

Deciphering the potential of LED lights in modulating morpho-physiological growth, specialized metabolites, and gene expression pattern in *Valeriana jatamansi* Jones

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Abstract

Valeriana jatamansi Jones is a well-known Himalayan medicinal rhizomatous herb due to the presence of therapeutically important bioactive metabolites. The precise manipulation of LED light quality and conditions acts as a powerful tool to sculpt plant growth, fine-tune photosynthetic performance, and boost phytochemical biosynthesis in controlled *in vitro* environments. Thus, in the present investigation, the effect of different LED lights (yellow, red, blue, and white as a control) on the growth, physiological responses, biochemical parameters, secondary metabolite accumulation, and gene expression pattern of *in vitro* raised *V. jatamansi* was systematically evaluated. Maximum shoot length (9.71 cm) was observed under blue light, whereas maximum leaf number (20.22) and root number (15.11) were found under white light. Red light promoted the maximum fresh weight in both leaf (1.42 g) and root (1.47 g) tissues. In root tissue, the highest total phenolic content (126.30 µg QE/mg DW) and flavonoid content (211.79 µg GAE/mg DW) were recorded under yellow and white light, respectively. Chlorophyll fluorescence measurements indicated stable photosynthetic efficiency across all light treatments. Peroxidase and catalase activity in root and leaf tissue were highest in white light conditions. The accumulation of valtrate was maximum under white and yellow light in leaf (0.090 mg/g DW) and root tissue (0.167 mg/g DW), respectively. Furthermore, qRT-PCR analysis showed significant upregulation of iridoid biosynthetic pathway genes (*GES*, *G10H*, and *7DLS*) under yellow and white light. This study highlights the potential of LED lights for optimizing growth and enhancing the production of bioactive metabolites in *V. jatamansi*, offering a sustainable approach for its conservation and future large-scale pharmaceutical applications under controlled *in vitro* conditions.

Introduction

Valeriana jatamansi Jones, a perennial rhizomatous medicinal herb belonging to the Caprifoliaceae family, is commonly referred to as Indian Valerian or Tagar. It predominantly grows in the Himalayan region at elevations between 900 and 3000 m above mean sea level (Bhatt et al. 2012). The roots and rhizomes contain bioactive molecules, including valerenic acid and valepotriates (valtrate, didrovaltrate, and acevaltrate), which are used for the treatment of various diseases (Singh et al. 2010). The subterranean tissue of the plant also contains essential oil, a prized aromatic substance extensively used globally. These essential oils comprise anxiolytic, antibacterial, antifungal, and sedative properties (Bos et al. 1997). The significant role of *V. jatamansi* in pharmaceuticals has made it one of the extensively harvested herbs in the Himalayan region. Overharvesting from wild habitats has led to high consumption, driving the need for cultivation and micropropagation to ensure a sustainable supply and production of metabolites (Pandey et al. 2020). Micropropagation has been extensively utilized for the swift and extensive propagation of plants to acquire uniform and pathogen-free planting material (Murthy et al. 2023). To establish an efficient *in vitro* production system, it is essential to select healthy explants, use optimum culture media, a suitable gelling agent, and plant growth regulators (Gupta et al. 2020).

Given the growing emphasis on optimizing *in vitro* culture conditions, exploring the role of LED lighting is crucial for refining protocols that enhance biomass production and metabolite synthesis. Because lighting strongly influences plant morphogenesis and secondary metabolism, understanding how different LED spectra affect *in vitro* cultures is crucial for developing efficient and sustainable production systems (Djangalina 2023). Therefore, investigating LED light regimes provides an opportunity to improve plant growth and maximize phytochemical yields under controlled conditions. Several studies have demonstrated that light has a direct impact on plant growth, physiology, and biochemical responses, promoting the biosynthesis of phytochemicals (Pang et al. 2023). But the synergistic or antagonistic effects of light are significantly influenced by light quality, intensity, duration of exposure, plant species, and cultivation conditions. The minimal heat output of LEDs reduces the possibility of overheating the *in vitro* cultivated tissues, hence providing a more secure and regulated growing environment (Attaya 2021; Barceló-Muñoz et al. 2021). Various forms of light have been utilized to generate secondary metabolites in different tissue culture techniques, encompassing shoot culture, adventitious and hairy root culture, callus, and cell suspension culture. Specifically, yellow, blue, and red-light treatments are frequently employed to enhance secondary metabolite production in many plant species (Wang et al. 2018; Gupta and Sood 2023; Partap et al. 2025a). A blend of red and blue light increases root number and shoot length under *in vitro* growth of *Cattleya walkeriana* (Nadal et al. 2023). Furthermore, the maximum salidroside content (3.12 mg/g DW) was recorded in the blue light-exposed callus culture of *Rhodiola imbricata* (Kapoor et al., 2018). Recently, a study demonstrated that the combined application of red light and precursor treatment (Vanillic acid) enhances the synthesis of picroside and precursor metabolites in *Picrorhiza kurroa* cell culture (Partap et al. 2025a). Also, the light spectrum and intensity significantly affect gene expression patterns and metabolic pathways by altering plant photosynthetic pigments and photoreceptor-mediated signaling. Additionally, numerous studies have shown that exposure to red and blue light affects gene expression related to secondary metabolism in plants (Li et al. 2017; Boonsongcheep et al. 2019). Blue light generally activates photoreceptors such as cryptochromes and phototropins, thus inducing oxidative stress responses and enhancing the synthesis of secondary metabolites. Conversely, red light primarily influences photosynthesis and cell elongation by activating phytochromes. Therefore, applying LED lights, either individually or in combination, is a promising approach to enhance plant growth and the accumulation of targeted bioactive molecules in *in vitro* plants.

Previous investigations have also effectively examined the influence of various biotic and abiotic elicitors on secondary metabolite biosynthesis in *V. jatamansi* under *in vitro* conditions (Shuang and Hong 2020; Pandey et al. 2022; Thakur et al. 2024b). However, despite growing evidence on how specific light spectra regulate physiological and molecular responses in diverse plant species, comparable studies in *V. jatamansi* remain scarce, leaving an important knowledge gap regarding the potential of LED illumination for its *in vitro* improvement. Thus, the current investigation aimed to assess the impact of different LED lights on plant growth, biomass yield, photosynthetic performance, valepotriate content, and gene expression patterns.

Materials and methods

Plant material and culture conditions

Nodal tissue was taken as an explant for establishing axenic shoot cultures. Explants were collected from primary hardened plants maintained in a walk-in growth chamber (22 ± 2°C, 65 ± 5% RH) at CSIR-IHBT, Palampur, India. Explants were initially washed under tap water for 15 minutes, then treated with Tween-

20 to remove dust. Surface sterilization was carried out inside a laminar flow cabinet using 0.1% bavistin and streptomycin sulfate for 5 minutes, followed by 70% ethanol for 30–60 seconds, and 0.06–0.1% HgCl₂. Each step was succeeded by 3–4 rinses with sterile distilled water to eliminate residues. The sterilized nodal segments were cultured on the Murashige and Skoog medium (MS) (Murashige and Skoog 1962) supplemented with 1 mg/L BAP, 3% sucrose, and 0.8% agar inside a laminar airflow chamber. The pH of the culture medium was set to 5.8 using 1 N sodium hydroxide (NaOH) or hydrochloric acid (HCl). The cultures were maintained in a sterile environment at a temperature of 25 ± 2°C, with a photoperiod of 16 hours of light and 8 hours of darkness, and under lighting conditions of photosynthetic photon flux density of 150 µmol m⁻² s⁻¹. The incubated cultures were kept under different LED lights, including red (650–670 nm), blue (450–490 nm), yellow (570–580 nm), and white (400–700 nm), as a control for 2 months, and were observed daily for plant growth (Fig. 1).

In vitro plant growth and biomass yield

The effect of different LEDs on *in vitro* raised *V. jatamansi* growth was investigated by various morphological parameters, including shoot length, leaf number, and root number, after 2 months of incubation. Furthermore, Biomass yield was assessed based on the fresh and dry weights of leaves, petioles, and roots. After 2 months of incubation, plantlets were harvested for further analysis.

Chlorophyll Fluorescence Parameters

To assess the influence of LED exposure on the photosynthesis of *in vitro* raised *V. jatamansi*, key chlorophyll fluorescence parameters were measured. These included the minimal fluorescence yield in the absence of actinic light (F₀), maximum fluorescence yield (F_m), potential quantum efficiency of PSII (F_v/F_m), proportion of open PSII centers (qP), non-photochemical quenching coefficient (qN), quantum yield of PSII photochemistry (ΦPSII), and electron transport rate (ETR). Measurements were taken on the third leaf from the top of *V. jatamansi* plants using a pulse-amplitude modulated (PAM) portable fluorometer (JUNIOR-PAM, Heinz Walz GmbH, Germany). The plants were acclimated in darkness for 30 minutes to facilitate the total oxidation of the PSII reaction center before measurements. Data were obtained from the adaxial surface of the leaf, excluding the central vein, utilizing a magnetic clip attached to the fluorometer via a fiber optic cable. Data acquisition was carried out using WinControl-3 software.

Estimation of Total Phenolic Content

The total phenolic content (TPC) was estimated by the Folin-Ciocalteu colorimetric procedure by Partap et al. (2020), calibrating against the gallic acid standards. Briefly, 100 µL of the methanolic extract was dissolved in 900 µL of 90% methanol. To this, 5 mL of reagent (1 FC: 9 DW) was added, followed by 4 mL of 7% sodium carbonate (Na₂CO₃). The mixture was kept at 40°C for 10 min in the dark using a hot air oven. The samples' absorbance was assessed in triplicate with a Synergy H1 Hybrid Multi-Mode Reader (BioTek, USA). The obtained data were represented in µg GAE (Gallic Acid Equivalents)/mg dry weight of leaves, based on a gallic acid calibration curve.

