Echinacea purpurea morphological and biochemical response to combined LED light and sucrose level in in vitro culture

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Abstract

Echinacea purpurea, a widely cultivated medicinal and ornamental plant, produces diverse biochemical compounds with immunostimulatory, anti-inflammatory, and antioxidant properties. Optimizing *in vitro*propagation conditions, particularly LED light and sucrose level, is essential to enhance plantlet quality and biochemical performance for large-scale production. The interaction between LED light and sucrose level significantly affected growth parameters, photosynthetic pigments, hydrogen peroxide (H_2O_2) content, catalase (CAT) activity, free amino acids (FAA), soluble protein, total soluble sugars (TSS), reducing sugars (RS), as well as total polyphenolic acids content (TPC) and total flavonoid content (TFC) of the plant. Blue-red and red LEDs combined with high sucrose levels (30 g·L^{-1}) promoted shoot elongation and induced oxidative stress, as reflected by elevated H_2O_2 and CAT activity. Conversely, white and blue light with moderate sucrose concentrations (10 and 20 g·L^{-1}) enhanced chlorophyll, TSS, and FAA levels, supporting balanced growth with lower stress indicators. Pigment production was more strongly influenced by light than by sucrose, while RS and antioxidant responses increased under stress-inducing conditions. TPC peaked under blue LEDs with 10 g·L^{-1} sucrose, whereas TFC accumulation was highest under moderate oxidative stress. Statistical interactions between LED light and sucrose were significant for multiple parameters, highlighting the synergistic role of these factors in shaping *in vitro* growth, metabolic activity, and biochemical composition of the plant. These results highlight the importance of fine-tuning light quality and sucrose levels to optimize growth and biochemical compounds production in *E. purpurea* micropropagation systems.

Key message

LED spectra and sucrose concentrations strongly affect morphology, pigments, oxidative stress markers, and biochemical production in *Echinacea purpurea in vitro*.

Introduction

Echinacea purpurea (L.) Moench, commonly referred to purple coneflower, is a herbaceous perennial plant from *Asteraceae* family, and widely cultivated for its ornamental and medicinal value. It is one of the most studied *Echinacea* species due to its rich profile of bioactive compounds, including polyphenolic acids, flavonoids, and essential oils, which contribute to its immunostimulatory, anti-inflammatory, and antioxidant effects (Bauer 1999; Zobayed and Saxena 2005). Due to its growing demand in the pharmaceutical and horticultural industries, efficient propagation methods are needed to ensure consistent plant quality and biochemical component. Large-scale culture systems, including bioreactor-based approaches, have also been explored for *E. purpurea* to enhance biomass and bioactive compound yields. For instance, Fan et al. (2021) demonstrated enhanced flavonoid and caffeic acid derivatives in co-cultured adventitious roots using bioreactors. Titova et al. (2024) also highlight successful scale-up of *E. purpurea* cell suspensions in mechanically stirred bioreactors, and Jeong et al. (2009) report application of airlift bioreactors to increase root biomass and caffeic acid derivative yield.

Micropropagation provides a powerful tool for the large-scale production of genetically uniform, disease-free plants under controlled conditions. It offers a high multiplication rate and reproducible performance, making it especially valuable for species with high commercial or medicinal relevance (Sharma et al. 2015). However, the success of *in vitro* propagation is highly dependent on several environmental and nutritional factors, particularly light and carbohydrate supply, which regulate plant development at both morphological and physiological levels.

Light is not only essential for photosynthesis but also acts as a key signal regulating plant morphogenesis, chlorophyll biosynthesis, stomatal behavior, and secondary metabolism. In traditional tissue culture systems, fluorescent lamps (FLs) have been used as the primary light source, but they possess limitations including high power consumption, heat production, and a non-specific spectral output. LEDs have become the preferred light source in *in vitro* culture due to their ability to provide narrow, tunable wavelengths that correspond precisely to plant photoreceptor sensitivities—such as red (~ 660 nm) for phytochrome activation and stem elongation, and blue (~ 450 nm) for chloroplast development and stomatal regulation. Recent studies have increasingly highlighted the benefits of LEDs in tissue culture, noting how spectral quality, light intensity, and LED design enhance morphological and physiological responses, including metabolite production (Cavallaro et al. 2023). LED irradiation also effectively stimulates metabolite synthesis during plant development and post-harvest (Livadariu et al. 2023). In addition, LED-based systems not only reduce energy costs but also allow precise control over morphogenesis and metabolite production *in vitro*, strengthening their role as an efficient alternative to conventional fluorescent lamps (Katsanou et. al. 2025). The ability to fine-tune light spectra using specific combinations of red, blue, and white LEDs allows researchers to modulate plant responses more precisely. Red light, for instance, enhances stem elongation and phytochrome-mediated responses, while blue light is essential for chloroplast development, stomatal regulation, and flavonoid synthesis (Bello-Bello et al. 2017). White light, composed of multiple wavelengths, can provide a balanced spectrum for comprehensive growth and pigment accumulation (Ptushenko et al. 2020). Recent work on toquilla palm (*Carludovica palmata*) further confirmed that blue-red LEDs promote seedling elongation, whereas red LEDs favor root development, highlighting the broad effectiveness of LED combinations in *in vitro* culture systems (Caamal-Velázquez et al. 2025).

In addition to light, sucrose plays a critical role in supporting plantlet growth under *in vitro* conditions, where autotrophic capacity is limited due to low CO_2 availability and often reduced photosynthetic activity. Sucrose serves as the primary source of carbon and energy, influences osmotic balance, and can act as a signaling molecule that modulates gene expression, hormone signaling, and redox homeostasis (Hazarika 2006; Gago et al. 2022). However, sucrose concentration must be carefully controlled: low concentrations may limit energy availability, while excessive sucrose can induce osmotic stress, repress photosynthesis, and trigger metabolic imbalances (Kwa et al. 1995; Keunen et al. 2013). Furthermore, sugars are increasingly recognized for their role in modulating stress responses and antioxidant activity, often acting as ROS scavengers or regulators of redox signaling pathways (Couée et al. 2006).

Given the commercial and pharmacological relevance of this species, optimizing its micropropagation conditions is of practical importance. We hypothesized that the interaction between LED spectrum and sucrose concentration would differentially regulate growth, photosynthetic pigments, stress responses, and biochemical compound accumulation in *E. purpurea*. To test this, we modulated light quality using different LED spectra in combination with varying sucrose levels under controlled *in vitro* conditions, aiming to identify optimal combinations for enhancing growth and biochemical performance.

Materials and methods

1.1. Experimental design

Axillary shoots of *E. purpurea* were used as explants. The explants were cultured on Murashige and Skoog (MS) medium supplemented with 0.35 mg·L⁻¹ 6-benzyladenine (BA), 0.1 mg·L⁻¹ indole-3-butyric acid (IBA), and 8 g·L⁻¹ BioAgar. Three sucrose concentrations were tested: 10, 20, and 30 g·L⁻¹. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 20 minutes under 110 kPa. For the rooting stage, shoots were transferred to MS medium containing 1 mg·L⁻¹ naphthaleneacetic acid (NAA). In both stages, five shoots were cultured per jar.

Cultures were maintained in a phytotron at 23 ± 1°C with a 16-hour photoperiod and a light intensity of 35 µmol·m⁻²·s⁻¹ photosynthetic photon flux density (PPFD). Four LED spectrum treatments were applied:

- White (85% white + 15% red) – control
- Blue (20% white + 60% blue + 20% red)
- Blue-Red (20% white + 40% blue + 40% red)
- Red (20% white + 20% blue + 60% red)

1.2. Evaluation of biometric and biochemical parameters

1.2.1. Biometric parameters

Biometric parameters were recorded at the sixth week of culture. For each treatment, twenty-five plants were evaluated to determine shoot length, number of leaves, number of roots, and root length. The average length and width of leaf blades, leaf area, and leaf perimeter were determined from the five largest leaves per treatment using ImageJ software (version 1.54g, National Institutes of Health, USA).

1.2.2. Biochemical analyses

Leaves were collected after six weeks of *in vitro* culture for biochemical analysis. Three biological replicates were taken per treatment. All absorbance measurements were performed using a UV-1601 PC spectrophotometer (Shimadzu, Columbia, MD, USA).

