Industrial Crops & Products 241 (2026) 122732

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Light/dark cycle lighting boost yield and centellosides content of vertically cultivated *Centella asiatica*

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ARTICLE INFO

Keywords:
Biological rhythm
Growth characteristics
Hydroponics
Light cycle
Medicinal plant
Photochemical reaction
Secondary metabolites

ABSTRACT

Centella asiatica (*C. asiatica*) is a representative medicinal plant rich in centellosides, which exhibit antioxidant, anti-inflammatory, and collagen synthesis-promoting activities. It is widely used as a high-value natural resource in functional cosmetics and pharmaceuticals for skin regeneration purposes. In this study, *C. asiatica* was cultivated in controlled environments to investigate the effects of different photoperiods (L, light) and cycle lengths (C) (L/D cycle) on yield performance and photochemical properties. The light treatments included CON (150 PPFD), C0L24 (100 PPFD), C2L8 (150 PPFD), and C4L4 (150 PPFD) per cycle, with all treatments adjusted to provide a fixed daily light integral (DLI) (8.64 $mol{\cdot}m^{-2}{\cdot}d^{-1}$). C0L24 produced the highest shoot fresh weight during the first half of the 6-week growing period, whereas the shoot dry weight was highest in both C0L24 and CON. The photosynthetic rate was highest in C2L8, whereas the efficiency of plant photosystem II and electron transport rate were highest in CON, followed by C0L24. Interestingly, the maximum photochemical efficiency of photosystem II showed similar trends among the treatments, ranging between 0.8 and 0.84. Chlorophyll content was highest in C0L24, followed by C2L8. Ascorbic acid concentration was highest in C2L8 and C4L4 under a short L/D cycle, whereas centellosides concentration was highest in C4L4 and lowest in C0L24. Overall, these

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Abbreviations: PAR, Photosynthetically active radiation; DLI, Daily light integral; L/D, Light/Dark; CON, 1 cycle per 24h, 16h light/8h dark; C0L24, 0 cycle per 24h, 24h light; C2L8, 2 cycles per 24h, 8h light/4h dark; C4L4, 4 cycles per 24h, 4h light/2h dark; PSII, Photosystem II; PPFD, photosynthetic photon flux density; SFW, Shoot fresh weight; SDW, Shoot dry weight; RFW, Root fresh weight; RDW, Root dry weight; ABTS, 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid; TP, Total phenol; TF, Total flavonoid; AOC, Antioxidant capacity; PCA, Principal component analysis; P_n, photosynthetic rate; φPSII, Photosystem II activity; ETR, Electron transport rate; Fv/Fm, Maximum quantum yield of PSII; NPQ, Non-photochemical quenching; CL, Continuous light; ROS, Reactive oxygen species.

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https://doi.org/10.1016/j.indcrop.2026.122732
Received 26 July 2025; Received in revised form 26 December 2025; Accepted 17 January 2026

results indicate that continuous lighting at a fixed DLI can effectively promote shoot growth in *C. asiatica* in vertical farms.

1. Introduction

Centella asiatica (L.) Urb, commonly known as Gotu kola, is a perennial herbaceous plant in Apiaceae family, distributed across tropical and subtropical regions (Devkota and Jha, 2013). It has been used for medicinal purposes and contains triterpene saponins, particularly madecassoside and asiaticoside, and sapogenins, such as madecassic and asiatic acids, all of which contribute to various health-promoting properties (Buraphaka et al., 2024). In particular, leaf extracts of *C. asiatica* are widely used in cosmetics and natural medicines due to their purported anti-inflammatory, wound-healing and skin regenerative (Eom et al., 2022). Consequently, the global market for *C. asiatica* has expanded rapidly in recent years, highlighting the need for more economical and sustainable cultivation strategies to meet the increasing consumer demands (McCaleb et al., 2000; Prasad et al., 2012). Moreover, as saponins serve as important indicator compounds in the pharmaceutical and cosmetic industries, practical research aimed at enhancing their production and utilization is important (Müller et al., 2013). Thus, previous studies have focused on adjusting environmental factors to promote the accumulation of bioactive compounds for the pharmacological efficacy of *C. asiatica*. Müller et al. (2013) reported that high photosynthetically active radiation (PAR) induces the accumulation of anthocyanins and saponins in *C. asiatica* and observed that older leaves are richer in sapogenins, whereas younger leaves contain higher levels of saponins.

Recent demographic surveys of agricultural workers have demonstrated that the aging of the farming population is becoming a serious problem around the world, including the United States, China, and Europe (Johr, 2012; Annunziata et al., 2024; Huang et al., 2024; Monnat, 2025). As population density increases more rapidly in urban areas than in rural areas and urbanization intensifies the pressure on agricultural land, the loss of agricultural land has become an ongoing issue (Jiang et al., 2012). Unpredictable global conditions, such as global warming and extreme weather, are expected to compromise crop production, thereby threatening the supply of food and other essential resources (Van der Velde et al., 2012; Lesk et al., 2016). Therefore, there is a growing need to address both land- and climate-related challenges to crop production while also addressing the issues posed by an aging agricultural workforce. One promising solution is the development of vertical farming designs that eliminate reliance on external natural environments (Despommier, 2010; Kabir et al., 2023). Vertical farming is a method of agricultural production that utilizes vertical space to increase productivity per unit of land and improve land-use efficiency (Eigenbrod and Gruda, 2015). Previous studies have reported that vertical farming enables the production of high-quality industrial crops in all seasons, and that crop yield and quality are often superior to those of crops grown in open fields (Park et al., 2019; Jin et al., 2023). However, because vertical farms depend on artificial light sources that consume significant amounts of energy, current vertical farming protocols must be designed to reduce energy consumption and optimize the cultivation environment to enhance market price competitiveness (Asseng et al., 2020).

The daily light integral (DLI) refers to the total amount of photosynthetic light received by a plant over a day. Although light quality and intensity are crucial factors for plant growth, DLI has been shown to have higher and more significant correlations with plant growth than these factors (Cui et al., 2021). As the use of artificial light in vertical farms has become more common in recent years, the optimization of DLI, a quantitative indicator of the light environment, has been highlighted as an important factor for improving crop productivity (Yang et al., 2024). These DLIs can be used as indicators for vertical farm design in terms of efficient light design and energy consumption optimization. The importance of DLI depends on numerous environmental factors, including the nature of the lighting sources; thus, DLI plays a crucial role in regulating plant growth and development (Gao et al., 2020). To date, considerable research has focused on investigating the relationship between the DLI and various plant characteristics. For example, in romaine lettuce, an increase in DLI resulted in a higher fresh weight and reduced nitrate accumulation in the biomass (Matysiak et al., 2022). In a separate study on spinach, the optimal DLI was determined to be 17.3 $mol\cdot m^{-2}\cdot d^{-1}$ based on assessments of fresh and dry weights, energy production, and photosynthetic rate (Gao et al., 2020). Dou et al. (2018) found that the growth and nutritional quality of sweet basil produced by vertical farming were optimal at a DLI of 12.9 $mol\cdot m^{-2}\cdot d^{-1}$, a level that is low enough to minimize the energy costs associated with cultivation. Moreover, it has been reported that extended photoperiod and weak lighting strategies at the same DLI offset weak light stress in lettuce and increase chlorophyll content, thereby enhancing light-use efficiency and promoting growth (Mao et al., 2019). Overall, optimizing the DLI can positively affect crop growth and save energy (Cui et al., 2021).

