



Effects of light intensity and temperature on growth and secondary metabolite production of *Centella asiatica* in vertical farms

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Abstract

The functional compounds in *Centella asiatica* L., namely four types of triterpene glycosides (asiatic acid, asiaticoside, madecassic acid, and madecassoside), are highly valuable in various industries. As potent antioxidants, there is a demand to explore conditions that enhance their production and functional compound content for their application in diverse environmental conditions. This study investigates the cultivation of *C. asiatica* in vertical farms, seeking conditions that yield higher concentrations of functional compounds through two light intensities (100 ± 10 and $300 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$) and three temperature levels (control: 24/20°C, High I: 30/26°C, and High II: 36/32°C). As the temperature increased, the content of functional substances (total phenolic content (TPC), total flavonoid content (TFC)) and antioxidant activities (measured by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) assays), as well as most growth parameters including fresh and dry weights of both above-ground and below-ground parts, increased. However, a decrease in all parameters was observed at High II (36/32°C), with all photochemical chlorophyll fluorescence parameters exhibiting lower values. This study identified a trend in which proline content increased as temperature decreased from the optimal growth temperature (30/26°C) for *C. asiatica*. Among the two light intensities used in the experiment, PFD 100 enhanced biomass production, whereas PFD 300 increased the content of beneficial secondary metabolites. Higher temperature conditions (specifically High I: 30/26°C) are necessary to enhance the yield of *C. asiatica*, while functional compounds can be improved by increasing the light intensity to PFD 300. These findings suggest that for cultivating *C. asiatica* on vertical farms, treatment with High I (30/26°C)_PFD 300 provides the optimal balance between biomass production and triterpene glycoside content, making it the most efficient cultivation condition for commercial applications.

Keywords Chlorophyll fluorescence · Optimal growth condition · Photochemical quenching · Phytochemicals

1 Introduction

Centella asiatica L. is a perennial plant that thrives in warm, humid regions, such as Madagascar, the Indian Ocean, and the southern parts of India (Rahajanirina et al. 2012; Seevaratnam et al. 2012). This plant is rich in beneficial compounds, primarily triterpene glycosides, such as asiatic acid, asiaticoside, madecassic acid, and madecassoside, with higher concentrations in the aerial parts than in the underground parts (Eom et al. 2022; Min et al. 2024). These substances are known for their antimicrobial, anticancer, and antioxidant activities and are extensively used in the pharmaceutical industry for the alleviation of skin inflammation, improvement of venous insufficiency, enhancement of memory, and treatment of psychiatric disorders (Bylka et al. 2014; Ratz-Lyko et al. 2016; Pal et al. 2018). In South

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Korea, the cultivation of *C. asiatica* is limited to small regions primarily due to its specific climatic requirements (Choi et al. 2020). The plant optimally grows in tropical and subtropical environments with consistent warm temperatures (20–35 °C), high humidity (70–90%), and abundant rainfall, conditions that are only present in limited southern coastal areas of the Korean Peninsula (Kim et al. 2019). *C. asiatica* is primarily native to tropical and subtropical regions, typically thriving in partial shade environments with light intensities ranging from 100 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and temperatures between 20 °C and 35 °C (Chandra et al. 2011; Bylka et al. 2014). These natural habitat conditions significantly influence its growth characteristics, with photoperiods of 12–14 h promoting optimal vegetative development and metabolite production (Runkle 2002). Under such conditions, the plant exhibits enhanced biosynthesis of triterpene glycosides, which are the primary bioactive compounds of interest (Song et al. 2022; Alcalde et al. 2023). Additionally, *C. asiatica* is susceptible to frost damage and cannot survive in regions experiencing prolonged cold periods, further restricting its cultivation potential in Korea's temperate climate with distinct seasonal variations. These geographical and climatic limitations result in a heavy reliance on imports of raw materials, causing supply instability and price fluctuations in the domestic market (Choi et al. 2020; Song et al. 2022).

Traditional cultivation methods of *C. asiatica* include open-field systems in tropical regions and greenhouse cultivation in temperate zones, both of which are highly dependent on seasonal and climatic conditions (Singh et al. 2010; Nguyen et al. 2019). Recent sustainable cultivation approaches have explored hydroponics, aeroponics, and tissue culture systems to enhance biomass and bioactive compound production while reducing environmental dependency (Nguyen et al. 2019; Hoang and Rehman 2022). However, these methods still face challenges in maintaining year-round production and standardizing medicinal compound content. Therefore, to ensure supply stability, methods for continuous and sustainable cultivation of *C. asiatica* must be explored. Traditional cultivation methods of *C. asiatica* include open-field systems in tropical regions and greenhouse cultivation in temperate zones, both of which are highly dependent on seasonal and climatic conditions (Singh et al. 2010; Nguyen et al. 2019). Recent sustainable cultivation approaches have explored hydroponics, aeroponics, and tissue culture systems to enhance biomass and bioactive compound production while reducing environmental dependency (Nguyen et al. 2019; Hoang and Rehman 2022). However, these methods still face challenges in maintaining year-round production and standardizing medicinal compound content. Therefore, to ensure

supply stability, methods for continuous and sustainable cultivation of *C. asiatica* must be explored.

The potential for growth regulation and the enhancement of functional substances through abiotic stress treatments in plant factories has been a consistent area of research (Oh et al. 2009; Ahmed et al. 2020). Precise control of complex environmental parameters in plant factory systems enables the modulation of biosynthetic pathways responsible for secondary metabolite production, potentially leading to enhanced accumulation of bioactive compounds in medicinal plants such as *C. asiatica* (Kim et al. 2019; Nguyen et al. 2019). The ability to control complex environmental factors within plant factories enables simultaneous treatment of additional or highly interrelated environmental factors, leading to more effective outcomes (Baek et al. 2016; Miyagi et al. 2017). This integrated environmental control is particularly significant for medicinal plants like *C. asiatica*, where the biosynthesis of secondary metabolites is directly influenced by the interplay of multiple environmental cues. For instance, the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, which are responsible for the synthesis of triterpene glycosides in *C. asiatica*, are regulated by both light-dependent transcription factors and temperature-sensitive enzymes (Wongshaya et al. 2020; Bergman et al. 2024). Light intensity influences the expression of key genes in the triterpene biosynthetic pathway by activating photoreceptors like phytochromes and cryptochromes, which subsequently affect the production of transcription factors that regulate the expression of genes involved in triterpene synthesis (Yadav 2020; Kamol et al. 2024). Similarly, temperature modulates enzyme activity and protein stability within these pathways, with optimal temperatures enhancing the catalytic efficiency of rate-limiting enzymes such as 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and squalene synthase (SQS), leading to increased triterpene accumulation (Alcalde et al. 2023). Consequently, the control of complex environmental factors in plant factories could assist in establishing cultivation conditions not only for plant growth but also for the enhancement of secondary metabolites (Nguyen et al. 2019; Ampim et al. 2022).

Photosynthesis is a crucial physiological process by which plants convert light energy into chemical energy, which is then transformed into biomass (Farquhar et al. 2001). The light absorbed by chlorophyll, which is not used for photosynthesis, is lost as heat or emitted as fluorescence (Baker and Rosenqvist 2004; Baker 2008). In plant factories, where conditions differ significantly from those in field cultivation, adjusting the environmental conditions to facilitate proper photosynthesis is important (Hang et al. 2019; Ouyang et al. 2020). The use of chlorophyll fluorescence imaging systems allows the acquisition of information on

chlorophyll fluorescence within the plant canopy through chlorophyll fluorescence parameters and images (Yao et al. 2018; Guidi et al. 2019). The sensitivity of the photosynthetic process to abiotic and biotic stress factors is crucial for assessing the changes in photosynthesis caused by these stresses (Choudhury et al. 2017; Gull et al. 2019; Gonzalez Guzman et al. 2022). Abiotic stress factors, such as light, high temperature, low temperature, and salinity can reduce photosynthesis and induce physiological changes in plants, leading to decreased growth and yield (He et al. 2018). Therefore, establishing a system that can quantitatively evaluate photosynthetic levels is important (Ashraf and Harris 2013). Song et al. (2022) investigated changes in the growth of *C. asiatica* by adjusting the light quality and intensity, whereas another researcher examined the effects of adding light quality on the elongation of petioles and increase in growth (Harakotr et al. 2024). In terms of temperature, a notable study explored the optimal temperature conditions for *C. asiatica* by examining the increase in leaf area (Hamid et al. 2002; Pipatsitee et al. 2023a). This reflects the major factors affecting photosynthesis, light intensity, and temperature in modern greenhouse production (Zhou et al. 2019). Evaluating factors associated with cultivation conditions is paramount for optimizing plant growth and development. Research on individual conditions for crop cultivation in plant factories is common; however, studies on combined environmental factors are rare. Specifically, enhancing the growth and physiological activity of *C. asiatica* by increasing the levels of key compounds, such as asiatic acid, asiaticoside, madecassic acid, and madecassoside has been less explored compared to other crops. The regulatory mechanisms governing triterpene glycoside biosynthesis in *C. asiatica* under different environmental conditions remain largely unexplored, particularly how the combined effects of light intensity and temperature influence both primary metabolism (growth and development) and secondary metabolism (bioactive compound production). Understanding these mechanisms is crucial for developing cultivation protocols that can simultaneously optimize biomass production and enhance the medicinal quality of the harvested material. Thus, optimal cultivation conditions must be established to boost growth and secondary metabolite production through combined environmental treatments. This study aimed to determine the best conditions for *C. asiatica* cultivation in plant factories, focusing on maximizing the production of its bioactive compounds by adjusting the light intensity and temperature.

2 Materials and methods

2.1 Plant materials and cultivation

C. asiatica was purchased from a specialized Centella cultivation farm located in Hapcheon, Korea, and transplanted into high beds filled with horticultural soil (Chorok-i, Non-gwoobio Co. Ltd., Suwon, Korea) in a plastic greenhouse at Jeonbuk National University, Jeonju, Korea. It was cultivated for two months using a leaf nutrient solution (Yamazaki 1982) at 1.5 dS m^{-1} . All treatments were sub-irrigated twice daily for 10 min each. New seedlings bearing four to six true leaves were cut to create uniform experimental plants, which were grown in a custom growth chamber for one week before the experiment. During the treatment period, temperature and relative humidity in the chamber ($65 \text{ cm} \times 65 \text{ cm} \times 60 \text{ cm}$, $l \times b \times h$) were maintained at $24/20^\circ\text{C}$ (day/night) and $50 \pm 5\%$, respectively.

