

Research Paper

Context matters: Photoperiodic light spectrum modulates UV-A-induced alkaloid profile and oxidative signaling in *Catharanthus roseus*

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ABSTRACT

Catharanthus roseus produces pharmacologically relevant terpenoid indole alkaloids (TIAs), including vinblastine (VLB) and vincristine (VCR). However, their low natural abundance makes it essential to optimize *C. roseus* photobiology toward enhanced specialized metabolism. This study explored the morphophysiological and biochemical responses of three *C. roseus* cultivars to ultraviolet-A (UV-A) supplementation (365 nm; 2 h; 3.6 kJ·m⁻²·day⁻¹) and examined how the photoperiodic light spectrum (WT–white; RB–red; blue) shapes these responses. UV-A had no significant effect on plant morphology but increased the photosynthetic rate (37%) and stomatal conductance (2.1-fold) under WT photoperiod. Under RB supplemented with UV-A (RB16+UVA2), plants showed higher malondialdehyde (MDA) levels and significantly higher total anticancer-related TIA concentrations. Notably, VCR in cv. ‘Cora Red’ reached 8.94 µg·g⁻¹ FW, representing a 4.4-fold increase compared with WT16+UVA2. Catharanthine (CAT) increased by 46% under RB16+UVA2, whereas anhydrovinblastine (AVLB) peaked under WT16+UVA2 (2.3-fold higher), indicating that photoperiodic spectrum modulates the UV-A-induced TIA biosynthesis. Both MDA and H₂O₂ correlated positively with total anticancer-related TIAs, and H₂O₂ was strongly associated with final VLB+VCR concentrations, highlighting their potential as biomarkers of alkaloid biosynthesis. These findings demonstrate that UV-A can function as a metabolic enhancer without compromising plant biomass, and that alkaloid biosynthesis is shaped by both cultivar and photoperiod light spectrum. Genotype selection is therefore critical, as cultivars differ markedly in their capacity to perceive UV-A and transduce it into enhanced alkaloid production. Optimizing light spectrum and genotype in vertical farming enables a scalable approach for molecular farming of anticancer alkaloids.

1. Introduction

Catharanthus roseus produces pharmaceutically valuable anticancer alkaloids, including vinblastine (VLB) and vincristine (VCR), yet their natural concentrations are extremely low, constraining cost-effective extraction and large-scale production (Nagy et al., 2023). Recent efforts to enhance their biosynthesis have focused on manipulating environmental conditions, particularly light quality, to optimize specialized metabolism in controlled environments (Livadariu et al., 2023).

The ability to precisely regulate light spectra, photoperiod, and elicitor exposure has positioned controlled-environment agriculture, including vertical farming (VF) and plant factory systems, as promising

platforms for the consistent process of production of high-value plant metabolites (Gruda et al., 2025; Sharma et al., 2026). Unlike open-field cultivation, these systems decouple biomass and metabolite production from natural environmental conditions, enabling precise selection of environmental inputs based on metabolic goals rather than just yield (Despommier, 2024; Erekaht et al., 2024).

Plants perceive and respond to light through specialized photoreceptors (Rai et al., 2020; Morales et al., 2025) that activate gene expression networks regulating growth and the production of plant specialized metabolites (PSM), including alkaloids. Ultraviolet (UV) radiation, which spans wavelengths from 100 to 400 nm, is naturally emitted by the sun and is well known for its dual role in plant

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physiology. At high doses – particularly in the UV-B and UV-C ranges – it can cause oxidative stress, leading to cellular damage or even cell death (Sánchez Correa et al., 2023; Cunningham et al., 2024; Shi and Liu, 2021). However, at low to moderate intensities, UV radiation can act as a beneficial signal, stimulating plant development (Silva et al., 2024) and PSM biosynthesis, including pharmaceutically valuable compounds (Loconsole and Santamaria, 2021). Within the UV spectrum, UV-A (315 – 400 nm) is the longest-wavelength and least energetic band. Although less studied than the higher-energy UV-B and UV-C, recent evidence suggests that UV-A can stimulate alkaloid biosynthesis in *C. roseus* without compromising vegetative growth when appropriately applied (Lourenço et al., 2025a). For instance, Lourenço et al. (2025a) reported that UV-A exposures of $10 \text{ kJ}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ (365 nm) applied during the final five days of production significantly increased upstream alkaloids, including catharanthine (CAT), vindoline (VDL), and anhydrovinblastine (AVLB), while maintaining plant biomass. Complementary evidence from detached leaf-based assays further supports the stimulatory role of UV-A: Fukuyama et al. (2025) showed that five days of UV-A radiation at 365 and 385 nm enhanced the anticancer alkaloid VLB, whereas shorter UV wavelengths (310 and 280 nm) and longer near-visible radiation (405 nm) did not elicit comparable responses. These findings suggest that VLB biosynthesis is not part of a generalized UV response, but is instead stimulated by a narrow UV-A wavelength range. However, in that study, UV-A intensity was applied in detached leaves, rather than to the whole-plant, and was only reported as irradiance ($\text{W}\cdot\text{m}^{-2}$), limiting direct quantitative comparison with whole-plant cultivation systems. Such discrepancies highlight that reported UV irradiances in the literature often reflect lamp output rather than biologically effective doses. Additionally, long-term plant exposure to UV-A during cultivation remains insufficiently defined. Moreover, although these studies collectively suggest that UV-A can act as a metabolic elicitor, the involvement of reactive oxygen species (ROS) has not been explicitly examined, leaving the mechanistic basis of UV-A-induced alkaloid biosynthesis still poorly characterized. Abiotic factors such as UV radiation often trigger signaling cascades involving ROS and phytohormones, including jasmonic and salicylic acid. These molecules regulate PSM biosynthesis, with ROS acting as essential signaling cues and, when excessive, causing cellular damage (Zhou and Chen, 2022; García-Muñoz et al., 2024). Elevated ROS can lead to lipid peroxidation, commonly assessed through malondialdehyde (MDA) accumulation (Kumari et al., 2021), whereas antioxidant responses help restore cellular homeostasis and may indicate elicitor efficacy (Raza et al., 2022). While these processes can influence alkaloid accumulation, they remain insufficiently studied in *C. roseus*.

Although several studies have examined the effects of light quality and intensity on TIA biosynthesis in *C. roseus* (summarized in Supplementary Table S1), their findings remain highly inconsistent. Ohashi et al. (2013) reported that red light (R) significantly promoted the production of monomeric alkaloids, such as CAT by 11% and VDL by 15%, relative to white (WT) light. In contrast, Quadri et al. (2025) found no significant increase of R over WT for CAT and VDL concentrations in leaf tissues, but observed a clear enhancement under a red:blue (R:B) 6:1 spectrum compared with WT and monochromatic R or B. Other work has similarly shown no consistent advantage of R over blue (B) or R:B (2:1) spectra for these monomeric alkaloids (Fukuyama et al., 2013). In addition to spectral composition, light intensity has also been shown to influence TIA accumulation; for example, Fukuyama et al. (2015) reported a peaked VDL concentration at moderate photosynthetic photon flux densities (PPFDs; $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Substantial discrepancies are also evident in the reported concentrations of the dimeric anticancer alkaloids VLB and VCR, with values varying enormously even under comparable growth conditions (Costa et al., 2008; Sottomayor et al., 2008; Carqueijeiro et al., 2013; Gao et al., 2022; Fukuyama et al., 2025; Li et al., 2025) (Supplementary Table S1). Together, these contrasting results highlight the absence of a clear consensus on the spectral requirements for enhancing anticancer alkaloid biosynthesis. They also

show the need for systematic studies that integrate photoperiodic spectra with supplemental UV-A, while explicitly accounting for cultivar-dependent metabolic responses.

Despite growing interest in UV-A as a mild elicitor of specialized metabolite enhancement, its influence on downstream anticancer alkaloids in *C. roseus* remains poorly understood, particularly in relation to the potential interference of the light spectrum applied during the photoperiod. Moreover, few studies have linked oxidative stress markers to the final yields of dimeric alkaloids, such as VLB and VCR (Sottomayor & Ros Barceló, 2003; Fouad et al., 2021). Advancing this knowledge would provide a critical foundation for optimizing elicitor application towards higher alkaloid biosynthesis.

This study investigates how UV-A supplementation influences morphological, physiological, and biochemical responses, and ultimately alkaloid profile, in three distinct *C. roseus* cultivars. We also examine whether the photoperiodic light spectrum, comparing R:B (3.3:1) with WT light radiation, modulates UV-A-induced responses and shapes the resulting alkaloid profile. In addition, we explore whether selected stress-related biochemical markers correlate with alkaloid accumulation, providing insights into the mechanisms underlying elicitor effectiveness. Based on this framework, we formulate the following hypotheses: (i) mild levels of UV-A supplementation throughout the growing cycle enhance TIA biosynthesis without compromising plant growth; (ii) the response to UV-A depends on the photoperiodic spectral composition (RB vs WT); (iii) cultivars differ in their physiological and metabolic responses to UV-A, reflecting genotype-specific plasticity. By addressing these questions, this study aims to optimize spectral and cultivar-specific strategies for efficient alkaloid production in vertical farming (VF) and plant molecular farming systems.