The plant circadian rhythm, or biological clock, helps predict and respond to environmental changes associated with the periodic day-night cycle caused by Earth's rotation (Liu et al., 2015). The ratio of the light period to the dark period within a given length of the cycle (photoperiod) is expressed as the light/dark (L/D) cycle, typically over a 24 h period (Dai et al., 2024). The biological clock synchronizes internal plant physiological and biochemical processes with the L/D cycle by regulating endogenous biological rhythms (Mao et al., 2019). In vertical farms isolated from the external environment, plants can be provided with a range of normal (L16/D8) and abnormal (L24/D12, L48/D24, L96/d48 and L120/D60) L/D cycles by continuous and intermittent irradiation via artificial lighting (Chen et al., 2022). A previous study on lettuce reported that a 12 h/12 h L/D cycle showed better growth compared with 6 h/6 h (L/D) or 3 h/3 h (L/D) cycles, with apparent differences also observed in leaf shape among the different cycles (Hang et al., 2019). Moreover, it has also been reported that the net photosynthetic rate and plant growth increased under a 6 h/6 h (L/D) photoperiod than under a shorter 3 h/3 h photoperiod. Another study by Chen et al. (2022) tested various L/D cycles, including 16 h/8 h, 24 h/12 h, 48 h/24 h, 96 h/48 h, and 120 h/60 h (L/D). They found that the dry weight and leaf assimilation efficiency of lettuce plants generally increased with longer L/D cycles, and that the L24/D12 condition exhibited the highest photosystem II (PSII) efficiency. Water absorption and root growth showed distinct patterns among the treatments (Chen et al., 2022), suggesting that lettuce plants may have achieved enhanced quality and more efficient energy utilization. Moreover, Zhou et al. (2020) underscored the significance of regulating the L/D cycle in vertical farms by emphasizing how its optimization can efficiently reduce the electricity costs associated with artificial lighting.

Although many studies have examined primary metabolite production by altering the L/D environments of various crops, similar studies on secondary metabolites are still lacking. Furthermore, while various studies have been conducted on the cultivation of *C. asiatica*, the effects of abnormal L/D cycles on its physiological and biochemical properties are poorly understood. Therefore, this study aimed to determine the effects of different L/D cycles with a fixed DLI on the growth of *C. asiatica* and the quality of its secondary metabolite content.

2. Materials and Methods

2.1. Plant material and growth conditions

The plant materials of *C. asiatica* used in this study were originally supplied by Kolmar B&H under a collaborative research agreement. The seedlings were maintained and vegetatively propagated for approximately one year in the controlled growth facility of Gyeongsang National University prior to the experiment. Before initiating the experiments, uniform-sized *C. asiatica* seedlings of uniform size with three leaves were selected and placed in distilled water for one week to promote rooting. The seedlings were then transplanted into a hydroponic pot (30 mm in diameter and 45 mm in height) using sponge cubes (35 × 35 × 30 mm, L × W × H) as a growth medium. The transplantation environment was maintained at a photosynthetic photon flux density (PPFD) of 100 μmol·m^{-2}·s^{-1} using a cool white LED light (DYT-18w; Dongyong Co, Daegu, Republic of Korea), with an air temperature of 25 ± 0.5 °C (mean ± standard deviation) and relative humidity of 60 ± 5 %. The treatments consisted of four different light/dark cycles (24 h). For each treatment, 36 plants were transplanted into a deep-flow hydroponic system consisting of a 12-hole cultivation panel (50 × 36 cm) placed on a bottom-irrigation tray (52 × 36 × 9 cm, L × W × H). The plant cultivation experiment was conducted for six weeks using the same cool white LEDs mentioned above, with four different light/dark cycles as treatments (Fig. S1). The air temperature and relative humidity were maintained at levels identical to those of the transplantation environment. Additionally, 10 L of Hoagland's solution (pH 6 ± 0.1, EC 2.0 ± 0.1 dS·m^{-1}) was applied immediately after transplantation and replaced every ten days.

2.2. Light/dark cycles treatments

All treatments were set at an identical DLI (8.64 mol·m^{-2}·d^{-1}) but had different cycle lengths, as follows: (1) 1 cycle per 24 h, 16 h light/8 h dark; 150 PPFD (CON; control), (2) 0 cycle per 24 h, 24 h light; 100 PPFD (C0L24), (3) 2 cycles per 24 h, 8 h light/4 h dark; 150 PPFD (C2L8), and (4) 4 cycles per 24 h, 4 h light/2 h dark; 150 PPFD (C4L4) (Fig. 1). The light intensity of the cultivation area was initially set to approximately 150 or 100 (± 5) PPFD by measuring six zones for each treatment using a quantum meter (LI-250A photometer; LI-COR, Lincoln, NE, USA) and then averaged. The samples were then monitored using a light spectrum analyzer (LI-180 Spectrometer; LI-COR, Lincoln, NE, USA). To minimize the potential effects of uneven light distribution between the treatment groups, the cultivation panels were rotated in a counterclockwise direction every two days. DLI was calculated using the following formula:

$$\text{DLI (mol·m}^{-2}\text{·d}^{-1}) = \text{PPFD (μmol·m}^{-2}\text{·s}^{-1}) \times (3{,}600 \times \text{photoperiod}) / 1{,}000{,}000$$

2.3. Plant growth properties

Five plants of uniform size were randomly selected from each treatment after two, four, and six weeks of the growing season, and direct measurements of growth characteristics were made as follows. The growth medium was removed, and the plants were washed under running tap water. The shoot and root parts were then separated for further growth analysis. Fresh shoot weight (including stolons) and root fresh weight were measured using an electronic microbalance (PX224KR/E; OHAUS, Parsippany, NJ, USA). The dry weights of the shoots and roots were measured after drying in a dry oven (WOF-155, DAIHAN, Gangwon, Republic of Korea) at 70°C for three days. Furthermore, for more detailed measurements, leaf length, width, area, and number of leaves were measured on fresh leaves using NIH ImageJ version 1.54 k (National Institutes of Health, Bethesda, MD, USA) from TIFF images captured by a camera (ZV-1F, Cannon, Tokyo, Japan). Finally, the fourth fully expanded leaf was selected to measure the leaf length, width, and area. All leaf data were derived from the same *C. asiatica* shoot.

2.4. Leaf-level gas exchange and chlorophyll fluorescence

Next, we measured photosynthetic rates at one, three, and five weeks

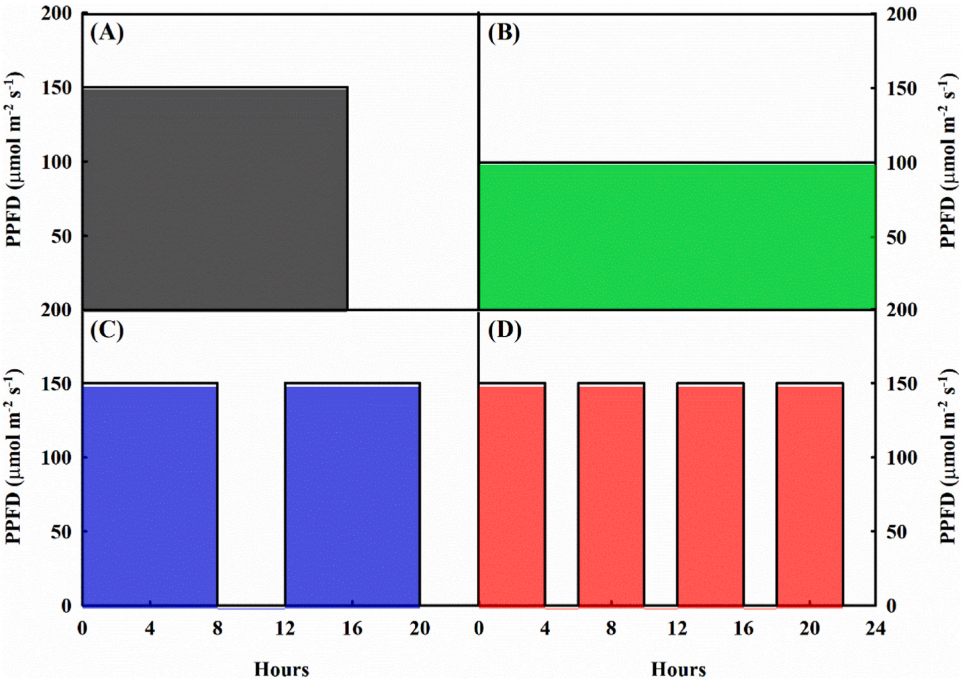

Fig. 1. Lighting strategies (A – D) applied to *C. asiatica*. All treatments had the same daily light integral (DLI: 8.64 mol·m^{-2}·d^{-1}), with photoperiods of 16, 24, 8, and 4 h for treatments A, B, C, and D, respectively.

of treatment using an LI-6400XT portable photosynthetic measurement system (LI-COR, Lincoln, NE, USA) equipped with a specialized extended reach Arabidopsis leaf chamber (0.79 cm^2) (6400–15; Li-Cor). Considering the chamber volume, four plants were measured per treatment, and the fourth fully expanded leaf was selected for the measurements. The measurement conditions were as follows: 25 ± 0.5°C of leaf temperature, 63 ± 2 % of relative humidity, 500 ± 10 $\mu mol \cdot mol^{-1}$ of CO_2 concentration, and 500 $\mu mol \cdot s^{-1}$ of flow rate respectively.

