vitro shoot proliferation of the Gisela 6 cherry rootstock

Treatment code number	Illumination type/source	Illumination lamps features	PAR intensity provision capacity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Light absorption wavelength spectrum (nm)	Spectral composition (%)	Spectral composition ratio
WFL-40 (BG)	WFL/PHILIPS	36 W/830 G13 1214 mm	40 (fix)	400–700	36%G: 32%B: 9%R: 9%Y: 9%O: 5%V ^a	4G:4B:1R:1Y:1O:½V
LED1-20 (BR) (20 $\mu\text{mol m}^{-2} \text{s}^{-1}$)/ LED2-40 (BR) (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$)/ LED4-120 (BR) (120 $\mu\text{mol m}^{-2} \text{s}^{-1}$)	LED/Magnus Clonekit Pro 120 cm	<ul style="list-style-type: none"> • Power consumption: 30 Watt/lightbar • PPFd: 293 μmol (distance 200 mm) • Height: at least 25–40 cm above canopy • Footprint: 60 × 120 cm • Light spectrum: RBW/RBS • Weight: 1.62 kg/lightbar • Dimensions: W1200XH45mm × 19mm 	0–120 (dimma- ble/adjustable)	430–690	63%R: 21%B: 7%G: 7%O: 2%Y ^a	9R:3B:1G:1O:¼Y
LED3-40 (BGYOR)	LED/Magnus Clonekit 120 cm	<ul style="list-style-type: none"> • Power consumption: 15 Watt/lightbar • PPFd: 85 μmol (distance 100 mm) • Height: at least 25 cm above canopy • Footprint: 60 × 120 cm • Light spectrum: RBW/RBS • Weight: 0.19 kg/lightbar • Dimensions: W1200XH17mm × 8mm 	40 (fix)	430–690	33%G: 22%B: 22%R: 11%O: 9%Y ^a	3G:2B:2R:1O:1Y

^aViolet (V), blue (B), green (G), yellow (Y), orange (O), red (R)

Light microscopy

Pieces of leaves from microshoots exposed to WFL-40 (BG), LED1-20 (BR), LED2-40 (BR), LED3-40 (BGYOR) and LED4-120 (BR) were prepared for chemical fixation, as previously reported (Dobrikova et al. 2021). In short, leaf segments were firstly immersed in a fixation solution containing 3% paraformaldehyde + 3% glutaraldehyde buffered with 0.05 M sodium cacodylate at pH 7.0 at room temperature for 6 h. Subsequently, the leaf pieces were post-fixed in a 2% osmium tetroxide solution similarly buffered for another 3 h. Following dehydration in an acetone series, leaf pieces

were treated with propylene oxide and were finally embedded in Durcupan ACM resin. Semi-thin sections (0.5–2 μm) that were obtained with the use of an ultramicrotome (LKB 8801A, Stockholm, Sweden) outfitted with a glass knife; were stained with a 0.5% (w/v) toluidine blue O solution, and observed under a Zeiss Axioplan light microscope equipped with a AxioCam MRc 5 digital camera (Zeiss, Berlin, Germany).

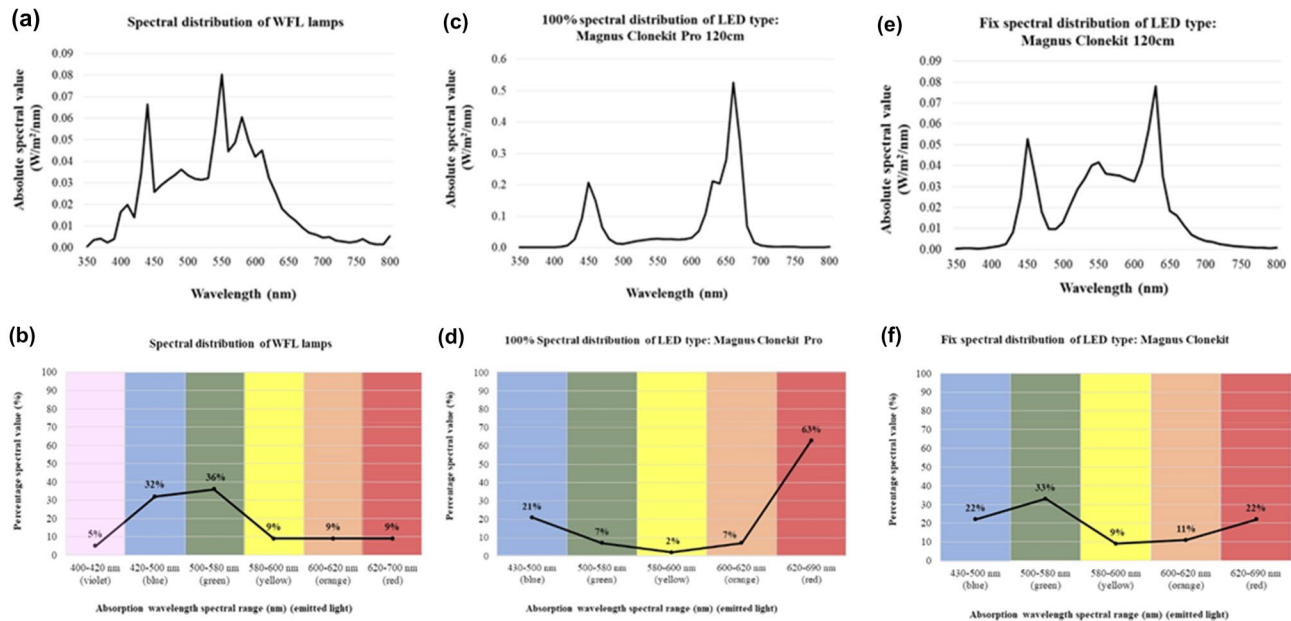


Fig. 1 Spectral distribution of WFL and the two LED types: Absolute spectral value (W/m²/nm) and absorption wavelength spectrum range (nm) per emitted light provided by **a, b** WFL [treatment: WFL-40