2. Material and methods

2.1. Plant material, growing conditions and experimental design

Seedlings of three *Catharanthus roseus* (L.) cultivars, bred by Syngenta Flowers (Enkhuizen, the Netherlands), were sourced from a commercial nursery (Group Roig – Viveros Pereira, Valencia, Spain) four weeks after sowing. The cultivars ‘Cora Red’ (‘C-Red’) and ‘Cora XDR Polka Dot’ (‘C-XDR-Polka’) were selected based on a previous cultivar comparative study (Lourenço et al., 2025b), which demonstrated the highest leaf anticancer alkaloids concentration in ‘C-Red’ and the highest biomass production in ‘C-XDR-Polka’ under VF systems. Additionally, ‘S-APR’ was selected since it is one of the most widely studied *C. roseus* cultivars, with available genetic mapping (Li et al., 2023). All cultivars were of the same chronological age, having been sown on the same date and grown under identical nursery conditions. Initial total dry weight was recorded ($0.088 \pm 0.028 \text{ g}$). In addition, the number of leaves larger than 2.0 cm (6.9 ± 1.2 leaves/plant) and plant height ($3.7 \pm 0.5 \text{ cm}$) were measured in the same set of seedlings, confirming a high degree of uniformity at the start of the experiment. Seedlings were transplanted into 8-cm pots containing a humus-based mix (50% humus, 17% peat, 17% perlite, 15% coco peat) enriched with a 16–11–10 + 2 Mg fertilizer and micronutrients. Plants were grown in controlled-environment chambers at $24.0 \pm 0.8^\circ\text{C}$ and 80% relative humidity, monitored at the canopy level using a LogTag HAXO-8 (Auckland, New Zealand), which uses an internal thermistor sensor for temperature measurement and a capacitive polymer sensor for relative humidity measurement. Temperature was continuously recorded at 4-min 30-s intervals throughout the experimental period, with the results presented in the supplementary material (Fig. S1). Plants were exposed to a 16-h photoperiod and a PPFD of $150 \pm 15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, measured at canopy level using a spectroradiometer (Optimum SRI-2000, Hsinchu, Taiwan). Under these growth conditions, four light-quality treatments were applied (i.e. two photoperiodic light spectra, with or without an extended 2-h UV-A exposure), resulting in RB16, RB16+UVA2, WT16, and WT16+UVA2 (Fig. 1). To characterize

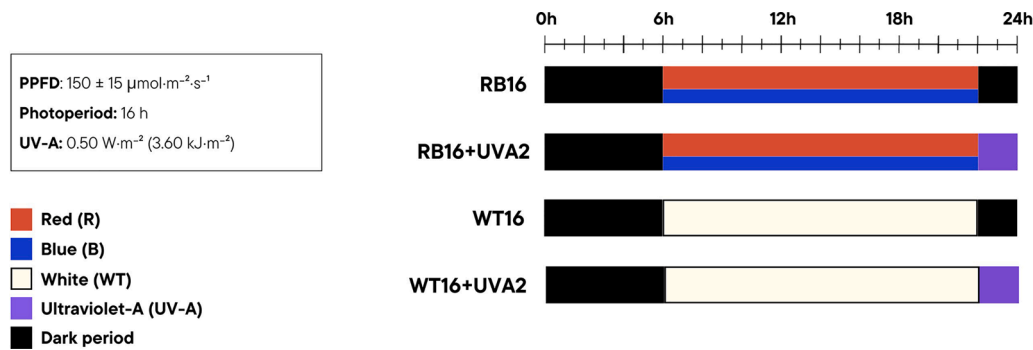


Fig. 1. Schematic representation of the four light treatments applied in controlled climate chambers throughout the *Catharanthus roseus* cultivation cycle. Red (R), Blue (B), and White (WT) bars represent the 16 h. Supplementary ultraviolet-A (UV-A) treatment applied for 2 h at the end of the photoperiod is also represented. General growth conditions: PPFD of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, UV-A with $0.50 \text{ W}\cdot\text{m}^{-2}$ irradiance corresponding to a $3.60 \text{ kJ}\cdot\text{m}^{-2}$ and peak at 365 nm, temperature maintained at $24.1 \pm 0.5 \text{ }^{\circ}\text{C}$, and relative humidity at $80 \pm 5\%$. The spectral photon distribution of R:B and WT light is detailed in Fig. 2.

the spectral composition of each treatment, the measured irradiance spectra were converted to photon flux density and subsequently normalized (normalized photon flux) to illustrate the relative spectral distribution (Fig. 2). PPFD from the photoperiodic light sources and supplementary UV-A were monitored weekly at the canopy level and adjusted as needed to ensure consistent intensity throughout the experiment and across treatments. In the RB16 treatment, plants received a red:blue spectrum (667 nm:450 nm) with a ratio of 3.3:1, delivered by LED panels (RX-TP12050-F5, 200 W, Koray Light, Seoul, Korea) (Figs. 1 and 2A). The R:B ratio was calculated from the non-normalized photon flux density spectrum by integrating emission across the 600–700 nm (red) and 400–500 nm (blue) wavebands using the trapezoidal rule. The same integration approach showed that red photons accounted for $\sim 70\%$ of total PAR, blue photons for $\sim 20\%$, and other wavelengths (500–600 nm) for $\sim 10\%$ of the delivered spectrum (Fig. 2A). The WT16 treatment corresponds to a polychromatic WT spectrum light provided by WT LEDs (RX-TP12050-F4, 200 W, Koray Light, Seoul, Korea) (Figs. 1 and 2B, Fig. S2). For the two treatments with UV-A supplementation, plants were exposed to the corresponding photoperiod light conditions but, at the beginning of the dark period, they received an additional 2 h of UV-A ($0.50 \text{ W}\cdot\text{m}^{-2}$ irradiance and total dose of $3.6 \text{ kJ}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) using a commercial UV-A fixture [nominal 350–380 nm, with a peak 365 nm; QTX, UK] whose emission spectrum and stability were verified weekly with a spectroradiometer [Optimum SRI-2000; Hsinchu, Taiwan] (Figs. 1 and 2C). This moderate daily UV-A dose was selected to provide a long-term stimulus throughout the production cycle, rather than a short-term high-irradiance stress treatment (Fukuyama et al., 2017, 2023). Measured spectra confirmed a narrow

UV-A emission centered at $\sim 365 \text{ nm}$ (Fig. S2). Residual off-band signal accounted for $< 3\%$ of the integrated spectral output. No detectable emission was recorded within the UV-B (280–315 nm) or UV-C ($< 280 \text{ nm}$) ranges.

To ensure uniform growth conditions, plants were arranged at a density of $156 \text{ plants}\cdot\text{m}^{-2}$, corresponding to 70 plants per light treatment per cultivar per experimental replicate. Plants were distributed evenly within each compartment and repositioned every three days to minimize border and positional effects. The experiment followed a split-plot design, with light spectrum assigned to main plots and cultivars randomized within each light treatment. All treatments included three independent experimental replicates allocated in different climate growth chambers. The full allocation of chambers and replicates is presented in Supplementary Fig. S3. The dataset consisted of 36 independent observations ($3 \text{ cultivars} \times 4 \text{ light spectra} \times 3 \text{ experimental replicates}$). For the analyses of the morphophysiological traits and alkaloid content, each experimental replicate included five randomly selected plants per cultivar and light spectra (Section 2.2). For the biochemical parameters and alkaloid concentration analyses (Section 2.3), each experimental replicate consisted of a pool of fresh leaf material from five randomly selected plants per cultivar and light spectra. Leaves were immediately frozen in liquid nitrogen and stored at $-80 \text{ }^{\circ}\text{C}$ until analysis.

The experiment ended 35 days after transplanting the rooted seedlings into the VF system. This represented a total cultivation period of 63 days (from sowing to harvest), and it corresponded to the full-bloom stage of development. As explained by Lourenço et al. (2025b), this timing was selected for its biological relevance, as previous studies have shown that alkaloid concentration may decline in older tissues (Mall

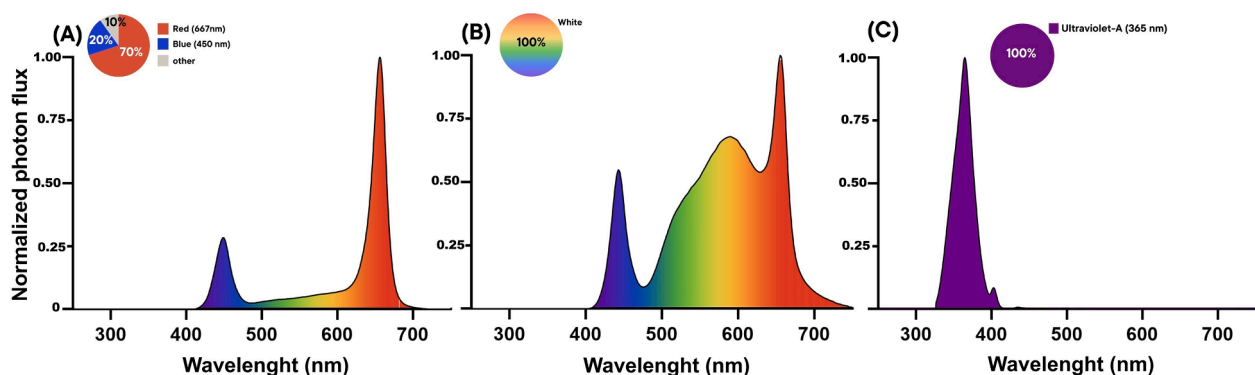


Fig. 2. Normalized photon flux density spectra of the light treatments. (A) red–blue (RB) spectrum with peaks at 667 nm (red) and 450 nm (blue). Photon-weighted integration across standard wavebands showed that the RB spectrum consisted of $\sim 70\%$ red (600–700 nm), $\sim 20\%$ blue (400–500 nm), and $\sim 10\%$ other wavelengths (500–600 nm). (B) Broad white polychromatic spectrum with major peaks at 655 nm (red), 580 nm (yellow/orange), and 445 nm (blue). (C) Ultraviolet-A spectrum with a peak at 365 nm. Spectra are shown as normalized photon flux for comparison of spectral shape; percentage values in the pie charts represent the relative photon contribution of each waveband.

et al., 2019; Hajibarat and Saidi, 2022). Therefore, prolonging the trial could result in lower alkaloid levels.