To assess plant photochemical efficiency in response to different light conditions, we measured chlorophyll fluorescence (Fo, Fm, Fv/Fm, ETR, and NPQ) at one, three, and five weeks after transplantation. Briefly, four plants were randomly selected from each treatment group, and the fully expanded fourth leaf was placed in the dark with a leaf clip holder (DLC-8; Heinz Walz GmbH, Effeltrich, Germany) for approximately 30 min before being measured using a PAM-2100 chlorophyll fluorescence meter (Heinz Walz GmbH, Effeltrich, Germany). To maximize the saturation of the PSII reaction center, a saturation pulse was applied. The measured light intensity was set to level 7, saturation light intensity was set to level 10, and modulation frequency was set to 20 kHz. Chlorophyll fluorescence parameters were defined as described by Mao et al. (2019).

Ft: Fluorescence yield measured at steady state.

Fo: Minimum fluorescence yield of dark-adapted samples when all PSII reaction centers are fully open or closed.

Fo′: Minimum fluorescence yield with all PSII centers fully open, adapted to preillumination conditions.

Fm: Maximum fluorescence yield of dark-adapted samples when all PSII reaction centers are fully open or closed.

Fm′: Maximum fluorescence yield reached by a saturation light pulse when pre-illuminated.

Fv/Fm: Measured maximum quantum yield of PSII under optimal conditions after dark adaptation [(Fm-Fo)/Fm].

ΦPSII: Effective quantum yield of PSII, which is close to the overall quantum yield of photosynthesis [(Fm'–Ft)/Fm'].

ETR: Relative apparent photosynthetic electron transport rate, measured in μmol electrons $m^{-2}s^{-1}$ ($\Phi PSII \times PAR \times 0.5 \times 0.84$).

NPQ: Non-photochemical quenching, reflecting the dissipated heat of the excitation energy from the antenna system [(Fm-Fm')/Fm'].

2.5. Bioactive compound and antioxidant analysis

To analyze the secondary metabolites, leaves from all treatments were dried, weighed, and pulverized using a blender. The concentrations of phenols, flavonoids, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ascorbic acid were measured using leaf samples collected at six weeks of cultivation. Total phenol (TP), total flavonoid (TF), and antioxidant capacity (AOC) were measured using the modified method described by Son and Oh (2013).

To measure the total phenol (TP) abundance, approximately 0.015 g of sample powder was extracted with 1.5 mL of 80 % (v/v) acetone, sonicated for 15 min, and then stored at 4°C for 12 h. The extracted samples were centrifuged at 1350 rpm for 2 min at 25°C, and the supernatant was retained for further analysis. Then, 135 μL of distilled water, 750 μL of 10 % diluted Folin-Ciocalteau reagent, 50 μL of the sample (or 50 μL of 80 % acetone as a control), and 600 μL of 7.5 % Na_3CO_3 were added to a microtube. The samples were then placed in a 45°C water bath for 15 min and cooled. The absorbance of the samples was measured at 765 nm using a spectrophotometer (Libra S32, Biochrom Ltd., Cambridge, UK). Simultaneously, a standard curve of phenol was generated using gallic acid, and the total phenol concentration from *C. asiatica* was expressed in milligrams of gallic acid equivalent per gram of dry leaf weight. Each biological replicate was measured in triplicate ($n = 3$ biological replicates × 3 technical replicates per treatment).

To analyze the total flavonoid (TF) concentration, approximately 0.05 g of dried leaf powder was extracted with 4 mL of 70 % (v/v) ethanol, vortexed, and sonicated for 15 min. The resulting supernatant was transferred to a microtube and centrifuged at 1350 rpm for 2 min at 25°C. To this supernatant, 1.25 mL of distilled water, 75 μL of 5 % $NaNO_2$, and 250 μL of sample were added (with 250 μL of 70 % ethanol used as a control). After 5 min, 150 μL of 10 % $AlCl_3$ was added to each sample. After 6 min, 500 μL of 1 M NaOH and 275 μL of distilled water were added. The sample was vortexed, allowed to rest for 5 min, and its absorbance was measured at 510 nm using a spectrophotometer (Libra S32, Biochrom Ltd., Cambridge, UK). The total flavonoid concentration of each sample was expressed in milligrams of (+)-catechin hydrate equivalent per gram of dry leaf weight ($n = 3$ biological replicates × 3 technical replicates per treatment).

To determine the antioxidant capacity (AOC) of the samples, 0.015 g of *C. asiatica* powder was extracted with 1.5 mL of 80 % (v/v) acetone solution. The resulting mixture was sonicated for 15 min and stored at −20°C in the dark. The mixture was centrifuged at 1000 rpm for 2 min at 25°C, and the resulting supernatant was used for further analysis. For the ABTS assay, 13.7 mg of ABTS was combined with 10 mL of distilled water to prepare a 2.5 mM ABTS stock solution to which 0.4 g of MnO_2 was added. The mixture was then stirred for 30 min. Subsequently, the mixture was filtered once using a paper filter and again using a 0.2-μm syringe filter. The resulting solution was placed in a conical tube in a 30°C water bath, and its absorbance was measured at 730 nm using a spectrophotometer (Libra S32, Biochrom Ltd., Cambridge, UK). The prepared ABTS solution was mixed with 5 mM PBS to adjust its absorbance to 0.7 (± 0.05). Then, 100 μL of the analytical sample and 1 mL of the sample mixture were mixed, vortexed, and the absorbance was measured after 1 min. The overall antioxidant capacity was expressed as millimoles of Trolox equivalent antioxidant capacity per gram of dry leaf weight ($n = 3$ biological replicates × 3 technical replicates per treatment) for each treatment.

Ascorbic acid concentrations were measured six weeks after transplantation. Briefly, 0.1 g samples of powdered leaves were extracted in 10 mL of distilled water. The samples were then centrifuged at 3000 rpm for 10 min at 4°C, and the resulting supernatant was used for further analysis. Ascorbic acid concentrations were measured using Ascorbic Acid Assay Kits (Biomax, Seoul, Republic of Korea) following the manufacturer's instructions. Results are expressed as mg g^{-1} DW ($n = 3$ biological replicates × 3 technical replicates per treatment).

Centelloside concentrations were analyzed using samples collected at two, four and six weeks. We used high-performance liquid chromatography (HPLC) to determine the concentrations of various cellular components, including centellosides (madecassoside, asiaticoside, madecassic acid, and asiatic acid), following a modified protocol described by Song et al. (2022). Briefly, 3 mL of 80 % MeOH was added to 0.05 g of dried leaf powder sample, and the extraction proceeded for 1 h in the dark before centrifugation. The supernatant was then extracted and filtered through a 0.45 μm PVDF filter. HPLC analysis was performed using an Agilent 1260 platform (Agilent Technologies Inc.) equipped with a Nova-Pak C18 (3.9 × 150 mm, 4 μm, Waters Corp., Milford, MA, USA) column. The two phases consisted of 100 % acetonitrile (solvent A) and 100 % distilled water (solvent B). Centelloside concentrations were analyzed at 210 nm using a UV detector while running a 20 %–100 % linear gradient at a flow rate of 1.0 $mL \cdot min^{-1}$ at 30°C for 25 min (Fig. 2). Each analysis was performed in triplicate to generate independent technical replicates. For the quantitative analysis of centellosides, external calibration curves were constructed using authentic standards of madecassoside, asiaticoside, madecassic acid, and asiatic acid (purity ≥ 98 %, Sigma-Aldrich). Stock solutions (10 mg mL^{-1}) of each standard were prepared in methanol and serially diluted to obtain ten concentration levels ranging from 0.1 to 1.0 mg mL^{-1} . Standard curves were generated by plotting the peak area against the concentration, with correlation coefficients (r^2) > 0.999 for all standards. The concentrations of individual centellosides in the samples were calculated from the respective external standard curves and expressed as mg g^{-1} dry weight (DW). Three biological replicates per