1.2.2.1. *Total chlorophyll a, chlorophyll b, chlorophyll (a + b), and carotenoids content*

Total chlorophyll a, chlorophyll b, chlorophyll (a + b), and carotenoids content were determined using the method of Lichtenthaler and Wellburn (1983). Fresh leaves (0.1 g) were ground in a mortar with quartz sand and 2.5 mL of 80% acetone. The extracts were filtered and brought to 25 mL with 80% acetoneAfter obtaining clear filtrates, absorbance was measured at 470, 646, 652, and 663 nm. Pigment concentrations were calculated using the following equations:

Chlorophyll a ($mg \cdot L^{-1}$) = 12.21 × A663–2.81 × A646

Chlorophyll b ($mg \cdot L^{-1}$) = 20.13 × A646–5.03 × A663

Total chlorophyll (a + b, $mg \cdot L^{-1}$) = 27.8 × A652

Carotenoids ($mg \cdot L^{-1}$) = (1000 × A470–3.27 × Chl a – 104 × Chl b) / 229

1.2.2.2. Hydrogen peroxide content

Hydrogen peroxide content was quantified following the method of Pick and Keisari (1980). Fresh leaves (0.1 g) were ground in a mortar with K-phosphate buffer. After mashing and pouring into plastic tubes, they were centrifuged for 20 min at 20,000 rpm at 4°C. The supernatants obtained were collected in glass tubes, from which the extract was taken for further steps. 0.1 mL of each extract was taken into glass tubes and made up to 0.5 mL with K-phosphate buffer. To the resulting solution, 0.5 mLof 0.1 M K-phosphate buffer and 1 mL of 1 M potassium iodide (KI) had to be added. The mixture was mixed and incubated in the dark for one hour. When the time had elapsed, the absorbance was measured at 390 nm

1.2.2.3. Catalase activity

Catalase activity was assessed according to the procedure described by Góth (1991). For this analysis, the supernatants obtained during H_2O_2 determination were utilized. The extracts (0.05 mL) were mixed with 0.45 mL of K-phosphate buffer (pH 7) and distributed into two groups of tubes. To the ‘A’ set, 1 mL of 0.1 M K-phosphate buffer was added, whereas the ‘B’ set received 1 mL of H_2O_2 solution in buffer (65 µM). Control tube ‘K’ was prepared by combining 1.5 mL of 0.1 M K-phosphate buffer, while tube ‘C’ contained 0.5 mL of 0.1 M K-phosphate buffer and 1 mL of H_2O_2 in buffer (65 µM). All samples were incubated in the dark for 10 minutes. After incubation, 1 mL of 32.5 mM ammonium molybdate was added to each tube and mixed thoroughly. The absorbance of the reaction mixture was then recorded at 405 nm.

1.2.2.4. Free amino acids

The determination of free amino acid content was performed using a modified version of the Rosen (1957) method. In this procedure, 0.2 mL of extract was mixed with 0.5 mL of 0.2 mM sodium cyanide prepared in acetate buffer (pH 5.3–5.4) and 0.5 mL of ninhydrin reagent. After mixing thoroughly, the

samples were incubated in a water bath at 100°C for 15 minutes. Following incubation, each warm tube was supplemented with 5 mL of isopropyl alcohol and left to cool. The absorbance of the resulting solution was then measured at 570 nm, using a standard curve prepared with leucine.

1.2.2.5. Total soluble proteins

Total soluble protein concentration was determined following the Bradford method (1976). For the assay, 0.1 mL of extract was added to 5.0 mL of Bradford reagent, and the mixture was incubated in darkness for 5 minutes. The absorbance was subsequently recorded at 595 nm.

1.2.2.6. Total soluble sugars content

Total soluble sugars were quantified using the colorimetric method described by Dubois et al. (1956). Samples were first homogenized in hot 80% ethanol, and the extracts were centrifuged at 20,000 × g for 20 minutes at 4°C. The resulting supernatant was transferred into resealable tubes and the volume was adjusted to 25 mL with 80% ethanol. For the assay, 100 µL of extract was combined with 1 mL of 5% phenol and 5 mL of concentrated sulphuric acid (96% H_2SO_4), followed by thorough mixing. The mixtures were incubated for 20 minutes, after which absorbance was recorded at 490 nm against a glucose standard curve.

1.2.2.7. Reduced sugars content

Reduced sugar content was determined following the method of Nelson (1944). Samples were homogenized in hot 80% ethanol, and the extracts were centrifuged at 20,000 × g for 20 minutes at 4°C. The supernatant was collected, transferred to resealable tubes, and the volume was adjusted to 25 mL with 80% ethanol. For the assay, 100 µL of extract was mixed with 1 mL of copper reagent and incubated in a water bath at 100°C for 20 minutes. After cooling in cold water, 1 mL of molybdenum–arsenic reagent was added, and the mixture was diluted to a final volume of 5 mL with distilled water. Absorbance was measured at 520 nm using a glucose standard curve.

1.2.2.8. Polyphenolic Acids Content

The determination of polyphenolic acids was performed using the colorimetric method with Arnow's reagent (Polskie Towarzystwo Farmakologiczne 1999; Polish Norm PN-91/R-87019). Absorbance was recorded at 490 nm with a Shimadzu UV-1601 PC spectrophotometer against the reference sample. The results were expressed as caffeic acid equivalents. For each treatment, three independent extracts were prepared, and each extract was analyzed in triplicate.

1.2.2.9. Flavonoids Content

Flavonoids content was measured using an aluminum chloride colorimetric method (Liu 2017). Fresh leaf extracts were mixed with 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH. Absorbance was recorded at 510 nm, and flavonoid concentration was calculated using a quercetin standard curve.

1.3. Data analysis

All measurements were performed using randomly selected plants. Statistical analysis and visualization were conducted using RStudio (v2023.03) and Microsoft Excel 365. Data were subjected to two-way analysis of variance (ANOVA) to evaluate the effects of light spectra, sucrose concentration, and their interaction. Differences between means were tested using Tukey's HSD test at $p \leq 0.05$. Data distribution and variance homogeneity were tested using the Shapiro-Wilk and Levene's tests, respectively. Biochemical results were expressed as mean ± SD (n = 3), while biometric data were reported as mean values for 25 replicates per treatment.

Results

2.1. Biometric parameters of Echinacea purpurea treated with various LED light and sucrose level in vitro conditions

Significant variation was observed in the biometric traits of *E. purpurea* under different LED light and sucrose concentrations (Table 1; Table 2; Fig. 1). The longest shoot were obtained under the Blue-Red (BR) LED with 30 g·L^{-1} sucrose, reaching an average of 5.33 cm. Slightly shorter but statistically similar heights were observed under Red LED (4.37–4.4 cm), whereas the shortest plants were found in White LED treatments (3.2–3.9 cm).

In terms of leaf traits, the highest number of leaves was produced under Blue LED at 30 g·L^{-1} sucrose (32 leaves per plant). This was followed closely by BR and Red treatments at 20–30 g·L^{-1}. In contrast, White LED combined with low sucrose (10 g·L^{-1}) resulted in the lowest leaf number. Leaf blade size (length and width) was also strongly affected by the spectral composition. BR light with 10 g·L^{-1} sucrose produced the longest and widest leaves, significantly larger than those developed under Red or Blue lights at high sucrose levels.

Root patterns showed a different result (Fig. 2). The greatest root length was recorded under BR + 20 g·L^{-1} sucrose (5.62 cm), followed by White and Red treatments at 30 g·L^{-1}. The number of roots was highest under Blue and Red LEDs with 20 g·L^{-1} sucrose (17–18 roots per plant), while BR and White treatments generally resulted in lower root numbers (< 6 roots/plant).

Table 1
Biometric parameters of *Echinacea purpurea* treated with various LED light and sucrose level *in vitro* conditions

LED spectrum	Sucrose Levels [g·L⁻¹]	Shoot length [cm]	Number of Leaves	Number of Roots	Length of Roots [cm]
White	10	3.24 ± 1.22 b	14.78 ± 5.07 d	5.75 ± 4.19 b	1.50 ± 0.41 cd
	20	3.92 ± 0.92 b	23.66 ± 6.96 abcd	18.75 ± 5.32 a	2.87 ± 0.75 bcd
	30	3.57 ± 0.57 b	18.80 ± 5.52 cd	4.00 ± 1.15 b	4.62 ± 2.98 ab
Blue	10	3.96 ± 0.81 ab	31.06 ± 10.40 a	6.00 ± 0.82 b	1.50 ± 0.41 cd
	20	3.82 ± 1.05 b	27.56 ± 7.15 abc	16.50 ± 4.43 a	3.00 ± 0.00 bcd
	30	4.03 ± 1.05 ab	31.98 ± 7.15 a	5.25 ± 1.26 b	3.37 ± 0.30 abcd
Blue-Red	10	4.01 ± 1.21 ab	18.59 ± 8.33 cd	4.75 ± 0.50 b	2.33 ± 0.58 bcd
	20	4.24 ± 1.00 ab	28.26 ± 6.76 ab	5.00 ± 4.69 b	5.62 ± 1.12 a
	30	5.33 ± 2.21 a	27.80 ± 6.99 abc	3.75 ± 1.26 b	4.33 ± 0.87 ab
Red	10	4.40 ± 0.76 ab	22.40 ± 5.89 bcd	4.75 ± 2.22 b	1.12 ± 0.30 d
	20	4.37 ± 1.12 ab	25.74 ± 10.70 abc	17.75 ± 5.19 a	3.04 ± 0.71 abcd
	30	4.19 ± 1.14 ab	30.08 ± 5.62 ab	4.25 ± 1.89 b	3.82 ± 0.67 abc
Means for Light Spectrum					
White		3.58 ± 0.96 b	19.08 ± 6.84 c	9.50 ± 7.75 a	3.00 ± 2.10 a
Blue		3.94 ± 0.94 ab	30.20 ± 9.51 a	9.25 ± 5.89 a	2.62 ± 0.89 a
Blue-Red		4.53 ± 1.64 a	24.89 ± 8.51 b	4.50 ± 2.61 a	4.10 ± 1.62 a
Red		4.32 ± 1.00 a	26.07 ± 8.21 ab	8.92 ± 7.23 a	2.66 ± 1.30 a