2.2 Experimental design

The experimental treatments were conducted in the growth chamber described in Sect. 2.1, equipped with warm white LED lights (L-PEC Co., Ltd., Jeollabukdo, Korea) and temperature control systems. Plants were subjected to a combination of two light intensities (100 ± 10 and $300 \pm 10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PFD) and three temperature regimes (control: $24/20^\circ\text{C}$, High I: $30/26^\circ\text{C}$, and High II: $36/32^\circ\text{C}$ day/night temperatures) (Fig. 1). The experimental parameters were meticulously established based on *C. asiatica*'s natural habitat characteristics and previous research findings. Ecological studies on *C. asiatica*'s natural habitats in tropical and subtropical regions (Bylka et al. 2014) have demonstrated that the species typically grows under partial shade conditions with light intensities ranging from 100 to $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and temperatures fluctuating between 20°C and 35°C . The most vigorous *C. asiatica* populations were found in areas with daytime average light intensities of $250\text{--}350 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and daytime average temperatures of $28\text{--}32^\circ\text{C}$, as reported in study (Author et al., 2020). Incorporating these natural habitat data alongside previous controlled environment cultivation studies (Song et al. 2022; Pipatsitee et al. 2023b), we established that $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ represents the lower threshold for adequate growth of *C. asiatica* in indoor vertical farming systems, while $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ corresponds to the optimal light intensity for maximizing bioactive compound accumulation without inducing photo-inhibition (Harakotr et al. 2024). Based on this evidence, these two representative light intensity levels (100 ± 10 and $300 \pm 10 \mu\text{mol m}^{-2} \text{ s}^{-1}$) were selected for this study. Temperature conditions were determined based on the physiological research conducted by Pipatsitee et al. (2023b),

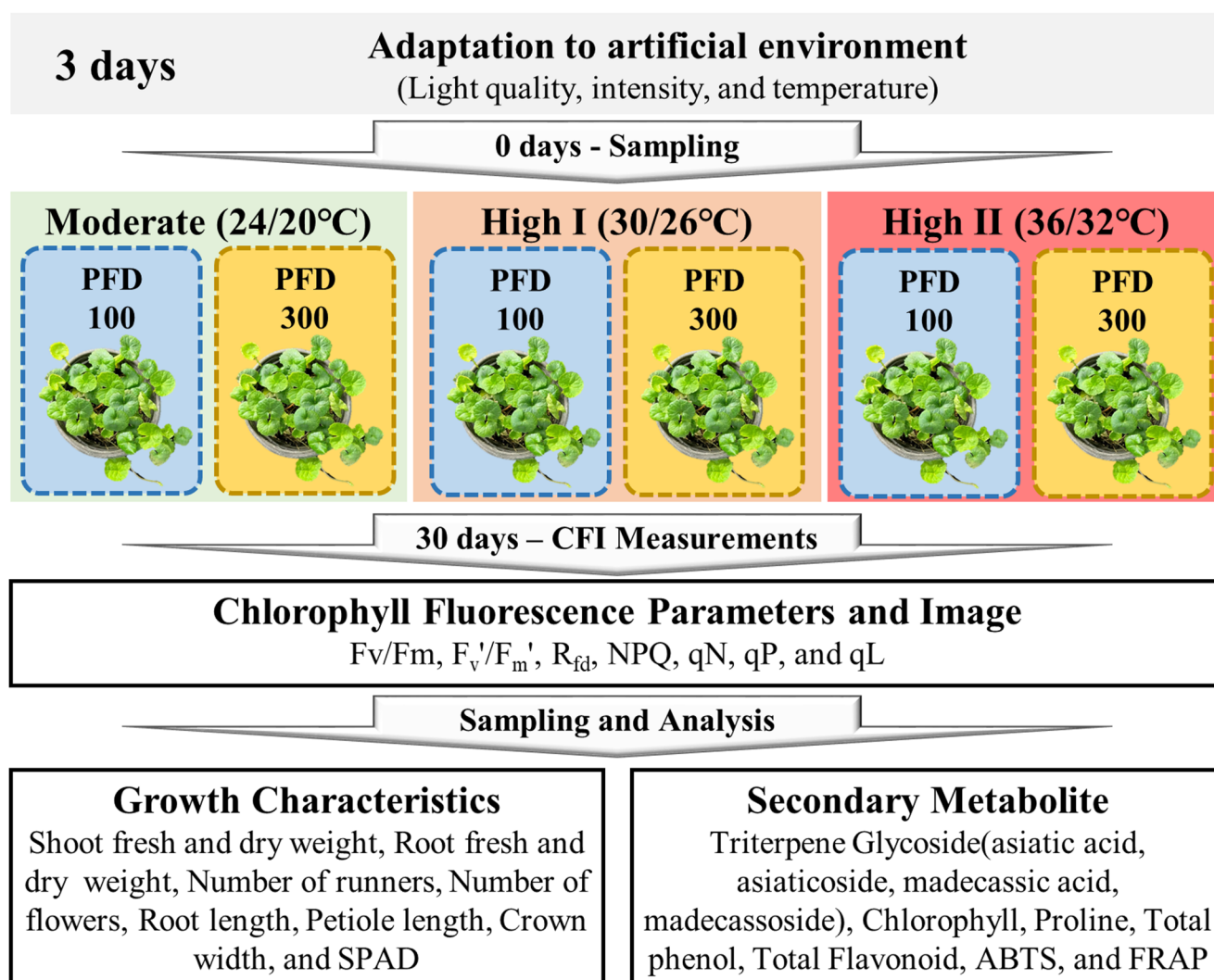


Fig. 1 Overview of individual experimental processes and measurement timeline

which identified 24/20°C (day/night) as optimal for biomass accumulation and secondary metabolite production in *C. asiatica* under controlled conditions. Consequently, this was established as the control temperature (24/20°C), while two additional temperature regimes were set to evaluate the plant's response to moderately elevated (High I: 30/26°C) and significantly elevated (High II: 36/32°C) temperatures that might occur in vertical farming environments. Across all temperature treatments, a consistent day-night temperature differential (DIF) of 4 °C was maintained to simulate *C. asiatica*'s natural habitat. According to Bylka et al. (2014), *C. asiatica*, native to tropical and subtropical regions, has adapted to day-night temperature fluctuations typically ranging from 3 to 7 °C, which significantly influence photosynthate distribution and respiratory efficiency. Research by Song et al. (2023) and Chandra et al. (2011) reported that moderate DIFs promote balanced vegetative growth and bioactive compound accumulation in *Panax ginseng* and

Cannabis sativa L. The 4 °C DIF was selected after integrating these previous findings with our preliminary experimental results. The photoperiod was set at 14/10 h (day/night) to reflect the seasonal average observed in *C. asiatica*'s natural habitats, and relative humidity was maintained at ambient levels ($50 \pm 5\%$) to account for natural environmental variations. After 30 days of cultivation, three plants from each light and temperature combination treatment category were harvested and separated into shoot and root parts. Each part was subsequently analyzed for growth parameters, major triterpene glycosides, and antioxidants (FRAP, ABTS, total phenol, and flavonoid content).

2.3 Growth measurements

The experiment was conducted over 30 d, with three plants per treatment randomly selected at 15-d intervals for a total of three times. The soil particles were removed and the roots

were washed before measuring the growth indicators of the shoots and roots. The biomass and dry weight (g) of the shoots and roots, petiole length (mm), crown width (mm), number of flowers (ea), number of runners (ea), root length (mm), and SPAD were investigated. This study describes the morphological structures and characteristics of *C. asiatica*, including leaves, petioles, flowers, and runners, with reference to the research by Noppradit et al. (2024) and Kandasamy et al. (2023). The biomass of the shoots, including leaves and petioles, and the dry weights of the shoots and roots were measured using a digital weighing machine (UX420H; Shimadzu Corp., Kyoto, Japan) after freeze-drying (ilShinBioBase Co., Ltd., Gyeonggi-do, Korea) for one week.

2.4 Analysis of triterpene glycoside content

The analysis of the four triterpene glycosides—asiatic acid, asiaticoside, madecassic acid, and madecassoside—was conducted using high-performance liquid chromatography (HPLC; Agilent 1200 series, Agilent Technologies, Santa Clara, CA, USA). The methodology was adapted from Song et al. (2022), ensuring the reproducibility and accuracy of the quantifications. All chemical reagents used in the analysis were sourced from Sigma-Aldrich Co. (St. Louis, MO, USA), providing high-grade purity to maintain consistency in experimental results. For this process, 50 mg of freeze-dried powdered *C. asiatica* was extracted with 5 mL of 80% methanol in a shaker for one hour. After extraction, the samples were centrifugation at 3,500 rpm for 10 min. The resultant clear supernatants were then passed through a 0.45 µm membrane filter and collected into 1.5 mL vials

in preparation for HPLC analysis. Each analysis involved injecting a 10 µL aliquot of the sample into the HPLC system, which utilized a diode array detector set to a wavelength of 292 nm for detecting peaks. The compounds were separated using a Nova-Pak C18 column with a mobile phase gradient that included solvent A (100% distilled water, HPLC grade) and solvent B (100% acetonitrile, HPLC grade) flowing at a rate of 1.3 mL/min. The elution program was designed to progressively increase the concentration of solvent B from 20 to 95% within the first 7 min, maintain that concentration for 15 min, return to 20% after 21 min, and hold it steady until 25 min. Calibration curves for the quantification of each triterpene glycoside were constructed using standard solutions provided by Sigma-Aldrich, with concentrations ranging from 25 to 200 µg/mL. These curves were based on the retention times and peak areas derived from the HPLC analysis, ensuring accurate quantification of the glycosides in the samples (Fig. 2.).

2.5 Chlorophyll fluorescence parameters and images

Using a Chlorophyll Fluorescence Imaging apparatus, specifically the Fluorcam FC 800-O/1010 (PSI, Drasov, Czech Republic), we successfully acquired both imagery and parameters related to chlorophyll fluorescence across 16 distinct types (Table 1). Prior to fluorescence measurements, the plants underwent a dark adaptation process for a duration of 20 min. Subsequently, the spatial separation between the camera and plant was meticulously calibrated to a fixed distance of 20 cm. After dark adaptation, the instrumental settings (Act 2 light, super light, Sensitivity,

Fig. 2 High-performance liquid chromatography (HPLC) chromatogram of four triterpene glycosides in the (a) standard mixture and (b) sample extract. 1: madecassoside; 2: asiaticoside; 3: madecassic acid; and 4: asiatic acid