2.2. Morphological and physiological measurements

Morphological and physiological measurements were performed following the protocols detailed in Lourenço et al. (2025b), with minor adjustments for the present experimental setup. Briefly, at the end of the cultivation period, five plants per experimental replicate (total of three experimental replicates) were harvested and separated into leaves, stems, and flowers. Fresh biomass was recorded immediately, leaf area was determined using a LI-3100C area meter (LI-COR, Lincoln, NE, USA), and all organs were oven-dried at 90 °C for 72 h to obtain dry biomass. Specific leaf area (SLA) was calculated as leaf area per unit leaf dry weight.

Physiological measurements were conducted on the youngest fully expanded leaf. Chlorophyll content was estimated with a SPAD-502 m (Konica Minolta, Tokyo, Japan). Gas exchange was assessed using an LI-6400XT infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA) operated at 400 $\mu\text{mol}\cdot\text{mol}^{-1}\text{CO}_2$, 350 $\mu\text{mol}\cdot\text{s}^{-1}$ airflow, 24.5 °C block temperature, $\approx 70\%$ relative humidity, and 170 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ cuvette PPFD, yielding net photosynthesis and stomatal conductance values.

2.3. Quantification of reactive oxygen species, oxidative stress, and alkaloid profile

2.3.1. Superoxide activity (O_2^-)

Leaf samples (100 mg) were homogenized in 2 mL of extraction buffer containing 0.01 M phosphate buffer (pH 7.8), 0.05% (w/v) nitro blue tetrazolium (NBT), and 10 mM sodium azide. After 1 h incubation at room temperature, samples were centrifuged at $13,000 \times g$ for 2 min at 4 °C. The supernatant was transferred to clean tubes, incubated at 85 °C for 10 min, and cooled on ice for 10 min. Absorbance was measured at 580 nm in a 96-well plate, using the extraction buffer as a blank. For each experimental replicate, three technical replicates were included. Results were expressed as absorbance units per g fresh weight.

2.3.2. Hydrogen peroxide concentration (H_2O_2)

Hydrogen peroxide concentration was quantified as described by Mariz-Ponte et al. (2018). Fresh leaf tissue (100 mg) was ground in 2 mL of 0.1% (w/v) trichloroacetic acid (TCA). The extracts were vortexed for 15 s and centrifuged at $12,000 \times g$ for 15 min at 4 °C. For the colorimetric reaction, 500 μL of the supernatant was mixed with 500 μL of 0.1 M phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. After 1 h of incubation in the dark, absorbance was recorded at 390 nm. Three technical replicates were analyzed for each experimental replicate. H_2O_2 concentration ($\mu\text{mol}\cdot\text{g}^{-1}\text{FW}$) was determined using a standard curve.

2.3.3. Total antioxidant capacity

Fresh leaf tissue (200 mg) was homogenized in 1 mL of 80% methanol at 4 °C, and the mixture was centrifuged at $12,000 \times g$ for 2 min. The resulting supernatants (900 μL) were stored at $-20\text{ }^\circ\text{C}$ until analysis. Radical scavenging activity was assessed using a modified 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($\text{ABTS}^{\bullet+}$) radical scavenging assay (Özgen et al., 2006). The $\text{ABTS}^{\bullet+}$ stock solution was generated by mixing 7 mM ABTS with 2.44 mM potassium persulfate and allowing the mixture to react for 16 h in the dark at 4 °C. A working solution was freshly prepared in 80% methanol and adjusted to an absorbance of 0.68–0.72 at 734 nm. A calibration curve was obtained using Trolox standards (25–175 μM). In a 96-well microplate, 20 μL of extract or Trolox standard was combined with 180 μL of $\text{ABTS}^{\bullet+}$ working solution. After 5 min incubation at 30 °C, absorbance was measured at 734 nm. For each experimental replicate, three technical replicates were analyzed. Results were expressed as μmol Trolox equivalents per g fresh weight ($\mu\text{mol TE}\cdot\text{g}^{-1}\text{FW}$).

2.3.4. Lipid peroxidation

Fresh leaf tissue (250 mg) was homogenized at 4 °C in 1.5 mL of 0.1% TCA and centrifuged at $12,000 \times g$ for 15 min at 4 °C. For the thiobarbituric acid (TBA) reaction, 250 μL of supernatant was mixed with 1 mL of 0.5% (w/v) TBA prepared in 20% (v/v) TCA. A blank was prepared similarly using 0.1% TCA instead of the sample extract. Mixtures were incubated at 95 °C for 30 min, chilled on ice for 10 min, and centrifuged at $10,000 \times g$ for 15 min. Absorbance of the supernatant was recorded at 532 and 600 nm. Thiobarbituric acid reactive substances (TBARS) levels were calculated according to Hodges et al. (1999) and expressed as nmol MDA per g fresh weight. Each experimental replicate included three technical replicates. Total alkaloid yield ($\text{mg}\cdot\text{plant}^{-1}$) was obtained by multiplying the measured concentration ($\text{mg}\cdot\text{g}^{-1}\text{FW}$) by total fresh biomass.

2.3.5. Alkaloid extraction and LC-MS methods

Alkaloid extraction and analysis were performed according to the methodology previously validated in Lourenço et al. (2025b), adapted from Carqueijeiro et al. (2016) and Jeong and Lim (2018). Briefly, 150 mg of frozen leaf powder was extracted with methanol, vortexed, sonicated, centrifuged, and filtered before injection. LC-ESI-SIM analysis was performed using a Finnigan Surveyor Plus HPLC (Thermo Scientific, Waltham, MA, USA) system with the gradient and ion-source settings described by Lourenço et al. (2025b). Alkaloids were identified based on retention time and extracted ion chromatograms (XICs) at their characteristic m/z values using authentic reference standards analyzed under identical conditions. Quantification was performed using external calibration curves. Method validation parameters, including ion targets, retention times, calibration ranges, correlation (R^2), and limits of detection and quantification (LOD/LOQ), are provided in Supplementary Table S2. Representative extracted ion chromatograms illustrating baseline separation of all target alkaloids are shown in Fig. S4.

2.4. Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate the effects of light spectrum, cultivar, and their interaction. Fisher's protected least significant difference (LSD) test was used for post-hoc comparison of means at $p = 0.05$. Residual normality was assessed using the Shapiro-Wilk test. Homogeneity of variances was evaluated using the Brown-Forsythe test. Although variance heterogeneity was observed for certain alkaloids (CAT, AVLb, and VLB), the use of a balanced experimental design with equal replication ensures that ANOVA remains robust despite these deviations (Box, 1954; Ageel et al., 2025).

Pearson correlation analyses were performed in GraphPad Prism® version 10.6.0 (GraphPad Software, San Diego, CA, USA) to assess associations between ROS, oxidative stress indicators, and alkaloid concentrations. All other statistical analyses were conducted using GenStat® version 22.1 (VSN International, Hemel Hempstead, UK), and graphical outputs were generated in GraphPad Prism® version 10.6.0.

3. Results

3.1. Plant morphology and physiology

None of the morphological traits was significantly influenced by the interaction between light spectrum and cultivar, and the light spectrum *per se* had no significant effect on these traits (Table 1). Instead, significant differences emerged among cultivars. 'C-Red' accumulated the highest total biomass, producing 18% more than 'C-XDR-Polka', and 65% more than 'S-APR'. This pattern was consistent across individual plant organs: leaf, stem, and flower dry weights were all greater in 'C-Red' compared with 'S-APR'. Pairwise contrasts, among cultivars from the same series, revealed that 'C-Red' and 'C-XDR-Polka' did not differ significantly in leaf and stem biomass. At the same time, 'C-Red' produced approximately 2-fold higher flower biomass than both other

Table 1

Morphological parameters of three *Catharanthus roseus* cultivars [Sunstorm Apricot (S-APR), Cora Red (C-RED), and Cora XDR Polka Dot (C-XDR Polka)] were measured on plants grown for 35 days under Red:Blue (RB) or white (WT) light spectra at a 16-h photoperiod, with and without additional 3.6 kJ·m⁻² UV-A corresponding to 2 h of ultraviolet-A (UV-A) at the end of the photoperiod. Mean values are based on three independent replicates, each composed of five plants. SEM represents the standard error of the mean calculated from the pooled residual variance of the ANOVA. Means followed by different letters differ significantly according to Fisher's protected LSD test at $p = 0.05$. Analysis of Variance was conducted, and p -values for the effects of light effect (P_L), cultivar effect (P_C), and interaction between light and cultivar ($P_{L \times C}$) are provided.