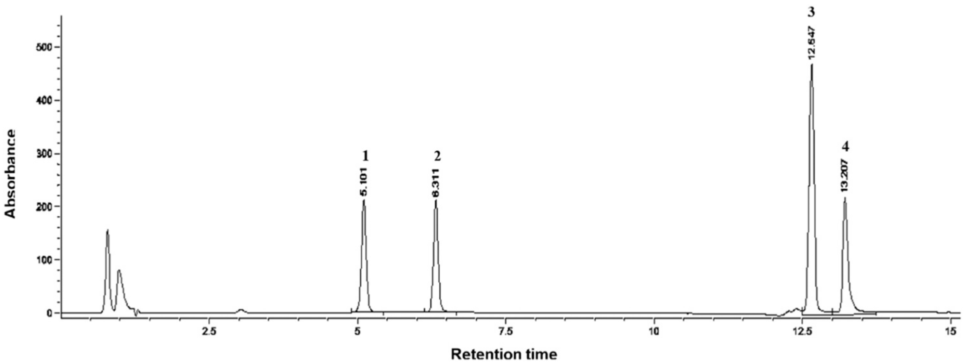

Fig. 2. High-performance liquid chromatography (HPLC) chromatogram of the standard: (1) madecassoside, (2) asiaticoside, (3) madecassic acid, and (4) asiatic acid.

treatment were analyzed, and each sample was measured in triplicate. The concentrations of TP, TF, AOC, ascorbic acid, and centellosides were determined from the same sample extracts.

2.6. Statistical analysis

Five independent biological replicates for each treatment were used to measure shoot fresh/dry weight, root fresh/dry weight, number of leaves and runners, leaf area, length, and width. Four independent biological replicates for each treatment were used to measure the leaf-level gas exchange and chlorophyll fluorescence. Three technical replicates were performed per treatment to measure the concentrations of TP, TF, AOC, ascorbic acid, and centellosides. Duncan's multiple range tests were used to evaluate the statistical significance of differences among treatment means ($p < 0.05$). All statistical analyses were performed using SAS version 9.45 (SAS Institute, Inc., Cary, NC, USA). All

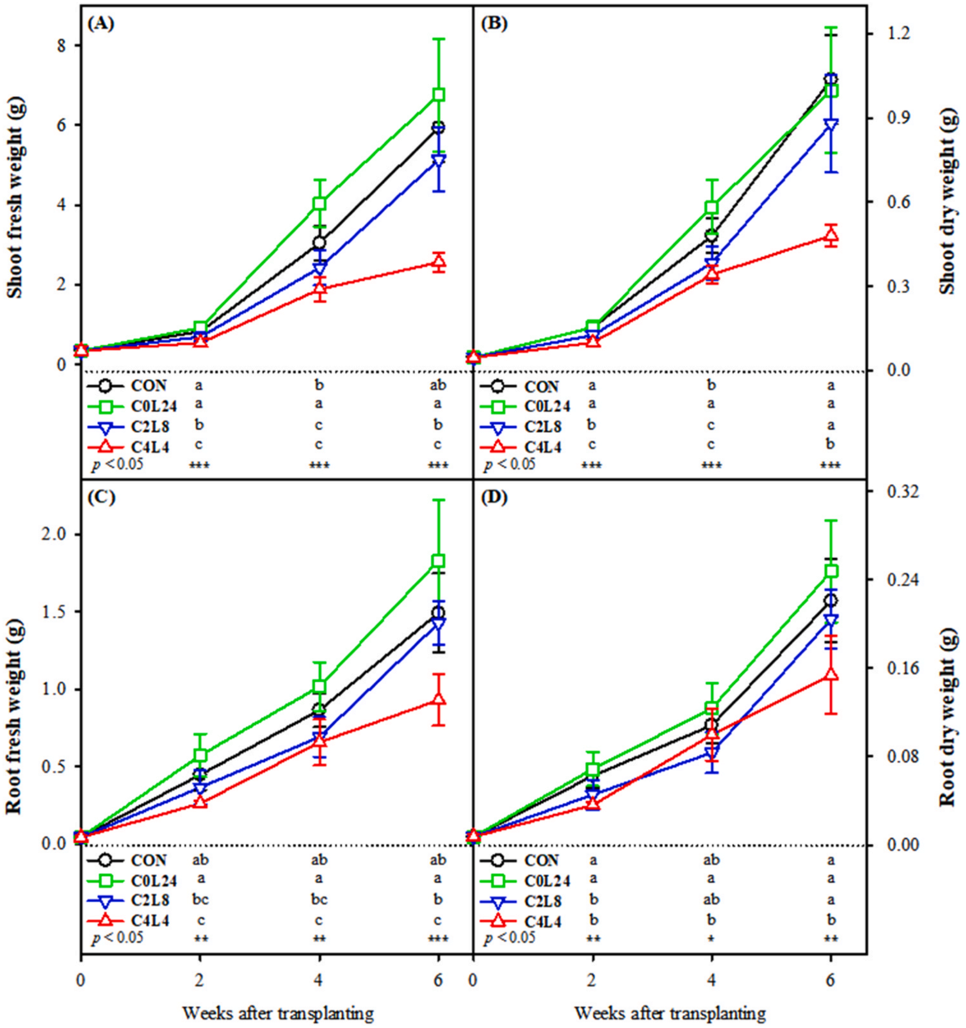

Fig. 3. Shoot fresh and dry weights of *C. asiatica* grown under different lighting strategies (A–D). Different letters above bars indicate significant differences among means (p < 0.05). Error bars represent standard deviation (n = 5).

figures and tables report mean ± standard deviation. To analyze relationships among variables in *C. asiatica* plants, principal component analysis (PCA) and heatmap were conducted using the FactoMine package in R software (version 4.1.1). PCA results highlighted the contributions of ADP, BDP, IER, and RF to each variable.

3. Results

3.1. Shoot and root characteristics

Light/dark (L/D) cycles greatly affect the growth and development of both shoots and roots (Fig. 3). *C. asiatica*, a positive correlation was observed between the light period and both fresh and dry weights of shoots and roots, with continuous light exposure promoting biomass accumulation. Specifically, the C0L24 treatment resulted in the highest shoot and root weights at two, four, and six weeks after transplantation among all treatments. However, at six weeks after transplanting, the root fresh weight and shoot and root dry weights of the C0L24, CON, and C2L8 treatments were not significantly different (Fig. 3B, C, and D). In addition, there was no statistically significant difference between the CON and C2L8 treatments at two, four, and six weeks after transplantation. Moreover, C4L4 treatment consistently exhibited the lowest shoot biomass throughout the cultivation period. In general, the treatment effects on both root fresh and dry weights showed trends similar to those observed in the shoots.

3.2. Leaf characteristics

L/D cycles significantly affected the leaf characteristics of *C. asiatica* (Fig. 4). Specifically, leaf characteristics increased linearly with the duration of the light period in this study. The C0L24 treatment exhibited the highest leaf area (134.9 cm^2) at six weeks after transplanting, which was 14 %, 25 %, and 60 % larger than that of the CON, C2L8, and C4L4 treatments, respectively (Fig. 4A). Overall, there was no significant difference in the number of leaves until four weeks after the light treatment. A similar trend persisted after six weeks of transplantation, with C0L24 showing the highest number of leaves (31.4), followed by CON (29.6), C2L8 (23.8), and C4L4 (14.6) (Fig. 4B). In addition, leaf length and width were consistently the greatest in the C0L24 treatment throughout the entire cultivation period (Fig. 4C and D). In contrast, the C4L4 treatment showed the lowest values for both parameters, whereas no significant differences were observed between the CON and C2L8 treatments.