(BG)], **c, d** the LED type ‘Magnus Clonekit Pro 120 cm’ [treatments: LED1-20 (BR), LED2-40 (BR), LED4-120 (BR)] and **e, f** the LED type ‘Magnus Clonekit 120 cm’ [treatment: LED3-40 (BGYOR)]

Statistical analysis

Data related to shoot proliferation parameters were analyzed by using the statistical package SPSS 17.0 (SPSS Inc., Illinois, New York, USA) and Analysis of Variance (one-way ANOVA). Descriptive data were compared using the Duncan’s multiple range test at a level of $P \leq 0.05$. Results are expressed as mean \pm standard error (SE). The first experiment included two light spectra of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ intensity, while the second one included three light intensities of the same spectrum. In both experiments, 56 explants/treatment (8 vessels/treatment \times 7 explants/vessel) were used. Data for shoot number, shoot length, shoot proliferation rate, FW, DW and FW/DW are means of 56 replicates (i.e., the explants), while data for percentage (%) of stressed explants are means of eight replicates (i.e., the vessels).

Mean values obtained by two independent experiments with five leaf samples per treatment per experiment were considered regarding chlorophyll fluorescence parameters, whereas three samples per treatment per experiment were considered for photosynthetic pigments analysis. Results are expressed as mean \pm standard error (SE). For chlorophyll fluorescence and photosynthetic pigment analysis, the Student’s *t*-test (StatView computer package, Abacus Concepts, Inc Berkeley, CA, USA) was used at a level of $P < 0.05$.

Results

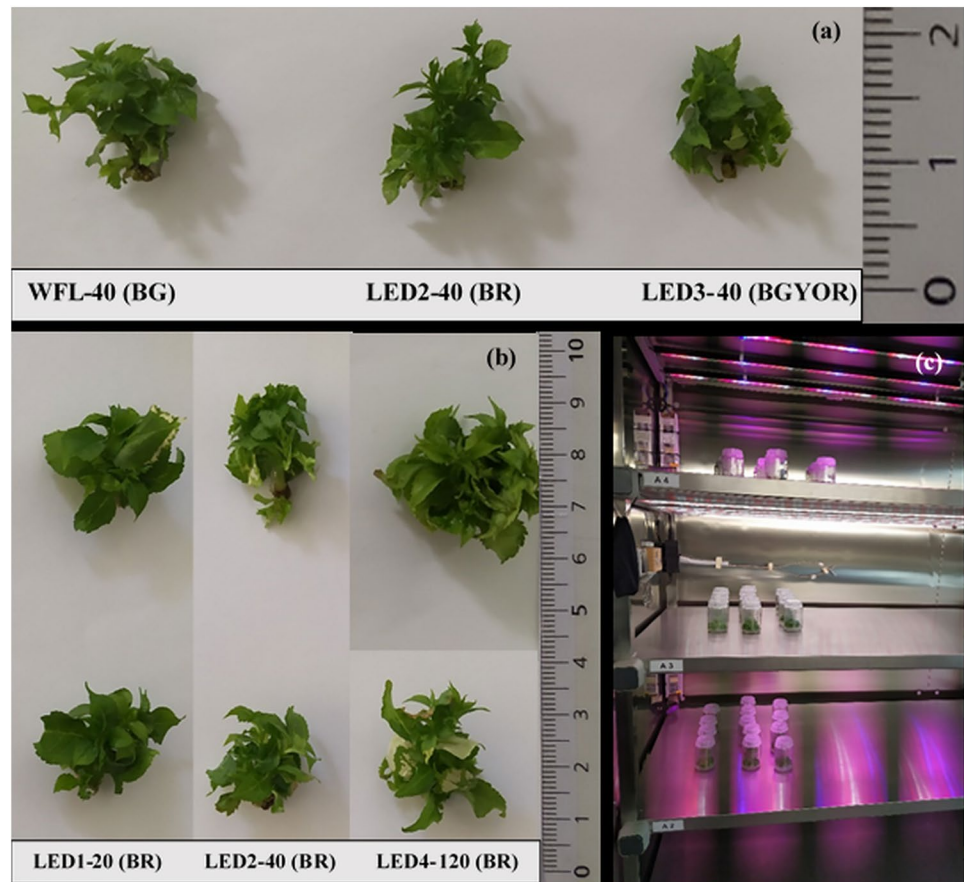
In vitro shoot proliferation

Under $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 100% multiple shoot formation was observed irrespective of spectrum in all three treatments. WFL-40 (BG) and LED2-40 (BR) gave similar shoot numbers (3.8–4.25), shoot heights (0.90–0.91 cm), proliferation rates (3.86–4.30) and percentages of stressed explants (5.36–10.72%). However, there was a noticeable increase in the FW and DW of explants as well as in the FW/DW ratio in LED2-40 (BR), differing significantly from WFL-40 (BG) and LED3-40 (BGYOR). The lowest percentage of explants with stress symptoms (5.36%) was recorded in LED2-40 (BR) and the highest (21.43%) in LED3-40 (BGYOR) differing significantly, while WFL-40 (BG) gave 10.72% stressed explants without a significant difference to the other treatments. There was a fourfold increase in the percentage of stressed explants between LED2-40 (BR) and LED3-40 (BGYOR)—with the latter—to be 4 \times times higher than the former one (Table 2; Fig. 2a).