Estimation of Total Flavonoid Content

The total flavonoid content (TFC) was quantified utilizing the aluminium chloride (AlCl₃) colorimetric protocol, as delineated by Partap et al. (2020). In brief, 0.3 mL of 0.5 M sodium nitrite (NaNO₂) and 0.3 mL of 0.3 M AlCl₃ were added to 100 µL of the methanolic extract. Then, 1.0 mL of a 1 M NaOH solution was introduced to each reaction mixture. After thoroughly mixing, the samples were incubated for 30 minutes. The absorbance of the samples was taken in triplicate with a microplate reader instrument (Synergy H1 Hybrid Multi-Mode Reader, BioTek, USA). The total flavonoid content was expressed in µg QUE (Quercetin Equivalents)/mg of dry weight, determined by comparing the sample absorbance to a calibration curve generated with standard quercetin.

Quantification of Antioxidant Enzyme Capacity

Extract Preparation

The enzymatic capacity of superoxide-dismutase (SOD), catalase (CAT), and peroxidase (POX) was evaluated in leaf and root tissues of *in vitro* raised *V. jatamansi*. Leaf and root samples (100 mg each) were pulverized in liquid nitrogen and extracted in 10 mL of chilled 50 mM sodium phosphate buffer with 1% polyvinylpyrrolidone. After centrifugation at 15,000 g for 10 min at 4°C, the supernatant was checked for enzymatic activity.

Superoxide Dismutase activity

The activity of superoxide dismutase (SOD) was assessed by its capacity to impede the photochemical reduction of Nitro-Blue Tetrazolium (NBT) by superoxide, per the methodology established by Beauchamp and Fridovich (1971). For the assay, 100 µL of enzyme extract was mixed with 50 mM sodium phosphate buffer (pH 7.8), 201 mM methionine, 1.72 mM NBT, 1% Triton X-100, and 30 µL of riboflavin. The mixture was placed in an illuminating chamber for 8 minutes inside a fluorescence lamp. The samples' absorbance was measured at 560 nm in triplicate with a Synergy H1 Hybrid Multi-Mode Reader (BioTek, USA). One unit of SOD activity corresponds to the measure of enzyme essential to prevent NBT reduction by 50%, and is reported in units (U) per gram of fresh weight (g FW).

Peroxidase activity

The Peroxidase (POX) enzymatic capacity was assessed according to the Malik and Singh (1980) procedure at 28–30°C. The mixture contained 3.5 mL of 50 mM sodium phosphate buffer (pH 6.5), 200 µL of enzyme extract, and 100 µL of freshly prepared O-dianisidine solution in methanol (1 mg/mL). Then, 200 µL of 0.2 M hydrogen peroxide (H₂O₂) was introduced. The rise in absorbance at 430 nm was monitored every 30 seconds for 3 minutes using a UV/Vis spectrophotometer (Thermo Scientific Inc, USA), and activity was reported as units (U) per gram of fresh weight (g FW).

Catalase Activity

The assay was based on the degradation of H₂O₂, following Aebi (1984). The assay mixture consisted of 1.5 mL of 100 mM sodium phosphate buffer (pH 7.0), 0.4 mL of distilled water, and 1 mL of 30 mM H₂O₂, to which 0.2 mL of enzyme extract was added. The decrease in absorbance at 240 nm was recorded every 15 seconds for 1 minute with a UV/Vis spectrophotometer (Thermo Scientific Inc, USA). Catalase activity was quantified based on the molar extinction coefficient of H₂O₂ and reported in (U) per gram of fresh weight (g FW).

Metabolites Quantification

Dried leaf and root tissues (100 mg) were ground into powder and extracted in 5 mL of methanol. The mixtures were shaken over-night, then sonicated at 45°C for 30 minutes. After sonication, samples were used for centrifugation at 1807 × g for 10 min at 4°C and filtered with 0.22-µm PVDF hydrophobic syringe filters (Moxcare, Ambala, India). Standard solutions (1 mg mL⁻¹ in methanol) were formulated, and valepotriates (valtrate, acevaltrate, didrovaltrate) were quantified with an Acquity™ UPLC H-Class system (Waters Corp, Milford, MA, USA).

RNA extraction, cDNA preparation, and qRT-PCR Analysis

The expression pattern of nine genes implicated in iridoid pathway, mainly *GES* (geraniol synthase), *10HGO* (10-hydroxygeraniol oxidoreductase), *G10H* (geraniol 10-hydroxylase), *IS* (iridoid synthase), *7DLS* (7-deoxyloganetic acid synthase), *7DLGT* (7-deoxyloganetic acid glucosyl transferase), *DL7H* (7-deoxyloganic acid hydroxylase), *SLS* (secologanin synthase), and *LAMT* (loganic acid O-methyltransferase), were determined through quantitative real-time PCR analysis to assess their expression profile in the leaf and root tissues of *in vitro* raised *V. jatamansi*. RNA extraction was performed from 100 mg of leaf and root tissue using TRIzol reagent (Invitrogen, Carlsbad, CA), and the extracted RNA was used for cDNA synthesis with the Verso First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA). RNA concentration was determined using a Nanodrop spectrophotometer, after which cDNA was prepared, standardized to 100 ng/µL, and employed for qRT-PCR (QuantStudio 3, Thermo Scientific). Gene-specific primers for biosynthetic pathway transcripts were designed according to Shuang and Chenshu (2020). qRT-PCR was carried out with an initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min for annealing and extension (Thakur et al. 2024a,b). Reactions were executed in triplicate, and relative expression was determined by the 2^{-ΔΔCt} approach, using GAPDH as the reference gene (Livak and Schmittgen 2001).

Statistical analyses

Data were analyzed statistically with SPSS software (version 25.0.0, IBM Corp., Chicago, USA). One-way ANOVA and Duncan's Multiple Range Test (DMRT) were applied for statistical analysis at a significance level of *P* ≤ 0.05. All the studies were done in triplicate.

Results

Effect of LEDs on Morphological Parameters and Growth Biomass

Light quality significantly influenced the *in vitro* morphology and biomass accumulation of *V. jatamansi* plants. Shoot height increased by 1.33-fold and 1.02-fold under white light compared to yellow and red light, respectively. Blue light-treated plants showed a 1.49-fold increase in shoot height compared to all other light treatments (Fig. 2a). Leaf number was highest under white light, with increases of 1.14-fold, 2.00-fold, and 1.06-fold compared to yellow, red, and blue light, respectively (Fig. 2b). Similarly, root number was maximum under white light, showing enhancements of 1.50-fold, 1.74-fold, and 1.07-fold relative to yellow, red, and blue light treatments, respectively (Fig. 2c). Leaf fresh weight was highest under red light, showing a 1.12-fold increase, while yellow and blue light resulted in 1.96-fold and 2.03-fold reductions, respectively, compared to white light (Fig. 3a). Petiole fresh weight was highest under white light, with increases of 1.84-fold, 1.03-fold, and 1.10-fold compared to yellow, red, and blue light conditions, respectively (Fig. 3c). Root fresh weight was highest under red light, with a 1.11-fold increase, whereas yellow and blue light caused 2.64-fold and 1.88-fold reductions, respectively, compared to white light (Fig. 3e). Red light resulted in a 2-fold increase in leaf dry weight, whereas blue light caused a 1.25-fold reduction compared to white light (Fig. 3b). Leaf fresh weight remained unchanged under yellow light as compared to white light. Petiole dry weight was 1.18-fold, 2.25-fold, and 2.18-fold higher under yellow, red, and blue light, respectively, compared to white light (Fig. 3d). Root dry weight increased by 1.07-fold and 2.33-fold under yellow and red light, respectively, while it remained unchanged under blue light compared to white light (Fig. 3f). The above-mentioned results concluded that light quality substantially affect the morphology and biomass of *V. jatamansi in vitro*, with white light promoting shoot and leaf growth, whereas red light facilitated biomass accumulation.

Effect of LEDs on Chlorophyll Fluorescence Parameters

V. jatamansi shows stable photosynthetic performance under different light treatments, indicating effective light adaptation and photoprotection. The initial fluorescence (Fo) value exhibited notable similarity across all light situations, ranging from a minimum of 36 in blue light to a maximum of 51 in yellow and red-light conditions (Fig. 4a). The maximum fluorescence (Fm) value achieved by the plants was consistently comparable across all light settings, ranging from 115 to 135 (Fig. 4b). The Fv/Fm ratio is a vital measure of photosynthetic efficiency. It denotes the highest attainable quantum efficiency of PS II, assuming all reaction centres are operational. Furthermore, the experimental plants in this study exhibit no substantial decrease in photochemical capacity, since all display a Fv/Fm ratio ranging from roughly 0.63 to 0.70 with no significant variation (Fig. 4c). Furthermore, no significant variation occurred in the ΦPSII, ETR, qP, and qN across the various experimental settings. This suggests that no physiological anomalies occurred across the treatments. Variation in

PAR wavelength influenced qP, qN, and ETR significantly, whereas ΦPSII values remained statistically unchanged (Fig. 5d). qP responded significantly at 190, 285, and 420 nm (Fig. 5b), qN across 90, 125, 190, 285, 420, and 625 nm (Fig. 5c), and ETR solely at 625 nm (Fig. 5a). Therefore, the variations are likely driven by dynamic photoprotection responses and light acclimation strategies to maintain photosynthetic stability under fluctuating light environments.

Effect of LEDs on Non-enzymatic Activity

Light conditions significantly influence antioxidant compounds in the *in vitro* culture of *V. jatamansi*. The total phenolic content (TPC) in leaf tissue was 1.01-fold higher under blue light, whereas it was 1.03-fold and 1.01-fold lower under red and yellow light, respectively, compared to white light. Similarly, in root tissue, TPC was 2.14-, 1.27-, and 1.22-fold higher under yellow, blue, and red light, respectively, than in white light. For total flavonoid content (TFC) in leaf tissue, yellow light resulted in a 1.01-fold increase, whereas red and blue light caused 1.41-fold and 1.47-fold reductions, respectively, compared to white light. In root tissue, blue, yellow, and red light led to 2.55-fold, 2.51-fold, and 1.06-fold increases, respectively, relative to white light conditions. (Table 1). These findings highlight the potential of blue and yellow light as effective spectral treatments for optimizing antioxidant metabolite production in *V. jatamansi*.