*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05

LED spectrum	Sucrose Levels [g·L⁻¹]	Shoot length [cm]	Number of Leaves	Number of Roots	Length of Roots [cm]
Means for Sucrose Levels					
10		4.23 ± 1.56 a	21.84 ± 8.37 b	5.31 ± 2.24 b	1.62 ± 0.60 b
20		4.14 ± 1.02 a	27.41 ± 8.44 a	14.50 ± 7.22 a	3.64 ± 1.37 a
30		3.90 ± 0.99 a	25.93 ± 9.83 a	4.31 ± 1.40 b	4.04 ± 1.51 a
Source of Variation					
Light spectrum x Sucrose Levels		0.00139	< 0.0001	< 0.0001	< 0.0001
Light Spectrum		0.000838	< 0.0001	0.169	0.0809
Sucrose Levels		1.157	0.00226	< 0.0001	< 0.0001
*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05					

Table 2
Biometric parameters of *Echinacea purpurea* leaves treated with various LED light and sucrose level *in vitro* conditions

LED spectrum	Sucrose Levels [g·L⁻¹]	Length of leaf blade [cm]	Width of leaf blade [cm]	Leave area [cm⁻²]	Leaf perimeter [cm]
White	10	1.35 ± 0.25 abc	1.54 ± 0.49 abc	1.23 ± 0.63 abc	4.79 ± 0.94 ab
	20	1.17 ± 0.51 abc	1.35 ± 0.33 abc	0.86 ± 0.30 bc	4.21 ± 1.23 bc
	30	0.88 ± 0.35 bc	1.59 ± 0.47 ab	0.81 ± 0.48 bc	4.05 ± 1.05 bc
Blue	10	1.51 ± 0.55 ab	1.60 ± 0.61 ab	1.5 ± 0.66 ab	5.14 ± 1.37 ab
	20	1.54 ± 1.00 ab	1.99 ± 0.89 a	2.04 ± 1.63 a	5.75 ± 2.63 a
	30	0.71 ± 0.27 c	0.83 ± 0.29 c	0.39 ± 0.18 c	2.55 ± 0.69 c
Blue-Red	10	1.75 ± 0.56 a	2.07 ± 0.34 a	2.23 ± 0.95 a	6.22 ± 1.07 a
	20	1.39 ± 0.23 abc	0.93 ± 0.24 bc	0.83 ± 0.35 bc	3.92 ± 0.64 bc
	30	1.32 ± 0.34 abc	1.14 ± 0.45 bc	0.91 ± 0.27 bc	4.08 ± 0.86 bc
Red	10	1.35 ± 0.60 abc	1.34 ± 0.29 abc	0.99 ± 0.33 bc	4.41 ± 0.94 abc
	20	1.46 ± 0.54 abc	1.36 ± 0.67 abc	0.86 ± 0.45 bc	4.60 ± 1.42 abc
	30	1.36 ± 0.53 abc	1.33 ± 0.47 abc	0.96 ± 0.52 bc	4.34 ± 1.28 abc
Means for Light Spectrum					
White		1.13 ± 0.42 a	1.49 ± 0.43 a	0.97 ± 0.51 a	4.35 ± 1.09 a
Blue		1.26 ± 0.76 a	1.47 ± 0.79 a	1.32 ± 1.21 a	4.48 ± 2.21 a
Blue-Red		1.49 ± 0.43 a	1.38 ± 0.61 a	1.33 ± 0.88 a	4.74 ± 1.36 a
Red		1.39 ± 0.54 a	1.34 ± 0.48 a	0.94 ± 0.43 a	4.45 ± 1.19 a
Means for Sucrose Levels					
10		1.49 ± 0.52 a	1.64 ± 0.51 a	1.49 ± 0.81 a	5.14 ± 1.25 a
20		1.39 ± 0.62 a	1.41 ± 0.69 ab	1.15 ± 0.99	4.62 ± 1.73 a

*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05

LED spectrum	Sucrose Levels [g·L⁻¹]	Length of leaf blade [cm]	Width of leaf blade [cm]	Leave area [cm⁻²]	Leaf perimeter [cm]
				ab	
30		1.07 ± 0.47 b	1.22 ± 0.49 b	0.77 ± 0.44 b	3.76 ± 1.19 b
Source of Variation					
Light spectrum x Sucrose Levels		0.00188	< 0.0001	< 0.0001	< 0.0001
Light Spectrum		0.078	0.449	0.113	0.358
Sucrose Levels		0.00191	0.00628	0.000366	0.000123
*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05					

2.2. Photosynthetic pigments

The chlorophyll and carotenoid contents in *E. purpurea* leaves *in vitro* varied significantly depending on the light spectrum and sucrose concentration (Table 3). White LED consistently supported the highest accumulation of chlorophyll pigments (14.53 mg·g⁻¹ d.w. for chlorophyll a and 5.16 mg·g⁻¹ d.w. for chlorophyll b), which were significantly higher than under blue, red, or blue-red LEDs. In contrast, red light produced the lowest levels of chlorophyll pigments, averaging 7.70 mg·g⁻¹ d.w. for chlorophyll a and 2.44 mg·g⁻¹ d.w.for chlorophyll b.

Among sucrose concentrations, 10 g·L⁻¹ generally supported higher chlorophyll content under white and blue LEDs, while 30 g·L⁻¹ tended to suppress pigment accumulation across all light spectra. Interestingly, BR (blue-red) spectrum combined with 20 g·L⁻¹ sucrose resulted in relatively stable chlorophyll retention, although it did not reach the levels seen under white light.

In terms of carotenoid content, the pattern was similar. The highest carotenoid concentration (3.50 mg·g⁻¹ d.w.) was observed under white LEDs with 10 g·L⁻¹ sucrose, mirroring the chlorophyll trends. Treatments with blue and red light produced considerably lower carotenoid levels.

Table 3

Chlorophyll a [mg·g^{-1} d.w.], chlorophyll b [mg·g^{-1} d.w.] chlorophyll a + b [mg·g^{-1} d.w.] and carotenoids [mg·g^{-1} d.w.] content of *Echinacea purpurea* leaves treated with various LED light and sucrose level *in vitro* conditions

LED spectrum	Sucrose Levels [g·L^{-1}]	Chlorophyll a [mg g^{-1} d.w.]	Chlorophyll b [mg g^{-1} d.w.]	Chlorophyll a + b [mg g^{-1} d.w.]	Carotenoids [mg g^{-1} d.w.]
White	10	14.31 ± 0.01 b	4.60 ± 0.01 b	3.19 ± 0.0161 a	3.50 ± 0.0088 a
	20	20.10 ± 0.04 a	7.73 ± 0.02 a	1.38 ± 0.0700 e	1.59 ± 0.0087 g
	30	9.17 ± 0.08 e	3.13 ± 0.04 d	2.12 ± 0.1280 b	2.88 ± 0.0069 b
Blue	10	13.52 ± 0.11 c	4.15 ± 0.06 c	0.88 ± 0.1790 j	1.27 ± 0.0153 j
	20	5.88 ± 0.08 j	1.99 ± 0.03 i	1.13 ± 0.1160 h	1.63 ± 0.0147 f
	30	7.57 ± 0.09 h	2.52 ± 0.06 g	1.97 ± 0.1740 c	2.76 ± 0.0103 c
Blue-Red	10	10.42 ± 0.09 d	3.18 ± 0.06 d	1.27 ± 0.1850 f	1.65 ± 0.0109 f
	20	8.70 ± 0.01 f	2.76 ± 0.01 f	1.28 ± 0.0161 f	1.81 ± 0.0034 e
	30	8.47 ± 0.02 g	2.92 ± 0.05 e	1.51 ± 0.0278 d	2.11 ± 0.0273 d
Red	10	8.33 ± 0.06 g	2.58 ± 0.03 g	1.08 ± 0.0000 i	1.39 ± 0.0109 i
	20	7.54 ± 0.02 h	2.35 ± 0.01 h	1.11 ± 0.0161 hi	1.52 ± 0.0033 h
	30	7.24 ± 0.01 i	2.40 ± 0.02 h	1.22 ± 0.0278 g	1.79 ± 0.0069 e
Means for Light Spectrum					
White		14.53 ± 4.73 a	5.16 ± 2.03 a	2.23 ± 7.8600 a	2.66 ± 0.8420 a
Blue		8.99 ± 3.48 b	2.89 ± 0.98 b	1.33 ± 4.9700 b	1.89 ± 0.6760 b
Blue-Red		9.20 ± 0.92 b	2.95 ± 0.19 b	1.35 ± 1.2100 b	1.86 ±