Under the same BR spectrum, multiple shoot formation to the 100% of the explants was achieved irrespective of light intensity (LED1-20, LED2-40, LED4-120). LED2-40 (BR) gave concurrently better results related to shoot number (3.8), shoot height (0.90 cm), proliferation rate (3.86), FW/DW ratio, FW and DW, as well as

Table 2 Effect of three different multiple light emitting composition wavelengths in full absorption spectrum under light intensity of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ on in vitro shoot proliferation parameters in theGisela 6 rootstock after 30 days of culture in MS medium enriched with $4.4 \mu\text{M}$ BA, $0.05 \mu\text{M}$ NAA, $0.3 \mu\text{M}$ GA₃, 20 g L^{-1} sucrose (pH 5.8) and 6 g L^{-1} Plant Agar

Treatment code	Number of new shoots/ explant	Shoot height (cm)	Proliferation rate	FW (g)	DW (g)	FW/DW ratio	Stressed explants (%)
WFL-40 (BG)	4.25 ± 0.18 a	0.91 ± 0.04 a	4.30 ± 0.19 a	0.308 ± 0.023 b	0.037 ± 0.003 b	8.34 ± 0.02 b	10.72 ± 5.23 ab
LED2-40 (BR)	3.80 ± 0.18 ab	0.90 ± 0.04 a	3.86 ± 0.18 ab	0.365 ± 0.025 a	0.046 ± 0.004 a	9.24 ± 0.50 a	5.36 ± 3.76 b
LED3-40 (BGYOR)	3.32 ± 0.18 b	0.79 ± 0.04 b	3.36 ± 0.19 b	0.310 ± 0.022 b	0.037 ± 0.003 b	8.37 ± 0.02 b	21.43 ± 6.04 a
P-values (one-way ANOVA)	0.002**	0.049*	0.002**	0.043*	0.047*	0.048*	0.032*

Means \pm SE with different letters in a column are statistically different according to Duncan's multiple range test at $P \leq 0.05$ In each column, *P-value ≤ 0.05 : statistically significant differences among treatments at a 5% level, **P-value ≤ 0.01 : statistically significant differences among treatments at an 1% level**Fig. 2** In vitro proliferation of Gisela 6 microshoots under the effect of a two absorption wavelength spectra including 400–700 nm (mainly blue-green, BG) provided by White Fluorescent Light [WFL-40 (BG)], and 430–690 nm provided by two LED types [mainly blue-red, BR/LED2-40 (BR) and blue-green-yellow-orange-red, BGYOR)/LED3-40 (BGYOR)] under $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, **b, c** blue-red (BR) absorption wavelength spectrum and three light intensities (20, 40, $120 \mu\text{mol m}^{-2} \text{s}^{-1}$) including three LED lamps [LED1-20 (BR), LED2-40 (BR) and LED4-120 (BR)]

the lowest percentage of stressed explants (5.36%). Stress symptoms in LED4-120 (BR) were more severe, intense, in higher number of explants (60.71%), being considerably increased compared to LED1-20 (BR) (17.86%) and LED2-40 (BR) (5.36%) (Table 3; Fig. 2b, c).

Changes in chlorophyll fluorescence parameters under different light conditions

Under constant intensity ($40 \mu\text{mol m}^{-2} \text{s}^{-1}$), all chlorophyll fluorescence parameters (Φ_{PSII} , F_v/F_m , Φ_{NPQ} , Φ_{NO} , NPQ, q_p , F_v'/F_m' , EXC) showed no significant difference under

Table 3 Effect of three light intensities (20, 40 and 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with the same wavelength absorption spectrum of 430–690 nm (mainly blue-red, BR at a 1:3 ratio) on in vitro shoot proliferation parameters in the Gisela 6 rootstock after 30 days of culture in MS medium enriched with 4.4 μM BA, 0.05 μM NAA, 0.3 μM GA₃, 20 g L⁻¹ sucrose (pH 5.8) and 6 g L⁻¹ Plant Agar

Treatment code	Light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Absorption wavelength spectrum	Number of new shoots/explant	Shoot height (cm)	Proliferation rate	FW (g)	DW (g)	FW/DW ratio	Stressed explants (%)
LED1-20 (BR)	20	BR	3.14 ± 0.18b	0.83 ± 0.04a	3.18 ± 0.18b	0.295 ± 0.014b	0.034 ± 0.002b	8.62 ± 0.01b	17.86 ± 5.88b
LED2-40 (BR)	40	BR	3.80 ± 0.18a	0.90 ± 0.04a	3.86 ± 0.18a	0.365 ± 0.025a	0.046 ± 0.004a	9.24 ± 0.50a	5.36 ± 3.76c
LED4-120 (BR)	120	BR	3.43 ± 0.17ab	0.65 ± 0.03b	3.43 ± 0.17ab	0.271 ± 0.027b	0.036 ± 0.004b	7.55 ± 0.01c	60.71 ± 14.48a
P-values (one-way ANOVA)			0.029*	0.000***	0.025*	0.010*	0.020*	0.000***	0.001**

Means ± SE with different letters in a column are statistically different according to Duncan's multiple range test at $P \leq 0.05$

In each column, *P-value ≤ 0.05 : statistically significant differences among treatments at a 5% level, **P-value ≤ 0.01 : statistically significant differences among treatments at a 1% level, ***P-value ≤ 0.001 : statistically significant differences among treatments at a 0.1% level

any treatment (WFL-40, LED2-40, LED3-40) irrespective of spectrum (BG, BR, BGYOR) (Table 4).