Measured parameter	Treatment	S-APR	C-RED	C-XDR-POLKA	Average light	p value
Total dry weight (g)	RB16	0.57	0.90	0.62	0.70	$P_{L \times C} = 0.362$
	RB16+UVA2	0.55	0.74	0.74	0.68	$P_L = 0.304$
	WT16	0.46	0.96	0.77	0.73	$P_C < 0.001$
	WT16+UVA2	0.44	0.75	0.71	0.63	SEM = 0.041
	Average cultivar	0.51^c	0.84^a	0.71^b		
Leaf dry weight (g)	RB16	0.38	0.60	0.45	0.48	$P_{L \times C} = 0.663$
	RB16+UVA2	0.35	0.49	0.51	0.45	$P_L = 0.145$
	WT16	0.34	0.57	0.58	0.49	$P_C < 0.001$
	WT16+UVA2	0.31	0.51	0.45	0.42	SEM = 0.029
	Average cultivar	0.34^b	0.54^a	0.50^a		
Stem dry weight (g)	RB16	0.060	0.111	0.092	0.088	$P_{L \times C} = 0.988$
	RB16+UVA2	0.061	0.096	0.085	0.081	$P_L = 0.577$
	WT16	0.059	0.108	0.100	0.089	$P_C < 0.001$
	WT16+UVA2	0.058	0.101	0.084	0.081	SEM = 0.0063
	Average cultivar	0.060^b	0.104^a	0.090^a		
Flower dry weight (g)	RB16	0.0449	0.0858	0.0258	0.0523	$P_{L \times C} = 0.113$
	RB16+UVA2	0.0328	0.0575	0.0642	0.0515	$P_L = 0.364$
	WT16	0.0173	0.0761	0.0354	0.0429	$P_C < 0.001$
	WT16+UVA2	0.0297	0.0616	0.0284	0.0399	SEM = 0.0055
	Average cultivar	0.0312^b	0.0703^a	0.0385^b		
Leaf area (cm ²)	RB16	117.0	205.4	134.6	152.3	$P_{L \times C} = 0.561$
	RB16+UVA2	106.8	172.8	155.4	145.0	$P_L = 0.586$
	WT16	107.5	173.9	152.1	144.5	$P_C < 0.001$
	WT16+UVA2	114.8	169.8	143.0	142.5	SEM = 7.13
	Average cultivar	111.5^c	180.5^a	146.3^b		
Specific leaf area (cm ² ·g ⁻¹)	RB16	315.2	342.6	303.4	320.4	$P_{L \times C} = 0.468$
	RB16+UVA2	323.0	359.4	307.9	330.1	$P_L = 0.052$
	WT16	322.3	309.8	285.6	305.9	$P_C = 0.009$
	WT16+UVA2	351.0	338.8	321.5	337.1	SEM = 6.65
	Average cultivar	327.9^a	337.6^a	304.6^b		

cultivars. Leaf area also varied significantly among cultivars. 'C-Red' exhibited the largest leaf area, exceeding 'C-XDR-Polka' by 23% and 'S-APR' by 62%. 'C-XDR-Polka' developed 31% more leaf area than 'S-APR', yet its significantly lower SLA (−7%) indicates a higher biomass investment per unit leaf area.

For the physiological traits assessed, a significant light spectrum × cultivar interaction was observed only for chlorophyll index (SPAD; Fig. 3A). Supplementary UV-A (3.6 kJ·m⁻²·day⁻¹) significantly reduced SPAD in 'C-Red', resulting in an average of 5% lower values, irrespective of the photoperiodic light spectrum. In 'C-XDR-Polka', SPAD declined by 9% under UV-A, but this reduction occurred exclusively under the WT spectrum. In contrast, in 'S-APR' UV-A supplementation had no impact on SPAD values under either spectrum. Photosynthetic rate and stomatal conductance were only significantly affected by the light quality treatment (Fig. 3B, C). Overall, the lowest photosynthetic activity was observed under WT16, with an average 29% reduction relative to RB16 light. Applying supplementary UV-A to plants grown under WT photoperiod light enhanced their photosynthetic rate, bringing it to the RB16 levels. Stomatal conductance followed a similar pattern, with plants grown under WT16 exhibiting the lowest values, and WT16+UVA2 boosting stomatal conductance in 81%.

Pearson's correlation analysis showed no significant associations between physiological parameters (net photosynthetic rate, stomatal conductance, and SPAD) and alkaloid concentrations – total TIAs or VLB+VCR (Fig. S5).

3.2. Oxidative stress markers and antioxidant capacity

Superoxide (O₂⁻) activity and hydrogen peroxide (H₂O₂) concentration differed significantly between cultivars, whereas the light spectrum did not impact these ROS levels (Fig. 4A, B). O₂⁻ activity reached the highest values in 'C-Red' and 'C-XDR-Polka', both having ~24% higher

levels than 'S-APR' (Fig. 4A). 'C-Red' consistently had the highest H₂O₂ concentration across all light spectra, nearly twice that of 'C-XDR-Polka' and 39% higher than 'S-APR' (Fig. 4B).

In contrast, the total antioxidant capacity, measured using the ABTS assay, showed no statistically significant differences among cultivars or light spectra (Fig. 4C). Lipid peroxidation, expressed as the concentration of MDA, showed significant differences among cultivars and across light conditions (Fig. 4D). 'C-Red' and 'C-XDR-Polka' exhibited 28% higher lipid peroxidation than 'S-APR'. In addition, supplementing RB-grown plants with 2 h UV-A light consistently increased lipid peroxidation in the three studied cultivars, leading to 30% higher levels compared with RB alone. A similar, though non-significant, trend was observed when UV-A supplementation was applied under WT photoperiodic light.

3.3. Alkaloid profiles

The total anticancer-related TIAs concentration (sum of CAT, VDL, AVLb, VLB, and VCR) in *C. roseus* leaves was significantly affected by both light spectrum and cultivar, with no significant interaction found among the two studied factors (Fig. 5A). RB16+UVA2 resulted in the highest TIA concentrations, with levels averaging ~24% higher than those observed under the three other spectra. Among cultivars, 'S-APR' consistently accumulated the lowest TIA concentrations, ~23% below both 'C-Red' and 'C-XDR-Polka'.

In contrast, focusing on the total anticancer alkaloids (VLb plus VCR), a significant interaction between light and cultivar was found (Fig. 5B). 'C-Red' under RB16+UVA2 exhibited the highest concentration, whereas the same cultivar under WT16+UVA2 showed the lowest levels, representing a ~2.6-fold reduction between these treatments. The same trend was observed for 'C-XDR-Polka', but it did not always show significant differences. Unlike the 'Cora' series, in 'S-APR', the

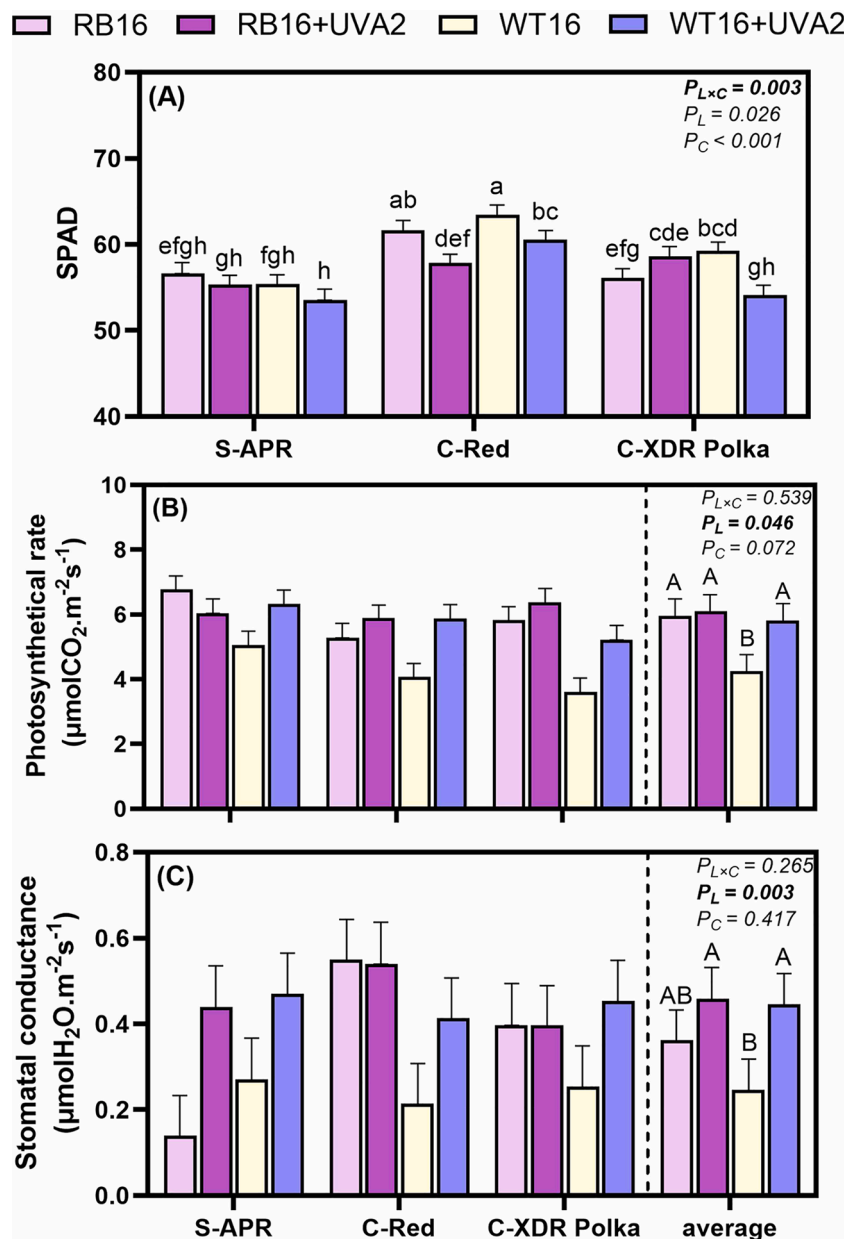


Fig. 3. Chlorophyll content (A), photosynthetic rate (B), and stomatal conductance (C) measured in three *Catharanthus roseus* cultivars after 35 days of cultivation under 16 h of red–blue (RB16), white (WT16), RB+UVA2, and WT+UVA2 light (details in Fig. 1). Cultivars tested were Sunstorm Apricot (S-APR), Cora Red (C-Red), and Cora XDR Polka Dot (C-XDR Polka). Mean values are based on three independent replicates ($n = 3$, each composed of five plants). Error bars represent the pooled standard error of the mean calculated from the ANOVA residual variance. *p*-values indicate the statistical significance of the main effects of light spectra (*PL*) and cultivar (*PC*), as well as their interaction (*PL* × *C*), based on ANOVA. Different letters above the bars indicate significant differences according to Fisher's protected LSD test ($p = 0.05$), with lowercase letters representing differences between the cultivar × light treatment interaction (panel A), and uppercase letters representing differences between light treatments (panel B and C).

higher values were obtained in plants grown under RB16 light with no UV-A supplementation.