*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05

LED spectrum	Sucrose Levels [g·L⁻¹]	Chlorophyll a [mg g⁻¹ d.w.]	Chlorophyll b [mg g⁻¹ d.w.]	Chlorophyll a + b [mg g⁻1 d.w.]	Carotenoids [mg g⁻¹ d.w.]
					0.2030 b
Red		7.70 ± 0.49 b	2.44 ± 0.10 b	1.13 ± 0.6310 b	1.57 ± 0.1740 b
Means for Sucrose Levels					
10		11.65 ± 2.51 a	3.63 ± 0.83 a	1.71 ± 3.7700 a	2.39 ± 0.4750 a
20		10.55 ± 5.85 a	3.71 ± 2.44 a	1.61 ± 9.6200 a	2.02 ± 0.9120 ab
30		8.11 ± 0.79 a	2.74 ± 0.31 a	1.21 ± 1.2500 a	1.57 ± 0.1080 b
Source of Variation					
Light spectrum x Sucrose Levels		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Light Spectrum		< 0.0001	< 0.0001	0.000101	0.00167
Sucrose Levels		0.0716	0.234	0.12	0.00767

*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05

2.3. Stress biomarkers and CAT activity

The content of hydrogen peroxide (H_2O_2) in *E. purpurea* leaves plantlets was significantly influenced by both the LED light spectrum and sucrose concentration (Table 4). The highest level of H_2O_2 accumulation (1940.3 μg·g⁻¹ d.w.) was observed under red LED illumination with 30 g·L⁻¹ sucrose, while the lowest concentration (550.8 μg·g⁻¹ d.w.) was recorded under white light at the low sucrose level.

Among the light treatments, red and blue-red LEDs elicited the strongest oxidative responses, as indicated by elevated H_2O_2 levels. In contrast, white LED light consistently resulted in the lowest oxidative stress.

Catalase (CAT) activity, a key enzymatic component of the antioxidant defense system, displayed an overall inverse trend relative to H_2O_2 accumulation. The highest CAT activity (2816.0 mkat·g⁻¹ d.w.) was recorded under white light with 30 g·L⁻¹ sucrose, whereas lower CAT activities were generally observed under red and blue LED treatments, particularly at lower sucrose concentrations.

Although CAT activity tended to increase in response to elevated H_2O_2 levels, the relationship between these two parameters was not strictly proportional across all treatments. For instance, under blue-red

LED with 10 g·L⁻¹ and white LED with 30 g·L⁻¹ sucrose, elevated CAT activity was accompanied by moderate to high H_2O_2 levels.

Table 4

H_2O_2 [μg·g^{-1} d.w.] and CAT [mkat g^{-1} d.w.] activity content of *Echinacea purpurea* leaves treated with various LED light and sucrose level *in vitro* conditions.

LED spectrum	Sucrose Levels [g·L^{-1}]	H_2O_2 [μg·g^{-1}d.w.]	CAT [mkat g^{-1}d.w.]
White	10	550.88 ± 45.60 e	1004.01 ± 237.00 ab
	20	925.15 ± 113.00 cde	757.16 ± 205.00 b
	30	994.15 ± 289.00 cde	2815.69 ± 73.70 a
Blue	10	764.91 ± 88.30 de	647.32 ± 29.50 b
	20	729.82 ± 129.00 de	1275.68 ± 25.50 ab
	30	1076.02 ± 124.00 bcde	1973.81 ± 170.00 ab
Blue-Red	10	1142.69 ± 273.00 bcd	2356.91 ± 1511.00 a
	20	1252.63 ± 240.00 bcd	2007.67 ± 82.00 a
	30	1583.63 ± 239.00 ab	1234.16 ± 39.00 ab
Red	10	872.51 ± 181.00 de	1659.14 ± 25.50 ab
	20	1421.05 ± 60.90 abc	2382.37 ± 53.10 a
	30	1940.35 ± 140.00 a	1454.89 ± 951.00 a
Means for Light Spectrum			
White		823.39 ± 259.00 b	1526.28 ± 987.00 a
Blue		856.92 ± 193.00 b	1299.27 ± 582.00 a
Blue-Red		1326.32 ± 295.00 a	1866.25 ± 906.00 a
Red		1411.31 ± 477.00 a	1832.13 ± 636.00 a
Means for Sucrose Levels			
10		832.75 ± 266.00 b	1416.82 ± 944.00 a
20		1082.16 ± 310.00 ab	1606.29 ± 666.00 a
30		1398.54 ± 441.00 a	1870.20 ± 758.00 a
Source of Variation			
Light spectrum x Sucrose Levels		< 0.0001	0.000417
Light Spectrum		0.000367	1.026
Sucrose Levels		0.00146	0.976

*The same letter in the lines indicates no difference between the means at a significance level of α = 0.05.

2.4. Free amino acids and soluble proteins content

The content of free amino acids in *E. purpurea* leaves plantlets was significantly influenced by the interaction between LED light spectrum and sucrose concentration (Table 5). The highest free amino acid level (753.0 mg·g⁻¹ d.w.) was observed under white light combined with 20 g·L⁻¹ sucrose, which was significantly higher than all other treatment combinations. In contrast, the lowest concentration (69.0 mg·g⁻¹ d.w.) was recorded under blue-red LED with 10 g·L⁻¹ sucrose.

Overall, white and blue LED treatments tended to promote greater free amino acid accumulation compared to red and blue-red light. Sucrose concentration alone did not exert a statistically significant effect on amino acid levels ($p = 0.791$).

Soluble protein content exhibited a contrasting trend. The highest protein concentration (56.95 mg·g⁻¹ d.w.) was recorded under red LED with 30 g·L⁻¹ sucrose, whereas the lowest values were consistently found in white and blue light treatments, regardless of sucrose level. Red LED consistently promoted higher protein accumulation, with a strong main effect of light spectrum ($p = 1.95 \times 10^{-11}$) and a significant interaction with sucrose ($p = 1.45 \times 10^{-11}$).

When averaged across treatments, white LED resulted in the highest mean free amino acid content (489.33 mg·g⁻¹ d.w.), followed by blue LED (375.00 mg·g⁻¹ d.w.). In contrast, red LED treatments produced the lowest average free amino acid level (179.50 mg·g⁻¹ d.w.) but the highest protein content (45.21 mg·g⁻¹ d.w.).

Table 5

Free amino acids [mg·g⁻¹ d.w.] and total soluble proteins [mg g^{-1} d.w.] content of *Echinacea purpurea* leaves treated with various LED light and sucrose level *in vitro* conditions.

LED spectrum	Sucrose Levels [g·L⁻¹]	Free Amino Acid [mg·g⁻¹d.w.]	Proteins [mg g – 1 d.w.]
White	10	253.0 ± 33 f	18.33 ± 6.06 c
	20	753.0 ± 25 a	14.02 ± 0.245 c
	30	462.0 ± 41 c	19.29 ± 0.367 c
Blue	10	650.0 ± 66 b	12.61 ± 0.00 c
	20	208.5 ± 20.5 de	19.15 ± 0.673 c
	30	266.5 ± 14.5 de	14.84 ± 0.662 c
Blue-Red	10	69.0 ± 17 f	18.30 ± 0.401 c
	20	204.5 ± 19.5 e	22.43 ± 3.62 b
	30	265.0 ± 7 de	23.21 ± 0.212 b
Red	10	301.0 ± 39 d	36.11 ± 2.08 b
	20	255.5 ± 27.5 de	42.57 ± 6.13 b
	30	210.0 ± 29 de	56.95 ± 10.7 a
Means for Light Spectrum			
White		489.33 ± 219.0 a	17.22 ± 3.89 b
Blue		375.00 ± 211.0 ab	15.53 ± 2.92 b
Blue-Red		255.50 ± 87.9 b	21.32 ± 2.93 b
Red		179.50 ± 48.3 b	45.21 ± 11.2 a
Means for Sucrose Levels			
10		318.25 ± 223.0 a	21.89 ± 9.12 a
20		355.38 ± 242.0 a	22.91 ± 12.9 a
30		300.88 ± 103.0 a	29.65 ± 17.2 a
Source of Variation			
Light spectrum x Sucrose Levels		< 0.0001	< 0.0001
Light Spectrum		< 0.0001	< 0.0001

*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05

LED spectrum	Sucrose Levels [g·L⁻¹]	Free Amino Acid [mg·g⁻¹d.w.]	Proteins [mg g − 1 d.w.]
Sucrose Levels		0.791	1.177
*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05			

2.5. Total Soluble Sugar and Reducing Sugar of Echinacea purpurea leaves

The content of total soluble sugars (TSS) in *E. purpurea* plantlets varied significantly depending on the interaction between LED light spectrum and sucrose concentration (Table 6). The highest TSS value (763.33 mg·g⁻¹ d.w.) was observed under blue LED combined with 30 g·L⁻¹ sucrose, while the lowest (630.00 mg·g⁻¹ d.w.) occurred under red LED with 10 g·L⁻¹ sucrose.