Taking into account the statistically increased FW and DW and decreased percentage of stressed explants of LED2-40 (BR) compared to WFL-40 (BG) and LED3-40 (BGYOR), chlorophyll fluorescence parameters under the same BR spectrum and different LED intensities (20, 40, 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were estimated. Under LED4-120 (BR), Φ_{PSII} decreased significantly compared to the other treatments while its highest value was observed under LED1-20 (BR), though not being significantly different compared to LED2-40 (BR). Φ_{NPQ} did not change under LED1-20 (BR) and LED2-40 (BR), but increased significantly under LED4-120 (BR) and the same pattern was also observed for Φ_{NO} (Fig. 3).

F_v/F_m was significantly decreased under LED4-120 (BR) but it did not differ under the other treatments, having a slight, non-significant, increase under LED1-20 (BR) (Fig. 4a). q_p did not differ under LED1-20 (BR) and LED2-40 (BR) and was only significantly decreased under LED4-120 (BR) (Fig. 4b). Under LED1-20 (BR), F_v/F_m was significantly increased compared to LED4-120 (BR) and slightly, non-significantly increased compared to LED2-40 (BR) (Fig. 4c). NPQ showed no significant difference under any treatment (Fig. 5a) and EXC was significantly increased under LED4-120 (BR) (Fig. 5b).

Photosynthetic pigment content under different light conditions

Under 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ intensity and irrespective of spectrum, photosynthetic pigments content including Chl a, Chl b, total chlorophyll Chl (a + b), Chl a/ Chl b and carotenoids

showed no significant difference under any treatment [WFL-40 (BG), LED2-40 (BR), LED3-40 (BGYOR)] (Table 5).

In the case of BR spectrum and different LED light intensities, Chl a and total chlorophyll content Chl (a + b) did not differ under any treatment (LED1-20, LED2-40, LED4-120). Chl b increased significantly under LED1-20 (BR) compared to LED2-40 (BR) that presented the lowest value, while under LED4-120 (BR), Chl b did not differ significantly compared to LED1-20 (BR) or LED2-40 (BR). The ratio Chl a/ Chl b increased significantly under LED2-40 (BR) compared to LED1-20 (BR) that presented the lowest value, while under LED4-120 (BR), Chl a/ Chl b had no significant difference compared to LED1-20 (BR) or LED2-40 (BR). Carotenoids reached the highest content under LED2-40 (BR), but without any significant difference compared to LED1-20 (BR) and they were found significantly decreased under LED4-120 (BR) having the lowest value (Table 6).

Leaf anatomy under different light conditions

Gisela 6 leaves are dorsiventral with the leaf parenchyma comprising of both palisade and spongy mesophyll. Leaf anatomy appeared to differ from the WFL-40 (BG)—control (Fig. 6a) in the microshoots growing under LED1-20 (BR) (Fig. 6b), LED3-40 (BGYOR) (Fig. 6d) and LED4-120 (BR) (Fig. 6e), while under LED2-40 (BR) illumination leaf anatomy was similar to that of observed in WFL-40 (BG) illuminated plants (Fig. 6c cf. 6a). A general trait of LED1-20 (BR), LED3-40 (BGYOR) and LED4-120 (BR) effects on leaf anatomy was the increase of the spongy mesophyll intracellular space i.e. a reduction in spongy mesophyll

Table 4 Chlorophyll fluorescence parameters of Gisela 6 leaves, under light intensity of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ and three different multiple light emitting composition wavelengths in full absorption spectrum provided by White Fluorescent Light (WFL) and two LED lamps (LED2 and LED3)

Treatment code	Absorption wavelength spectrum	Light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Φ_{PSII}	F_v/F_m	Φ_{NPQ}	NPQ	q_p	F_v/F_m'	EXC	
WFL-40 (BG)	BG	40	0.389 ± 0.091 a	0.636 ± 0.136 a	0.136 ± 0.034 a	0.476 ± 0.094 a	0.075 ± 0.024 a	0.667 ± 0.052 a	0.583 ± 0.128 a	0.647 ± 0.130 a
LED2-40 (BR)	BR	40	0.431 ± 0.131 a	0.683 ± 0.103 a	0.114 ± 0.024 a	0.456 ± 0.125 a	0.068 ± 0.025 a	0.668 ± 0.133 a	0.638 ± 0.094 a	0.653 ± 0.129 a
LED3-40 (BGYOR)	BGYOR	40	0.346 ± 0.082 a	0.576 ± 0.043 a	0.124 ± 0.006 a	0.531 ± 0.084 a	0.065 ± 0.010 a	0.619 ± 0.110 a	0.548 ± 0.037 a	0.799 ± 0.141 a
P-values (one-way ANOVA)			0.389 ns	0.222 ns	0.313 ns	0.442 ns	0.678 ns	0.656 ns	0.282 ns	0.578 ns

Means \pm SE denoted by different letters are statistically different ($P < 0.05$)

In each column, ns: non-significant statistically differences among treatments at a 5% level (P -value > 0.05)

cell density (Fig. 6b, d, e *cf.* Figure 6a, c). The reduction of the spongy mesophyll cell density was more obvious under LED1-20 (BR) lighting (Fig. 6b) followed by LED3-40 (BGYOR) and LED4-120 (BR) (Fig. 6d and e; respectively). Under LED4-120 (BR) lighting, the upper epidermal cells also increased in size, (and by this the whole upper epidermis appeared thicker) and were filled with a material which was intensively stained (arrows in Fig. 6e). Palisade mesophyll cell structure did not appear to be affected under LED1-20 (BR), LED3-40 (BGYOR) and LED4-120 (BR) (Fig. 6b, d, e) nevertheless, under LED1-20 (BR) and LED3-40 (BGYOR) lighting dark stained material seemed to be accumulated inside the cells (Fig. 6b, d).