Table 1
Effect of monochromatic LED lights on Total phenolic content (TPC) and Total flavonoid content (TFC) in *in vitro* raised *V. jatamansi*

Light	Tissue	TFC (µg QE/mg DW)	TPC (µg GAE/mg DW)
White	Leaf	125.79 ± 2.46a	64.38 ± 3.14a
	Root	211.79 ± 2.46a	58.90 ± 3.38a
Yellow	Leaf	128.30 ± 1.44a	63.47 ± 3.32a
	Root	84.91 ± 2.88b	126.30 ± 2.18a
Red	Leaf	88.68 ± 0.94b	62.10 ± 3.80a
	Root	199.69 ± 3.50a	72.37 ± 1.00a
Blue	Leaf	85.22 ± 1.66b	65.30 ± 3.36a
	Root	83.01 ± 2.50b	75.11 ± 3.98a
Different superscript lowercase letters in numbers indicate statistically significant differences between the means (p < 0.05)			

Effect of LEDs on Enzymatic Activity

The POX assay results revealed significant differences among the light treatments. In leaf tissue, POX activity increased by 11.85-fold under yellow light, 2.84-fold under blue light, and 1.92-fold under red light compared to white light (Fig. 6a). In root tissue, POX activity under white light was 7.02-fold, 1.33-fold, and 2.44-fold higher compared to yellow, blue, and red-light conditions, respectively (Fig. 6b). Catalase activity varies significantly under different light treatments. In leaf tissue, white light induces a 9.34-fold, 4.10-fold, and 7.44-fold higher activity compared to yellow, blue, and red-light conditions, respectively (Fig. 6c). In root tissue, white light enhances catalase activity by 2.07-fold, 1.92-fold, and 2.68-fold compared to yellow, blue, and red-light conditions, respectively (Fig. 6d). Light exposure markedly affects SOD activity in both leaf and root tissues. In leaf tissue, activity under red, blue, and yellow light was 1.07-, 1.05-, and 1.07-fold higher, respectively, compared to white light (Fig. 6e). Conversely, in root tissue, SOD activity under blue, red, and yellow light was 1.08-, 1.02-, and 1.01-fold higher, respectively, compared to white light (Fig. 6f). Light quality significantly influenced antioxidant enzyme activities in *V. jatamansi*, with yellow light enhancing POX in leaves, while white light consistently promoted higher catalase activity, and red and blue light slightly increased SOD activity.

Effect of LEDs on Valepotriate Content

Light conditions affect valtrate accumulation in the leaf and root tissues of *in vitro* raised *V. jatamansi*. UHPLC analysis revealed that in leaf tissue, valtrate content under white light was 1.04-fold, 1.32-fold, and 2.09-fold higher compared to yellow, blue, and red-light conditions, respectively (Fig. 7a). Similarly, in root tissue, valtrate content was 1.14-fold higher under yellow light, while decreases by 1.47-fold and 1.22-fold under red and blue light, respectively, compared to white light (Fig. 7b). However, acevaltrate and didrovaltrate were not detected in any leaf or root samples across all light conditions. Overall, the study concludes that light quality significantly influences valtrate accumulation in *V. jatamansi*, with white light favouring its production in leaves and yellow light in roots.

Effect of LEDs on Gene Expression Profile

The transcript levels of genes involved in the iridoid biosynthesis route were quantified in leaf and root tissues of *V. jatamansi* incubated in various light conditions. The expression pattern of *GES*, *10HGO*, *G10H*, *IS*, *7DLS*, *7DLGT*, *DL7H*, *SLS*, and *LAMT* genes was evaluated. Under yellow light, increased expression of *GES*, *10HGO*, and *7DLGT* was observed in leaf tissue (Fig. 8a), while in root tissue, *GES*, *10HGO*, *LAMT*, *SLS*, and *DL7H* showed elevated expression (Fig. 8b). Under red light, *10HGO*, *LAMT*, and *7DLGT* were highly expressed in leaf tissue, whereas in root tissue, all genes except *GES*, *LAMT*, and *SLS* showed higher expression. Under blue light, *10HGO*, *LAMT*, *7DLS*, and *7DLGT* were highly expressed in root tissue, while *10HGO*, *G10H*, *IS*, *LAMT*, *7DLS*, and *DL7H* showed increased expression in leaf tissue. Under white light, all nine genes exhibited similar expression patterns in both leaf and root tissues. These results suggest that light conditions modulated the expression of iridoid biosynthetic genes in *V. jatamansi*, with gene-specific upregulation observed under yellow, red, and blue light.

Correlation analysis

The Pearson correlation analysis (PCA) shown in Table 2 examines morphological parameters, biomass yield, antioxidant, and enzymatic activities, valtrate content, and gene expression patterns in *in vitro* *V. jatamansi* leaf (L) and root (R) tissues under white, yellow, red, and blue light conditions. The correlation coefficients for the top variables were significant at both the 1% and 5% levels, with positive and negative values. Significant positive correlations include shoot Length (SL) and *7DLSL* (0.966). Root fresh weight (RFW) correlates with TFC R (0.963), and leaf number (LN) has a strong correlation with *ISR* (0.977). Root number (RN) shows a strong correlation with *SLSR* (0.978), and petiole dry weight (PDW) correlates with *10HGOL* (0.960) and *LAMTR* (0.970). TPC R exhibits a strong correlation with *GESL* (0.955) and *SLSL* (0.959). *TPCL* is correlated with *SLSR* (0.953). *POXL* correlates with *GESL* (0.977), *7DLGTR* (0.988), and *SLSL* (0.971). CAT L has a strong correlation with CAT R (0.982). *GESL* correlates strongly with *G10HR* (0.969), *7DLGTR* (0.977), and *DL7HR* (0.966). *GESR* is correlated with *DL7HR* (0.957), and *10HGOL* correlates with *ISL* (0.964). *G10HR* shows strong correlations with *DL7HL* (0.972) and *SLSL* (0.954). *ISR* is correlated with *SLSR* (0.981), and *7DLGTR* correlates with *SLSL* (0.987).

Pearson correlation analysis for morphological parameters, biomass yield

Variables	SL	LN	RN	LFW	PFW	RFW	LDW	PDW	RDW	TPC L	TPC R	TFC L	TFC R	SOD L	SOD R
SL	1	0.242	0.544	-0.267	0.515	-0.077	-0.325	0.592	-0.205	0.669	-0.520	-0.718	-0.255	0.861	0.211
LN		1	0.846	-0.539	-0.129	-0.486	-.958*	-0.616	-.982*	0.880	0.018	0.496	-0.335	0.092	-0.81
RN			1	-.172	.416	-.046	-.737	-.332	-.744	.882	-.512	.151	.025	.182	-.69
LFW				1	.668	.981*	.753	.063	.679	-.596	-.632	-.076	.974*	-.582	.044
PFW					1	.799	.289	.351	.298	.097	-.993**	-.482	.697	.071	.016
RFW						1	.696	.162	.642	-.467	-.766	-.205	.963*	-.440	.062
LDW							1	.465	.988*	-.907	-.191	-.370	.587	-.304	.629
PDW								1	.594	-.170	-.260	-.982*	-.110	.699	.902
RDW									1	-.861	-.192	-.501	.496	-.153	.736
TPC L										1	-.182	.025	-.442	.521	-.48
TPC R											1	.405	-.687	-.049	.094
TFC L												1	.066	-.743	-.80
TFC R													1	-.643	-.17
SOD L														1	.491
SOD R															1
POX L															
POX R															
CAT L															
CAT R															
VAL L															
VAL R															
GES L															
GES R															
10HG0 L															
10HG0 R															
G10H L															
G10H R															
IS L															
IS R															
7DLS L															
7DLS R															
7DLGT L															
7DLGT R															
DL7H L															

“*” significant at 5%, “**” significant at 1% level

SL; Shoot length, LN; Leaf number, RN; Root number, LFW; Leaf fresh weight, PFW; Petiole fresh weight, RFW; Root fresh weight, LDW; Leaf dry weight, PDW; dismutase root, POX L; Peroxidase leaf, POX R; Peroxidase root, CAT L; Catalase leaf, CAT R; Catalase root, VAL L; Valtrate leaf, VAL R; Valtrate root, GES L; G synthase leaf, IS R; Iridoid synthase root, 7DLS L; 7-deoxyloganetic acid synthase leaf, 7DLS R; 7-deoxyloganetic acid synthase root, 7DLGT L; 7-deoxyloganetic acid synthase root, LAMT L; Loganic acid O-methyltransferase leaf, LAMT R; Loganic acid O-methyltransferase root

Variables	SL	LN	RN	LFW	PFW	RFW	LDW	PDW	RDW	TPC L	TPC R	TFC L	TFC R	SOD L	SOD R
<i>DL7HR</i>															
<i>SLS L</i>															
<i>SLS R</i>															
<i>LAMTL</i>															
<i>LAMTR</i>															
“*” significant at 5%, “**” significant at 1% level															
SL; Shoot length, LN; Leaf number, RN; Root number, LFW; Leaf fresh weight, PFW; Petiole fresh weight, RFW; Root fresh weight, LDW; Leaf dry weight, PDW; dismutase root, POX L; Peroxidase leaf, POX R; Peroxidase root, CAT L; Catalase leaf, CAT R; Catalase root, VAL L; Valtrate leaf, VAL R; Valtrate root, <i>GES L</i> ; <i>G synthase</i> leaf, <i>IS R</i> ; <i>Iridoid synthase</i> root, <i>7DLS L</i> ; <i>7-deoxyloganetic acid synthase</i> leaf, <i>7DLS R</i> ; <i>7-deoxyloganetic acid synthase</i> root, <i>7DLGT L</i> ; <i>7-deoxyloganetic acid synthase</i> root, <i>LAMTL</i> ; <i>Loganic acid O-methyltransferase</i> leaf, <i>LAMTR</i> ; <i>Loganic acid O-methyltransferase</i> root															

Negative correlations were also evident. LN negatively correlates with leaf dry weight (LDW) (0.958), Root dry weight (RDW) (0.982), and *10HGO R* (0.952). Petiole fresh weight (PFW) correlates with *GES L* (0.970), *7DLGT R* (0.984), and *SLS L* (0.962). PDW strongly correlates with TFC L (0.982), and TFC L correlates with *IS L* (0.964). POX R correlates with *10HGO R* (0.971) and *LAMTR* (0.966). CAT L correlates with *G10HL* (0.987). Overall, the correlation patterns imply that LED light quality modulates a tightly interconnected network of growth, defense, and secondary metabolism, where enhanced expression of key biosynthetic genes supports valepotriate production, while red and yellow light favor biomass and antioxidant capacity, respectively.