Among white LED treatments, all values (688.33–756.67 mg·g⁻¹ d.w.) were relatively high and showed no significant differences, indicating consistent sugar accumulation. The application of 20 and 30 g·L⁻¹ sucrose consistently enhanced TSS content compared to 10 g·L⁻¹, particularly under blue and white light.

Under blue-red and red LEDs, sugar content varied more markedly. In red LED treatments, TSS increased progressively with sucrose concentration, ranging from 630.00 to 675.00 mg·g⁻¹ d.w.. Conversely, under blue-red light, TSS peaked at 20 g·L⁻¹ sucrose but declined slightly at 30 g·L⁻¹. When averaged across all treatments, the highest mean TSS was recorded under white light (718.56 mg·g⁻¹ d.w.), while the lowest occurred under blue-red light (662.11 mg·g⁻¹ d.w.). Across sucrose concentrations, both 20 and 30 g·L⁻¹ resulted in significantly higher TSS levels than 10 g·L⁻¹.

For reducing sugars (RS), significant differences were observed among treatments. The highest RS content was found under white LED with 30 g·L⁻¹ sucrose (131.33 mg·g⁻¹ d.w.), followed closely by red LED with the same sucrose level (127.67 mg·g⁻¹ d.w.). These values were significantly greater than those recorded under other treatments. The lowest RS levels were observed under blue-red and blue LEDs at 10 g·L⁻¹ sucrose (60.33 and 63.00 mg·g⁻¹ d.w., respectively).

When averaged across all treatments, sucrose concentration had the strongest influence on reducing sugar content ($p < 2 \times 10^{-16}$), with a clear decline in RS as sucrose levels increased from 10 to 30 g·L⁻¹. This trend may reflect enhanced sugar utilization or metabolic conversion under higher external carbon supply. Although reducing sugar contents differed significantly between specific light × sucrose combinations, mean RS values across different LED spectra were not statistically different ($p = 0.194$).

Table 6

Total soluble [mg glucose · g d.w^{-1}] and reduced sugars [mg glucose · g d.w.$^{-1}$] content of of *Echinacea purpurea* leaves treated with various LED light and sucrose level *in vitro* conditions.

LED spectrum	Sucrose Levels [g·L^{-1}]	Total Soluble Sugar [mg g − 1 d.w.]	Reducing Sugar [mg g − 1 d.w.]
White	10	688.33 ± 12.2 abc	83.67 ± 3.06 ef
	20	756.67 ± 15.8 ab	118.33 ± 2.08 b
	30	710.67 ± 3.06 abc	131.33 ± 0.58 a
Blue	10	647.67 ± 0.58 c	63.00 ± 2.00 g
	20	655.00 ± 1.00 c	107.67 ± 4.04 c
	30	763.33 ± 40.7 ab	97.67 ± 0.58 d
Blue-Red	10	650.67 ± 3.06 c	60.33 ± 4.04 g
	20	706.67 ± 2.52 abc	82.67 ± 1.53 ef
	30	675.00 ± 9.85 bc	125.00 ± 2.65 ab
Red	10	630.00 ± 28.6 c	76.00 ± 1.00 fg
	20	681.33 ± 1.00 bc	85.33 ± 4.04 e
	30	763.00 ± 73.2 ab	127.67 ± 4.16 a
Means for Light Spectrum			
White		718.56 ± 31.8 a	111.11 ± 21.4 a
Blue		668.56 ± 35.1 ab	89.44 ± 20.4 a
Blue-Red		662.11 ± 28.6 b	89.33 ± 28.6 a
Red		688.67 ± 68.3 ab	96.33 ± 24.0 a
Means for Sucrose Levels			
10		654.17 ± 28.4 b	120.42 ± 10.2 a
20		699.92 ± 43.4 a	98.50 ± 15.9 b
30		699.33 ± 54.9 a	70.75 ± 14.1 c
Source of Variation			
Light spectrum x Sucrose Levels		< 0.0001	< 0.0001
Light Spectrum		0.000933	0.194
*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05			

LED spectrum	Sucrose Levels [g·L⁻¹]	Total Soluble Sugar [mg g − 1 d.w.]	Reducing Sugar [mg g − 1 d.w.]
Sucrose Levels		0.000428	< 0.0001
*the same letter in the lines indicates no difference between the means at a significance level of α = 0.05			

2.6. Polyphenolic Acid and Flavonoid

The accumulation of polyphenolic acids in *E. purpurea* was significantly influenced by the interaction between LED light spectrum and sucrose concentration (Table 7). The highest polyphenolic acid content was observed under blue light combined with 10 g·L⁻¹ sucrose, reaching 101.00 mg·g⁻¹ d.w., which was significantly higher than all other treatments. This was followed by blue-red with 30 g·L⁻¹ (85.78 mg·g⁻¹ d.w.) and blue with 20 g·L⁻¹ (82.44 mg·g⁻¹ d.w.). In contrast, the lowest value (20.78 mg·g⁻¹ d.w.) was recorded under blue-red with 10 g·L⁻¹ sucrose, followed by red with 10 g·L⁻¹ (39.99 mg·g⁻¹ d.w.).

A declining trend in polyphenolic acid content was evident under blue light as sucrose concentration increased, while the opposite pattern was observed under blue-red light, where polyphenolic levels increased with higher sucrose concentrations. When averaged across sucrose levels, the blue light spectrum resulted in the highest mean polyphenolic acid content (82.30 mg·g⁻¹ d.w.), which was significantly higher than the value recorded under white light (48.56 mg·g⁻¹ d.w.). Although sucrose concentration alone had no significant effect on polyphenolic acid levels ($p = 0.376$), its interaction with light spectrum was highly significant ($p < 2e - 16$).

For flavonoid content, the highest accumulation was found under blue-red light with 10 g·L⁻¹ sucrose (407.86 mg·g⁻¹ d.w.), followed by the same spectrum with 20 and 30 g·L⁻¹ (372.80 and 363.56 mg·g⁻¹ d.w., respectively). The lowest flavonoid level was recorded under white light with 10 g·L⁻¹ sucrose (139.73 mg·g⁻¹ d.w.).

Among all spectra, blue-red induced the highest average flavonoid accumulation (381.41 mg·g⁻¹ d.w.), followed by red (270.91 mg·g⁻¹ d.w.) and blue (249.72 mg·g⁻¹ d.w.), while white light produced the lowest mean (207.96 mg·g⁻¹ d.w.). No statistically significant differences were found among sucrose levels when considered alone ($p = 0.386$), but the interaction between light and sucrose was highly significant ($p < 0.001$).

Table 7
Polyphenolic acids [mg·g⁻¹d.w.] and flavonoids [mg·g⁻¹d.w.] of *Echinacea purpurea* leaves treated with various LED light and sucrose level *in vitro* conditions

LED spectrum	Sucrose Levels [g·L⁻¹]	Polyphenolic Acid [mg·g⁻¹d.w.]	Flavonoid [mg·g⁻¹d.w.]
White	10	62.00 ± 0.33 e	139.73 ± 18.2 f
	20	41.11 ± 0.19 f	259.50 ± 3.64 de
	30	42.56 ± 0.19 f	224.66 ± 0.577 e
Blue	10	101.00 ± 0.00 a	225.60 ± 0.00 e
	20	82.44 ± 0.19 c	257.00 ± 14.8 de
	30	63.44 ± 0.19 d	266.56 ± 2.77 d
Blue-Red	10	20.78 ± 0.19 h	407.86 ± 3.52 a
	20	76.44 ± 0.19 d	372.80 ± 0.693 ab
	30	85.78 ± 0.19 b	363.56 ± 7.16 b
Red	10	39.99 ± 1.53 g	268.20 ± 3.12 d
	20	62.22 ± 0.19 d	315.20 ± 25.0 c
	30	82.00 ± 1.00 c	229.33 ± 7.57 e
Means for Light Spectrum			
White		48.56 ± 10.1 b	207.96 ± 3.89 c
Blue		82.30 ± 16.3 a	249.72 ± 2.92 bc
Blue-Red		61.00 ± 30.4 ab	381.41 ± 2.93 a
Red		61.19 ± 18.5 ab	270.91 ± 11.2 b
Means for Sucrose Levels			
10		55.78 ± 31.3 a	260.35 ± 102.0 a
20		65.56 ± 16.6 a	301.12 ± 51.1 a
30		68.44 ± 17.9 a	271.03 ± 58.5 a
Source of Variation			
Light spectrum x Sucrose Levels		< 0.0001	8.24e-08
Light Spectrum		0.0116	< 0.0001
Sucrose Levels		0.376	0.386

2.7. Correlation analysis among morphological and biochemical parameters

The Pearson correlation heatmap (Fig. 3) revealed several strong and significant relationships among morphological and biochemical parameters of *in vitro E. purpurea*. Growth parameters, including shoot length, number of leaves, and root length, were highly positively correlated with each other. For instance, shoot length was strongly correlated with the number of roots (r = 1.00), number of leaves (r = 0.88), and root length (r = 0.29).