Examining individual alkaloid compounds (Fig. 6), CAT mirrored total TIA trends in terms of light spectrum response, with ~1.5-fold higher levels under RB16+UVA2 compared to the other spectra, but no cultivar effect was found (Fig. 6A). In contrast, VDL was strongly cultivar-dependent, being approximately 50% lower in 'S-APR' than in 'C-Red' and 'C-XDR-Polka', regardless of the light treatment (Fig. 6B). AVLb showed no cultivar effect but was enhanced by WT16+UVA2 (~40% increase relative to other treatments) (Fig. 6C). Finally, concerning the specific anti-cancer alkaloids, significant light and cultivar interactions were detected for VLB and VCR. VLB accumulation peaked under RB16+UVA2 in both 'C-Red' and 'C-XDR-Polka', averaging ~1.8-

fold higher than under the other light regimes, which did not differ from each other (Fig. 6D). A different response was observed in 'S-APR' with plants grown under WT light presenting a lower VLB concentration when compared with WT+UVA2, which in turn did not differ from the other light treatments. The largest VCR production occurred in 'C-Red' under RB16+UVA2, followed by RB16 alone; these two treatments did not differ from each other (Fig. 6E). Nonetheless, in this cultivar, a UV-A supplementation in plants grown under WT light led to a 72% decrease in VCR levels.

When expressed as total alkaloid content per plant (sum of CAT, VDL, AVLb, VLB, and VCR), only cultivar differences were evident: 'C-Red' and 'C-XDR-Polka' produced nearly twice as much as 'S-APR' (Fig. 7A). In contrast, the anticancer alkaloid content (VLb + VCR) revealed a

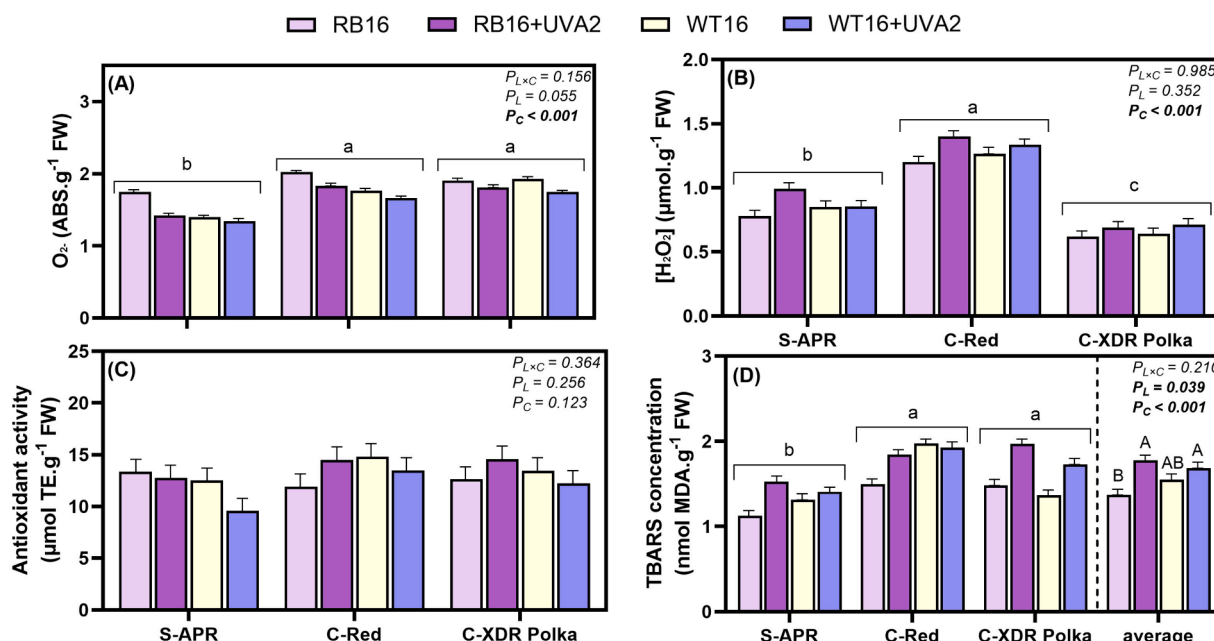


Fig. 4. Effect of spectral composition on different reactive oxygen species (ROS), total antioxidant capacity, and lipid peroxidation in three *Catharanthus roseus* cultivars after 35 days of cultivation under 16 h of red–blue (RB16), white (WT16), RB16 + UV-A 2 h, and WT16 + UV-A 2 h light. (A) superoxide (O_2^-) concentration expressed as absorbance at 580 nm per gram of fresh weight; (B) Hydrogen peroxide (H_2O_2) concentration; (C) total antioxidant capacity measured by the ABTS method and expressed as μ mol Trolox equivalents (TE) per gram of fresh weight; (D) thiobarbituric acid reactive substances (TBARS) concentration, expressed as nmol malondialdehyde (MDA) per gram of fresh weight, indicating lipid peroxidation levels. Cultivars tested were Sunstorm Apricot (S-APR), Cora Red (C-Red), and Cora XDR Polka Dot (C-XDR Polka). Mean values are based on three independent replicates ($n = 3$, each composed of five plants). Error bars represent the pooled standard error of the mean calculated from the ANOVA residual variance. *p*-values indicate the statistical significance of the main effects of light spectra (*PL*) and cultivar (*PC*), as well as their interaction ($PL \times C$), based on ANOVA. Different letters above the bars indicate significant differences according to Fisher's protected LSD test ($p = 0.05$), with lowercase letters representing differences between cultivars (panel A, B and D), and uppercase letters representing differences between light treatments (panel D).

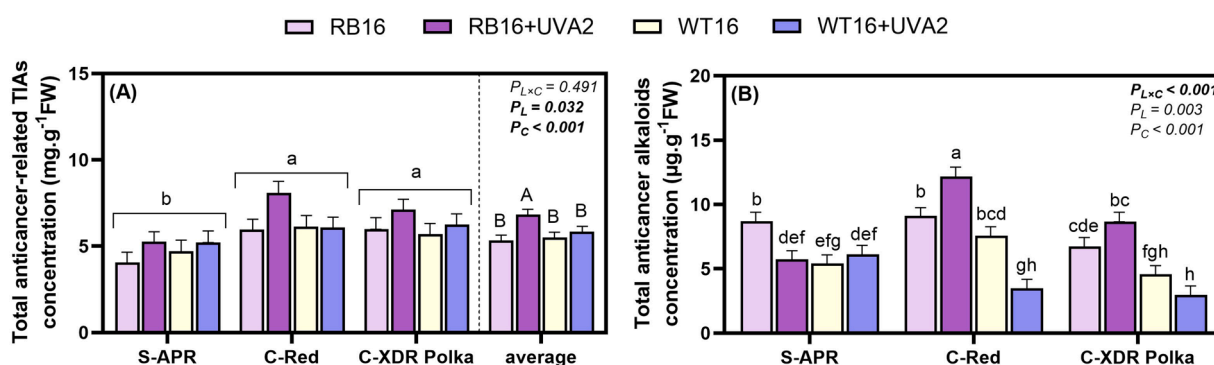


Fig. 5. Total anticancer-related TIAs concentration [measurements included catharanthine (CAT), vindoline (VDL), anhydrovinblastine (AVLB), vinblastine (VLB), and vincristine (VCR)] (A), along with the concentration of anticancer alkaloids (VLB and VCR) (B), of three *Catharanthus roseus* cultivars after 35 days of cultivation under 16 h of red–blue (RB16), white (WT16), RB16 + UV-A 2 h, and WT16 + UV-A 2 h light. The cultivars tested were Sunstorm Apricot (S-APR), Cora Red (C-Red), and Cora XDR Polka Dot (C-XDR-Polka). Mean values are based on three independent replicates ($n = 3$, each composed of five plants). Error bars represent the pooled standard error of the mean calculated from the ANOVA residual variance. *p*-values indicate the statistical significance of the main effects of light spectra (*PL*) and cultivar (*PC*), as well as their interaction ($PL \times C$), based on ANOVA. Different letters above the bars indicate significant differences according to Fisher's protected LSD test ($p = 0.05$). In panel A, the lowercase letters represent differences between cultivars and the uppercase letters represent differences between light treatments. In panel B, lowercase letters represent differences between the cultivar $\times$ light treatment interaction.

significant interaction among the studied factors (Fig. 7B). ‘C-Red’ was the cultivar that showed the highest content, reached under RB16 with or without UV-A supplementation, whereas ‘C-XDR-Polka’ significantly benefited from UV-A supplementation if the photoperiodic light was RB, producing up to 94% more than under the other light spectra. ‘S-APR’ responded best to RB light without UV-A supplementation, yielding ~66% more than under its other treatments.