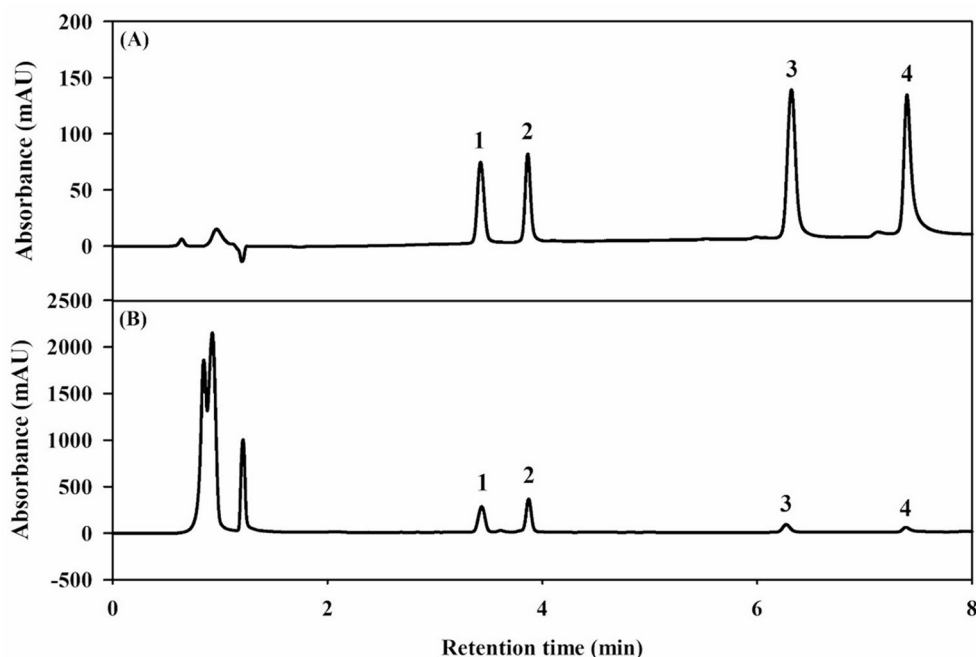


Table 1 List of the abbreviations and their descriptions used in this study

Parameter	Formula	Description
F_v/F_m	$(F_m - F_0)/F_m$	Maximum quantum yield of PSII photochemistry measured in the dark-adapted state
F_v'/F_m'	$(F'_m - F'_0)/F'_m$	Exciton transfer efficiency from antenna pigments to the reaction center of photosystem II (PSII) in the light-adapted state
Y(II)	$(F'_m - F_s)/F'_m$	Effective quantum yield of photochemical energy conversion in PSII
NPQ	$(F_m - F'_m)/F'_m$	Non photochemical quenching of maximum fluorescence
qP	$(F'_m - F_s)/(F'_m - F'_0)$	Photochemical quenching of PSII
qN	$(F_m - F'_m)/(F_m - F'_0)$	Coefficient of non-photochemical quenching of variable fluorescence
qL	$qP \times F_0/F_s$	Coefficient of photochemical quenching of variable fluorescence based on the lake model of PSII
Y(NO)	$1/[NPQ + 1 + qL(F_m/F_0 - 1)]$	Quantum yield of non-regulated energy dissipation in PSII
Y(NPQ)	$1 - \phi_{PSII} - \phi_{NO}$	Quantum yield of regulated energy dissipation in PSII
Rfd	$(F_m - F_s)/F_s$	Ratio of fluorescence decline

and Shutter) were finely tuned to ensure a homogenous distribution of fluorescence emissions across the foliage at an intensity level of 1500–1600. The criteria for chlorophyll fluorescence measurements were adhered rigorously to and remained invariant throughout the analysis of all plants.

2.6 Chlorophyll and proline analysis

For the analysis of chlorophyll, the method of Shin et al. (2020) was adapted and applied to this study. Freeze-dried samples (0.05 g) were suspended in 10 mL of methanol and extracted for 6 h at room temperature with continuous shaking at 150 rpm. The extract was centrifuged at $2,500 \times g$ for 15 min, and the absorbance of the supernatant was measured at 652 and 665 nm using a microplate reader (Multiskan, Thermo Scientific Inc.). The contents of chlorophyll a and b were calculated according to the path length-corrected formula.

Proline content was determined using an improved analytical method adapted from Shin et al. (2020). Freeze-dried powdered samples (0.05 g) were mixed with 5 mL of 3% sulfosalicylic acid and extracted by shaking at 150 rpm for 1 h. The extract was centrifuged at $2500 \times g$ for 12 min and

filtered through a 0.2- μ m filter. Equal volumes (500 μ L) of the filtered supernatant, acetic acid, and acid ninhydrin were simultaneously combined in a 15 mL tube, mixed, and then incubated in a water bath at 95 °C for 60 min. The reaction mixture was rapidly cooled on ice, followed by the addition of 1 mL of toluene and shaking for 30 s, then centrifuged at 3,600 rpm for 10 min. The absorbance of the toluene layer (200 μ L) was measured at 520 nm, and proline content was quantified using a standard L-proline curve with a linear range of 0–100 μ g mL⁻¹.

2.7 Total phenol and total flavonoid content

Total polyphenol content (TPC) was determined by extracting 50 mg of powdered sample with 1.5 mL of 80% methanol (MeOH), followed by incubation in a water bath at 50 °C with agitation at 150 rpm for 1 h (Bhandari et al. 2022). Following extraction, the mixture was centrifuged at 14,000 rpm for 10 min at a temperature of 4 °C. The resultant extract was then filtered through a membrane filter with a 0.45 μ m pore size, preparing it for total phenol and flavonoid content analyses. In summary, 200 μ L of the supernatant was diluted with 600 μ L of distilled water in a 2 mL tube. To this, 200 μ L of Folin–Ciocalteu reagent (Sigma-Aldrich) was added. The mixture was then vigorously mixed, followed by incubation in a 27 °C water bath for 5 min. Subsequently, 200 μ L of 15% sodium carbonate (Sigma-Aldrich) was introduced to the solution, which was then left to incubate under dark conditions at room temperature (25 °C) for an hour. After another round of centrifugation at 12,000 rpm for 10 min at 4 °C, the absorbance of a 200 μ L aliquot of the extract was measured at 760 nm using a microplate reader (Multiskan GO; Thermo Fisher Scientific Inc., Waltham, MA, USA). A standard curve was generated using gallic acid (Sigma-Aldrich) concentrations ranging from 10 to 100 μ g/mL, and the results were reported as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g⁻¹ DW).

According to Shin et al. (2021), the total flavonoid content (TFC) was analyzed using the same extract previously employed for total phenolic content (TPC) assessment. In a condensed procedure, 200 μ L of the sample extract was combined with 800 μ L of water in a 2 mL tube. To this mixture, 60 μ L of 5% sodium nitrite solution from Sigma-Aldrich was added. After a 5-min interval, 60 μ L of 10% aluminum chloride solution and 400 μ L of 1 M sodium hydroxide solution, both sourced from Sigma-Aldrich, were introduced simultaneously. The absorbance of a 200 μ L aliquot of the extract was measured at 510 nm using a microplate reader (Multiskan Go; Thermo Fisher Scientific Oy, Vantaa, Finland). For quantification purposes, a standard curve was established using concentrations ranging from 10

to 100 µg/mL of catechin hydrate (Sigma-Aldrich), which facilitated the calculation of TFC expressed in milligrams of catechin hydrate equivalent per gram of dry weight (mg CE g⁻¹ DW).

2.8 Measurement of antioxidant activities

Bhandari et al. (2022) employed two distinct methodologies to ascertain the antioxidant capabilities of *C. asiatica*: ferric reducing antioxidant power (FRAP) assay and the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Antioxidant activity was evaluated using the same supernatant derived for TPC and TFC analyses. To obtain the FRAP solution, a mixture comprising 300 mM acetate buffer (blending 3.1 g of sodium acetate trihydrate with 16 mL of acetic acid, adjusted to pH 3.6), 10 mM 2,4,6-Tris (2-pyridyl)-s-triazine dissolved in 40 mM HCl, and 20 mM ferric chloride hexahydrate (all from Sigma-Aldrich) was prepared in a volumetric ratio of 10:1:1. Following this preparation, 50 µL of the supernatant was amalgamated with 950 µL of the freshly prepared FRAP solution and subsequently incubated at 37 °C for a duration of 10 min. The absorbance of a 200 µL reaction mixture sample was then measured at 593 nm employing a microplate reader. A standard curve was established using various concentrations (0–1000 µM) of Trolox (Sigma-Aldrich), facilitating the determination of antioxidant capacity, which is denoted as µM Trolox equivalent antioxidant capacity per gram of dry weight (µM TE g⁻¹ DW). For the ABTS assay, ABTS radical cation (ABTS⁺) was prepared by mixing 7.4 mM ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (Sigma-Aldrich) and 2.4 mM potassium persulfate (Sigma-Aldrich) solution at equal ratio (v/v) in the dark for 16 h at room temperature (25 °C). The solution was then diluted with MeOH to obtain an absorbance of approximately 0.90 at 734 nm. Fifty microliters of supernatant was mixed with 950 µL of ABTS⁺ solution, vortexed, and stored in the dark at room temperature (25 °C) for 2 h. The absorbance of the reaction mixture (200 µL) was measured at 734 nm using a microplate reader. Trolox (100–1000 µM) was used to generate a standard curve by applying the same method as in the sample. The results were then expressed as µM TE g⁻¹ DW.

2.9 Statistical analysis

All statistical analyses were conducted using RStudio software, version 3.4 (RStudio Inc., Boston, MA, USA). The growth parameters are reported as the mean of four biological replicates, while the concentrations of triterpene glycosides, total polyphenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (assessed

by FRAP and ABTS assays) are presented as the mean of three technical replicates. Differences among data were evaluated using Two-way ANOVA, and significant effects were further analyzed using Duncan's multiple range test. Additionally, heatmap analysis was employed to visualize patterns and correlations among all datasets. Figures were generated using SigmaPlot12 software (Systat Software Inc., San Jose, CA, USA).

3 Results

3.1 Growth characteristics

In the cultivation of *C. asiatica*, the growth parameters exhibited significant differences based on the levels of photosynthetic photon flux density (PPFD) and temperature (Figs. 3 and 4). Specifically, a notable effect was observed across different temperature levels [Moderate (24/20 °C), High I (30/26 °C), and High II (36/32 °C)] compared to the applied light intensities PPFD 100 and PPFD 300. Visually, the High I and II conditions resulted in an increased number of shoots and runners compared with the moderate condition, with all PPFD 300 treatments showing some leaf senescence, as opposed to those under PPFD 100 (Fig. 3). The effects of temperature and light intensity on aerial fresh weight, aerial dry weight, and number of runners were significant, with the highest values across all growth parameters observed at 30d_High I_300, whereas the lowest values were recorded at 30d_High II_100 (Fig. 3A, B,D). An increase in light intensity led to an increase in the number of flowers; however, the highest temperature conditions under PPFD 100 resulted in the lowest values. The SPAD values were not significantly different among the experimental treatments. Visually, an increase in exposure to high temperatures compared to moderate conditions resulted in decreased root growth, a notable change in root color to brown, and disintegration (Fig. 4). The fresh root weight, measured by washing off the soil at the end of the experiment on day 30, was significantly lowest in the 30d_High II_100 treatment (Fig. 4A). A similar outcome was observed for root dry weight, with stem diameter showing lower values in the 30d_High II_100 treatment than that in the other treatments (Fig. 4B, E). Root length was highest in the 30d_Moderate_PPFD 300 treatment (24.39 mm), which was significantly higher than most other treatments (Fig. 4C). The 30d_High I_PPFD 300 treatment also showed relatively high root length (13.78 mm), though not statistically different from 30d_Moderate_PPFD 100. The petiole length showed variation across treatments with the highest value observed in the 30d_High I_PPFD 100 treatment (79.17 mm), though statistically similar to