3.3. Leaf-level gas exchange

Overall, the photosynthetic rate (P_n) was not significantly different between CON and C2L8, but it was highest after three weeks of

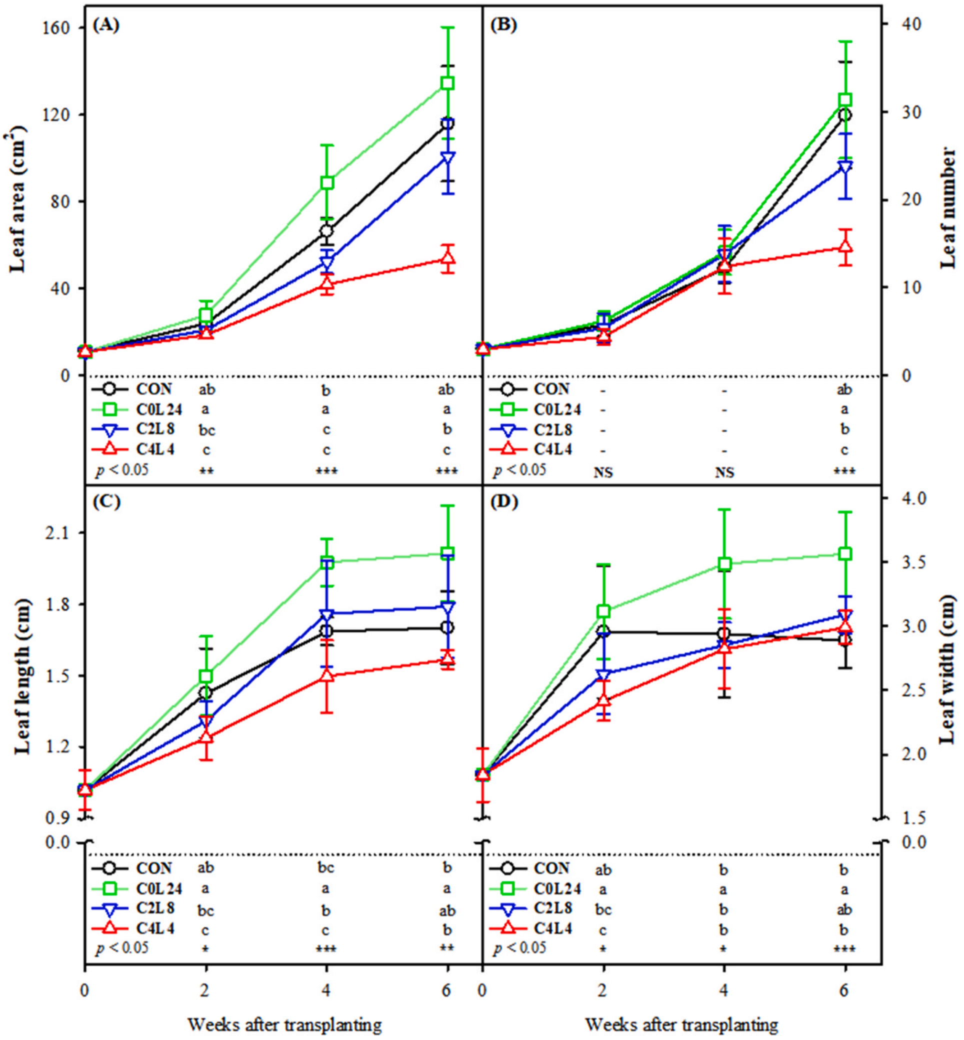

Fig. 4. Leaf characteristics of *C. asiatica* under lighting strategies A–D. Different letters above bars indicate significant differences among means ($p < 0.05$). Error bars represent standard deviation ($n = 5$).

transplanting, with C0L24 showing the lowest value (Fig. 5A). One week and six weeks after transplanting, we did not observe significant differences in intracellular CO_2 concentration (C_i) (Fig. 5B) among the different treatments. However, C0L24 and C2L8 showed the highest levels, whereas C4L4 exhibited the lowest at three weeks after transplanting. After five weeks, these values were either maintained or slightly decreased; however, no significant differences were observed among the treatments. Stomatal conductance (G_s) and transpiration rate (T_r) followed similar trends, with C2L8 showing relatively high values at one and three weeks after transplanting. By three weeks onward, a general increase in all treatments was observed, with C0L24, C2L8, and CON showing relatively elevated values, whereas C4L4 showed lower G_s and T_r. After five weeks, G_s and T_r of C0L24 decreased and increased in C4L4, respectively. However, no significant differences were observed among the treatments.

Although C2L8 exhibited a relatively high leaf-level gas exchange rate, no significant differences were observed at six weeks after transplanting, and this was not reflected in shoot or root growth.

3.4. *Chlorophyll fluorescence, antioxidant capacity, and centellosides concentration*

The photochemical characteristics of *C. asiatica* were affected by the L/D cycle treatment (Fig. 6). Fv/Fm did not show significant differences among the treatments, but it increased after one to three weeks of transplantation (Fig. 6A). It was then maintained at a value between 0.8 and 0.84 for three to five weeks after transplanting. The quantum efficiency of photosystem II activity (φPSII) and electron transport rate (ETR) showed similar trends, increasing from one to three weeks after transplanting, but no significant differences were observed among the treatments (Fig. 6B and C). However, after five weeks of transplanting, φPSII and ETR levels were maintained in the C0L24 and CON groups, whereas they declined in the C2L8 and C4L4 groups. The CON had the highest values, whereas C2L8 and C4L4 had the lowest values. NPQ decreased during the growing period (Fig. 6D). No significant differences were observed at one and five weeks after transplanting, but at three weeks after transplanting, C2L8 and C4L4 had the highest NPQ content, while CON had the lowest.

Changes in antioxidant activity by L/D cycles are shown in Table 1. TP levels were highest in the C4L4 and CON conditions and lowest in the C0L24 group. In contrast, TF was highest in C4L4, C2L8, and CON and lowest in C0L24, whereas ABTS was high in C4L4, with no significant differences among the remaining treatments. Finally, the ascorbic acid content was highest in C2L8, followed by C4L4, and lowest in C0L24.

The concentrations of centellosides (madecassoside, asiaticoside, madecassic acid, and asatic acid) decreased with increasing L/D cycle length (Fig. 7). Madecassoside and asiaticoside showed a similar trend, with the highest levels in C4L4 throughout the growing period, followed