Photosynthetic pigment contents also exhibited clear internal consistency, as chlorophyll a was perfectly correlated with total chlorophyll a + b (r = 1.00), and strongly with chlorophyll b (r = 0.98). Additionally, chlorophyll a was positively correlated with shoot length (r = 0.34) and number of leaves (r = 0.30), indicating a moderate link between pigment accumulation and shoot growth. Carotenoids showed a very strong positive correlation with chlorophyll a (r = 0.95) and b (r = 0.97).

Regarding stress-related parameters, H_2O_2 content exhibited positive correlations with CAT activity (r = 0.70), phenolic acids (r = 0.56), and flavonoids (r = 0.64). Reducing sugars also correlated positively with CAT (r = 0.58) and H_2O_2 (r = 0.42). In contrast, free amino acid content was negatively correlated with CAT (r = − 0.47) and H_2O_2 (r = − 0.36), while being moderately positively correlated with chlorophyll a (r = 0.42) and shoot length (r = 0.18). Lastly, soluble proteins showed a moderate positive correlation with CAT activity (r = 0.37) and a weak negative correlation with flavonoids (r = − 0.370).

Discussion

The specific conditions of *in vitro* culture, including controlled light and supplementary sugars, substantially influence plant growth as well as physiological and biochemical responses (Batista et al. 2018; Shah et al. 2019). *In vitro* plants, unlike their naturally grown counterparts, are exposed to unique stressors that alter their metabolism, stress responses, and pigment biosynthesis (Zhang et al. 2022; Espinosa-Leal et al. 2022). This study examined the impact of different LED spectrum and sucrose concentrations in *E. purpurea in vitro*. We found that both LED spectrum and sugar levels significantly affected plant growth, pigment content, stress responses, enzymatic activity, and biochemical production of the *in vitro* plant.

The combination of LED spectrum and sucrose concentration significantly influenced the biometrics parameters of *E. purpurea in vitro*, particularly shoot length, leaf traits, and rooting traits (Table 1 and Table 2). However, rooting traits was more dependent on sucrose concentration and showed limited response to LED spectrum.

The longest shoots were observed under red-blue LED and 30 $g \cdot L^{-1}$ sucrose, surpassing other treatments. This suggests that combining red and blue light with high sucrose enhances stem elongation, possibly through activation of photoreceptors (phytochromes and cryptochromes) and

energy supplied by sucrose, which together facilitate hormone transport and cell expansion (OuYang et al. 2015; Wu et al. 2025). The significant interaction highlights the synergistic effect of light and sugar on stem growth.

The highest leaf number was recorded under blue LED with 30 g·L⁻¹ sucrose, suggesting that blue light stimulates leaf initiation by modulating growth signals. Blue light is known to promote compact growth by suppressing stem elongation and enhancing cytokinin activity (Kong and Zheng 2023). In contrast, red and white LEDs resulted in fewer leaves, indicating distinct morphogenic responses under varying spectral qualities.

Leaf area was also influenced by both factors, with the largest leaves found under blue LED and 20 g·L⁻¹ sucrose. This combination may promote stomatal development and photosynthetic capacity, as reported in studies of Chrysanthemum plantlets *in vitro* that show blue LED light increasing stomatal traits and net photosynthetic rate (Xu et al. 2020) However, leaf area decreased under blue light with 30 g·L⁻¹ sucrose, possibly due to feedback inhibition or sugar-induced osmotic stress (Yavari et al. 2021).

The light spectrum did not significantly affect root number or root length, while sucrose concentration played a more prominent role. The longest roots were obtained under red-blue LED with 20 g·L⁻¹ sucrose. Sucrose supports root development by acting as both an energy source and signaling molecule that modulates hormone transport (Sakr et al. 2018; Mishra et al. 2022). Suboptimal or excessive sucrose concentrations impeded root growth, indicating a narrow optimal range.

Chlorophyll, the principal pigment responsible for light absorption in photosynthesis, serves as an important indicator of photosynthetic efficiency. Carotenoids contribute both to light harvesting and photoprotection by shielding chlorophyll molecules from oxidative damage (Simkin et al. 2022).

In this study, chlorophyll content was significantly affected by light spectrum, while sucrose concentration alone had no significant effect. The highest chlorophyll level was observed under white LED with 10 g·L⁻¹ sucrose, suggesting that full-spectrum light combined with low sucrose favors chloroplast development and photosynthetic capacity. This condition likely activated both red and blue photoreceptors, which regulate chlorophyll biosynthesis (Kochetova et al. 2022). Across treatments, white LED produced the highest mean chlorophyll content, indicating a positive effect on pigment accumulation *in vitro*.

The results showed clear differences in the accumulation of chlorophyll a, chlorophyll b, and total (a + b) under different LED spectrum and sucrose levels. Across all treatments, chlorophyll a was consistently higher than chlorophyll b, which is expected since chlorophyll a is the primary photosynthetic pigment directly involved in the reaction centers of both photosystems, while chlorophyll b functions mainly as an accessory pigment that extends the absorption spectrum and transfers energy to chlorophyll a (Ke et al. 2020).

Carotenoid content, on the other hand, was significantly influenced by both light and sucrose concentration, as well as their interaction. The maximum carotenoid level was found under white LED with 10 g·L⁻¹ sucrose, indicating that photoprotective mechanisms are also enhanced under balanced light and carbon conditions. Elevated carotenoid levels were also observed under white and blue LEDs with 30 g·L⁻¹ sucrose, suggesting that carotenoids accumulated in response to stress induced by high sugar concentration and intense light. Similar stress-induced carotenoid responses have been reported previously (Mortain-Bertrand et al. 2008; Jeandet et al. 2022).

A significant interaction between light and sucrose was observed for both pigments, suggesting that pigment biosynthesis is tightly regulated by both photoreceptor signaling and sugar availability. Notably, under blue-red LEDs, increasing sucrose concentrations slightly increased carotenoid content while chlorophyll remained comparatively low, indicating a potential metabolic trade-off favoring stress mitigation over photosynthetic pigment accumulation (Meijer 2023).

Hydrogen peroxide (H_2O_2) is one of the most prevalent reactive oxygen species (ROS) in plants, functioning as a signaling molecule at low concentrations but becoming cytotoxic when excessively accumulated (Quan et al. 2008). In this study, H_2O_2 content varied significantly in response to LED spectra and sucrose levels, with a strong interaction effect. The highest H_2O_2 level was recorded under red LED with 30 g·L⁻¹ sucrose, followed by blue-red LED with 30 g·L⁻¹. These results suggest that narrow-band red and blue-red spectra, particularly when combined with high sucrose levels, enhance oxidative stress, possibly by accelerating cellular respiration and activating light-responsive metabolic pathways (Stamford et al. 2023).

Interestingly, H_2O_2 levels decreased under high sucrose concentrations (30 g·L⁻¹). This reduction may reflect a metabolic shift from ROS generation to detoxification or sugar-induced feedback suppression of oxidative processes (Hassanpour 2022). Overall, the highest mean H_2O_2 content was observed under red LEDs followed by blue-red, blue, and white.

To mitigate oxidative damage, plants activate antioxidant defense enzymes such as catalase (CAT), which decomposes H_2O_2 into water and oxygen. CAT activity in this study was significantly influenced by the interaction of light spectrum and sucrose concentration, although neither factor alone had a statistically significant effect. The highest CAT activity was unexpectedly observed under white LED with 10 g·L⁻¹ sucrose despite only moderate H_2O_2 levels. This suggests that CAT regulation may not solely depend on ROS levels but also involve light-induced signaling pathways independent of oxidative cues.

High CAT activity was also observed under blue-red LED with 30 g·L⁻¹ sucrose and red LED with 20 g·L⁻¹, both of which coincided with elevated H_2O_2 concentrations. These responses indicate that *E. purpurea* mounted a robust enzymatic antioxidant defense in response to combined light and sugar stress. In contrast, low CAT activity was recorded under blue LED with 20 g·L⁻¹, corresponding to a lower H_2O_2 level.

The combined data suggest that catalase activity in *E. purpurea* is tightly regulated by both external stress (light and sucrose) and internal ROS signaling. The correlation between high H_2O_2 and increased CAT activity in most treatments supports its role as a core component of the oxidative stress response, consistent with findings in *Arabidopsis* and maize (Mhamdi et al. 2010; Liu et al. 2018). Moreover, the strong CAT response under white LED suggests a possible photoreceptor-mediated pathway that primes antioxidant capacity even under relatively moderate ROS levels. These findings highlight the dual role of light and sucrose as both metabolic resources and stress-modulating factors in *in vitro* cultures.