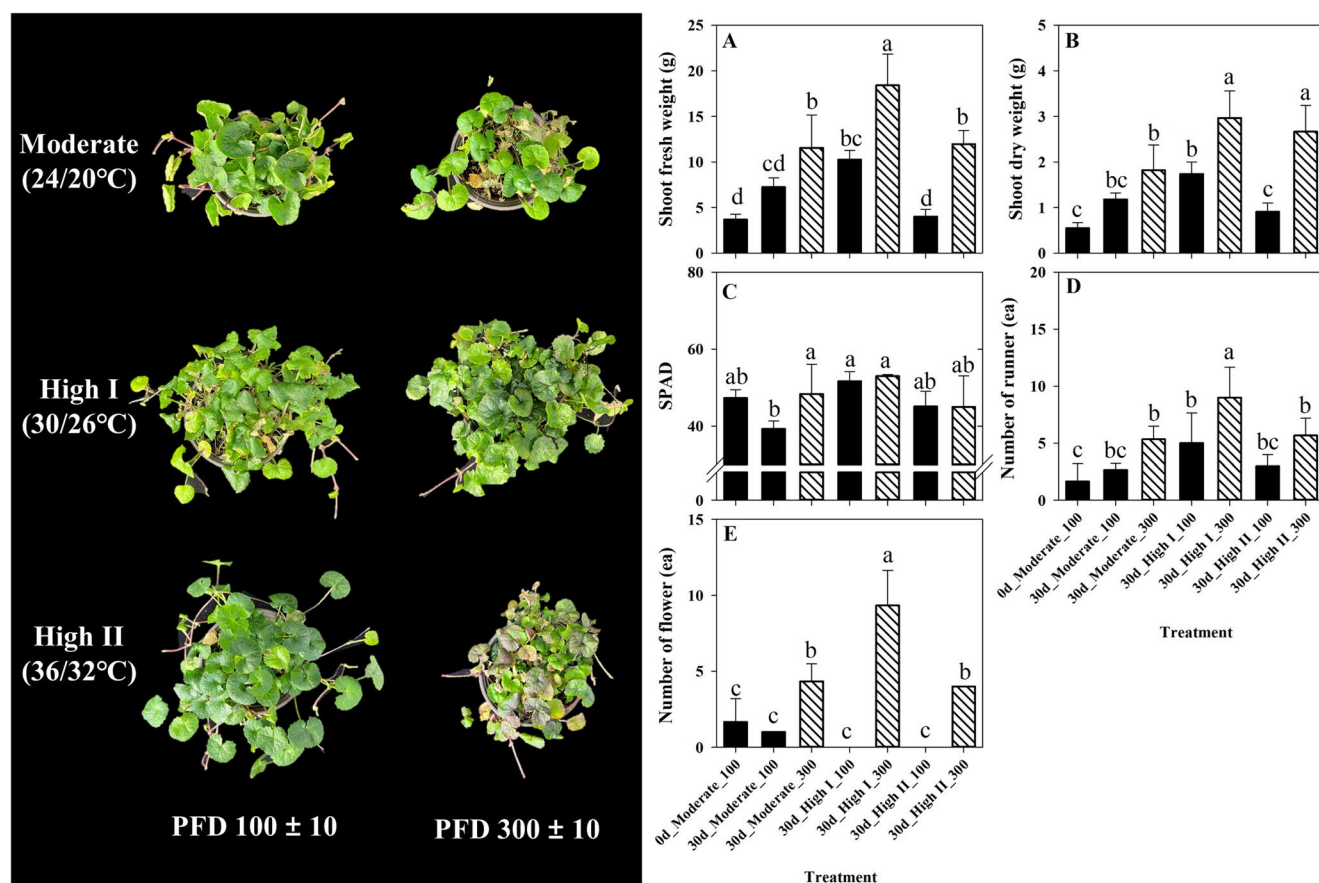


Fig. 3 Appearance of top view and growth parameters [shoot fresh weight (A), shoot dry weight (B), SPAD (C), number of runners (D), and number of flowers (E)] of *C. asiatica* L. grown under light intensity (PPDF 100 and 300) and temperature [Moderate (24/20°C), High

I (30/26°C), and High II (36/32°C)] treatment on the 30th d. Each bar represents the mean $\pm$ SD of three biological replicates in growth parameters. Different letters within a figure indicate a significant difference at $p < 0.05$ by Duncan's multiple range test

several other treatments including 0d_Moderate_PDF 100, 30d_High I_PDF 300, and 30d_High II_PDF 100 (Fig. 4D).

3.2 Chlorophyll fluorescence parameters and images

We observed that the ratios of major parameters indicating the partitioning of light energy used in photosynthesis, $Y(NO)$, $Y(NPQ)$, and $Y(II)$, varied more significantly with temperature than with light intensity (Fig. 5). $Y(II)$, which represents the fraction of light energy utilized in photosynthesis, showed the most significant proportion at 30d_Moderate 300 (0.25 ± 0.0206) and the lowest at 30d_High II 100 (0.07 ± 0.0096). As the temperature increased, $Y(NO)$ also increased, reaching its highest ratio in 30d_High II 100 (0.45 ± 0.0480). $Y(NPQ)$ recorded its lowest value at 30d_High II 100 (0.48 ± 0.0403), whereas it was relatively higher at 30d_High I 100 (0.65 ± 0.0037), indicating that temperature treatments have a profound effect on these photosynthetic efficiency parameters.

Under the High II (36/32°C) condition, images for most chlorophyll fluorescence parameters (Fv/Fm , NPQ, $Y(II)$, qL) indicated a decrease in the efficiency levels of light energy utilized in photosynthesis (Table 2). At High II, both PFD 100 (0.66 ± 0.06) and PFD 300 (0.65 ± 0.1) exhibited a decrease in the most commonly used fluorescence parameter, Fv/Fm , which reflects the quantum yield of primary photochemical reactions at open PSII reaction centers in the dark-adapted state. A significant reduction was also observed in Fv'/Fm' , Rfd, NPQ, qN , qP , and qL at High II compared to other temperature treatments. Most parameters showed significant differences across temperature treatments, whereas a significant relationship was observed only for parameters other than Fv/Fm and Fv'/Fm' for light treatments. A significant interaction was observed only for Rfd, NPQ, and qP between the temperature and light treatments.

The chlorophyll fluorescence parameters were visually interpreted using images for each parameter. Chlorophyll Fluorescence Images (CFI) were based on color changes corresponding to the fluorescence levels of each measured parameter. Within the range of chlorophyll fluorescence

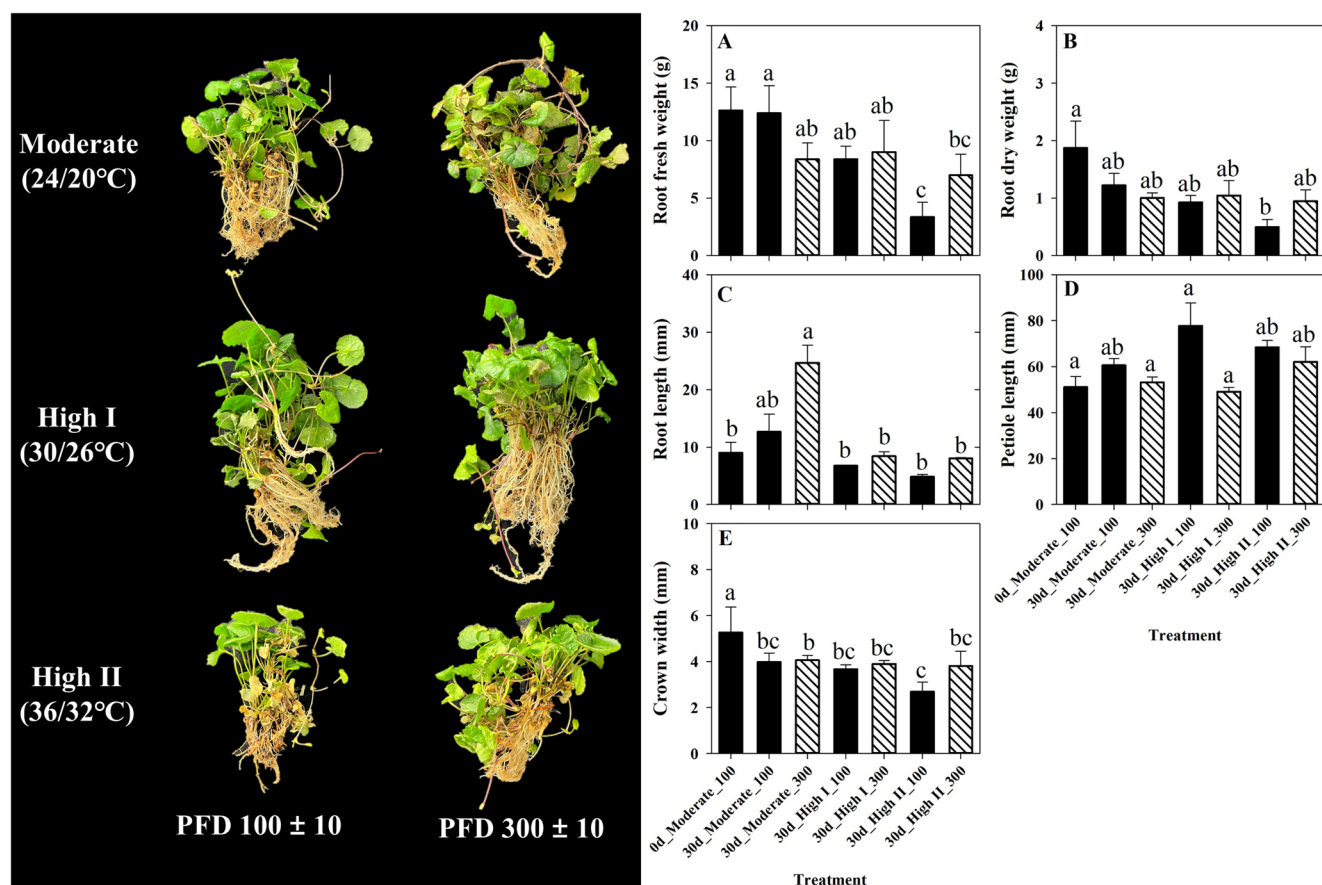


Fig. 4 Appearance of phenotype and growth parameters [root fresh weight (A), root dry weight (B), root length (C), petiole length (D), and crown width (E)] of *C. asiatica* L. grown under light intensity (PPDF 100 and 300) and temperature [Moderate (24/20°C), High I (30/26°C), and High II (36/32°C)] treatment on the 30th d. Each bar represents the mean ± SD of three biological replicates in growth parameters. Different letters within a figure indicate a significant difference at $p < 0.05$ by Duncan's multiple range test