Discussion

Valeriana jatamansi Jones is a renowned medicinal plant recognized for its numerous attributes linked to secondary metabolites, particularly iridoids and valepotriates. Several studies have investigated antioxidant compounds, secondary metabolite accumulation, and gene expression patterns in *V. jatamansi*, using both tissue culture and wild-grown samples (Jugran et al. 2015; Pandey and Pant 2020; Pandey et al. 2022; Gehlot et al. 2022; Bahukhandi et al. 2023; Thakur et al. 2024a,b). The integration of tunable LED lighting into plant tissue culture is emerging as a strategic approach to optimize growth conditions and enhance the accumulation of valuable metabolites (Sakulsathaporn et al. 2025; Partap et al. 2025a). Light functions not only as the primary energy source for photosynthesis but also as a key environmental signal that regulates plant growth, morphogenesis, and metabolic pathways. To perceive and respond to diverse wavelengths, plants have evolved at least five major classes of photoreceptors: phytochromes, which detect red and far-red light; cryptochromes and phototropins, which sense blue and UV-A light; F-box flavin-binding proteins, which also respond to blue/UV-A regions; and UVR8, which is specialized for UV-B perception (Bae and Choi 2008). Each of these photoreceptors initiates distinct signaling cascades upon activation, modulating pigment biosynthesis, developmental programs, and stress responses. Collectively, these mechanisms enable plants to fine-tune their physiological, developmental, and metabolic adaptations in response to changing light spectra (Kong and Okajima 2016). Our findings highlight, for the first time, that tailored LED light regimes can effectively modulate growth, photosynthesis, metabolite accumulation, and molecular responses in *in vitro* cultures of *V. jatamansi*, offering a basis for optimized propagation and metabolite production.

Unlike conventional light sources, LEDs allow precise control over light quality, intensity, and duration, which are critical for optimizing *in vitro* plant responses. Several previous studies highlighted the critical role of LED light in morphological traits improvement under *in vitro* conditions. In our study, the maximum shoot length (9.71 cm) was achieved in blue light. The maximum leaf (20.22) and root number (15.11) were recorded in white light. Comparable patterns have been noted in other species. In the Silk Banana variety *Nanjanagud Rasabale*, the highest shoot length was found in blue light supplemented with cytokinin (Waman et al. 2016). The superior shoot elongation in blue light may reflect the stimulation of cryptochrome- and phototropin-mediated signaling in the shoot apex. These pathways are known to interact with the gibberellin biosynthetic pathway, particularly up-regulating *GA20-oxidase*, a key enzyme in the conversion of GA precursors to active gibberellins (Fukuda 2013). Elevated GA levels modulate transcription factors that activate cell-elongation genes, leading to taller shoots. In contrast, white light's broad spectrum regulates photoreceptor activation, enhancing photosynthesis and auxin-cytokinin signaling, which promotes leaf and root formation.

The spectral composition of light can significantly influence biomass production by affecting photosynthesis. In our study, the maximum fresh weight of leaf (1.42 g) and root (1.47 g) was observed in red light conditions, while the fresh weight of petiole (1.09 g) was found to be maximum in white light conditions. In case of dry weight of leaf (0.10 g), petiole (0.061 g), and root (0.063 g), red light was best suitable. The lowest leaf fresh weight (0.62 g) and dry weight (0.04 g) were found in blue light conditions. In the root tissue lowest fresh weight (0.50 g) and dry weight (0.027 g) were found in blue and white light conditions. Our results are consistent with previous investigations. Seyedi et al. (2024) indicated that in *Thymus vulgaris*, maximum fresh weight (31.45 g) was found in the red and blue light combination, while the minimum (10.08 g) was in blue light. The red-light exposure yielded the maximum dry weight (2.87 g), whereas the blue light treatment produced the lowest dry weight (0.97 g). Similarly, in *Malus domestica* rootstock M9, enhanced mean fresh and dry weight was observed under red light exposure (Muleo and Morini 2008). In red light, there is higher fresh and dry biomass due to the promotion of starch accumulation in the tissue (Li et al. 2010). Hung et al. (2016) also reported enhanced fresh weight (251.4-292.5 mg explant⁻¹) in *Vaccinium ashei* under red light conditions. Red LED increased the accumulation of biomass (3.56 ± 0.04 g L⁻¹) in *S. chirayita* shoot cultures (Gupta and Sood 2023). Red light is efficiently absorbed by chlorophyll a and b, the primary pigments involved in photosynthesis. This results in greater light energy utilization for carbon fixation, which directly contributes to increased biomass accumulation (Massa et al. 2008). Similarly, Wu et al. (2023) reported that a 30-day exposure to red light led to the highest fresh and dry weight accumulation in the leaves, stems, and roots of *Lonicera macranthoides*. In *Stevia rebaudiana*, red light resulted in the highest proliferation rate, while a combination of red and blue light enhanced shoot elongation (Ramírez-Mosqueda et al. 2017). Bello-Bello et al. (2016) also

stated that fluorescent lamps and white light resulted in enhanced shoot biomass, plant height, leaf, and root number in *Vanilla planifolia*. The higher biomass under red light can be attributed to its efficient absorption by chlorophyll a and b, which enhances photosynthetic carbon fixation and promotes starch accumulation in tissues (Massa et al. 2008). In contrast, blue light favors shoot elongation over biomass accumulation. Rihan et al. (2022) demonstrated that specific wavelengths strongly influence plant morpho-physiological traits, consistent with our findings that blue light promotes shoot elongation, white light favors leaf and root formation, and red light enhances fresh and dry biomass, highlighting the wavelength-dependent allocation of growth in *V. jatamansi*.

LED lights enhance antioxidant compounds production in *in vitro* raised plants by activating photoreceptor pathways, upregulating biosynthetic genes, and mimicking stress cues that stimulate secondary metabolism. These antioxidant secondary metabolites, synthesized by plant species to mitigate the reactive oxygen species (ROS) produced under stressful conditions, are recognized for their extensive biological activity (Yousuf et al. 2017). In our study, the highest total flavonoid content (TFC) ($\mu\text{g QE/mg DW}$) in leaf tissue was observed under yellow light (128.30), whereas maximum TFC was recorded under white light (211.79) in root tissue. In leaf tissue, the highest total phenolic content (TPC) ($\mu\text{g GAE/mg DW}$) was observed under blue light (65.30), whereas maximum TPC was recorded under yellow light (126.30) in root tissue. Our results are consistent with earlier reports; likewise, continuous exposure to white light (24 hours) significantly enhanced TPC (47.43 mg/g) and TFC (9.41 mg/g) in *in vitro* callus cultures of *Lepidium sativum* (Ullah et al. 2019). Treatment of yellow light in *Epimedium pseudowushanense* exhibited increased flavonoid content (Yang et al. 2019). Similarly, Kim et al. (2006) highlighted that light-induced synthesis of Malonyl CoA and Coumaroyl CoA significantly contributes to the increased production of phenolic compounds. Manivannan et al. (2015) reported that in *in vitro* shoot cultures of *Rehmannia glutinosa*, blue LED treatment led to higher total phenolic content in leaf extracts than root extracts. Fazal et al. (2016) demonstrated that green and yellow lights increased TPC (112.52 g/100 ml) and TFC (9.64 g/100 ml) in callus culture of *Prunella vulgaris*. The enhanced production under specific wavelengths can be attributed to the light-mediated activation of phenylpropanoid pathway enzymes, particularly phenylalanine ammonia-lyase (PAL), which catalyzes the first step in the biosynthesis of phenolic and flavonoid compounds (Ahmadi et al. 2020). Blue light is known to activate PAL and related transcription factors, promoting phenolic accumulation in leaves. In contrast, yellow and white lights may stimulate alternative signaling pathways and photoreceptors that enhance flavonoid synthesis in roots. Red and green lights, although not as productive, may partially affect metabolite synthesis by moderately stimulating photosynthesis, ROS signaling, and secondary metabolism. Overall, these findings indicate that light spectrum, intensity, and duration, along with tissue-specific regulatory mechanisms, collectively determine the accumulation of phenolic and flavonoid compounds in *V. jatamansi*, highlighting the importance of optimizing light quality for targeted metabolite enhancement.

The activity of antioxidant enzymes was assessed, as they indicate how they react to oxidative stress and are crucial in the intricate mechanisms of ROS removal (Reische et al. 2002). LED lighting elicits varying degrees of photooxidative stress and alterations in the antioxidant defense mechanism (Gupta and Sahoo 2015). In our study, the highest POX activity in leaf tissue was recorded under yellow light (54.51 U/g), while the lowest was observed under white light (4.60 U/g). In contrast, root tissue showed maximum POX activity under white light (93.45 U/g) and minimum under red light (38.23 U/g). Similarly, catalase activity was highest under white light in both leaf (4.02 U/g) and root (2.93 U/g) tissues. The lowest catalase activity in leaf tissue was observed under yellow light (0.43 U/g), whereas in root tissue, it was lowest under red light conditions (1.09 U/g). SOD activity was highest under red light (1.86 U/g) and the lowest under white light (1.73 U/g) in leaf tissue. For root tissue, maximum activity was observed under blue light (1.75 U/g), while the lowest was observed under white light (1.61 U/g). These findings are consistent with previous reports. For example, Gnasekaran et al. (2021) demonstrated that blue light (460 nm) significantly enhances superoxide dismutase activity in *Halia Bara* plantlets, while Fazal et al. (2016) observed that yellow light maximizes antioxidant enzyme activity, including superoxide dismutase and peroxidase, in callus cultures of *Prunella vulgaris*. Similarly, Hassanpour (2022) reported elevated SOD and catalase activity in *Hyoscyamus reticulatus* callus cultures under red-blue, white, and dark conditions. Red-light significantly influenced POX enzymatic activity in the callus culture of *A. absinthium* (Tariq et al. 2014). The differential responses of antioxidant enzymes to light spectra in *V. jatamansi* may be explained by light-mediated regulation of ROS signaling and photoreceptor-activated transcriptional networks. Yellow and blue lights boost ROS removal in leaves, white light strengthens root antioxidants, whereas red light increases leaf SOD activity through mild ROS production and red-light photoreceptor control of defense enzymes. Overall, the spectral lights modulate enzymatic activities, reflecting the tissue-specific adaptation of antioxidant compounds to optimize protection against oxidative stress under varying light conditions.