Pearson's correlation analysis revealed several significant associations among alkaloid concentrations and biochemical stress indicators in

C. roseus (Fig. 8). CAT and VDL showed positive correlations with lipid peroxidation – MDA ($r = 0.37$, $p = 0.03$; and $r = 0.44$, $p = 0.008$, respectively). O_2^- concentration correlated positively with VDL ($r = 0.63$; $p < 0.001$), while H_2O_2 correlated positively with VCR ($r = 0.39$; $p = 0.02$). Total concentration of the studied TIA displayed positive correlations with both H_2O_2 and MDA ($r = 0.38$, $p = 0.02$; and $r = 0.54$, $p = 0.001$, respectively), and the combined concentration of the anticancer alkaloids VLB and VCR was positively correlated with H_2O_2 ($r = 0.37$; $p = 0.03$).

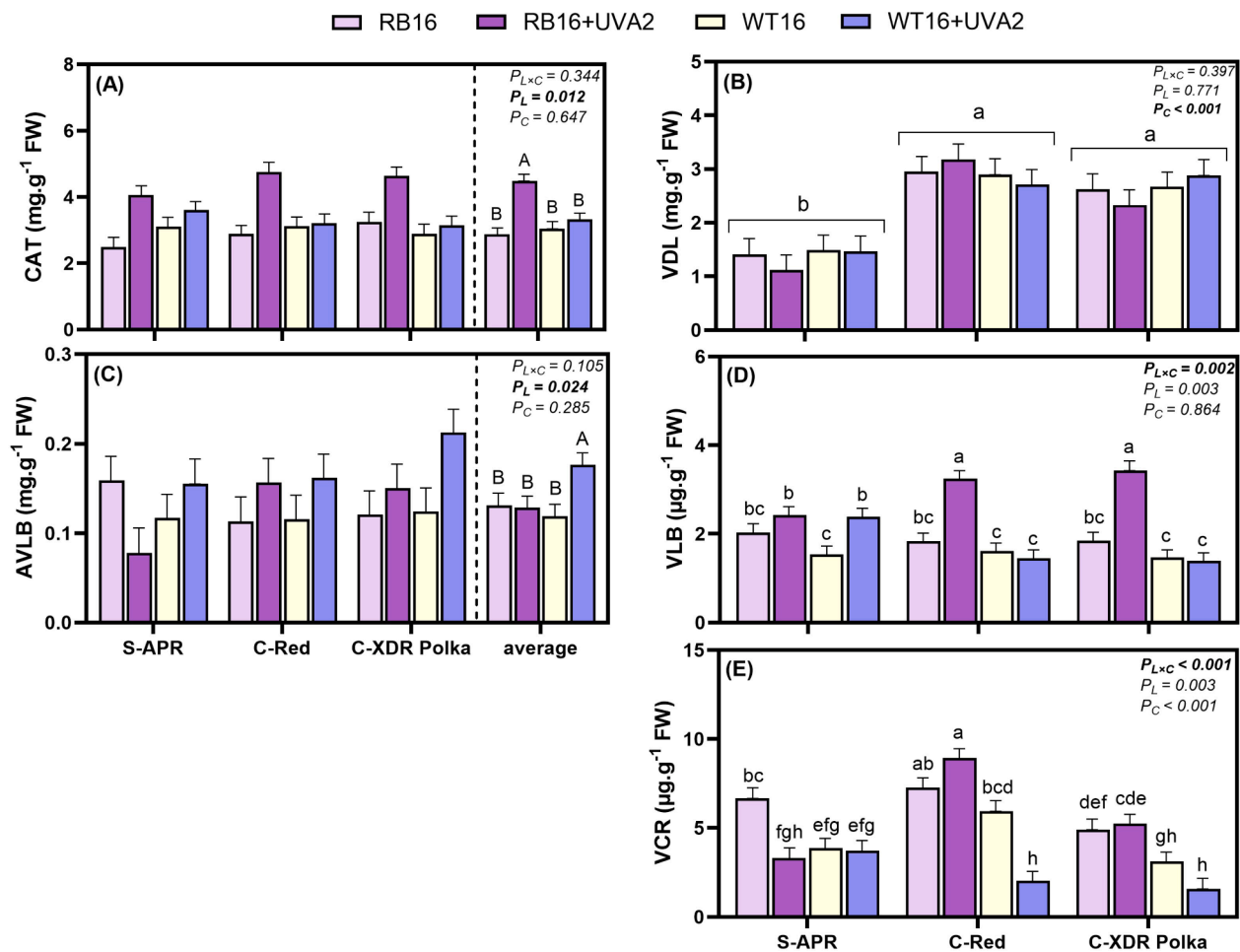


Fig. 6. Concentration of anticancer-related TIA [Catharanthine (CAT) (A), vindoline (VDL) (B), anhydrovinblastine (AVLB) (C), vinblastine (VLB) (D), and vincristine (VCR) (E)] per gram leaf fresh weight (LFW) of three *Catharanthus roseus* cultivars after 35 days of cultivation under 16 h of red-blue (RB16), white (WT16), RB16 + UV-A 2 h, and WT16 + UV-A 2 h light. The cultivars tested were Sunstorm Apricot (S-APR), Cora Red (C-Red), and Cora XDR Polka Dot (C-XDR-Polka). Mean values are based on three independent replicates ($n = 3$, each composed of five plants). Error bars represent the pooled standard error of the mean calculated from the ANOVA residual variance. p -values indicate the statistical significance of the main effects of light spectra (PL) and cultivar (PC), as well as their interaction ($PL \times C$), based on ANOVA. Different letters above the bars indicate significant differences according to Fisher's protected LSD test ($p = 0.05$). In panel A and C, the uppercase letters represent differences between light treatments. In panel B, the lowercase letters represent differences between cultivars. In panel D and E, lowercase letters represent differences between the cultivar $\times$ light treatment interaction.

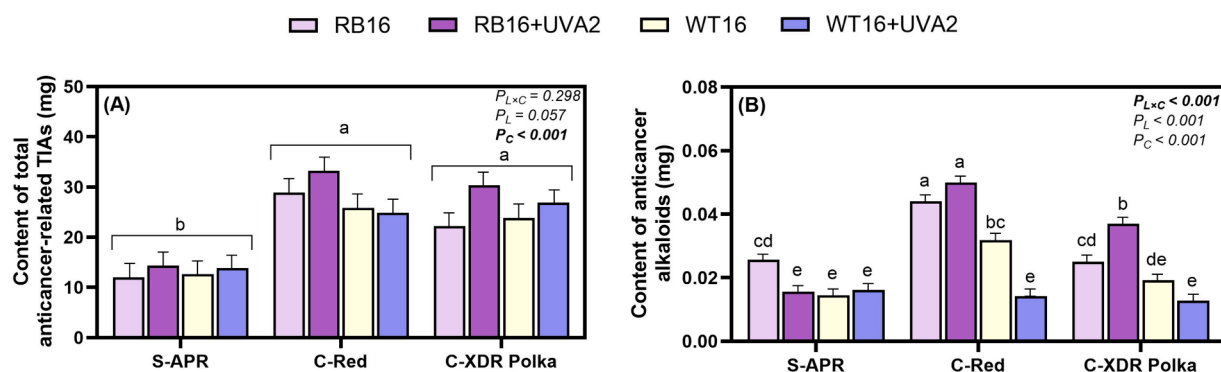


Fig. 7. Total anticancer-related TIAs content [measurements included catharanthine (CAT), vindoline (VDL), anhydrovinblastine (AVLB), vinblastine (VLB), and vincristine (VCR)] (A), along with the content of anticancer alkaloids (VLB and VCR) (B), of three *Catharanthus roseus* cultivars after 35 days of cultivation under 16 h of red-blue (RB16), white (WT16), RB16 + UV-A 2 h, and WT16 + UV-A 2 h light. The cultivars tested were Sunstorm Apricot (S-APR), Cora Red (C-Red), and Cora XDR Polka Dot (C-XDR-Polka). Mean values are based on three independent replicates ($n = 3$, each replicate composed of five plants). Error bars represent the pooled standard error of the mean calculated from the ANOVA residual variance. p -values indicate the statistical significance of the main effects of light spectra (PL) and cultivar (PC), as well as their interaction ($PL \times C$), based on ANOVA. Different letters above the bars indicate significant differences according to Fisher's protected LSD test ($p = 0.05$). In panel A, the lowercase letters represent differences between cultivars. In panel B, lowercase letters represent differences between the cultivar $\times$ light treatment interaction.