Fig. 5 Appearance of partitioning of light energy according to light intensity (PPDF 100 and 300) and temperature [Moderate (24/20°C), High I (30/26°C), and High II (36/32°C)] treatment on the 30th d in *C. asiatica* L. [White: Rapid light curves of effective PSII quantum ($Y(II)$), hatch: quantum yield of regulated energy dissipation ($Y(NPQ)$), and Gray: quantum yield of nonregulated energy dissipation ($Y(NO)$)]. Each bar represents the mean ± SD of four biological replicates in chlorophyll fluorescence parameters. Different letters within a figure indicate a significant difference at $p < 0.05$ by Duncan's multiple range test

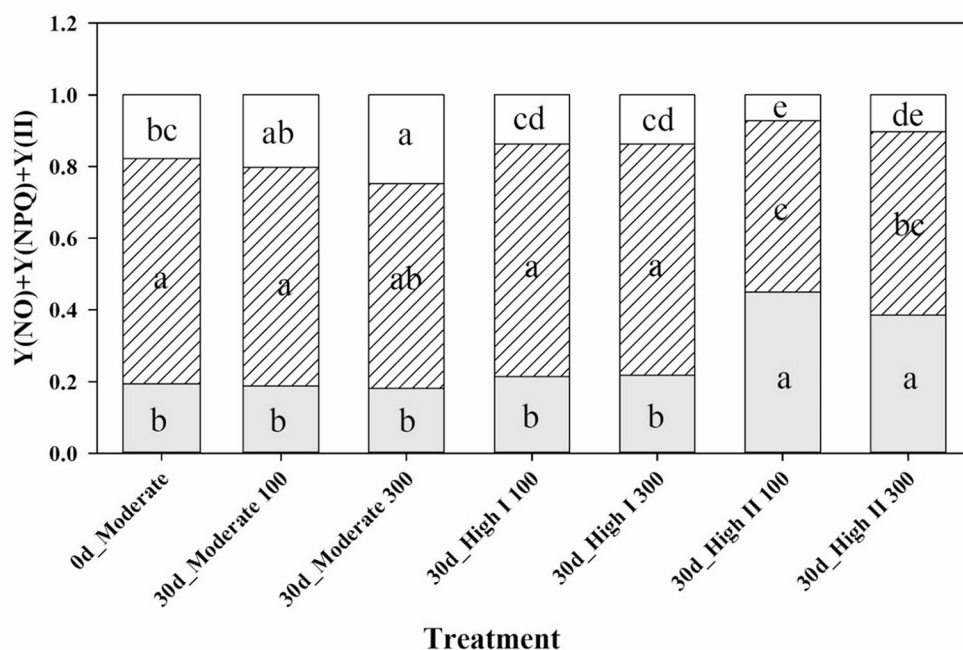
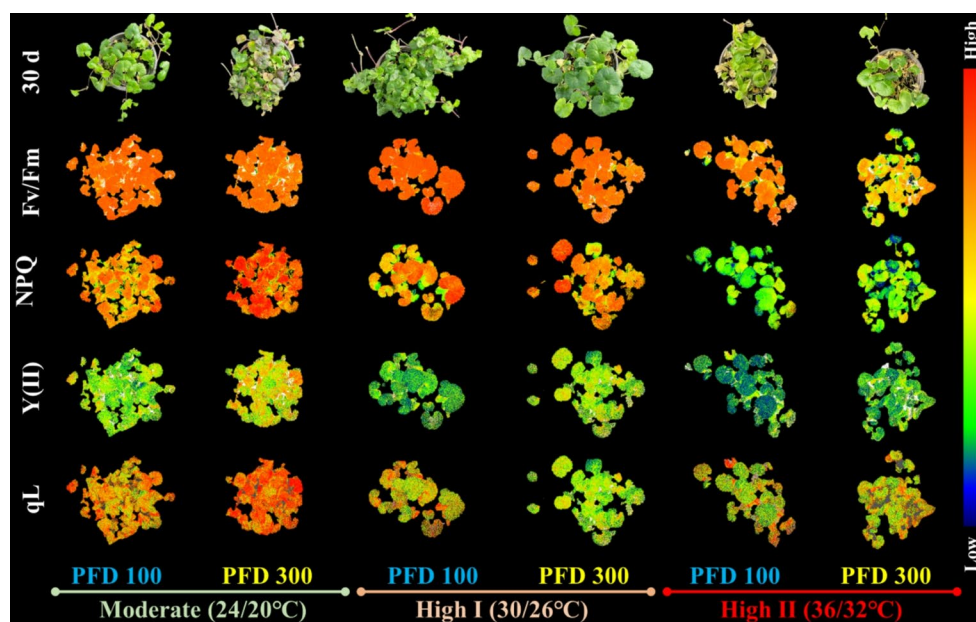


Table 2 Effects of light intensity (PFD) and temperature on the chlorophyll fluorescence parameters of the *C. asiatica* L. after 30 d of the treatment

Treatment		Chlorophyll fluorescence parameters						
Light intensity (PFD) [L]	Temperature [T]	Fv/Fm	Fv'/Fm'	Rfd	NPQ	qN	qP	qL
100 PFD	Moderate (24/20 °C)	0.81±0.00 ^z a ^y	0.65±0.01 a	1.69±0.25 a	1.31±0.17 a	0.66±0.04 a	0.31±0.04 a	0.71±0.04 a
	High I (30/26 °C)	0.82±0.00 a	0.68±0.01 a	1.38±0.10 b	1.17±0.08 a	0.63±0.02 a	0.20±0.02 b	0.54±0.03 b
	High II (36/32 °C)	0.66±0.06 b	0.54±0.01 b	0.87±0.09 c	0.68±0.07 b	0.56±0.03 b	0.13±0.12 c	0.26±0.04 c
300 PFD	Moderate (24/20 °C)	0.79±0.0 a	0.59±0.01 ab	2.11±0.17 a	1.60±0.12 a	0.73±0.01 a	0.42±0.04 a	0.77±0.03 a
	High I (30/26 °C)	0.81±0.0 a	0.67±0.0 a	1.29±0.19 b	1.1±0.14 b	0.62±0.03 b	0.20±0.02 b	0.55±0.05 b
	High II (36/32 °C)	0.65±0.1 b	0.51±0.13b	0.95±0.04 c	0.80±0.06 c	0.61±0.07 b	0.19±0.00 b	0.38±0.09 c
	T	16.55 ^{***}	11.85 ^{***}	78.51 ^{***}	75.88 [*]	15.30 [*]	112.10 ^{***}	121.24 ^{**}
	L	0.32 ^{NS}	1.22 ^{NS}	4.39 ^{***}	6.72 ^{***}	4.43 ^{***}	24.02 ^{***}	8.31 ^{***}
	T X L	0.02 ^{NS}	0.39 ^{NS}	5.23 [*]	4.13 [*]	1.90 ^{NS}	7.12 ^{**}	2.00 ^{NS}

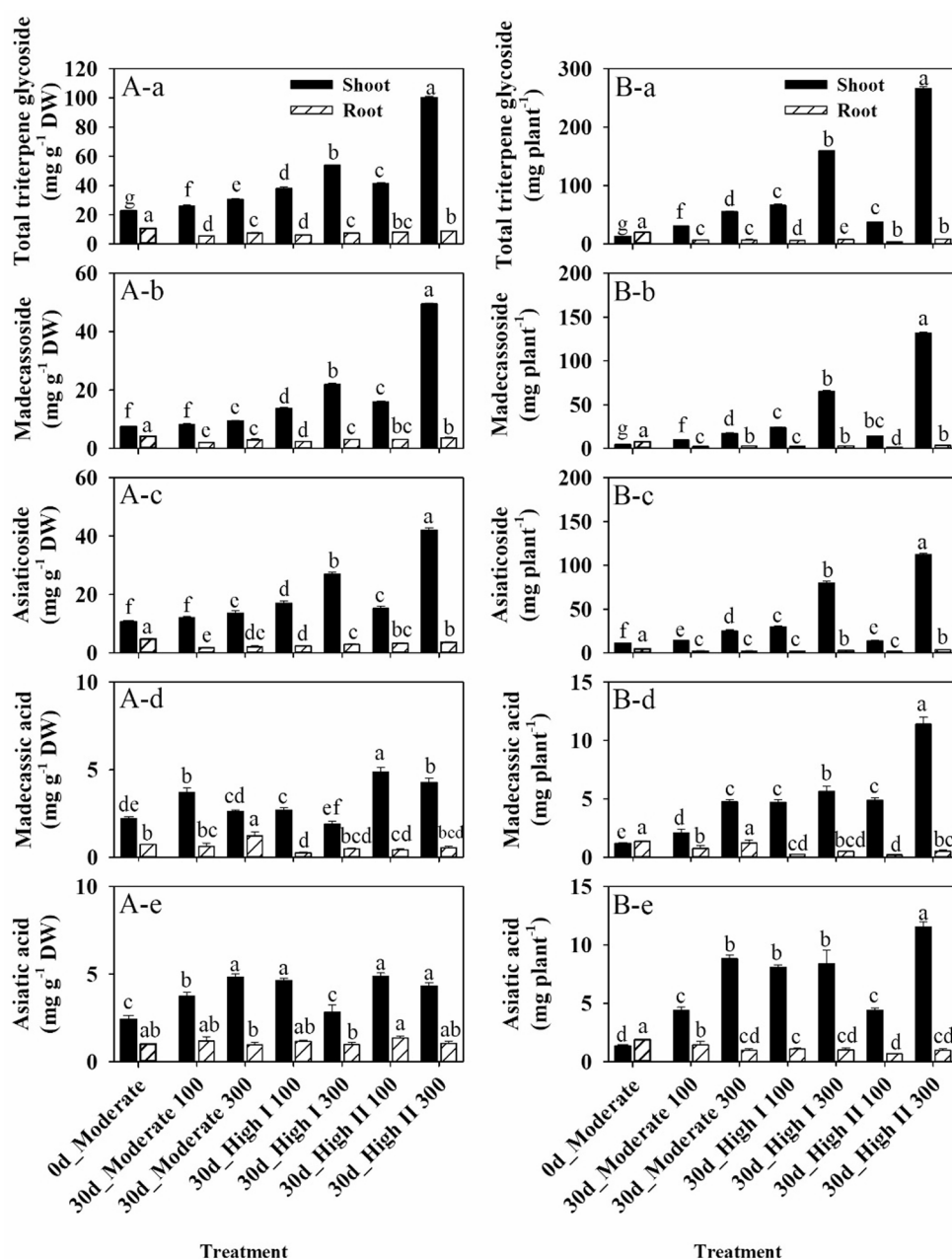
^zData are presented as mean of four replicates^yDifferent letters in the same column indicate significant differences based on Duncan's multiple-range test ($p < 0.05$)^{NS}Non-significant, ^{*}Significant at $p < 0.05$, ^{**}Significant at $p < 0.01$, ^{***}Significant at $p < 0.001$ **Fig. 6** Appearance of chlorophyll fluorescence images according to light intensity (PPDF 100 and 300) and temperature [Moderate (24/20 °C), High I (30/26 °C), and High II (36/32 °C)] treatment on the 30 days. The color bar situated on the right side serves as an indicator for the numeric values associated with each chlorophyll fluorescence parameter, delineating a spectrum from low to high. Refer to Table 1 for descriptions of each CF parameter

levels affected by the combined light intensity and temperature treatments, a color scheme was established, where blue represents the lowest fluorescence intensity and red indicates the highest intensity (Fig. 6). Notably, the images for Y(II) turned blue for the 30d_High I PFD 100, 30d_High II 100, and 30d_High II 300 samples, reflecting a decrease in the corresponding values. Similarly, a decrease in activity was observed in the Y(II) images for 30d_High II 100, indicating a uniform drop in efficiency.