Abiotic factors like light stress can affect the photosynthetic apparatus, particularly PSII, leading to ROS production, photoinhibition, and impaired repair mechanisms (Sghaier-Hammami et al. 2023). Plants counteract this by dissipating excess energy as heat or reducing electron transport rate (Moustaka and Moustakas 2023). Chlorophyll fluorescence serves as a non-invasive indicator of photosynthetic performance, especially PSII efficiency (Goltsev et al. 2016). The Fv/Fm ratio reflects PSII's energy conversion efficiency, with healthy plants typically showing values around 0.75; lower values suggest stress-related photochemical decline (Maxwell and Johnson 2000; Kalaji and Guo 2008). In our investigation, the Fv/Fm ratio ranged from 0.63 to 0.70, indicating that the plantlets experienced mild photoinhibition, which led to lessened photochemical efficiency and PSII activity. Likewise, Guo et al. (2023) noted that Fv/Fo and Fv/Fm ratios decreased in the leaves of *in vitro*-grown *Dendrobium nobile* plantlets exposed to red and blue light. Whereas Ouzounis et al. (2015) also observed reduced Fv/Fm ratios in orchids under monochromatic red light compared to blue light. Plants preferentially absorb blue and red-light wavelengths over green and yellow light wavelengths (Wang et al. 2022). Despite these variations, other chlorophyll fluorescence parameters, including ΦPSII , Y(II), qP, and ETR, remained largely stable across all LED treatments, with only qN showing significant variation. This stability implies that *V. jatamansi* maintained efficient photochemical performance, likely through the activation of photoprotective mechanisms such as non-photochemical quenching (qN) and regulation of electron transport. Collectively, these results suggest that while specific lights can slightly affect PSII efficiency, *V. jatamansi* exhibits effective photoprotective and regulatory responses that preserve overall photosynthetic performance under diverse LED light conditions.

The influence of light on plant metabolism is often facilitated by photoreceptors that convey downstream signal transduction, regulating the gene expression of biosynthetic pathways that impact metabolic processes. Monochromatic and blended LED lights, through their distinct wavelengths, stimulate diverse photoreceptors and activate signaling pathways that modulate plant metabolism, thereby enhancing the biosynthesis of bioactive compounds in *in vitro* cultures. Numerous studies have confirmed the impact of diverse light spectra on the synthesis of secondary metabolites in medicinal plants. For example,

Gupta and Sood (2023) reported that blue LED light resulted in maximum amarogentin content ($8.025 \mu\text{g mg}^{-1} \text{DW}$) in *S. chirayita* shoot cultures. Exposure of the root culture of *Hypericum perforatum* to red light leads to a maximum yield of total hypericins ($9.61 \pm 0.3 \mu\text{g/g}$) (Sobhani Najafabadi et al. 2019). In our study, the highest valtrate content (mg/g DW) in leaf tissue was observed under white light (0.090), and the minimum was under blue light (0.043). Similarly, in root tissue, the highest valtrate content was recorded under yellow light (0.167), and the minimum was under red light (0.099). These results are consistent with previous studies showing that white light enhances secondary metabolite production: for example, Ullah et al. (2019) reported that continuous 24-hour white light exposure significantly increased phytochemical synthesis in *Lepidium sativum*, and in wheat sprouts, white light (380 nm) favored epicatechin biosynthesis ($616.56 \mu\text{g/g DW}$) (Cuong et al. 2019). Likewise, in *Agastache rugosa*, exposure to white light resulted in a 2.85-, 2.72-, 1.72-, and 1.71-fold increase in rosmarinic acid content, and a 1.79-, 1.57-, 1.32-, and 1.27-fold increase in tilianin content compared to red, blue, green, and orange light treatments, respectively (Park et al. 2020). Wang et al. (2018) indicated that exposure to yellow light resulted in a considerably elevated number of soluble sugars and polysaccharides in *Anoectochilus roxburghii*. The observed difference in valtrate accumulation may be attributed to light-mediated regulation of secondary metabolite pathways. White light, containing a broad spectrum of wavelengths, can simultaneously activate multiple photoreceptors, enhancing the expression of key enzymes in the valepotriate biosynthetic pathway. Yellow light may similarly stimulate carbohydrate metabolism and increase the availability of soluble sugars, providing precursors for secondary metabolite synthesis in roots. Collectively, these results highlight the critical role of optimized light spectra in promoting the biosynthesis of pharmaceutically valuable secondary metabolites in tissue culture systems.

Furthermore, light modulates the biosynthetic pathways of secondary metabolites via specialized receptors, phytochromes, and cryptochromes. Therefore, by altering light quality, one can affect the production of plant metabolites through gene expression across many metabolic pathways (Li et al. 2017). However, limited data are available on gene expression responses to light; therefore, a major aim present investigations was to evaluate the influence of LED treatment on secondary metabolite production in tissue culture and the expression of related biosynthetic genes. Previous studies have reported that monochromatic light or combinations of different light treatments significantly influence gene expression levels in tissue culture plants of *Scrophularia kakudensis* (Manivannan et al. 2021), *Curculigo latifolia* (Muslihatin et al. 2024), *Salvia atropatana*, and *S. bulleyana* (Grzegorzczak-Karolak et al. 2025). In our study, in leaf tissue, *GES*, *G10H*, *LAMT*, *SLS*, and *DL7H* genes exhibited up to 3.21-, 2.45-, 1.62-, 1.79-, and 2.06-fold increases in expression in yellow light compared to white light. In root tissue under yellow light, the genes *GES*, *G10H*, *DL7H*, and *7DLGT* showed 2.43-, 2.47-, 2.32-, and 2.37-fold higher expression, respectively. Under red light, *10HGO*, *LAMT*, and *7DLGT* showed 1.78-, 1.72-, and 1.30-fold higher expression in root tissue, while *G10H*, *10HGO*, *IS*, *DL7H*, *7DLS*, and *7DLGT* exhibited 2.45-, 1.71-, 1.61-, 1.70-, 1.44-, and 2.74-fold higher expression, respectively, in leaf tissue. Under blue light, *10HGO*, *LAMT*, *7DLS*, and *7DLGT* showed increased expression levels of 1.31-, 1.50-, 2.50-, and 1.19-fold, respectively, in root tissue. In leaf tissue, *G10H*, *10HGO*, *IS*, *LAMT*, *DL7H*, and *7DLS* showed 2.19-, 1.90-, 1.67-, 1.34-, 2.00-, and 2.46-fold higher expression compared to white light. These results are consistent with earlier studies demonstrating stress treatment-induced regulation of valepotriate pathway genes. For example, Thakur et al. (2024a) reported upregulation of *GES*, *G10H*, *10HGO*, *IS*, *LAMT*, *SLS*, and *7DLS* in the aerial parts of *V. jatamansi* under drought stress. In a subsequent study, Thakur et al. (2024b) observed overexpression of *GES*, *G10H*, *IS*, *LAMT*, and *SLS* in endophyte-treated cell suspension cultures of *V. jatamansi*. *G10H*, a cytochrome P450 monooxygenase, is a crucial regulating enzyme in terpenoid biosynthesis Collu et al. (2001); thus, its enhanced expression may elevate iridoid biosynthesis. A similar observation was made in our study, where *G10H* showed increased expression under yellow light in both root and leaf tissues, which may contribute to the enhanced accumulation of valtrate content. *GES* is recognized as an essential enzyme in the terpenoid route. *GES* is widely utilized for the exogenous synthesis of geraniol, an important intermediary molecule in this pathway (Vasilev et al. 2014). In our study, an increased expression of *GES* was found under yellow light in both leaf and root tissue, which may account for the enhanced content of valtrate. Likewise, *7DLS* exhibited a 2.50-fold increase in root and a 2.46-fold increase in leaf tissue under blue light compared to white light. Increased expression of *7DLS* was observed in the leaf as compared to the root of *Catharanthus roseus* (Salim et al. 2014). Yang et al. (2019) also reported that yellow light significantly overexpresses of flavonoid biosynthesis genes, specifically *CHS1*, *F3H1*, *PT_5*, and *raGT_5*, which probably contributes to the increased metabolite production in *E. pseudowushanense*. The differential expression patterns of iridoid biosynthetic pathway genes in *V. jatamansi* under white, yellow, red, and blue LED lights are likely mediated by light-specific photoreceptors and downstream signaling. Yellow light may activate specific photoreceptors and signaling pathways that induce transcription factors regulating genes like *GES*, *G10H*, *LAMT*, *SLS*, *DL7H*, and *7DLGT*, resulting in higher expression in both leaf and root tissue. Red light, sensed by phytochromes, appears to preferentially enhance the expression of *10HGO*, *LAMT*, and *7DLGT* in root tissue, while also moderately upregulating several genes in leaves, reflecting tissue-specific regulation. Blue light, sensed by cryptochromes and phototropins, promotes genes like *7DLS* and *10HGO* in root and leaf tissue, suggesting its role in stimulating downstream iridoid pathway enzymes. Overall, these results indicate that LED light quality modulates the expression of key biosynthetic genes in a spectral and tissue-dependent manner, likely through photoreceptor-mediated signal transduction, transcription factor activation, and enhanced metabolic flux toward valepotriate synthesis.