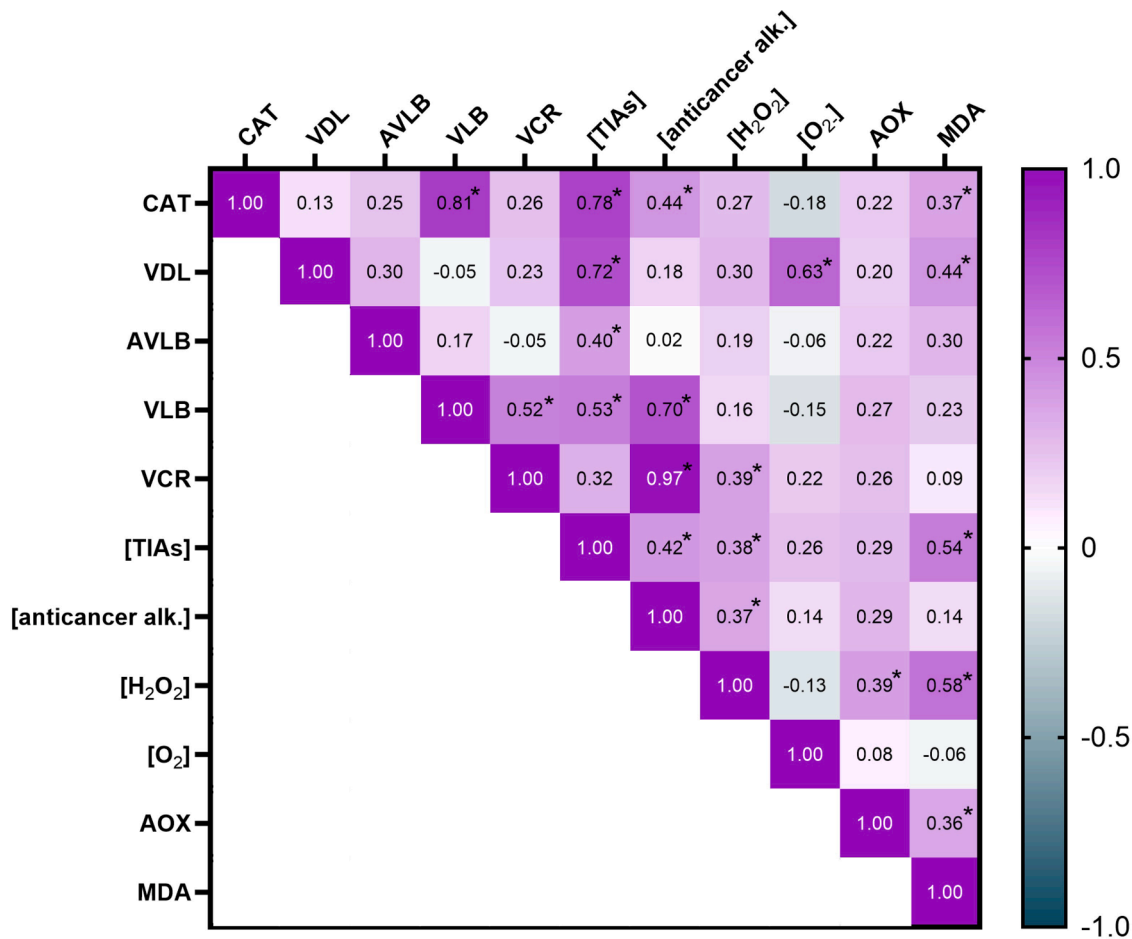


Fig. 8. Pearson's correlation coefficients (r) among alkaloid concentrations and biochemical stress indicators in *Catharanthus roseus* across light treatments and cultivars. The color gradients represent the magnitude and direction of the correlations (purple = positive; blue = negative), with * indicating significant correlations at $p = 0.05$. Measured alkaloids include monomeric [CAT] catharanthine, [VDL] vindoline, [AVLB] anhydrovinblastine, and dimeric [VLB] vinblastine and [VCR] vincristine. Total anticancer-related alkaloid concentration is expressed as [TIAs] and total anticancer alkaloids as [anticancer alk.]. Physiological stress markers include hydrogen peroxide (H₂O₂), superoxide (O₂⁻), total antioxidant capacity (AOX), and lipid peroxidation (MDA).

4. Discussion

4.1. Spectral composition modulates physiological performance, but plant morphology remains unaffected

This study demonstrated that the *C. roseus* genotype shaped all the studied morphological traits, with no significant contribution from photoperiod light or UV-A supplementation (Table 1). These findings align with hypothesis (iii), which proposed that cultivar identity would be the dominant driver of morphological outcomes due to genotype-specific growth habits. In contrast, the physiological plant responses were mostly modeled by light spectral composition (Fig. 3). Among the three cultivars tested, 'C-Red' consistently outperformed the other ones (in terms of total biomass, flower production, and leaf area), with 'S-APR' significantly lagging behind across most parameters. These differences in final DW are partly explained by inherent genetic variation among cultivars, which strongly influences vigor and developmental patterns. Altogether, the results confirm 'C-Red' as a high-performing genotype for controlled-environment cultivation and highlight substantial intraspecific variability, reinforcing the central role of genotype selection in determining performance under VF conditions (Lourenço et al., 2022, 2025b).

UV-A (315–400 nm) is increasingly recognized as a photomorphogenic signal due to its proximity in terms of wavelength to the visible blue light (400–500 nm), enabling activation of similar photoreceptor pathways that can enhance plant biomass, compactness, and overall

vigor (Sonjaroan et al., 2024; Qian et al., 2021). Despite these documented effects, the UV-A dose applied ($3.6 \text{ kJ}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) did not promote additional growth and even showed a slight decreasing trend in 'C-Red'. In this genotype, the tendency towards lower total dry weight under UV-A supplementation may indicate a subtle shift in resource allocation from primary growth toward specialized metabolism, consistent with known trade-offs between growth and alkaloid biosynthesis (Le Bot et al., 2009; Isah, 2019). This interpretation aligns with the increased alkaloid levels observed under RB16+UVA2 in later sections.

Although the photoperiod spectrum did not influence final biomass, clear differences emerged in photosynthetic activity (Fig. 3B). Photosynthetic rates were lower under WT compared with RB light, consistent with earlier findings in *C. roseus* 'Titan', where WT light resulted in reduced leaf fresh weight relative to monochromatic R (Ohashi et al., 2013). The higher photosynthetic rates observed under RB agree with the well-established high quantum efficiency of R and B wavelengths (McCree, 1972; Ke et al., 2024). Nevertheless, WT LEDs are increasingly adopted in VF primarily for operational reasons, including improved visual assessment of crops and worker comfort, rather than higher photosynthetic performance (Mitchell et al., 2012; Bugbee, 2016).

Interestingly, in the three studied cultivars, UV-A supplementation increased photosynthetic rates only in plants grown under polychromatic (WT) light (Fig. 3B). This differential response likely reflects differences in baseline photoreceptor excitation between spectral backgrounds. Under RB conditions, blue-light-responsive

photoreceptors such as cryptochromes and phototropins are already strongly activated by the blue component of the growth light, potentially saturating downstream signaling pathways controlling stomatal opening and photosynthetic regulation (Christie, 2007; Hogewoning et al., 2010; Kaiser et al., 2019). In contrast, under WT light, where blue photon enrichment is lower and spectrally diluted, supplementary UV-A can provide an additional effective stimulus for these photoreceptors (Verdaguer et al., 2017; Jenkins, 2017). Consequently, UV-A supplementation under WT enhances stomatal conductance and carbon assimilation, whereas under RB it confers little additional benefit. The enhancement of gas-exchange activity under WT16+UVA2 (Fig. 3C), therefore, suggests that UV-A contributed positively to photosynthetic regulation even without affecting plant biomass.

4.2. UV-A effect on alkaloid biosynthesis is modeled by the photoperiodic light spectrum

The biosynthesis and accumulation of total anticancer-related TIAs in *C. roseus* were strongly influenced by both cultivar and spectral composition (Fig. 5A). In line with our first hypothesis, UV-A supplementation enhanced TIA accumulation without compromising plant biomass. A similar response was observed for the valuable dimeric alkaloids VLB and VCR (Fig. 5B), although this effect was restricted to the 'Cora' cultivar series. Oxidative stress markers provide further insight into these plant responses. While neither photoperiodic light nor UV-A supplementation significantly alters ROS concentrations (Fig. 4A and B), UV-A significantly increased MDA under RB light by 30% (Fig. 4D), indicating lipid peroxidation and the onset of moderate oxidative stress. These findings suggest that UV-A exposure may act as a mild metabolic elicitor rather than a severe stressor, stimulating specialized metabolism without significantly reducing biomass. This is in line with the concept of a controlled plant elicitation, which can serve as a signal to activate specialized metabolism (Sharma et al., 2012; Noctor and Foyer, 2016). Moreover, recent evidence shows that at a molecular level, light spectral composition, particularly blue and near-UV wavelengths, upregulate transcription factors that can activate key biosynthetic enzymes, including phenylalanine ammonia-lyase (PAL) and downstream pathway genes, ultimately promoting the accumulation of specialized metabolites (Wu et al., 2025). In *C. roseus*, UV-B and blue light have been shown to induce TIA biosynthetic genes including tryptophan decarboxylase (TDC) and strictosidine synthase (STR), whereas red light regulates enzymes of the VDL pathway, including D4H and DAT (Aerts and De Luca, 1992; Vázquez-Flota and De Luca, 1998; Zhang et al., 2021). Additional UV-responsive modulation has been reported for enzymes involved in strictosidine conversion and alkaloid dimerization, including PRX1 (Zhong et al., 2021). However, many of these findings derive from stress-type elicitation or suspension culture systems rather than spectral optimization under controlled photon flux conditions. Therefore, although the present results are consistent with photoreceptor-mediated transcriptional regulation of the TIA pathway, direct validation through gene expression or enzyme activity profiling under whole-plant UV-A supplementation remains to be done.