3.3 Triterpene glycoside content

The results revealed that on day 30, *C. asiatica* cultivated under High II_300 conditions exhibited the highest levels of bioactive compounds, both individually and in terms of total triterpene glycoside content (Fig. 7A). This finding aligns with the observations that higher light conditions across various temperature treatments correlate with an increase in total triterpene glycoside content. Triterpene glycosides were predominantly located in the aerial parts of the plant rather than in the underground parts. Among the triterpene

Fig. 7 Changes of light intensity (PDF 100 and 300) and temperature [Moderate (24/20 °C), High I (30/26 °C), and High II (36/32 °C)] treatment on the total triterpene glycoside (a), madecassoside (b), asiaticoside (c), madecassic acid (d), and asiatic acid (e) of in the shoot and root of *C. asiatica*. after 30 days. The left side represents mg g⁻¹ DW (A), and the right side represents mg plant⁻¹ (B). Each bar represents the mean $\pm$ SD of three biological replicates in triterpene glycoside. Different letters within a figure indicate a significant difference at $p < 0.05$ by Duncan's multiple range test



glycoside contents analyzed per gram, madecassoside and asiaticoside constituted the majority, whereas madecassic acid and asiatic acid were present in lower proportions (Fig. 7A-a). On day 30 under the High II_300 treatment, the highest levels of madecassoside and asiaticoside in the aerial parts were 49.48 ± 0.38 and 42.09 ± 0.73 mg/g DW, respectively, whereas the lowest levels observed at 30d_Moderate_100 were 8.16 ± 0.52 and 12.05 ± 0.37 mg/g DW, respectively (Fig. 7A-b, c). Furthermore, the underground parts showed significantly lower content of all four triterpene glycosides than the aerial parts, with madecassoside and asiaticoside constituting the majority. The lowest content in the shoot at 30d_Moderate_100 (2.01 ± 0.16 mg/g DW) was

4.07 times less than that in the shoot (8.16 ± 0.52 mg/g DW) and the maximum difference was 13.55 times between the shoot (49.48 ± 0.38 mg/g DW) and root (3.65 ± 0.17 mg/g DW) at 30d_High II_300.

To determine the content of triterpene glycosides per plant, the amounts measured in mg/g were converted into actual dry weights (mg) of the aerial and underground parts (Fig. 7B). The results for the aerial parts showed a trend similar to the per gram mg basis for all individual and total triterpene glycoside content. Conversely, the underground parts exhibited a tendency for all four triterpene glycoside components to decrease upon exposure to higher temperatures (Fig. 7B). The interaction between light intensity and

temperature had a significant impact on the triterpene glycoside content (madecassoside, asiaticoside, madecassic acid, and asiatic acid), indicating their substantial influence on the levels of bioactive compounds in *C. asiatica*.

3.4 TPC, TFC, antioxidant activities, chlorophyll, and proline content

As the temperature and light intensity increased, a general enhancement was observed in total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (measured via FRAP and ABTS assays) (Table 3). Under PFD 300 conditions, TPC exhibited significant differences, with the order being High I (30/26°C) (30.63 ± 0.32 mg GAE g⁻¹ DW) > High II (36/32°C) (30.52 ± 1.96 mg GAE g⁻¹ DW) > Control (26.58 ± 0.25 mg GAE g⁻¹ DW), indicating that higher light intensities, particularly at elevated temperatures, contributed to an increase in phenolic content. Similarly, the TFC results showed that higher light intensity had a significant effect compared to the temperature treatments alone.

The range for Ferric Reducing Antioxidant Power (FRAP) was noted to be between 106.62 ± 1.96 and 195.13 ± 13.81 μmol TE g⁻¹ DW (Table 3), whereas the range for the ABTS assay extended from 181.31 ± 10.92 to 296.65 ± 23.04 μmol TE g⁻¹ DW. Both markers of antioxidant capacity were significantly higher in the 30d_High II 300 treatment group, indicating that this treatment enhanced the antioxidant properties of the plants the most. Conversely, the lowest values for these antioxidant metrics were observed under the 30d_Moderate 100 condition, suggesting that lower temperatures and light intensity conditions were less conducive to the accumulation of these bioactive compounds.

After 30 days, chlorophyll content varied significantly with temperature and light conditions (Table 3). The High I temperature treatment under PFD 100 treatment showed the highest chlorophyll concentration (6.60 ± 0.07 mg g⁻¹ DW), increasing 5.8% from initial values. Conversely, High II temperature caused substantial chlorophyll reductions at both light intensities, with decreases of 43.4% at PFD 100 and 54.3% at PFD 300. Higher light intensity (PFD 300) consistently produced lower chlorophyll levels across all temperature treatments compared to PFD 100. Despite these variations in total chlorophyll content, the Chl a/b ratio remained relatively stable (2.6–3.1) across all conditions.

Proline content in plant shoots varied significantly across different treatments after 30 days (Table 3). Control accumulated the highest proline concentration (2.46 ± 0.05 mg g⁻¹ DW), while initial control (day 0) exhibited the lowest values (0.87 ± 0.06 mg g⁻¹ DW). Control under higher light intensity (PFD 300 μmol m⁻² s⁻¹) also demonstrated high proline content (2.30 ± 0.04 mg g⁻¹ DW). Bot High I

Table 3 Effects of light intensity (PFD) and temperature on the chlorophyll fluorescence parameters of the *C. asiatica* after 30 d of the treatment

Treatment		Second metabolites								
Date	Light intensity (PFD)	Temperature	Total phenol (mg GAE g ⁻¹ DW)	Total flavonoid (mg CE/g DW)	FRAP (μmol TE g ⁻¹ DW)	ABTS (μmol TE g ⁻¹ DW)	Chl a (mg g ⁻¹ DW)	Chl b (mg g ⁻¹ DW)	Total Chl (mg g ⁻¹ DW)	Proline (mg g ⁻¹ DW)
0 d	100 PFD	Moderate (24/20 °C)	21.79±0.35 ^{xy}	23.06±1.22 cd	129.07±1.33d	199.88±5.13 cd	4.56±0.09b	1.68±0.03b	6.24±0.11b	0.87±0.06f
		Moderate (24/20 °C)	19.03±0.07d	21.21±0.78d	106.83±1.19e	181.31±10.92d	3.46±0.07d	1.06±0.08d	4.52±0.08d	2.46±0.05a
		High I (30/26 °C)	21.93±0.64c	25.46±0.77c	131.23±0.92d	213.94±2.81c	4.81±0.05a	1.79±0.02a	6.60±0.07a	1.20±0.01e
30 d	100 PFD	High II (36/32 °C)	18.16±0.10d	21.48±0.32d	106.62±1.96e	196.15±4.68cde	2.57±0.06f	0.96±0.04d	3.53±0.03f	1.61±0.09d
		Moderate (24/20 °C)	26.58±0.25b	29.71±3.09b	166.45±1.59c	256.15±1.10b	2.98±0.03e	0.97±0.05d	3.95±0.09e	2.30±0.04b
		High I (30/26 °C)	30.63±0.32a	34.95±1.58a	183.89±2.16b	296.05±1.36a	4.13±0.16c	1.35±0.11c	5.48±0.27c	1.20±0.04e
300 PFD	100 PFD	High II (36/32 °C)	30.52±1.96a	36.40±0.85a	195.13±13.81a	296.65±23.04a	2.10±0.03 g	0.75±0.02e	2.85±0.01 g	1.81±0.04c

^aData are presented as mean of four replicates

^bDifferent letters in the same column indicate significant differences based on Duncan's multiple-range test ($p < 0.05$)

temperature treatments showed nearly identical proline levels under different light intensities ($1.20 \pm 0.01 \text{ mg g}^{-1} \text{ DW}$ at PFD 100 and $1.20 \pm 0.04 \text{ mg g}^{-1} \text{ DW}$ at PFD 300). The High II temperature treatments displayed intermediate values, with $1.61 \pm 0.09 \text{ mg g}^{-1} \text{ DW}$ at PFD 100 and $1.81 \pm 0.04 \text{ mg g}^{-1} \text{ DW}$ at PFD 300.

3.5 Heatmap analysis

Figure 8 comprehensively analyzes the relationships between the growth, chlorophyll fluorescence, and bioactive compound profiles of *C. asiatica* cultivated under various temperature and light intensity conditions in a vertical farm environment. Heatmap tree analysis revealed two principal clusters. The upper cluster includes branches most closely associated with two specific light intensities (100 ± 10 and $300 \pm 10 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and three temperature regimes (control: 24/20°C, High I: 30/26°C, and High II: 36/32°C). The cluster on the left features branches that characterize the relationships between growth parameters, chlorophyll fluorescence parameters, and bioactive compounds. The harvest periods on Days 0 and 30 were significantly correlated with the factors determined by ANOVA. Except for the key bioactive compound, triterpene glycoside (root) madecassic acid, the related substances clustered together. A notable correlation was observed between the number of runners and flower occurrence, with significant findings at a PFD of 100. This suggests that the relevance of light intensity treatments to photosynthesis and bioactive substances is greater than that of temperature treatments.

4 Discussion

In the present study, we explored the optimal cultivation conditions for *C. asiatica* cultivated on a vertical farm with a combined treatment of temperature and light intensity, focusing on four types of triterpene glycosides, growth characteristics, photosynthetic capacity, and secondary metabolites in each sample. Vertical farming offers less variability than unpredictable outdoor environmental conditions and has advantages, such as year-round production, plant growth predictability, and tailored production planning. Therefore, this study demonstrated the importance of achieving production goals by artificially manipulating various environmental factors to produce crops suitable for specific purposes.

4.1 Effect of light intensity and temperature on growth characteristics

Across ten quantitative morphological characteristics, significant increases in aerial fresh weight, dry weight, and number of flowers were observed with differences in light intensity, whereas an increase in high-temperature conditions led to a significant decrease in underground fresh weight, dry weight, and root length. Through the analysis of growth characteristics, we found that petiole length, number of runners, leaf area, and fresh weight increase related to *C. asiatica* cultivation were associated with previously reported studies on light intensity and temperature conditions in vertical farms (Rahajanirina et al. 2012; Song et al. 2022; Harakotr et al. 2024). In particular, this study showed the results for leaf area and fresh weight under relatively high light intensity conditions at the same temperature. This has implications for agricultural management to achieve high yields per unit area in limited vertical farm spaces (Song et al. 2022). Phenotypic characteristics that vary with the cultivation environment are influenced by interactions between various environmental factors and cultivation management methods. Moreover, *C. asiatica* showed a tendency towards increased biomass with higher light intensities; however, previous studies have confirmed that not all temperature conditions are optimal for crop growth (Rahajanirina et al. 2012; Harakotr et al. 2024). Thus, by incrementally increasing the temperature suitable for *C. asiatica* cultivation during growth and enhancing the PFD, a cultivation strategy that effectively increases both biomass and dry weight can be developed and applied. Our experiments confirmed that the trends in the actual fresh weight and dry weight increase or decrease involved both temperature and light intensity in the combined treatments of *C. asiatica*.