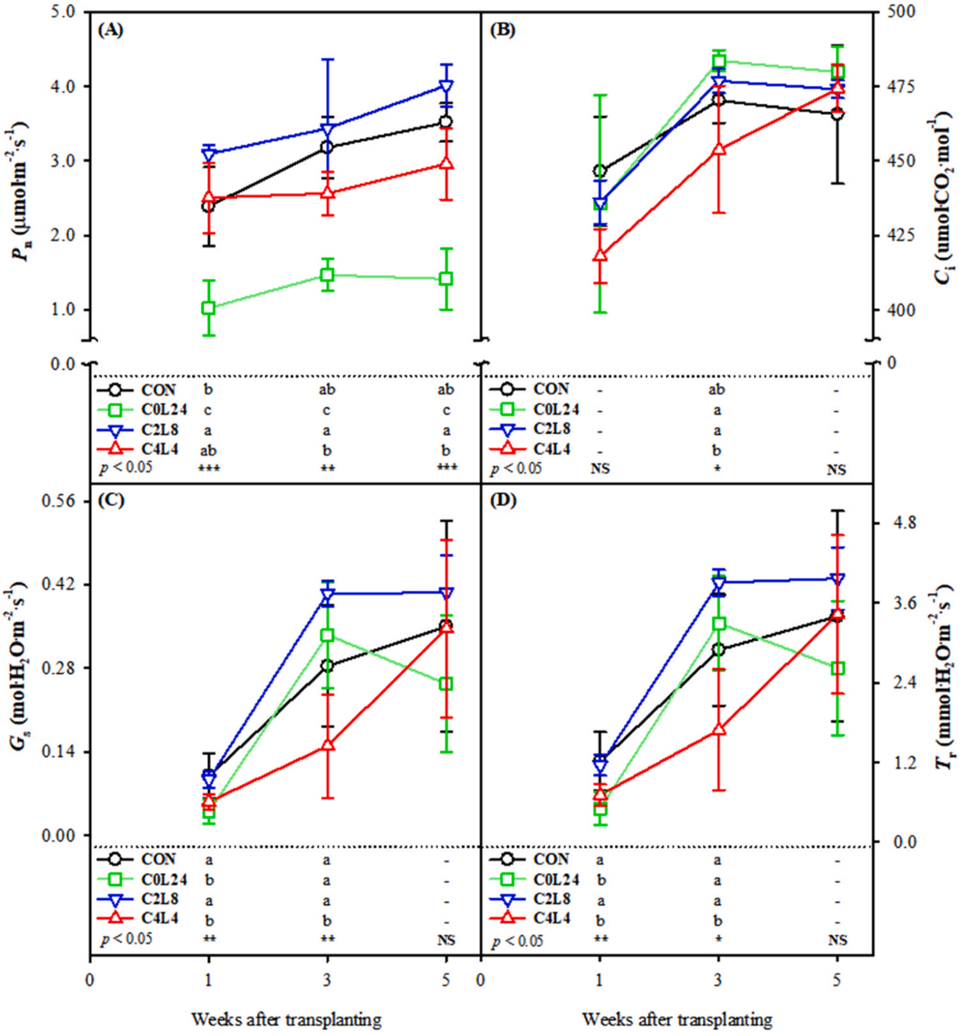

Fig. 5. Photosynthetic characteristics of *C. asiatica* under various lighting strategies: (A) net photosynthetic rate, (B) intercellular CO₂ concentration, (C) stomatal conductance, and (D) transpiration rate. Different letters above bars indicate significant differences among means ($p < 0.05$). Error bars represent standard deviation ($n = 4$).

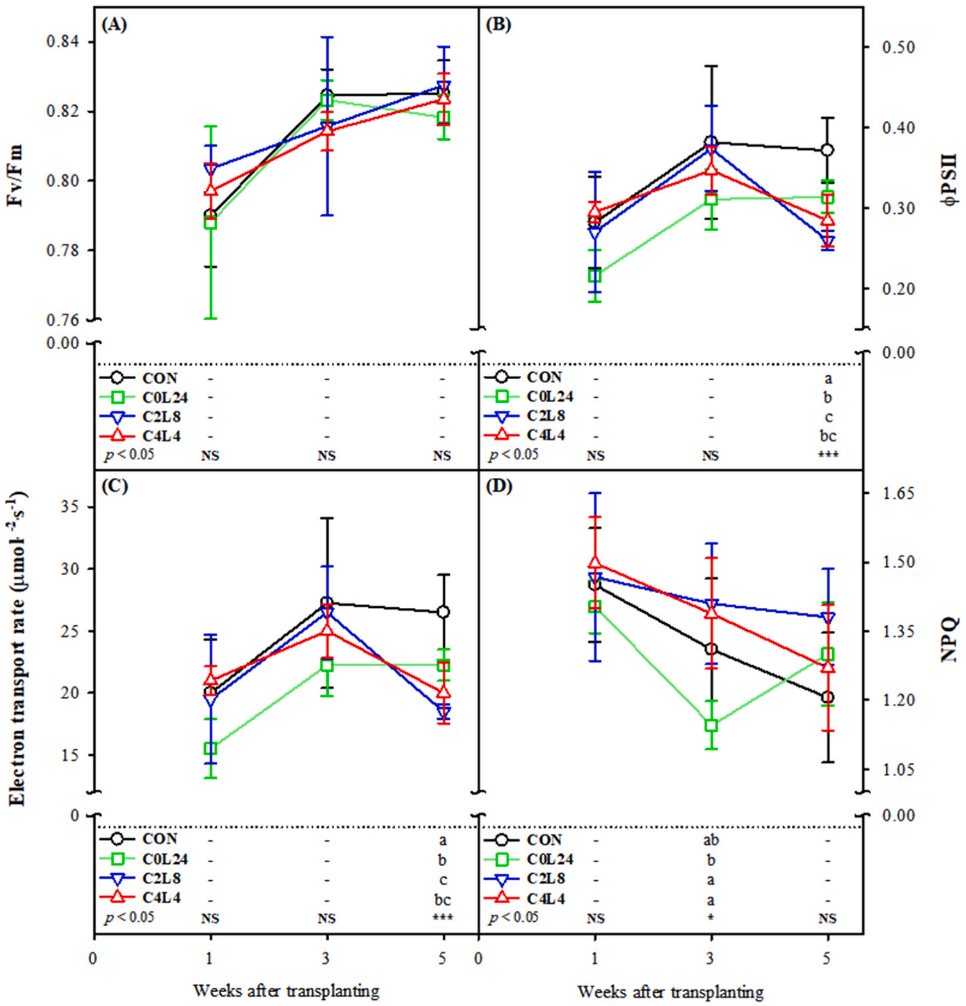

Fig. 6. Chlorophyll fluorescence parameters of *C. asiatica* under various lighting strategies: (A) maximum PSII quantum yield (Fv/Fm), (B) quantum yield of photosystem II (ΦPSII), (C) electron transport rate (ETR), and (D) non-photochemical quenching (NPQ). Different letters above bars indicate significant differences among means ($p < 0.05$). Error bars represent standard deviation ($n = 4$).

Table 1

Effect of various light treatments on total phenol (TP) and total flavonoid (TF) concentrations, antioxidant activity (ABTS assay), and ascorbic acid concentration of *C. asiatica* at 6 weeks of cultivation.

Treatment (cycle/light)	TP (mg GAE·g^{-1} DW)	TF (catechin-eq mg·g^{-1})	ABTS (Trolox mM)	Ascorbic acid (mM)
CON (C1L16)	25.33 ± 0.71[z] a[y]	10.46 ± 0.31 a	15.65 ± 0.03 b	0.77 ± 0.00c
C0L24	20.65 ± 0.33c	9.45 ± 0.35 b	15.73 ± 0.04 b	0.66 ± 0.03 d
C2L8	23.00 ± 0.54 b	10.41 ± 0.20 a	15.66 ± 0.05 b	1.08 ± 0.04 a
C4L4	25.68 ± 0.22 a	10.44 ± 0.02 a	15.86 ± 0.05 a	0.93 ± 0.05 b
Significant	***	**	**	***

[z] Mean ± S.D.

[y] Statistical significance of differences between the means of two treatments was determined by Student's *t*-test ($n = 3$). ***, $p < 0.001$

by CON, C2L8, and C0L24 (Fig. 7A and B). However, C0L24 gradually decreased during the growing period. Madecassic acid content was highest in the C4L4 and lowest in C0L24 at two weeks after transplanting but decreased after four weeks of transplanting (Fig. 7C). At four and six weeks after transplanting, madecassic acid was not detected in C0L24, whereas CON showed the highest levels, followed by C4L4 and C2L8. Asiatic acid content was highest in C2L8 and lowest in C0L24 at two weeks after transplanting (Fig. 7D). However, at four weeks, C4L4 had the highest asiatic acid content, followed by CON, C2L8, and C0L2. At six weeks, C4L4 and CON were the highest. Overall, C0L24 consistently exhibited the lowest centellosides levels throughout the growing period.

3.5. Correlation analysis

The results of the growth analysis showed that C0L24 showed the best growth, followed by CON, C2L8, and C4L4. In contrast, the antioxidant content showed the opposite trend. To understand this trend more accurately, PCA was performed (Fig. 8A). PC1 and PC2 together accounted for 63.9 % of the total variation, with PC1 and PC2 explaining 44.3 % and 19.6 % of the variation, respectively. The positive (+) direction of PC1 was strongly correlated with the antioxidant index, and the negative (–) direction of PC1 was closely related to the growth index. C0L24 showed a close correlation with growth-related indices, and C4L4 was close to antioxidant activity. Meanwhile, the CON and C2L8 treatment groups showed photochemical efficiency and balance between growth and substances and showed an intermediate position.