Soluble proteins and free amino acids (FAAs) are useful indicators of plant metabolism, especially during stressful periods (Møller and Kristensen 2004; Batista-Silva et al. 2019). When plants are under osmotic, oxidative, or nutritional stress, these compounds often increase (Rao et al. 2016). In this study, both light and sugar had a big effect on FAA levels. The highest FAA was reported with blue LEDs that had 10 $g \cdot L^{-1}$ of sucrose and white LEDs that had 20 $g \cdot L^{-1}$ of sucrose. These conditions likely enhanced the plant's capacity to assimilate nitrogen and manage stress.

Amino acids not only form proteins, but they also help control ROS, serve as building blocks for other chemicals, and protect cells from stress (Trovato et al. 2021). A greater FAA under white or blue light and a low sugar level shows that the plant was well-balanced between growing and being able to handle stress. Moderate sugar stops osmotic problems, while blue light helps the body take up nitrogen. Potato plantlets had similar results, showing that perfect light conditions and adding sugar can help free amino acids develop up (Zhang 2020).

On the other hand, red and blue-red LEDs with a lot of sugar had the lowest FAA levels. These treatments also exhibited significant levels of $H2O_2$ and CAT, which means that when the plant is under stress, it uses nitrogen more for defense (such antioxidant enzymes) than for making amino acids. Plants that are under stress have been shown to redirect amino acids to make compounds that are associated to stress, such as proline or polyphenolics (Phang et al. 2010).

The highest concentration of soluble protein under red LED was 30 $g \cdot L^{-1}$ sucrose. This finding aligns with the research conducted by Ahmad et al. (2024), which demonstrated that the soluble protein levels in *Dendrobium officinale* tissue culture seedlings significantly increased when subjected to red LED light. The discovery that elevated CAT levels corresponded with increased protein levels substantiates this idea. Antioxidant enzymes likely constituted a segment of the protein reservoir in these treatments.

It's noteworthy to observe that high-stress treatment had more protein, whereas low-stress one had more FAA. This indicates that with increasing stress levels, the body may transition from storing nitrogen as amino acids to using it in proteins.

Carbohydrates, especially soluble sugars, are important sources of energy and can also help reduce stress. They serve as signals in stress responses, bolster antioxidant systems, and facilitate the maintenance of water balance (Afzal et al. 2021). The light spectrum and the amount of sucrose both had a big effect on the TSS levels in our study. The white LED with 20 $g \cdot L^{-1}$ sucrose and the blue LED

with 30 g·L⁻¹ sucrose exhibited the highest total solid state (TSS). It's likely that these conditions helped the plants store energy and get used to the *in vitro* surroundings.

Soluble sugars can protect proteins and membranes from damage in addition to turning on genes that are connected to stress (Couée et al. 2006). The white LED light seems to help make sugar and photosynthesis, especially when used with a little bit of sucrose. *Eutrema japonicum* and *Lactuca sativa* had analogous results, with white LED light enhancing sugar accumulation either independently or in combination with UV (Alrajhi et al. 2023; Ruamrungsri et al. 2024).

On the other hand, red and blue-red LEDs made the most reducing sugars (RS), such fructose and glucose, especially when there was 30 g·L⁻¹ sucrose in the air. This might mean that stress made more sucrose turn into simple sugars. RS reduces oxidative damage by enhancing antioxidant systems, including the pentose phosphate pathway, which produces NADPH (Cherkas et al. 2020). The fact that RS levels were high in the same treatments with high H_2O_2 and CAT (like red LED + 30 g·L⁻¹) shows that they play a role in stress signaling and protection.

The varied effects of RS and TSS show how the plant uses and stores sugar in different ways depending on the situation. Plants that are less stressed (such those with white or blue LEDs and low sucrose) have greater TSS, which helps them grow and stay stable. But RS levels go up when stress levels go up (as when red/blue-red LEDs are used with high sucrose), which presumably helps protect against stress. According to comparable research (Zamljen et al. 2022), stress increased RS and antioxidant activity in *Capsicum annuum*.

Statistics showed that both light and sucrose had big effects on TSS and their interaction was very important. TSS was more affected by the light spectrum, whereas sucrose had a bigger effect on RS. This shows that light and sugar control different parts of sugar metabolism, such how it breaks down and is stored.

Flavonoids and polyphenolic acids are important secondary metabolites that protect plants from oxidative stress, especially when there are stressors inside or outside the plant (Kumar et al. 2023). In our study, we specifically measured polyphenolic acids, and found that the light spectrum had a strong effect on both total polyphenolic acids content (TPC) and total flavonoid content (TFC), while sucrose had a smaller but still noticeable effect. This shows that light has a bigger role than sugar in controlling how these compounds are produced.

The highest TPC was observed under blue LED with 10 g·L⁻¹ sucrose, indicating that blue light may enhance the phenylpropanoid pathway responsible for polyphenolic acids biosynthesis (Zoratti et al. 2014).

In contrast, the highest TFC was recorded under blue-red LED with 10 g·L⁻¹ sucrose, followed by blue-red with 20 and 30 g·L⁻¹. These results suggest that blue-red light strongly stimulates flavonoid biosynthesis, possibly through the combined activation of cryptochrome and phytochrome signaling pathways.

Statistical analysis confirmed that light spectrum significantly affected both TPC and TFC, while the interaction between light and sucrose was significant for both parameters. However, sucrose alone had no significant effect on either TPC or TFC. This suggests that while sugars may serve as metabolic precursors for polyphenolics, their effect is only evident when coupled with specific light qualities.

The Pearson correlation heatmap (Fig. 3) revealed several strong and significant relationships among morphological and biochemical parameters of *in vitro E. purpurea*, showing that treatments promoting shoot elongation also favored root proliferation and overall vegetative development. This coordinated growth pattern reflects the joint regulation of shoot and root development by sugar availability and hormonal signaling, particularly auxins and cytokinins, which are known to control biomass partitioning between above and belowground tissues (Ahmad et al. 2023).

Photosynthetic pigment contents also displayed strong internal consistency, as chlorophyll a was perfectly correlated with total chlorophyll a + b and closely associated with chlorophyll b. The positive relationships of chlorophyll a with shoot length and leaf number indicate that pigment accumulation contributes to growth performance. Carotenoids were also tightly linked with both chlorophyll a and b, reflecting the coordinated regulation of light-harvesting pigments under different light and sucrose regimes. Such associations emphasize the integrated functioning of the photosynthetic apparatus, where chlorophylls and carotenoids act together to balance energy capture with photoprotection, thereby preventing oxidative damage while sustaining growth (Simkin et al. 2022).

Stress-related parameters revealed further connections. H_2O_2 levels were positively correlated with CAT activity, phenolic acids, and flavonoids, showing that oxidative stress was accompanied by activation of both enzymatic and non-enzymatic antioxidant defenses. Reducing sugars also showed positive relationships with CAT and H_2O_2, highlighting the involvement of carbohydrate metabolism in maintaining redox balance. This pattern supports the dual role of H_2O_2 as both a damaging oxidant and a signaling molecule that stimulates antioxidant defenses (Sachdev et al. 2021), while sugars may further contribute to stress adaptation by supplying energy and modulating ROS signaling (Afzal et al. 2021).

By contrast, free amino acid content showed negative associations with both CAT and H_2O_2, but a moderate positive link with chlorophyll a and shoot length. This suggests that amino acids function as adaptive nitrogen reserves under less stressful conditions, but when stress intensifies, nitrogen is redirected from amino acid pools toward protein and enzyme synthesis to reinforce defense mechanisms (Farhan et al. 2024). Soluble proteins, in turn, were positively correlated with CAT activity but negatively linked with flavonoids, reflecting a trade-off in nitrogen allocation between protein biosynthesis and secondary metabolite production. Such allocation patterns are consistent with the growth–defense balance concept, where plants shift resources either toward structural growth and metabolism or toward protective compounds depending on environmental conditions (Figueroa-Macías et al. 2023).

Conclusions

This study demonstrated that both LED light and sucrose concentration significantly influenced the morphological and biochemical responses of *E. purpurea* cultured *in vitro*. Different combinations of light quality and sucrose levels distinctly affected the growth, photosynthetically active pigments biosynthesis, oxidative stress markers, nitrogen metabolism, and the accumulation of polyphenolic acids and flavonoids. Carotenoid accumulation and catalase activity were closely associated with oxidative stress, while polypolyphenolic acids and flavonoids acted as additional non-enzymatic defense compounds. The coordinated increase in H_2O_2, CAT, and polyphenolic compounds suggests that *E. purpurea* activates both enzymatic and non-enzymatic antioxidant systems in response to stress conditions. These results provide practical guidance for optimizing micropropagation protocols of E. *purpurea*, for example by using white or blue LEDs with moderate sucrose to support balanced growth, or red/blue-red LEDs with higher sucrose to enhance biochemical accumulation.