Furthermore, this study examined the influence of day-night temperature differential (DIF) on the growth and secondary metabolite production in *C. asiatica*. A consistent DIF of 4 °C was maintained across all treatment conditions (Moderate: 24/20°C, High I: 30/26°C, High II: 36/32°C) to mimic the diurnal temperature fluctuations that *C. asiatica* experiences in its natural habitat. Native to tropical and subtropical regions, *C. asiatica* has typically adapted to day-night temperature variations, which affect the plant's photosynthate allocation and respiratory efficiency (Coltalti et al. 2020; Harakotr et al. 2024). The consistent DIF of 4 °C used in this study represents a practical approach for commercial vertical farming operations, balancing energy efficiency with physiological requirements. In tropical native habitats of *C. asiatica*, natural DIF ranges from 3 to 7 °C, with the plant exhibiting optimal growth within this range (Song et al. 2023; Yang et al. 2023). Our selection of a 4 °C DIF was based on preliminary trials and literature

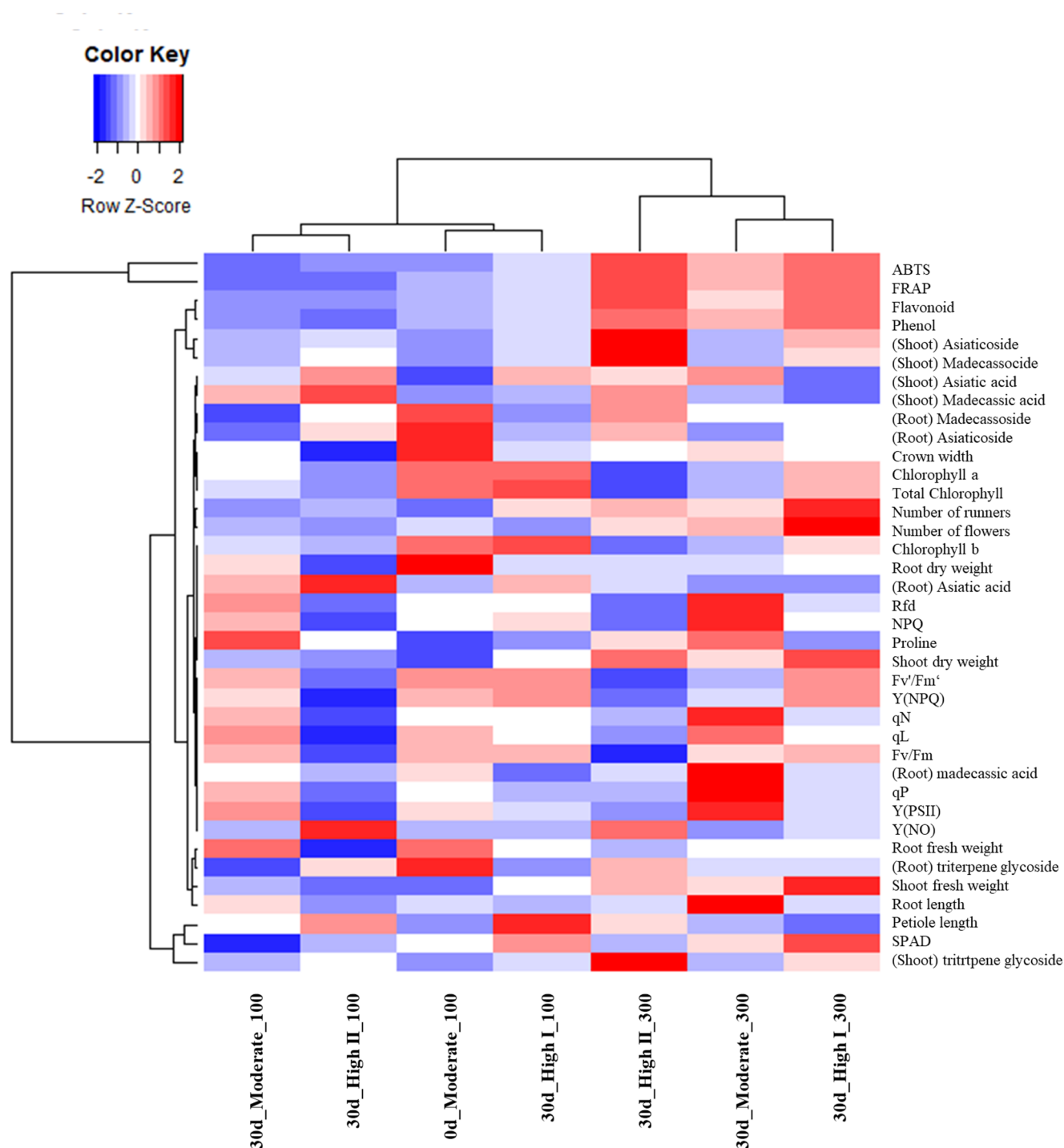


Fig. 8 Heatmap analysis of the effects of light intensity (PFD 100 and 300) and temperature (Moderate [24/20°C], High I [30/26°C], and High II [36/32°C]) on *C. asiatica*. Red color represents negative whereas blue represent positive status. Color intensity were propor-

tional to the correlation coefficients, which are shown in the legend to the right. FRAP: ferric reducing antioxidant power; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

suggesting that moderate DIFs promote balanced vegetative growth and bioactive compound accumulation in medicinal plants (Bylka et al. 2014; Alcalde et al. 2023). Our results suggest that the 4 °C DIF used across all temperature regimes provided consistent diurnal cues for metabolic

regulation, allowing us to isolate the effects of absolute temperature levels on plant response. This DIF appears particularly effective when combined with the High I (30/26°C) temperature regime and high light intensity (PFD 300), as evidenced by optimal triterpene glycoside production under

these conditions. While our experimental design maintained a constant DIF to focus on the effects of absolute temperature levels, future studies should investigate how varying DIF conditions influence the growth and functional compound accumulation in *C. asiatica*. Vertical farming systems, with their capacity for precise environmental control, offer significant potential for optimizing both productivity and bioactive compound content through DIF manipulation.

4.2 Effect of light intensity and temperature on chlorophyll fluorescence

Vertical farms are characterized by consistent energy use, predictable plant growth, and flexibility in production planning. Although an external light environment can introduce variability in photosynthesis, which directly affects crop productivity, vertical farms can provide a uniform light environment. Thus, identifying the optimal photosynthetic conditions for producing optimal and maximum biomass of the target crop is crucial. In this study, we established low- (PFD 100) and high-light (PFD 300) environments for *C. asiatica*, characterized by its regional traits, and utilized a chlorophyll fluorescence imaging system for nondestructive measurements of photosynthesis levels. We observed that light intensity and temperature influenced the chlorophyll fluorescence parameters (Fig. 5; Table 2). Y(II), Y(NO), and Y(NPQ), components of light energy partitioning, signify not only the fraction of absorbed light energy directly used for photosynthesis, but also its use in other processes, summing up to an absolute value of 1 (Kalaji et al. 2014). Our study showed significantly lower results for most chlorophyll fluorescence parameters at High II (36/32 °C) under both PFD 100 and PFD 300 compared to the Moderate control, suggesting that unsuitable high temperatures decrease the fraction of light energy not used for photosynthesis, leading to its release as heat or chlorophyll fluorescence (Baker 2008). A decrease in Fv/Fm under light intensity-related conditions was observed, consistent with previous research indicating that high temperature (Hou et al. 2016; Bhandari et al. 2018) and high light (Kwack et al. 2021) conditions could affect the potential photosynthetic capacity of crops cultivated on vertical farms. Fv'/Fm', acquired in light conditions and typically used similarly to Fv/Fm (0.78–0.83) obtained in dark adaptation, shows relatively lower values (Maxwell and Johnson 2000).

Y(NPQ), qN, and NPQ represent non-photochemical quenching mechanisms, indicating the level of light energy dissipated as heat rather than being used for photosynthesis (Baker 2008; Murchie and Lawson 2013). As shown in Table 2, these parameters, along with Fv/Fm, exhibited significant decreases under high temperature conditions (High II, 36/32°C) compared to moderate (24/20°C) and High I

(30/26°C) temperature treatments at both light intensities. The decline in NPQ-related parameters under extreme heat stress can be attributed to temperature-induced damage to the photosynthetic apparatus, particularly the thylakoid membranes and the proteins involved in the xanthophyll cycle (Tardy and Havaux 1997). When exposed to temperatures exceeding physiological thresholds, the structural integrity of PSII and associated light-harvesting complexes may be compromised, impairing both photochemical efficiency (reflected in reduced Fv/Fm) and the capacity for regulated energy dissipation (reflected in reduced NPQ values) (Hossain et al. 2011). This concurrent decrease in both photochemical and non-photochemical parameters indicates a comprehensive impairment of the photosynthetic regulatory mechanisms under severe heat stress, rather than a compensatory relationship (Sharma et al. 2015). These findings suggest that while *C. asiatica* can maintain effective photoprotection mechanisms at moderately elevated temperatures (30/26°C), exposure to higher temperatures (36/32°C) overwhelms these protective capacities, potentially leading to chronic photoinhibition and reduced productivity, as previously reported in studies of heat stress effects on photosynthetic apparatus (Yamori et al. 2014; Kalaji et al. 2016). The decrease in Rfd, indicating the rate of fluorescence decline and qL (lake model), which expresses the complex reaction of photosystem II, signified a decrease in the net photosynthetic rate, indicating a significant reduction in photosynthetic levels during the treatment period. These findings illustrate the differences based on the sensitivity of the parameters measured using chlorophyll fluorescence, supporting the notion that light energy efficiency varies depending on environmental factors.

4.3 Effect of light intensity and temperature on triterpene glycoside

To operate vertical farms with optimal efficiency, a complex combination of environmental factors must be considered to increase the growth rate, yield, and secondary metabolite content of crops. In the present study, four triterpene glycosides (madecassoside, asiaticoside, madecassic acid, and asiatic acid) were investigated. On day 30, the madecassoside and asiaticoside contents were higher in the aerial parts than in the underground parts, with the highest content observed in the 30d_High II 300. This finding is consistent with previous reports showing that the aerial parts (leaves and petioles) contain higher levels of the four functional substances than the roots (Hamid et al. 2002). Previous research related to the light intensity of *C. asiatica* (Song et al. 2022) confirmed that the content of the four types of triterpene glycosides increased with increasing light intensity, which is similar to the results of this study. Some

bioactive components originate from complex physiological responses induced by light intensity and temperature. Previous studies have identified that the most crucial factors affecting the synthesis of major triterpene glycosides and other metabolites *C. asiatica* are annual temperature variations and precipitation. In particular, variations in the content of major metabolites are closely related to geographical changes due to climate change (Shukurova et al. 2021).