Discussion

The quality and quantity of light in which plants are exposed play key role in their growth and development through photosynthesis and photosynthetic pigment synthesis (Gao et al. 2020), also affecting leaf anatomical traits. In the present study light quality and light quantity as a combined effect factor affected the in vitro development of Gisela 6. Explants were better suited under LED2-40 (mainly BR at a 1:3 ratio). Red-blue LED mixtures have been connected with higher shoot numbers, longer internodes, higher proliferation rates and biomass yields, and leaf expansion (Li et al. 2010). Specifically, the higher ratio of red to blue light seems to be beneficial (Yudina et al. 2022) due to higher light penetration in the canopy and increased light absorption (Sarlikioti et al. 2011), thus causing the formation of more photosynthetic products (Zheng et al. 2008).

The positive effect of blue-red LED on height of Gisela 6 microshoots could be attributed to: (1) the stimulation of endogenous gibberellins involved in mitosis and cell proliferation (Manivannan et al. 2015) by regulating gibberellic acid biosynthesis (Ouyang et al. 2015), (2) the induced expression of genes which inhibit auxin action and stimulates stem elongation (Li et al. 2017), or (3) the high effectiveness of the green light inclusion to the spectrum in boosting stem lengthening at an early stage (Folta et al. 2005).

In some species light intensities above the optimum, can result in the degradation of photosynthetic pigments content (Zhou et al. 2008) Moreover, the non-adequate diffusion of the excess energy in the xanthophyll cycle-induced by high light intensities- [observed by the significantly lower carotenoids content (Junior et al. 2020) in Gisela 6 under LED4-120 (BR)] can led to a decline in proliferation performance (Lin 2008). Indeed, there is an inverse correlation between increasing light availability and decreasing plant height attributed in apical dominance

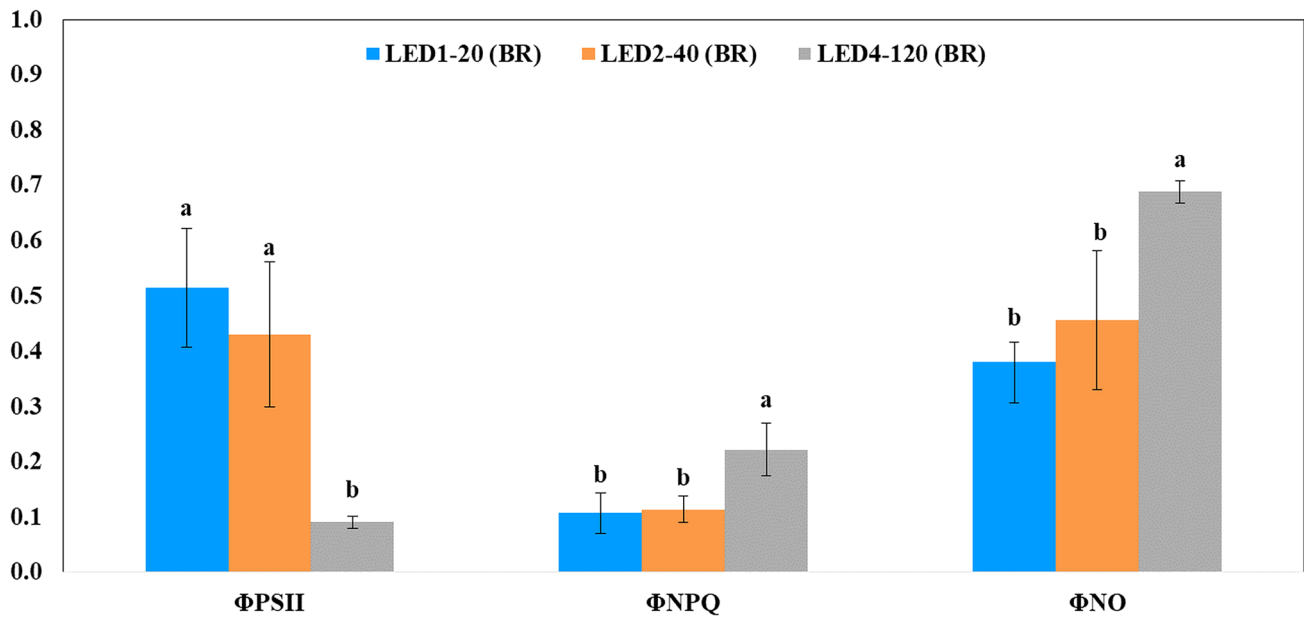


Fig. 3 Changes in the effective quantum yield of PSII photochemistry (Φ_{PSII}), the quantum yield of regulated non-photochemical energy loss in PSII (Φ_{NPQ}) and the quantum yield of non-regulated energy loss (Φ_{NO}), in Gisela 6 leaves under blue-red (BR) absorption wave-

length spectrum and three light intensities [$20 \mu\text{mol m}^{-2} \text{s}^{-1}$ /LED1-20 (BR), $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ /LED2-40 (BR) and $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ /LED4-120 (BR)]. Error bars are standard errors. Columns denoted by different letters indicate statistically significant differences ($P < 0.05$)