To study the relationships between growth parameters, antioxidant activity, secondary metabolite content, and gene expression in *V. jatamansi* under different LED light spectra, correlation analysis was performed. Significant positive correlations between shoot length and genes such as *7DLSL*, *7DLGTL*, and *GESL* suggest that the transcriptional activation of valepotriate biosynthetic genes contributes to shoot elongation. Similar correlation pattern for valtrate content in leaves and expression of *GESL* and *10HGO* confirms accumulation of that iridoid derivative was directly linked to the upregulation of key pathway genes. Leaf fresh weight showed a strong positive correlation with total flavonoid content, indicating that flavonoids may play a protective role in sustaining leaf growth under light-induced oxidative stress. Moreover, positive associations between biomass traits (LFW, PFW, RFW) and antioxidant enzymes imply that efficient ROS scavenging under red light promotes cellular homeostasis, which favors biomass accumulation. Whereas some parameters show negative correlations with some parameters, suggesting trade-offs between primary growth and the allocation of resources to specialized metabolism. Likewise, shoot length was negatively correlated with TPC R and TFC L, implying that the diversion of carbon skeletons toward polyphenol biosynthesis can occur during the rapid elongation growth period. Similarly, the negative relationship between catalase and other antioxidant enzymes (SOD, POX) points to a complementary regulation of ROS detoxification, where different enzymes may dominate depending on the cellular redox status. Overall, these findings support the hypothesis that LED light quality creates a complex network linking to photoreceptor-mediated signaling, antioxidant defense, and secondary metabolite biosynthesis pathway. Where yellow light likely enhances phenolic and iridoid pathways by modulating gene expression and ROS signaling, while

red light favors biomass through improved photosynthetic efficiency and oxidative balance. Understanding these inter-relationships is crucial for optimizing light regimes to maximize both growth and phytochemical yields in *V. jatamansi*, including other medicinal plants.

Conclusion

The findings of this research indicated that different LED light conditions considerably influenced the growth, antioxidant activity, chlorophyll fluorescence characteristics, secondary metabolite accumulation, and gene expression patterns of *in vitro* raised *V. jatamansi*. Among the employed lights, the red light was suitable for biomass enhancement, while the yellow light was best for higher antioxidant activity and valtrate content accumulation. These findings highlight that integrating *in vitro* culture techniques with targeted LED light treatments represents a sustainable and efficient strategy to optimize both biomass and secondary metabolite production in *V. jatamansi*. In the future, combining different LED light spectra with elicitor applications and precursor feeding strategies could be an effective approach to enhance plant growth and promote the biosynthesis of phytochemicals in medicinally important plant species (Thakur et al. 2021; Rattan et al. 2022; Partap et al. 2025a,b).

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

AK: Framed the experimental design and execution of all experiments, Data analysis and investigation, and original manuscript writing. **Kanika:** Execution of the experiments. **SR:** Execution of the experiments. **AS:** Execution of the experiments. **ANK:** Metabolite data analysis and investigation. **DK:** Metabolite data analysis and investigation. **ARW:** Conceived the concept and framed the experimental design, Experimental data analysis and investigation, original manuscript writing and editing.

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Data availability:

Data will be made available on request

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Figures

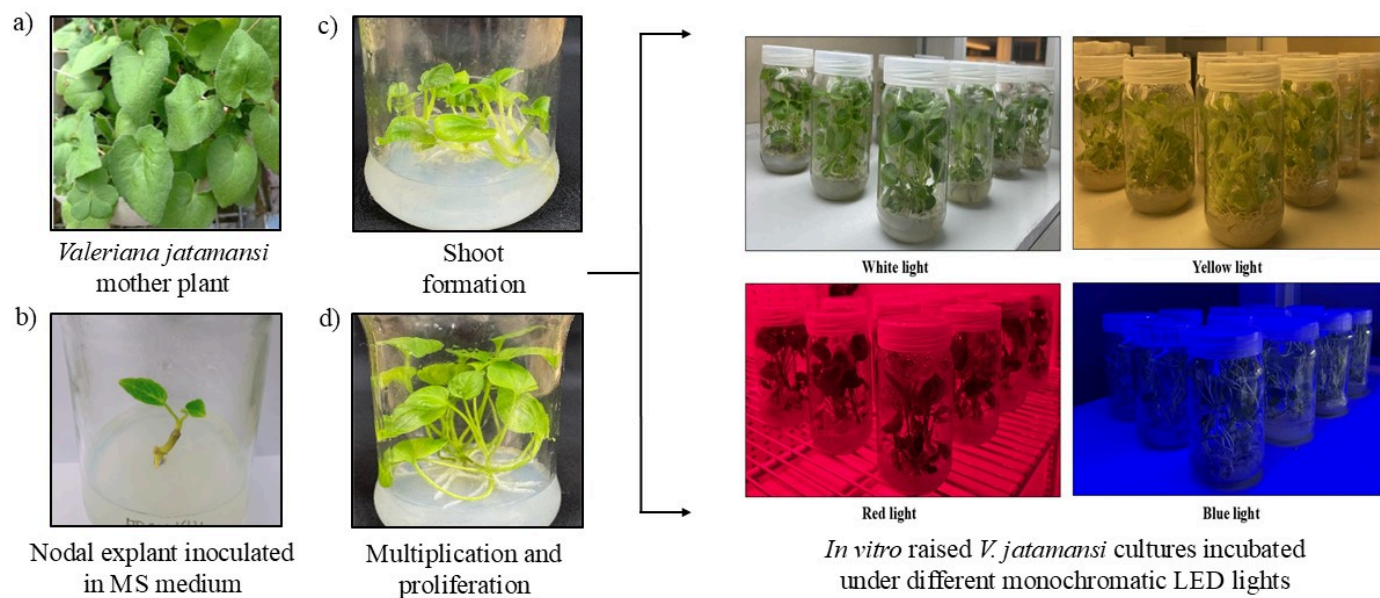


Figure 1

Establishment of *in vitro* culture of *Valeriana jatamansi* and incubated under different LED lights

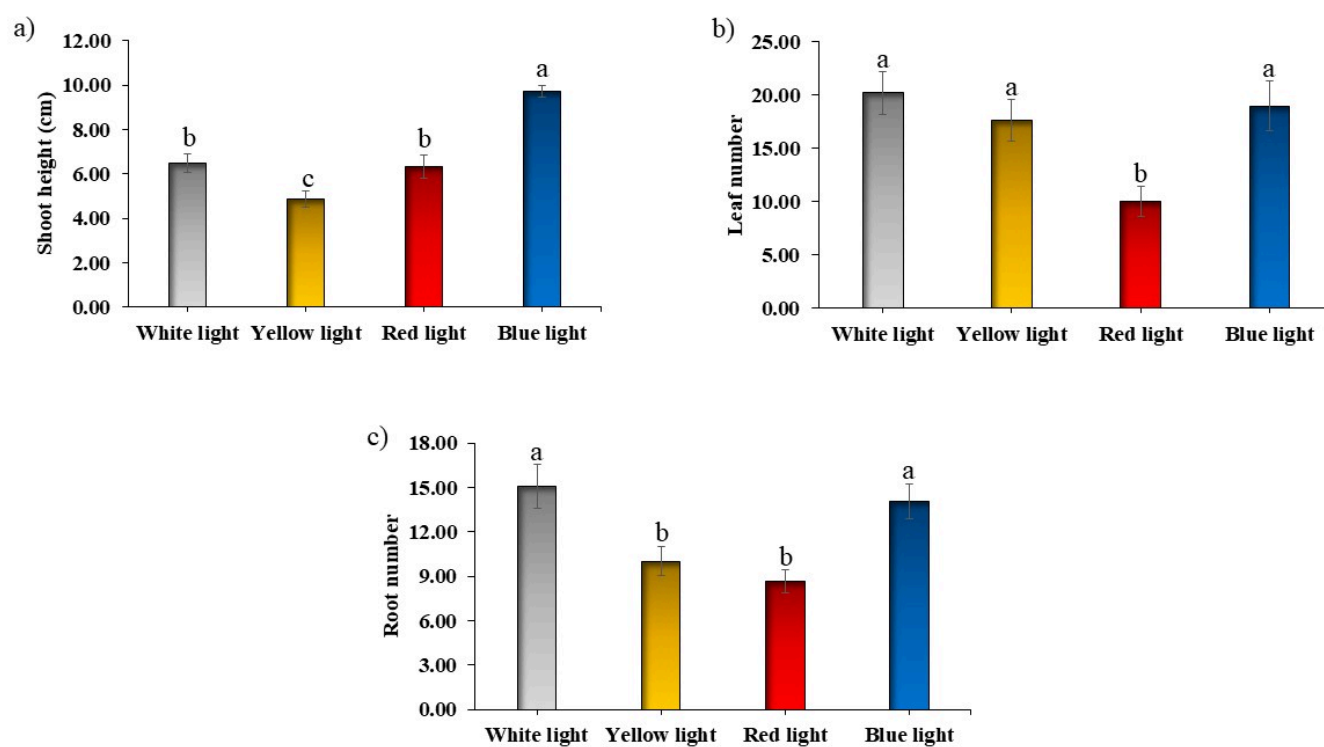


Figure 2

Effect of LED lights on morphological traits of *in vitro* raised *V. jatamansi*. a) Shoot height, b) Leaf length, and c) Root number. Bars designate mean $\pm$ standard error (SE) ($n=36$). Different superscript lowercase letters in a column indicate statistically significant differences between the means ($p < 0.05$)