4.4 Effect of light intensity and temperature on TPC, TFC, antioxidant activities, chlorophyll, and proline content

When plants are exposed to environmental factors that induce a certain level of stress, their antioxidant systems enhance the content of specific compounds, thus playing a crucial protective role (Yoon et al. 2020). Secondary metabolites, such as phenolic and flavonoid compounds act as effective antioxidants and an increase in their content in plants signifies an activated defense response. This study demonstrates that phenolic and flavonoid compounds can increase the concentration of phenolic compounds in *C. asiatica* plants due to heat stress caused by temperature. Similarly, the accumulation of phenolic compounds in *C. asiatica*, lettuce, and cabbage is an adaptation to heat stress (Argosubekti 2020; Camejo et al. 2020). According to Song et al. (2022), the activity level of antioxidant metabolism is related to light intensity and quality, confirming that light intensity is associated with the activation of antioxidant metabolism. Corresponding research indicates that the results showing an increase in phenolic compound content due to temperature are similar to the findings of other studies, demonstrating similar outcomes.

Antioxidant capacity refers to the ability to inhibit oxidation and is widely used to express the health-promoting potential of plants or their products. Two methods (ABTS and FRAP) were used to assess antioxidant activity. As shown in Table 3, light intensity had a more pronounced effect on antioxidant activities than temperature. Under high light conditions (PFD 300), FRAP values ranged from 166.45 to 195.13 $\mu\text{mol TE g}^{-1}\text{DW}$, while under low light conditions (PFD 100), values were significantly lower (106.62 to 131.23 $\mu\text{mol TE g}^{-1}\text{DW}$) across all temperature treatments. Similarly, ABTS values under high light (256.15 to 296.65 $\mu\text{mol TE g}^{-1}\text{DW}$) were consistently higher than those under low light (181.31 to 213.94 $\mu\text{mol TE g}^{-1}\text{DW}$). This light-dependent increase in antioxidant capacity corresponded with the observed trends in TPC and TFC, suggesting that enhanced light intensity is a primary driver in activating antioxidant defense mechanisms in *C. asiatica*. Within the same light intensity group, temperature did influence antioxidant activity, with the High I (30/26°C) and

High II (36/32°C) treatments generally showing higher values than the Moderate (24/20°C) treatment under high light conditions. These findings align with Song et al. (2022), who demonstrated that light intensity is strongly associated with the activation of antioxidant metabolism in plants. The significant enhancement of antioxidant capacity under high light conditions provides valuable insights for optimizing cultivation parameters in vertical farming systems aimed at maximizing bioactive compound production in *C. asiatica* (Devi et al. 2017).

Significant results were also observed for the major photosynthetic pigments, Chl a and Chl b, on the last day of treatment. A decrease in chlorophyll content indicates damage to the chloroplast membrane and structure, photo-oxidation of chlorophyll, increased chlorophyll activity, and inhibition of chlorophyll biosynthesis owing to water deficiency (Faseela et al. 2020; Shin et al. 2021). Studies related to chlorophyll damage due to light intensity have shown that high light intensity in vertical farms leads to a decrease in chlorophyll not only in *C. asiatica* but also in various other research findings. The range of light intensities for cultivating leafy green varies widely and significant differences may exist between crops and cultivation facilities, underscoring the importance of identifying the appropriate light intensity for crop cultivation. Furthermore, Bhandari et al. (2018) demonstrated that chlorophyll biosynthesis in crops grown at optimal growth temperatures is closely related to photosynthesis, implying that temperature is involved in plant physiological systems.

Proline, which is prevalent in plants, accumulates under stress conditions, such as light, temperature, and water stress, and plays a crucial role in maintaining homeostasis within the plant (Bhandari et al. 2018; Shin et al. 2020, 2021; Tonhati et al. 2020). While the typical growth temperature range for horticultural crops is identified to be between 20 and 29 °C, necessitating higher temperature conditions for some crops, in this study, High II (36/32 °C) was verified as a high-temperature treatment. The study demonstrated that Moderate (24/20 °C), deemed the control condition, exhibited a significantly higher increase. This finding suggests that this temperature, when assessed against the climatic environment of *C. asiatica*'s native region, might be considered a lower temperature, leading to an increase in proline content, which is consistent with the outcomes of Plengmuankhae and Tantitadapitak (2015). This study highlights the importance of maintaining an optimal growth temperature for *C. asiatica* when cultivated on vertical farms, indicating that careful temperature management is crucial for optimizing plant health and productivity.

The physiological mechanisms underlying elevated secondary metabolite production under high temperature and high light conditions are multifaceted (Zobayed et al. 2005;

Zhang et al. 2021). Plants exposed to environmental stress conditions activate their defense systems as an adaptive response. High light intensity can potentially exceed the photosynthetic capacity of plants, creating energy imbalances within the photosystems. Similarly, high temperatures can disrupt cellular homeostasis by affecting membrane fluidity and protein stability (Devi et al. 2017). In response to these stressors, plants typically enhance their secondary metabolite biosynthesis pathways as part of their defense strategy. The increased production of phenolic and flavonoid compounds observed in our study of *C. asiatica* under varying light and temperature conditions aligns with this protective mechanism. These compounds serve dual roles: they act as antioxidants that help maintain cellular redox balance and provide photoprotection by dissipating excess light energy (Cazzaniga et al. 2013). The triterpene glycosides in *C. asiatica*, synthesized through the mevalonate pathway, are likely upregulated under stress conditions as part of the plant's overall stress response (Yendo et al. 2014). This metabolic shift represents an ecological adaptation that enables plants to withstand suboptimal environmental conditions while simultaneously producing higher concentrations of bioactive compounds valuable for medicinal applications.

4.5 Effect of light intensity and temperature on heatmap

Heatmap analysis allows for the visual examination of changes within a data matrix under various treatment conditions, enabling the evaluation of how specific treatments affect stress responses in plants. In this study, the relationships between light intensity and temperature treatments in *C. asiatica* within a vertical farm were elucidated, revealing the interconnectedness among various factors. These outcomes reflect the factors that increase productivity and growth characteristics as well as the four types of triterpene glycosides. This is in line with the findings from the heatmap analysis of responses to light intensity and temperature, demonstrating similarities with the results reported by Mohi-Ud-Din et al. (2021). Under the light intensity treatments, close clusters were shown between PFD 300 and PFD 100, indicating a negative correlation between various functional compounds. These results confirm that the similarities between light intensity and temperature treatments are higher than the relationships among different factors, highlighting the distinct influence of each environmental condition on the biochemical and physiological responses of the plant.

4.6 Integration of growth characteristics, photosynthetic parameters, and secondary metabolite production

The comprehensive analysis of our findings reveals several interconnected relationships between environmental conditions and plant responses in *C. asiatica*. High light intensity (PFD 300) combined with moderate to high temperatures (24/20°C and 30/26°C) created optimal conditions for both biomass accumulation and bioactive compound synthesis. This relationship demonstrates how photosynthetic capacity, as measured by chlorophyll fluorescence parameters, directly influences both primary growth and secondary metabolism. Specifically, treatments that maintained higher Fv/Fm and Y(II) values (indicative of efficient photosynthetic machinery) corresponded with increased biomass production and elevated levels of triterpene glycosides, particularly madecassoside and asiaticoside. The observed correlation between antioxidant capacity (ABTS and FRAP values) and triterpene glycoside content suggests a coordinated stress response mechanism. Under high light conditions (PFD 300), plants simultaneously increased their antioxidant defense systems and triterpene production, indicating that these biochemical pathways may share regulatory elements or signaling cascades. This coordination likely represents an ecological adaptation strategy where resources are allocated to both growth and defense when environmental conditions are favorable for photosynthesis but potentially stressful in terms of light intensity (Rahajanirina et al. 2012). Furthermore, the inverse relationship between proline accumulation and optimal growth conditions provides a physiological indicator of plant stress levels across treatments (Shin et al. 2020). The integrative view of these parameters demonstrates that *C. asiatica* cultivation in vertical farming can be optimized by balancing light-induced photosynthetic productivity with temperature-regulated metabolic processes. This balance point, as our data suggests, occurs at PFD 300 and 30/26°C (High I), where both growth parameters and bioactive compound production reached their highest levels without triggering excessive stress responses such as proline accumulation or photosynthetic inhibition. From a practical cultivation perspective, our findings on DIF have significant implications for commercial vertical farming of *C. asiatica*. While maintaining a constant 4 °C DIF across treatments allowed us to standardize one variable, the interaction between DIF and absolute temperature levels warrants further investigation. Based on our results and existing literature, we propose that vertical farming operations could implement a dynamic DIF strategy throughout the cultivation cycle: using smaller DIFs (2–3 °C) during early vegetative growth to promote compact, bushy growth habits, and transitioning to moderate

DIFs (4–6 °C) during the maturation phase to enhance triterpene glycoside accumulation. This approach would leverage the precision environmental control capabilities of vertical farming systems to optimize both growth parameters and bioactive compound production. Furthermore, implementing strategic DIF manipulation could potentially reduce overall energy consumption while maintaining or even enhancing product quality. For operations primarily focused on biomass production, maintaining our established 4 °C DIF with the High I temperature regime (30/26 °C) and high light intensity (PFD 300) would be recommended. For operations prioritizing bioactive compound concentration, exploring slightly higher DIFs (5–6 °C) during the final weeks of cultivation could potentially enhance triterpene glycoside accumulation by introducing mild stress conditions that stimulate secondary metabolite production pathways (Zobayed et al. 2005).

5 Conclusion

In vertical farming, identifying the optimal growth conditions that efficiently increase the biomass of crops traditionally cultivated in external environments is crucial. Efforts to cultivate plants, such as *C. asiatica* on vertical farms have been sparse, with previous studies largely focusing on single environmental factors. This study presents a novel approach for the effective cultivation *C. asiatica* in a vertical farming setting. The results showed that by appropriately regulating the temperature and light intensity, the aerial biomass of *C. asiatica* could be increased and plants with high levels of the four types of triterpene glycosides could be obtained. High light intensity positively affected major quantitative growth characteristics and increased biomass, whereas combined treatments of temperature and light intensity resulted in significant increases in key functional substances. Chlorophyll fluorescence analysis highlighted the importance of optimal temperature and light intensity for crops, and the increase in secondary metabolites (such as TPC, TFC, antioxidant activity (FRAP and ABTS analysis), chlorophyll, and proline content) demonstrated that the environmental conditions within vertical farms significantly affected the increase in secondary metabolites. This study offers a novel approach for applying complex environmental treatments to the effective cultivation of *C. asiatica* on vertical farms. Finally, future research should explore the multidimensional studies necessary for effective cultivation of *C. asiatica* on vertical farms.

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Data availability Data will be made available on request.

Declarations

Competing interest 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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