Abbreviations

PGRs – plant growth regulators

LED - Light-Emitting Diode

TSS - Total Soluble Sugars

RS - Reducing Sugars

FAA - Free Amino Acids

CAT - Catalase

H_2O_2 - Hydrogen Peroxide

ROS - Reactive Oxygen Species

TPC - Total Polyphenolic Content

TFC - Total Flavonoid Content

Chl – Chlorophyll

Car – Carotenoids

Declarations

Data Availability

The data supporting the findings are available from the corresponding author upon request.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Autor's Contribution

All authors contributed to this study's design and conception. NS collected the data and performed the experiments and laboratory work. NS conducted statistical analyses. MM assisted analysis of biochemical. NS wrote the manuscript. AP and MM revised the manuscript. All authors read and approved the final version of manuscript.

Ethics Declaration

Not applicable.

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Figures

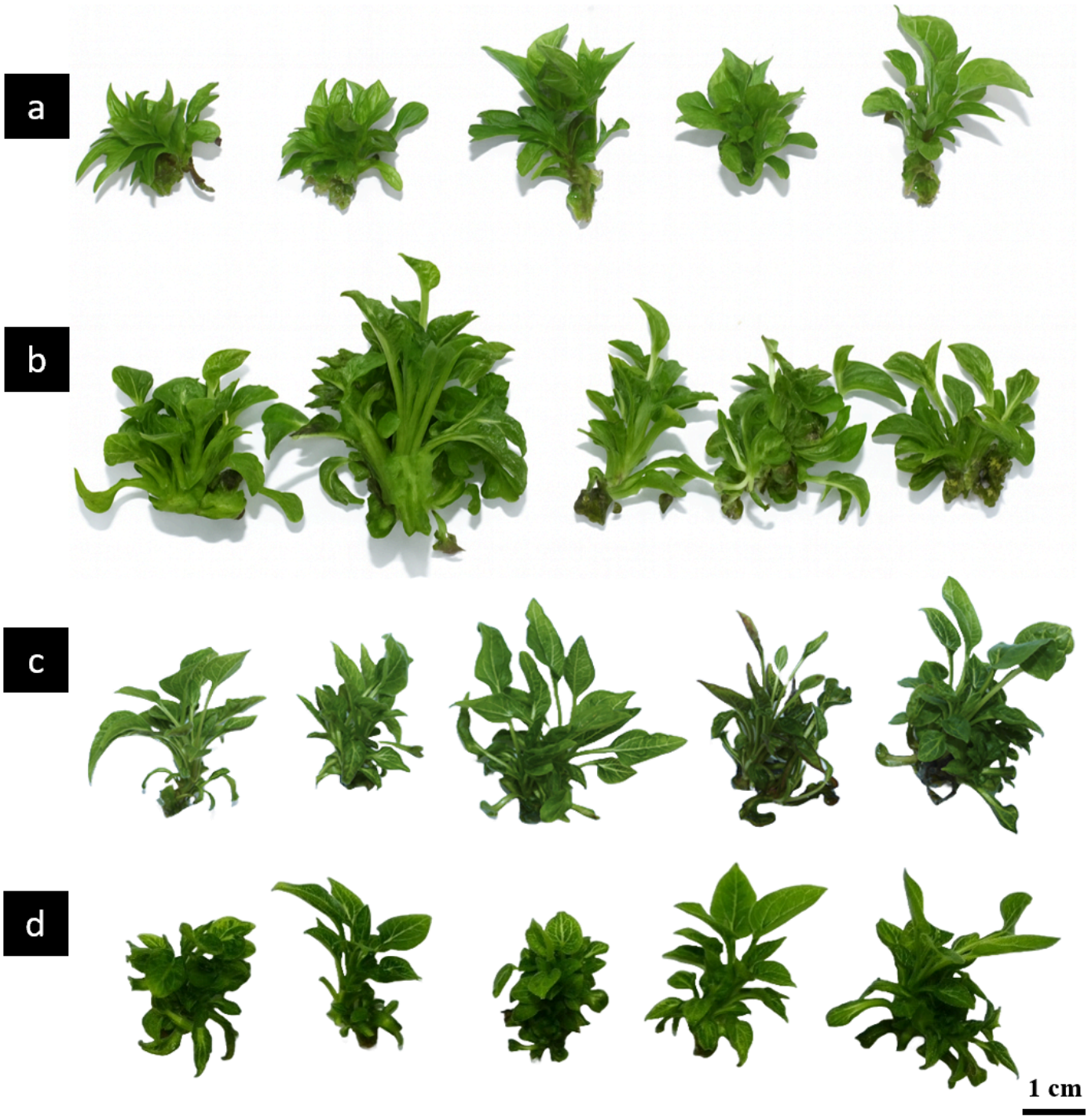

Figure 1

In vitro Echinacea purpurea under white LED with 10 g·L⁻¹ sucrose (a), blue LED with 30 g·L⁻¹ sucrose (b), Blue-Red LED with 30 g·L⁻¹ sucrose (c), Red LED with 10 g·L⁻¹ sucrose (d).

Figure 2

In vitro rooting *Echinacea purpurea*under white LED (a), blue LED (b), Blue-Red LED (c), Red LED (d) with different sucrose concentration.

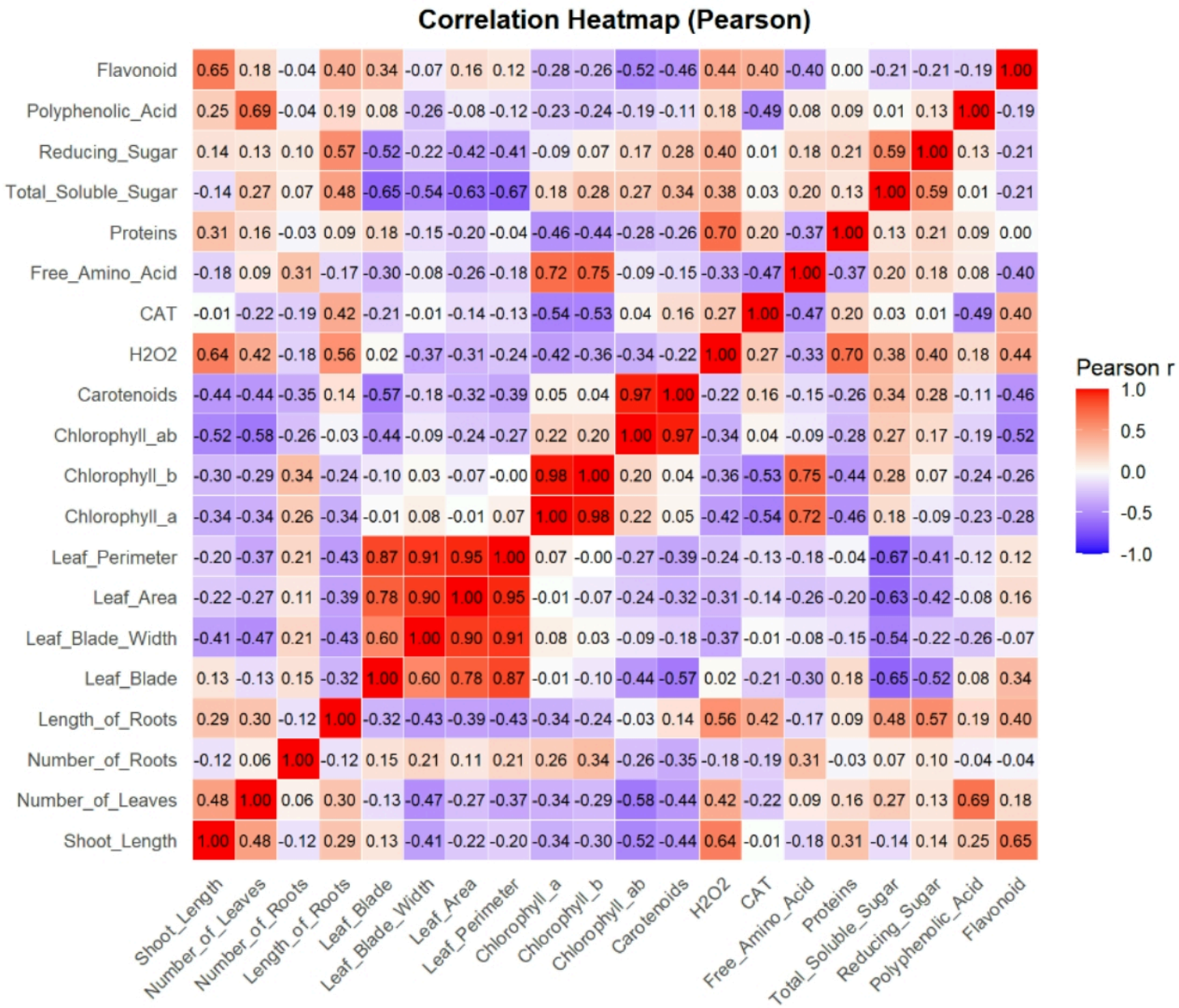

Figure 3

Correlation Matrix of Morphological and Biochemical Levels of *Echinacea purpurea* treated with various LED light and sucrose level *in vitro* conditions