due to increased levels of endogenous auxin and/or shade avoidance syndrome and etiolation induced by other plant growth regulators (PGRs) such as gibberellic acid and brassinosteroids (Vanneste and Friml 2009). In Gisela 6, there was a significant decline in Chl b content and a significant increase in the Chl a/ Chl b ratio when light intensity increased from 20 to $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ while the further threefold increase to $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ caused a considerable decrease in the carotenoid content (BR spectrum). Any alterations in the photochemistry of photosystem, especially those concerning the content of PSII: PSI ratio and the size of PSII light-harvesting antenna, can be mirrored by the modifications in Chl a/ Chl b ratio (Xu et al. 2020) and carotenoids content, since carotenoids function as photo-sensitizers exhibiting a scavenging action against the increased production of reactive oxygen species (ROS) (Lin et al. 2020). Therefore, the higher Chl a/b ratio under LED2-40 (BR) as compared to LED1-20 (BR) suggests more profound adaptability of light, greater electron transportation capability of chlorophyll and stronger activity of the enzyme involved in the Calvin cycle (Xu et al. 2020).

On one hand, plants with lower chlorophyll content [i.e., Chl b in the case of LED2-40 (BR)] can use

photosynthetic centers more efficiently than plants with excessive chlorophyll [i.e., Chl b, LED1-20 (BR)], thus higher content of these pigments does not necessarily indicate higher photosynthetic efficiency (Saebo et al. 1995), while on the other hand, high light intensity [i.e., LED4-120 (BR)], can contribute to ROS emancipation and cell damage (Moustakas 2022). Our data showed that Φ_{PSII} , q_p and F_v/F_m' were substantially higher in LED1-20 (BR) and LED2-40 (BR), compared to LED4-120 (BR) and this fact can be ascribed based on the PSII function model either to the fraction of open PSII reaction centers (q_p) or to the efficiency of these centers (F_v/F_m') (Genty et al. 1989). There is a close relationship between avoidance of excess energy in chloroplasts and satisfactory activation of photoprotective mechanisms (Moustakas et al. 2020) as it was observed under LED1-20 (BR) and LED2-40 (BR), implying that lower light intensities contribute to impeding of surplus stimulation energy at PSII. Also, Gisela 6 explants exhibited a considerable higher excess excitation energy (EXC) and Φ_{NO} under LED4-120 (BR) that could not be offset by the augmented photoprotective energy diffusion (Φ_{NPQ}), which may result in the proportional raise of singlet oxygen ($^1\text{O}_2$) production, which is a ROS,

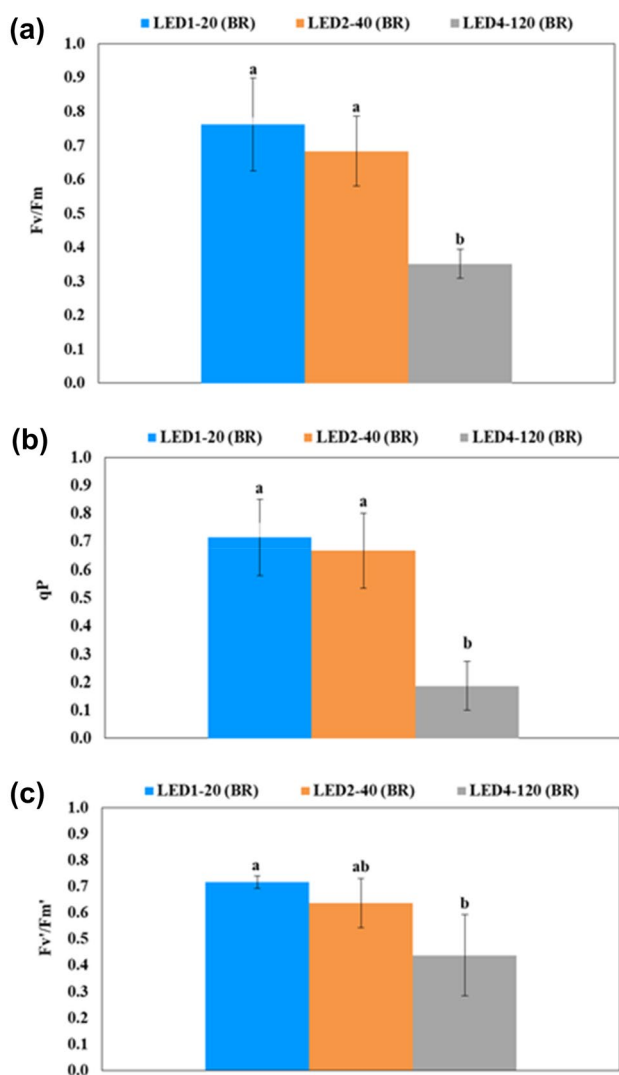


Fig. 4 Changes in **a** the maximum efficiency of PSII photochemistry (F_v/F_m), **b** the fraction of open PSII reaction centers pool (q_p) and **c** the efficiency of open PSII reaction centers (F_v/F_m'), in Gisela 6 leaves under blue-red (BR) absorption wavelength spectrum and three light intensities [$20 \mu\text{mol m}^{-2} \text{s}^{-1}$ /LED1-20 (BR), $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ /LED2-40 (BR) and $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ /LED4-120 (BR)]. Error bars are standard errors. Columns denoted by different letters indicate statistically significant differences ($P < 0.05$)

provoking concrete damage to cells (Moustaka et al. 2020; Sperdouli et al. 2021).