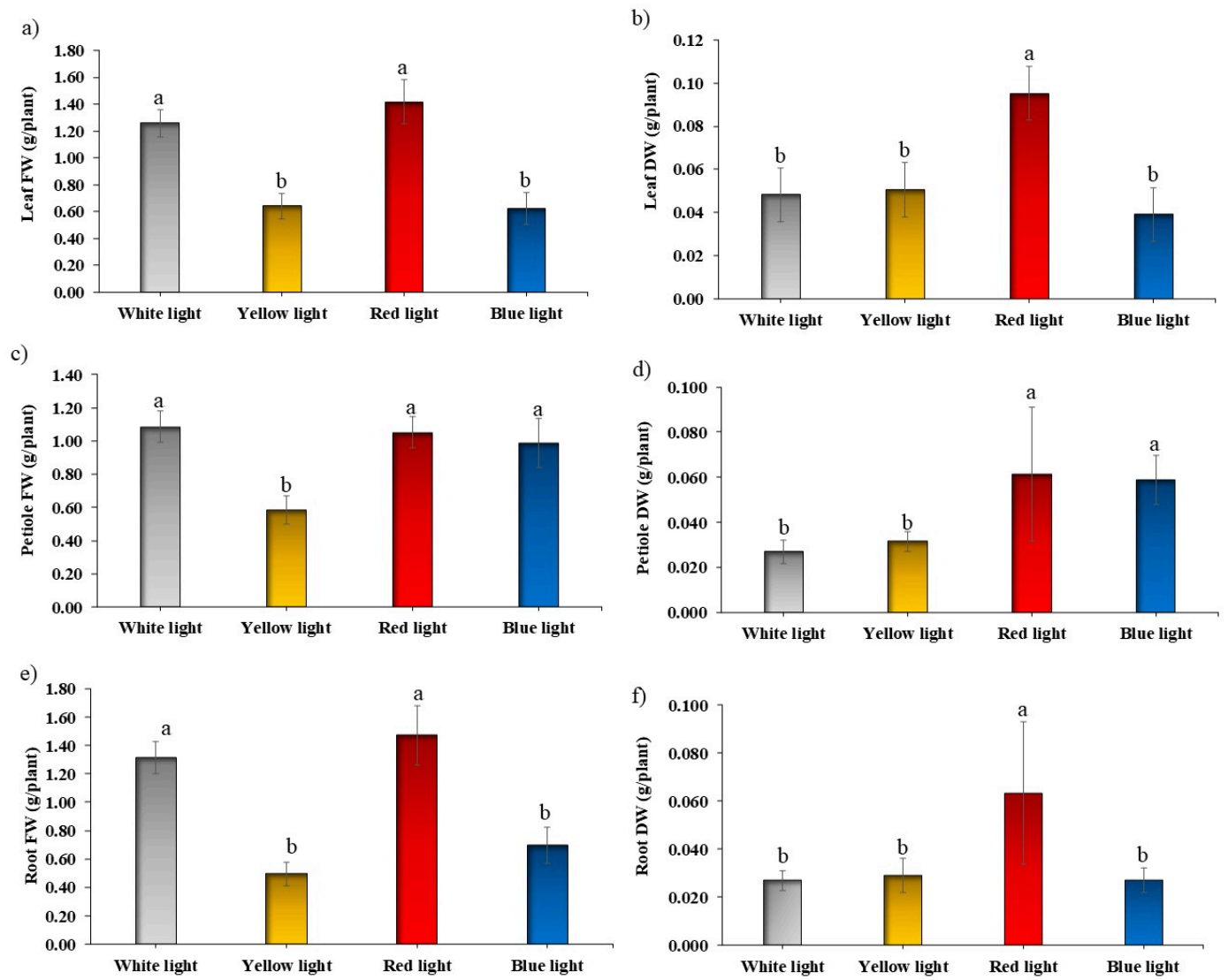


Figure 3

Effect of LED lights on biomass yield (Leaf, Petiole, and Root) in *in vitro* raised *V. jatamansi*. a) Leaf fresh weight, b) Leaf dry weight, c) Petiole fresh weight, d), Petiole dry weight, e) Root fresh weight, and f) Root dry weight. Bars designate mean $\pm$ standard error (SE) ($n = 33$). Different superscript lowercase letters in a column indicate statistically significant differences between the means ($p < 0.05$)

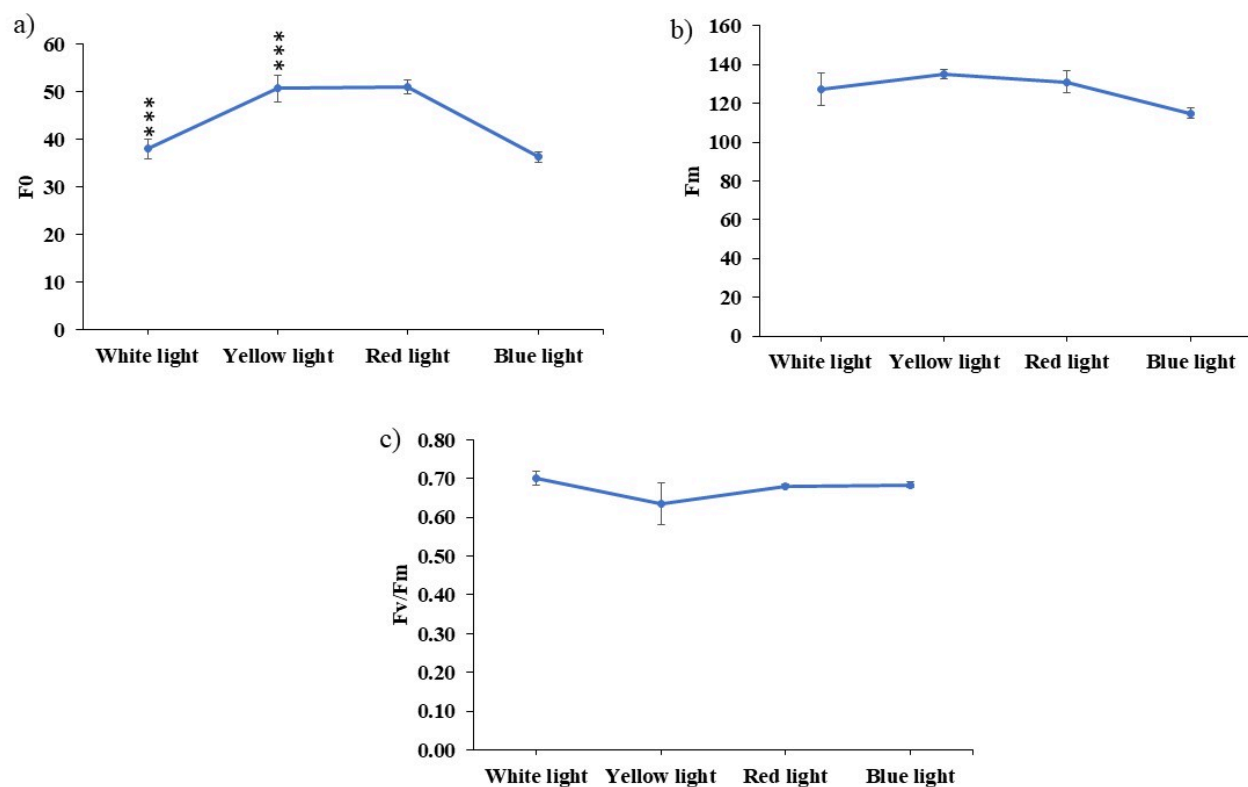


Figure 4

Effect of LED lights on Chl fluorescence parameters in *in vitro* raised *V. jatamansi*. a) Comparative initial fluorescence (Fo), b) Comparative maximum fluorescence (Fm), and c) Comparative potential quantum efficiency of photosystem II (Fv/Fm). The black color (*) indicates a statistically significant difference ($p < 0.05$) from the control condition

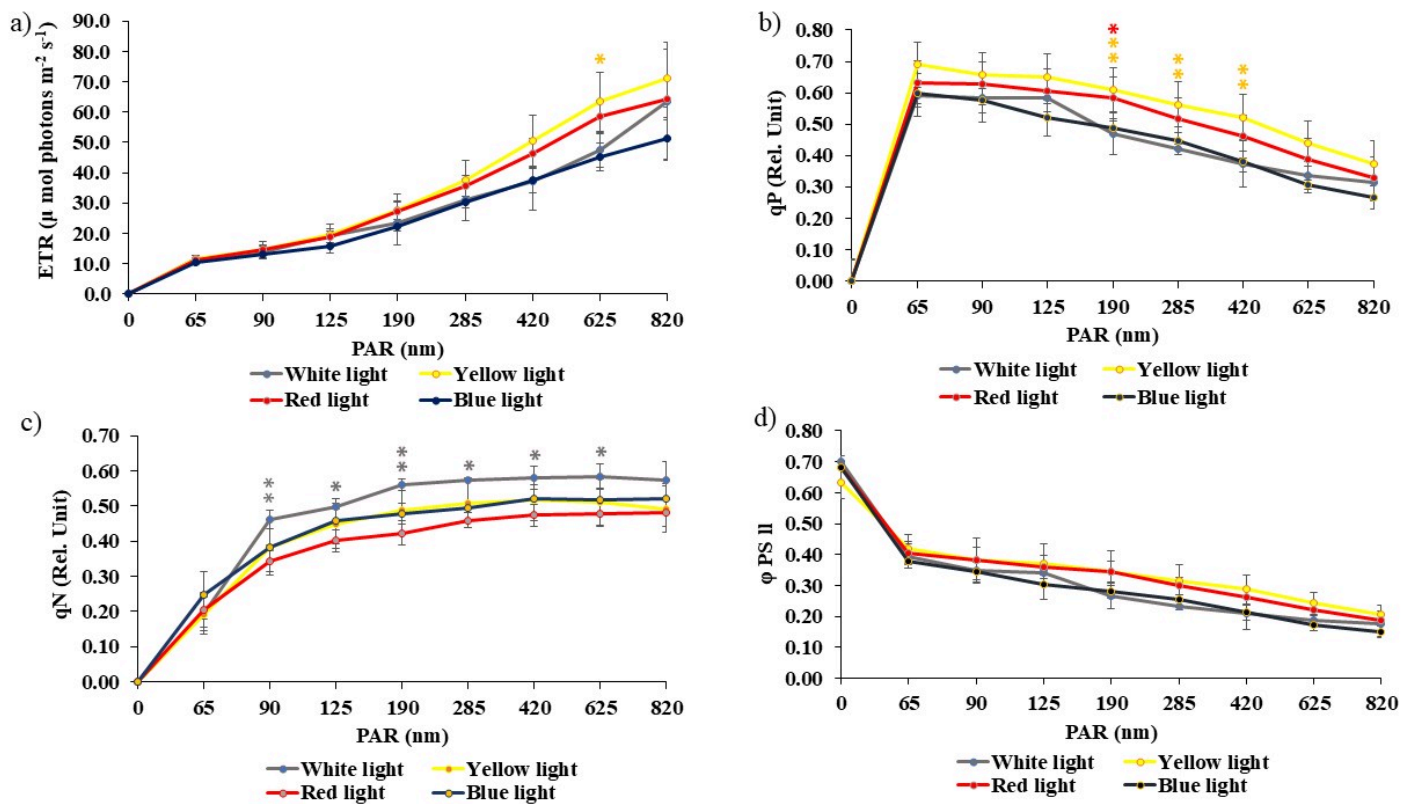


Figure 5

Effect of LED lights on Chl fluorescence parameters in *in vitro* raised *V. jatamansi*. a) The rate of electron transport (ETR), b) Coefficient of photochemical quenching (qP), c) Coefficient of non-photochemical quenching (qN), and d) Effective quantum yield of PS II (Φ PSII). The colored (*) indicates a statistically significant difference ($p < 0.05$) between different light conditions