ROS biosynthesis patterns were largely cultivar-dependent: H_2O_2 was consistently higher in 'C-Red', while O_2^- was elevated in both 'C-Red' and 'C-XDR-Polka' (Fig. 4A and B). These are the same cultivars that also produced the greatest TIA concentrations, suggesting that intrinsic ROS status may contribute to cultivar-specific metabolic responsiveness. Such ROS accumulation is consistent with a potential signaling role rather than oxidative damage (Noctor and Foyer, 2016; Mittler, 2017; Sharma et al., 2012; Rao et al., 2025), particularly given the absence of biomass penalties under UV-A supplementation (Table 1). However, in the absence of direct measurements of antioxidant enzyme activities, this interpretation remains provisional. While total antioxidant capacity did not show compensatory changes (Fig. 4C) we acknowledge that the ABTS assay provides a non-specific estimate and may not fully capture in vivo redox dynamics. Therefore, the present results support a correlative

association between ROS markers and alkaloid induction rather than a causal demonstration of controlled oxidative signaling.

To better understand the aforementioned relationships, a Pearson's correlation analysis was conducted between the studied biochemical markers and the alkaloid concentrations (Fig. 8). Total TIA production correlated positively with both H_2O_2 and MDA ($r = 0.38$; $p = 0.02$ and $r = 0.54$; $p = 0.001$, respectively), reinforcing their potential as predictive biomarkers for alkaloid induction. Notably, H_2O_2 also correlated positively with the final concentrations of the dimeric anticancer alkaloids VLB and VCR ($r = 0.37$; $p = 0.03$, respectively), supporting its role as a reliable indicator of downstream biosynthetic activity. H_2O_2 has been previously identified as a key signaling molecule in monoterpene indole alkaloid biosynthesis, mediating oxidative stress (Matsuura et al., 2014). Among the analyzed alkaloids, VCR showed the strongest association with total anticancer-related TIA levels ($r = 0.97$; $p < 0.001$), confirming its potential as a biochemical proxy for overall pathway activation (Lourenço et al., 2025b). While O_2^- did not correlate with total TIA or anticancer alkaloid concentration, its positive association with VDL suggests a possible involvement in early pathway steps. This interpretation is supported by evidence from *C. roseus* cell cultures, where elicitor-induced O_2^- production was shown to be necessary for PAL activation and CAT biosynthesis, whereas H_2O_2 played a less direct role in triggering these initial reactions (Xu and Dong, 2005). Together, these findings indicate that distinct ROS species may regulate different branches of the TIA pathway, with O_2^- acting upstream and H_2O_2 reflecting broader pathway activation and metabolic output. Nevertheless, these correlations do not establish causality, and the relatively low coefficients of determination indicate that ROS markers account for only part of the variability in alkaloid production, suggesting the involvement of additional regulatory mechanisms. Future work integrating antioxidant enzyme profiling and redox homeostasis markers will therefore be required to clarify the mechanistic role of specific ROS species in TIA regulation.

The moderate rise in MDA under RB16+UVA2 coincided with a significant increase in CAT, contributing to the higher TIA pool observed under this treatment (Fig. 4D, Figs. 5A, and 6A). This pattern suggests that mild membrane perturbation induced by UV-A may help redirect metabolic flux toward upstream precursors. Moreover, the individual alkaloids responded differently to spectral cues: AVL was preferentially enhanced under WT16+UVA2 (Fig. 6C), whereas CAT increased under RB16+UVA2 (Fig. 6A). This spectrum-dependent differentiation in TIA responses further supports hypothesis (ii). This represents an important finding, indicating that the composition of the photoperiodic light spectrum may selectively activate distinct branches of the TIA pathway, possibly via differential regulation of biosynthetic enzymes. Future research is needed to better explore these mechanisms.

Importantly, in the present study, UV-A supplementation was applied within a 16 h photoperiod, indicating that its effects likely occurred within an already active photoreceptor network. Cryptochromes, which absorb in the blue/UV-A range, function both as light-quality sensors and as regulators of circadian and photoperiodic pathways, interacting with phytochromes to coordinate developmental and metabolic processes (Ponnu and Hoecker, 2022). Therefore, UV-A treatment can induce the elicitation of specialized metabolites, and these responses to UV-A are also modulated by light-spectrum-dependent signaling cascades. However, because transcriptomic and enzyme-activity analyses were beyond the scope of this work, the proposed mechanistic links remain hypothetical and warrant targeted investigation in future studies.

Genotypic differences further highlight the importance of cultivar-specific strategies in alkaloid production. 'C-Red' achieved the highest yields of VLB and VCR under RB16 + UVA2 and RB16 through superior alkaloid accumulation, while 'C-XDR-Polka' also reached high anticancer yields under RB16+UVA2 (Fig. 6D and E). In contrast, 'S-APR' consistently displayed limited responsiveness, producing lower concentrations and yields across treatments, likely reflecting a restricted

capacity to upregulate TIA biosynthesis in response to light cues. These cultivar-specific patterns directly confirm hypothesis (iii) and reinforce earlier reports of strong intraspecific variability in *C. roseus* specialized metabolism (Kumar et al., 2013; Al-Andal, 2023; Lourenço et al., 2025b) and illustrate the complexity of genotype $\times$ environment interactions. Such physiological plasticity may arise from differences in photoreceptor sensitivity or transcriptional regulation, as suggested in other species (Monteagudo et al., 2021). Similar genotype-dependent responses to RB and UV-enriched spectra have been documented in tomato and gerbera (Ke et al., 2024; Lim et al., 2023), showing the need to consider intra-specific diversity when designing light-based elicitation strategies for optimized TIA production. Although the molecular basis of this variability remains largely unexplored in commercial cultivars, available genomic resources for *C. roseus* (Li et al., 2023) reveal extensive diversity in genes associated with TIA biosynthesis, transcriptional regulation, and stress signaling. It is therefore plausible that differences among cultivars may arise from variation in transcription factor networks, promoter sensitivity to light-regulated pathways, or redox signaling capacity. However, comparative analyses of photoreceptor gene expression, antioxidant enzyme activity, or pathway-specific transcript abundance among these cultivars have not yet been reported. Targeted transcriptomic and enzymatic profiling would be required to determine whether differences in photoreceptor sensitivity, ROS homeostasis, or key biosynthetic enzymes underlie the observed phenotypic divergence.

Beyond their mechanistic relevance, the spectrum- and genotype-dependent responses identified in this study have direct implications for the economic feasibility of anticancer alkaloid production in controlled-environment systems. In particular, the identification of light regimes and cultivars that selectively enhance downstream dimeric alkaloids without compromising biomass provides a critical biological foundation for economically viable plant molecular farming strategies. Within VF platforms, where energy and infrastructure costs remain dominant constraints (Benke and Tomkins, 2017), integrating cultivar-specific metabolic responsiveness with spectral optimization may therefore represent a key pathway toward scalable and economically sustainable production of high-value pharmaceutical compounds. Importantly, the UV-A supplementation applied in this study was limited to a short daily exposure (2 h), suggesting that metabolite enhancement can be achieved without substantially increasing total light energy input. In commercial VF systems, where lighting represents the highest operational cost, short-duration spectral interventions that increase target compound concentration per unit biomass may improve metabolite yield per kWh consumed. Thus, coupling genotype selection with temporally optimized UV-A supplementation may enhance both biological efficiency and economic feasibility of anticancer alkaloid production.

5. Conclusions

This study demonstrates that supplementing *C. roseus* with UV-A at $3.6 \text{ kJ}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ throughout the production cycle does not impact biomass production. In contrast, physiological and several biochemical responses were strongly shaped by light spectral composition. Among the spectral treatments tested, RB16+UVA2 consistently enhanced total anticancer-related TIA concentrations across the three studied cultivars. Moreover, in 'C-Red' it increased the concentration of the anticancer alkaloids (VLB and VCR) without reducing its biomass. These results highlight that daily short-term, low-intensity UV-A can function as a mild metabolic elicitor under specific spectral and genotypic contexts, supporting the hypothesis that UV-A supplementation promotes TIA biosynthesis without imposing growth penalties. Moderate oxidative shifts (reflected by higher MDA, O_2^- , and H_2O_2) aligned with increased alkaloid pools, especially VDL and total TIAs, suggest that ROS and lipid peroxidation are potential biomarkers associated with alkaloid biosynthesis in *C. roseus*. Nonetheless, the exact link between ROS homeostasis

and alkaloid biosynthesis remains to be fully established. Spectral specificity also emerged at the pathway level: RB16+UVA2 favored CAT accumulation, whereas WT16+UVA2 favored AVLb, indicating that the photoperiodic spectrum can selectively modulate distinct branches of the TIA pathway. Genotype played a decisive role in determining alkaloid output: 'C-Red' maximized anticancer alkaloids under RB16+UVA2 and RB16, 'C-XDR-Polka' also responded strongly to RB16+UVA2, whereas 'S-APR' showed limited responsiveness, consistent with cultivar-specific metabolic plasticity. Overall, R:B light combined with daily UV-A supplementation, throughout the cultivation period, represents a promising strategy for increasing anticancer alkaloid accumulation in selected *C. roseus* cultivars under controlled-environment conditions.

Data availability

The data are contained within the article.

CRediT authorship contribution statement

Marisa S.C. Lourenço: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Andreia Garrido:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Victor A.P. Freitas:** Validation, Supervision, Resources, Methodology. **Ep Heuvelink:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis. **Susana M.P. Carvalho:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

Plant material, facilities, and logistical support were provided by Viveiros Vitor Lourenço. One author is affiliated with this company. The company had no involvement in the study design, data collection, analysis, interpretation, or in the decision to publish the results.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2026.114803](https://doi.org/10.1016/j.scienta.2026.114803).

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