It has been stated that blue light addition to the LED spectrum is important for normal anatomical leaf development (Zheng and Van Labeke 2017). The leaf mesophyll structure changes observed could be due to the fluctuation of both palisade and spongy mesophyll tissues, the relative cell density of which is directly controlled by the light spectrum (Borsuk et al. 2022). Palisade mesophyll cells allow light diffusion inside the chloroplasts, while spongy mesophyll cells, enhance light scattering increasing light

capture (Ustin and Jacquemoud 2020). Commonly, it has been stated that during in vitro culture of many plants, mesophyll structural changes occur due to the specific light spectrum used (Ma et al. 2021). For example Macedo et al. (2011) showed that in in vitro cultured *Alternanthera brasiliana* plants, blue LED light increased the thickness of palisade vs. spongy mesophyll tissue, while, red LED lighting reduced total leaf thickness, with the most severe effects to be observed in the spongy mesophyll tissue. In accordance, leaf blades of Gisela 6 microshoots developed under LED light that contained either high intensity (LED4) or low intensity (LED1) Blue-Red spectrum produced suppressed spongy mesophyll, while LED2 ($40 \mu\text{mol m}^{-2} \text{s}^{-1}$ intensity) had the same effect to the leaf anatomy as WFL did. The noted spongy mesophyll suppression could be reflecting the microshoot leaf response to radiation stress (Wellman 1983). The manifested protective mechanism could be mirrored by the low numbers of spongy mesophyll cells, and the leaves structural adaptations to radiation (Ustin and Jacquemoud 2020) could explain the observed anatomical changes. However, this reduction can be also due to the lowered photosynthetic activity (Li et al. 2008), as observed in LED4 lighting conditions (Fig. 5). Moreover, during micropropagation, epidermal tissue thickness is dependent on the light quality and of the concentration of the different growth regulators which the culture medium contains (Cioć and Pawłowska 2020). Controlling these external factors, one could counterbalance the harmful effects of the increased moisture occurring inside the culture vessels i.e. the epidermal thinning (Hazarika 2006). LED4 lighting applied during multiplication of microshoots of Gisela 6, increased the thickness of the adaxial epidermis of the leaves, (desirable for plants growing inside culture vessels; Hazarika 2006), however LED4 light stressed the microshoots as already reported in Table 2.

Conclusions

In the Gisela 6 cherry rootstock in vitro culture, LED2-40 (BR) proved to be the most appropriate lighting [$40 \mu\text{mol m}^{-2} \text{s}^{-1}$, multiple emitting LED light wavelengths (red-blue-green-yellow-orange) within the full absorption spectrum (430–690 nm) at a 9R:3B:1G:1Y:1O ratio]. Under LED2-40 (BR), higher proliferation rate and biomass yields, a better PSII functionality, an increased Chl b and carotenoids content were reported. Moreover, leaf anatomy showed similar features compared to WFL-40 (BG). In this study, the replacement of common WFLs with LEDs (430–690 nm, mainly blue-red, 1:3 ratio) is an economically viable option contributing to better plant metabolism. The manipulation of

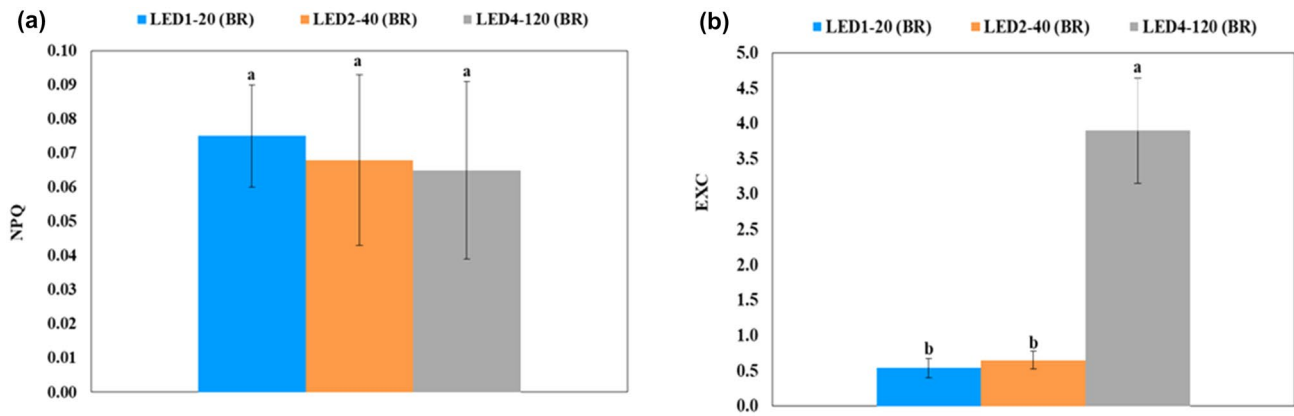


Fig. 5 Changes in **a** the non-photochemical quenching (NPQ) and **b** the excess excitation energy (EXC), in Gisela 6 leaves under blue-red (BR) absorption wavelength spectrum and three light intensities [20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ /LED1-20 (BR), 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ /LED2-40 (BR)

and 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ /LED4-120 (BR)]. Error bars are standard errors. Columns denoted by different letters indicate statistically significant differences ($P < 0.05$)

plant growth/development, photosynthesis and morphology in a controlled environment can be achieved with the use of LED technology through the improvement of the qualitative (spectrum) and the quantitative (intensity) parameters of conventional growing.