Similarly, correlation analysis confirmed the PCA results, and growth and antioxidant indices showed a clear negative correlation (Fig. 8B). High positive correlations were observed among growth indices, such as

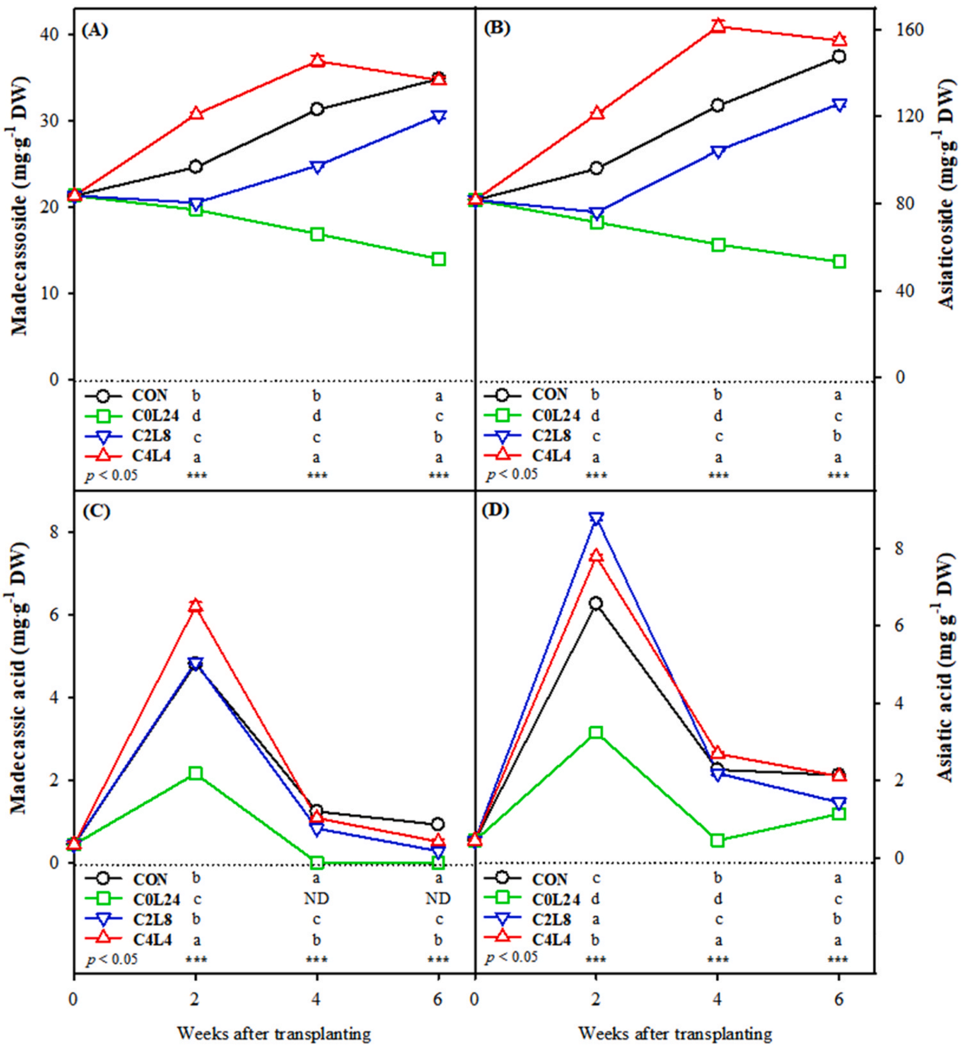

Fig. 7. Centellosides content in *C. asiatica* under different lighting strategies: (A) madecassoside, (B) asiaticoside, (C) madecassic acid, and (D) asiatic acid. Different letters above bars indicate significant differences among means ($p < 0.05$). Error bars represent standard deviation ($n = 3$).

SDW-LN (0.91), SFW-LA (0.87), and SDW-RDW (0.80). Strong positive correlations were also observed among the antioxidant indices, such as AA-TP (0.97), AS–TP (0.94), and MAS–TF (0.88). However, SDW-ABTS (-0.65), LA-AS (-0.6), and LA–TP (-0.55) showed consistent negative correlations between growth and antioxidant index. In addition, photochemical indices (Fv/Fm, Yield, PSII, ETR, etc.) did not share a clear directionality with growth and antioxidant indices in general and tended to show neutral or independent responses.

4. Discussion

4.1. Shoot and root characteristics

Understanding how light and dark periods control plant traits is important for effective crop cultivation, since light conditions strongly determine the yield and quality of agricultural products (Dai et al., 2024). This control is achieved through the biological clock within plants, which is an internal time-regulation mechanism that adjusts biological activities to match changes in the external environment, such as light and dark cycles (Lumsden, 1998). These adjustments facilitate plant survival and adaptation by regulating physiological growth and development through circadian rhythms (McClung, 2006). In general, the biological clock is characterized by two properties: it is both endogenous, that is, it functions without external influence, and inducible, that is, it can be synchronized in response to specific environmental stimuli, such as light conditions (Dai et al., 2024). In this study, the synchronization of *C. asiatica* under various L/D cycle treatments may be explained by the entrainment of its endogenous circadian clock to photoperiodic cues. In a previous study, a lighting strategy characterized by slightly reducing light intensity and increasing photoperiod at a fixed DLI promoted the growth of lettuce through a mechanism that increased chlorophyll in response to low light levels (Mao et al., 2019). Another study reported that when the light period was extended to 24 h in hydroponically grown *Artemisia princeps*, growth and secondary metabolite content increased (Hata and Kawamura, 2023). Furthermore, Zhou et al. (2020) reported that when the photoperiod of lettuce was increased from 3 h to 12 h, the photosynthetic capacity increased and improved the biomass. In contrast, this study confirmed that *C. asiatica* biomass decreased as the light period was shortened (Fig. 3). Urairi et al. (2017) reported that an increased L/D ratio resulted in increased starch content in lettuce during the dark period, which caused a decrease in the growth rate. Chatterton and Silvius (1979) showed that leaves exposed to short photosynthetic periods exhibited higher starch accumulation than those exposed to longer photosynthetic periods, whereas residual dry weight tended to decrease. Although carbohydrate levels were not directly measured, the observed low biomass under C4L4 treatment likely reflects the 4 h light/4 h dark cycle being suboptimal for both photosynthetic carbon gain and the