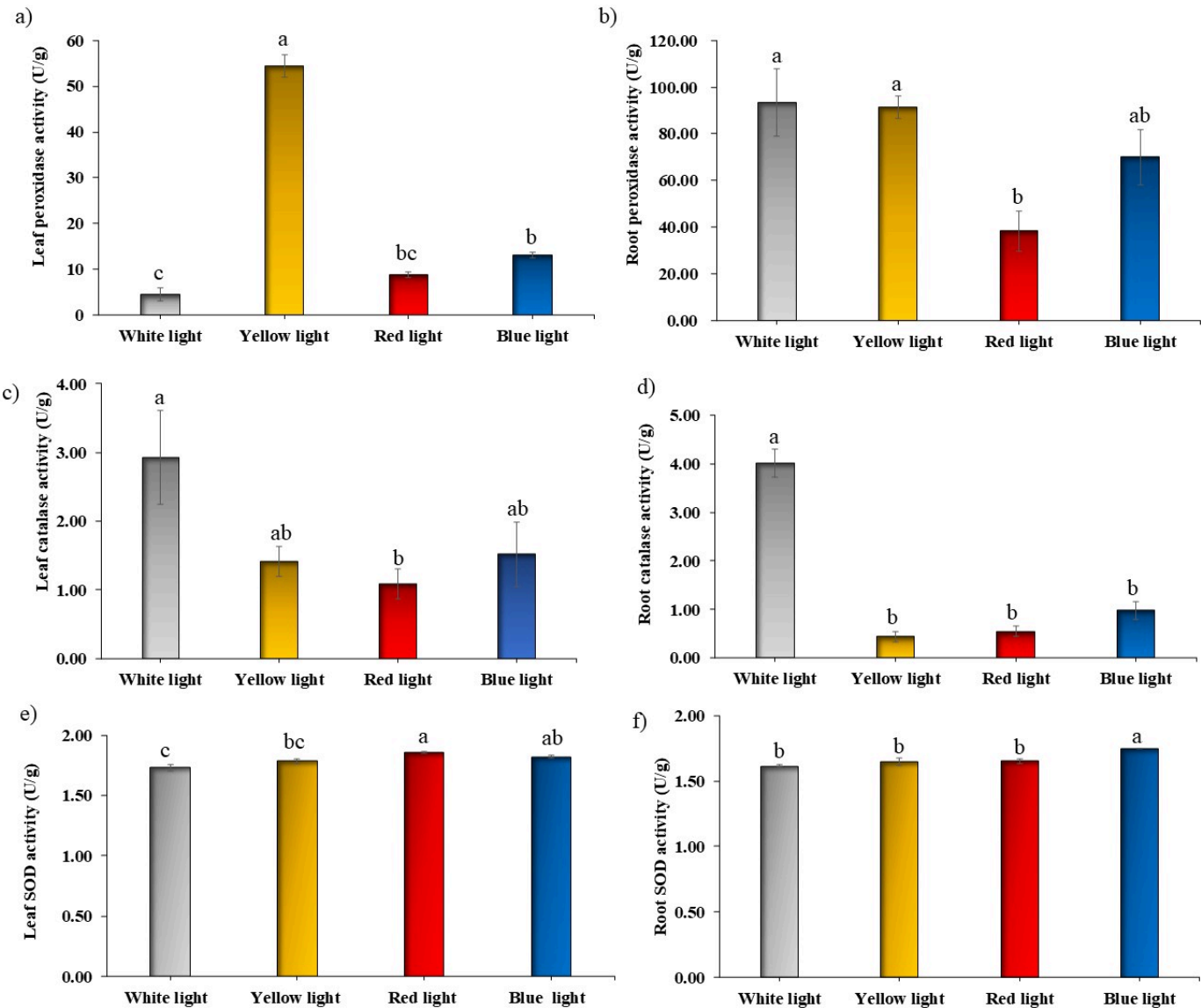


Figure 6

Effect of LED lights on the Enzymatic activity in *in vitro* raised *V. jatamansi*. a) Leaf peroxidase activity, b) Root peroxidase activity, c) Leaf catalase activity, d) Root catalase activity, e) Leaf superoxide dismutase (SOD) activity, and f) Root superoxide dismutase (SOD) activity. Different superscript lowercase letters in a column indicate statistically significant differences between the means ($p < 0.05$)

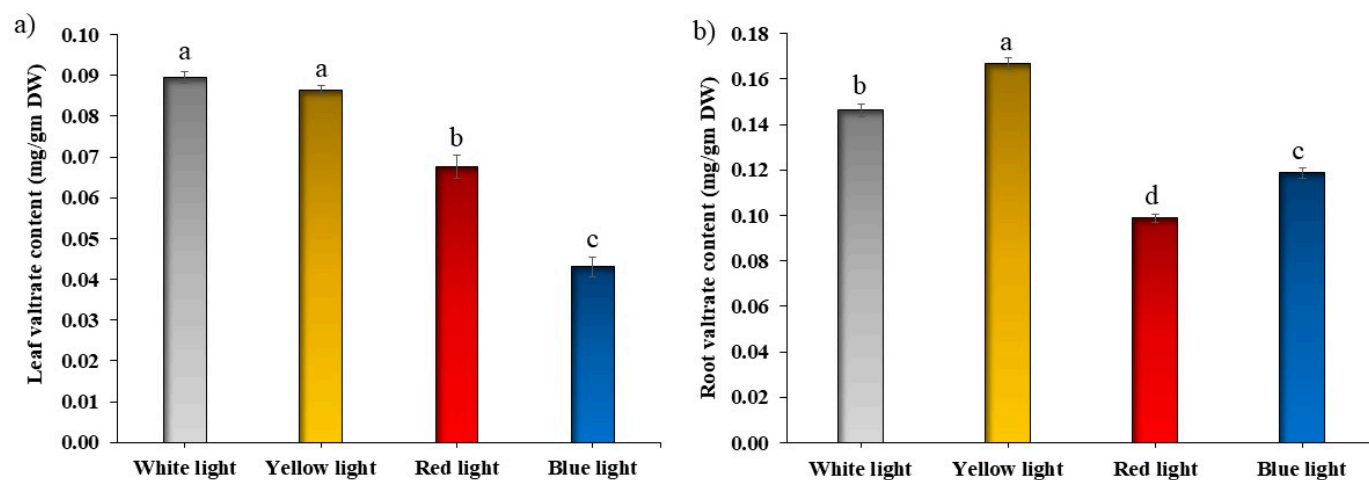


Figure 7

Effect of LED lights on Valtrate content in *in vitro* raised *V. jatamansi*. a) Leaf valtrate content, and b) Root valtrate content. Different superscript lowercase letters in a column indicate statistically significant differences between the means ($p < 0.05$)

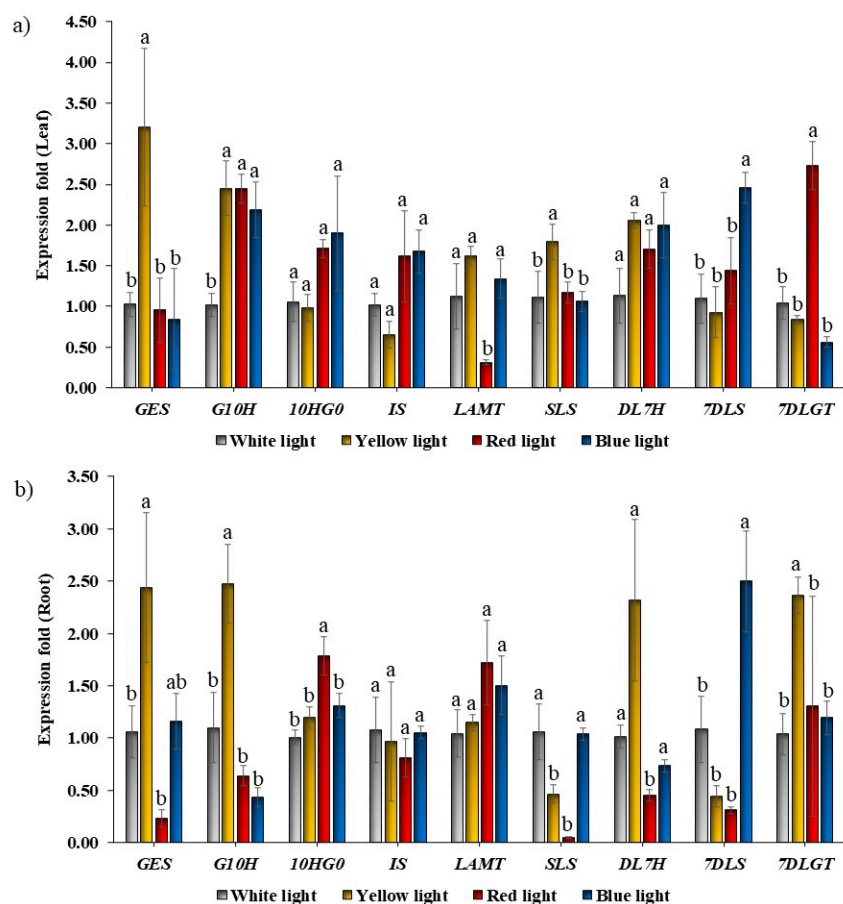


Figure 8

Effect of LED lights on iridoid biosynthesis pathway genes in *in vitro* raised *V. jatamansi*. a) Gene expression in leaf, and b) Gene expression in root. Different superscript lowercase letters in a column indicate statistically significant differences between the means ($p < 0.05$)