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Table 5 Photosynthetic pigment content in Gisela 6 leaves, under light intensity of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and three different multiple light emitting composition wavelengths in full absorption spectrum provided by White Fluorescent Light (WFL) and two LED lamps (LED2 and LED3)

Treatment code	Absorption wavelength spectrum	Light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Total Chl (a+b) (mg g^{-1})	Chl a/ Chl b	Carotenoids (mg g^{-1})
WFL-40 (BG)	BG	40	0.013 \pm 0.001 a	0.010 \pm 0.001 a	0.024 \pm 0.001 a	1.249 \pm 0.108 a	0.136 \pm 0.005 a
LED2-40 (BR)	BR	40	0.014 \pm 0.001 a	0.012 \pm 0.003 a	0.026 \pm 0.003 a	1.173 \pm 0.344 a	0.143 \pm 0.008 a
LED3-40 (BGYOR)	BGYOR	40	0.013 \pm 0.001 a	0.012 \pm 0.004 a	0.024 \pm 0.005 a	1.221 \pm 0.393 a	0.129 \pm 0.012 a
P-values			0.319 ns	0.619 ns	0.463 ns	0.940 ns	0.137 ns

Means \pm SE denoted by different letters are statistically different ($P < 0.05$)

In each column, ns: non-significant statistically differences among treatments at a 5% level (P -value > 0.05)

Table 6 Photosynthetic pigment content in Gisela 6 leaves, under the same light wavelength absorption spectrum (BR, 430–690 nm) and three different LED intensities (20, 40, 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (LED1, LED2, LED4)

Treatment code	Absorption wavelength spectrum	Light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Total Chl (a+b) (mg g^{-1})	Chl a/Chl b	Carotenoids (mg g^{-1})
LED1-20 (BR)	BR	20	0.013 \pm 0.001 a	0.016 \pm 0.001 a	0.029 \pm 0.001 a	0.777 \pm 0.038 b	0.138 \pm 0.006 ab
LED2-40 (BR)	BR	40	0.014 \pm 0.001 a	0.012 \pm 0.003 b	0.026 \pm 0.003 a	1.173 \pm 0.344 a	0.143 \pm 0.008 a
LED4-120 (BR)	BR	120	0.013 \pm 0.001 a	0.014 \pm 0.005 ab	0.026 \pm 0.005 a	1.083 \pm 0.462 ab	0.128 \pm 0.008 b
P-values			0.265 ns	0.019*	0.553 ns	0.046*	0.037*

Means \pm SE denoted by different letters are statistically different ($p < 0.05$)

In each column, ns: non-significant statistically differences among treatments at a 5% level (P -value > 0.05), * P -value ≤ 0.05 : statistically significant differences among treatments at a 5% level

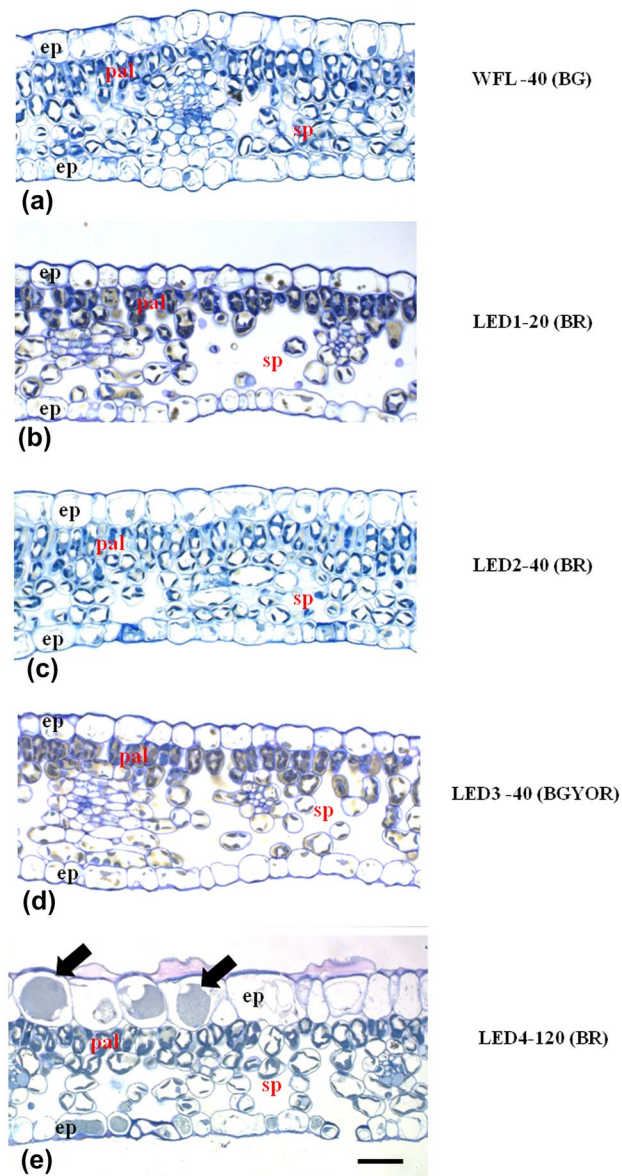


Fig. 6 Leaf cross sections of microshoot leaves grown under WFL-40 (BG) (a), LED1-20 (BR) (b), LED2-40 (BR) (c), LED3-40 (BGYOR) (d) and LED4-120 (BR) (e), lighting conditions, stained with toluidine O. Arrows in e, indicate intensely stained material. *Ep* epidermis, *pal* palisade mesophyll, *sp* spongy mesophyll. Scale bar: 20 μm

Authors contributions Conceptualization, experimental design, validation, supervision, project administration, funding acquisition, writing—review & editing were provided by KG. Investigation, implementation of the experiments, data processing, formal analysis, data curation, writing—original draft, review & editing were performed by VS, IS, and IDA. In particular, in vitro shoot proliferation was performed by VS, photosynthetic pigments content and chlorophyll fluorescence parameters were estimated by IS, and scanning light microscopy images of leaf anatomy was obtained by IDA. All authors read and approved the final manuscript.

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Data availability This manuscript has no associated data.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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