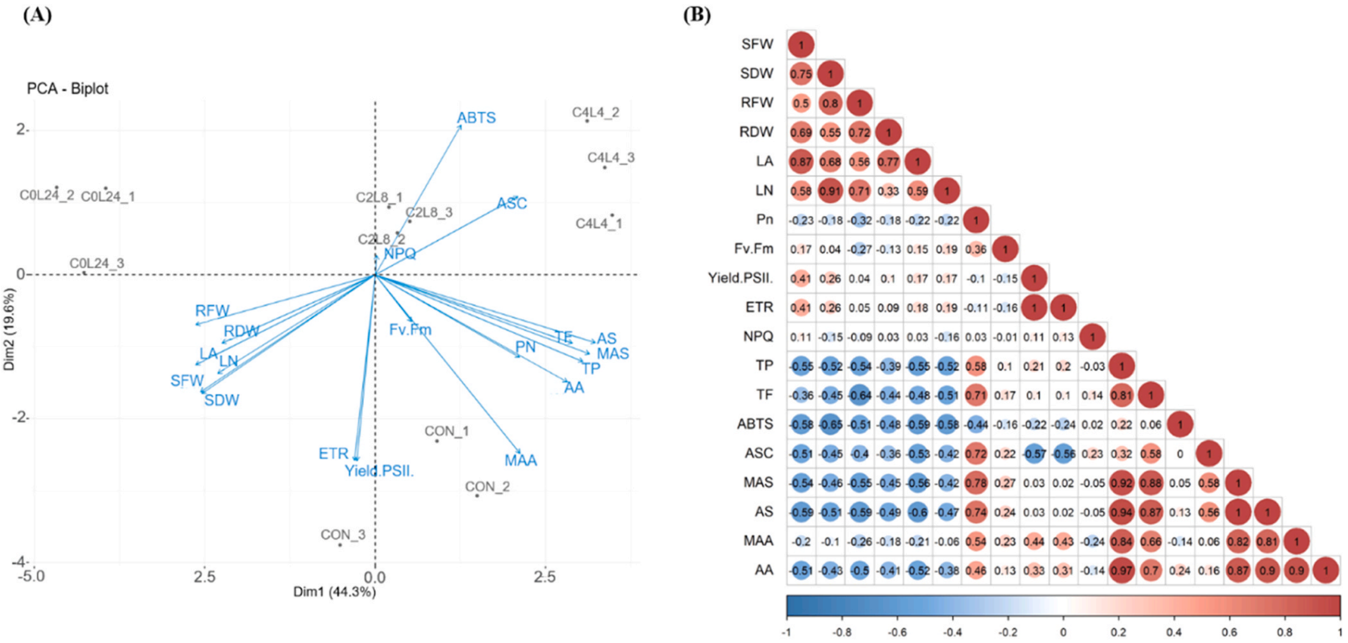

Fig. 8. Centellosides Multivariate principal component analysis (PCA) of the growth characteristics and secondary metabolites of *C. asiatica* under different lighting strategies. (A) Correlation plots and PCA scatterplots summarizing the metabolic relationships between conditions and variables treatments at different lighting strategies (n = 3). (B) Heatmap of Pearson correlation between variables in *C. asiatica* under different lighting strategies. Abbreviations: SFW: shoot fresh weight; SDW: shoot dry weight; RFW: root fresh weight; RDW: root dry weight; LA: leaf area; LN: number of leaves; Pn: net photosynthetic rate; Fv.Fm: maximum PSII quantum yield; Yield.PSII: quantum yield of photosystem II; ETR: electron transport rate; NPQ: non-photochemical quenching; TP: total phenol; TF: total flavonoid; ABTS: ABTS assay; ASC: ascorbic acid; MAS: madecassoside; AS: asiaticoside; MAA: madecassic acid; AA: asiatic acid. The significance level for the model was set at $p < 0.05$.

subsequent metabolic utilization of photosynthates for growth. Urairi et al. (2017) found that shorter light periods led to greater allocation to root production, which aligns with the results of the present study (data not shown). In addition, continuous light treatment (CL), C0L24 did not cause any physiological disorders in *C. asiatica*. CL treatments are generally tolerated by plants with specific genotypes. Moreover, in the absence of sufficient tolerance, overall plant growth can be severely impaired under continuous light (CL) conditions owing to symptoms such as leaf chlorosis, scorching, and necrosis (Sysoeva et al., 2010). For instance, Velez-Ramirez et al. (2014) reported that tomato yield increased by up to 20 % when a CL tolerance gene was identified and introduced into the breeding program. As observed in this study, *C. asiatica* may possess an inherent tolerance to continuous light or exhibit reduced sensitivity to circadian rhythms, allowing it to utilize light efficiently under prolonged photoperiods without apparent physiological damage. Another possible explanation is that the light intensity used in this experiment was not high enough to induce photodamage, allowing *C. asiatica* to tolerate the 24-hour photoperiod. Moreover, this species is typically found in shaded environments, which may contribute to its inherent tolerance to continuous low-light intensity conditions (Mathur et al., 2000).

Therefore, this study confirms that the biomass content of *C. asiatica* effectively increased as the light period increased, particularly under CL. Taken together, these findings may be valuable for guiding the regulation of plant circadian clocks in space farms containing light environments that differ from those on Earth, providing fundamental knowledge for space food production (Dodd et al., 2005).

4.2. *Leaf characteristics*

Most plants are sensitive to seasonal changes, especially day length, and seasonal differences can trigger the decision to engage in reproductive growth (Jackson and Thomas, 1997). In general, plants detect light signals through photoreceptors, such as phytochromes, phototropins, and cryptochromes. These regulate plant circadian rhythms and can transmit signals to the rest of the plant, thereby allowing it to adapt to its growing environment (Jackson, 2009). Plants induce a variety of photomorphogenetic changes in response to light stimulation (Mawphlang and Kharshiing, 2017). Thus, especially in space-limited production systems, such as vertical farms, cultivation strategies that integrate leaf morphogenesis and photosynthetic efficiency should be established to maximize productivity. In this study, longer light periods were associated with increased leaf sizes. This was especially true in the C0L24 group, consistent with a previous study showing that extending light beyond 16 h of CL conditions positively affected microgreen biomass accumulation and photosynthetic activity (Shibaeva et al., 2022). Hang et al. (2019) reported a comparison of 12, 6, and 3 h photoperiods, and found that longer photoperiods generally increased biomass and leaf area in lettuce. Moreover, comparisons of 6 h and 3 h treatments revealed that growth increased because 6 h was closer to (if still shorter than) the circadian rhythm. Thus, C0L24 treatment likely affected both photoperiod and light intensity. A previous study reported that leaf area decreased in shaded plants compared to that in plants grown under direct, natural light (Lichtenthaler et al., 1981). Moreover, Palmer and van Iersel (2020) reported that at low PPFD levels, leaf length increased in two lettuce cultivars, resulting in reduced leaf overlap and increased light interception. Therefore, we suggest that, unlike other treatments, C0L24 may compensate for the low light intensity of 100 μmol·m^{-2}·s^{-1} by expanding its leaves to capture more light. Larger leaves increase the total area capable of receiving light, thereby leading to an increase in both fresh and dry weights (Fig. 3). Overall, these results demonstrate the effectiveness of C0L24 and CON in increasing the biomass of *C. asiatica*.

4.3. *Leaf-level gas exchange*

In general, photosynthesis is a critically important metabolic process in plants because it is required for nutrient accumulation and assimilation. Previous studies have reported that the photosynthetic rate is not the only factor influencing crop growth and leaf area (Hang et al., 2019). For example, there may be differences in the respiration period when the plant is in the dark. In most plants, the amount of carbon used during photosynthesis is determined by the balance between photosynthesis and dark respiration (Amthor, 1984). This is primarily because plants photosynthesize in the presence of light to accumulate the carbon and energy required for their growth and development. In contrast, during the night, plants rely on previously stored carbon reserves to support their growth and metabolic processes (Sulpice et al., 2014). This process is driven by cellular respiration, which occurs in the mitochondria. Briefly, ATP is generated via oxidative phosphorylation, which causes the release of CO_2 and the reduction of O_2 to water, which can be used for cell maintenance and growth (Millar et al., 2011). Previous studies have reported that the starch content in grapevine leaves decreases with increasing darkness duration compared to control leaves (Chanishvili et al., 2004). These findings suggest that insufficient dark periods may limit respiratory carbon turnover and affect overall carbon balance. Although respiration rates are known to be strongly influenced by temperature, the results of this study suggest that the duration of the L/D cycle may also play a role in regulating respiration. Here, C2L8 had a higher photosynthetic rate than CON, but the duration of the dark period was shorter. It is possible that under short-dark conditions, plants cannot efficiently generate and accumulate assimilation products through photosynthesis during light periods, although additional experiments focused on respiration and starch content are required to conclusively demonstrate this. Chlorophyll is an important pigment for photosynthesis and plays a direct role in it. In this study, except for C0L24, the chlorophyll content showed a trend similar to the effects of the photosynthetic rate (Fig. S2). However, despite the high chlorophyll content of C0L24, it exhibited a low photosynthetic rate. Previous studies have reported that under CL, photosynthate production exceeds photosynthate utilization, resulting in increased starch accumulation in leaves along with low photosynthate utilization (Sysoeva et al., 2010). However, in this experiment, photosynthesis was measured using a light source in a cultivation environment, not artificial light, and there was a difference in light intensity of approximately 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ between C0L24 and other treatments. As a result, there was a large difference in the measured photosynthetic rate, but we found no significant difference in G_s, T_r, and C_i during the latter half of the growth period. These indices may be related to characteristics such as the stomatal volume, size, and density. Therefore, there are limitations in interpreting these results with the current experimental results. However, it was confirmed that the duration of the L/D cycle affected these characteristics. In addition, when measured with a photosynthetic device under artificial light, we confirmed that the photosynthetic rate of C0L24 was slightly higher than that of C4L4 (Fig. S3 and Table S1). Therefore, although photosynthesis is a critical metabolic process in plants, the photosynthetic rate was not the sole factor affecting biomass accumulation in *C. asiatica* in this study, as we hypothesized.

4.4. *Chlorophyll fluorescence*

The Fv/Fm value indicates the maximum photoconversion efficiency of PSII. Most healthy C3 plant species have an Fv/Fm value of approximately 0.8–0.84; however, this value decreases significantly following exposure to stress (Björkman and Demmig, 1987; Baker, 2008). In this study, the Fv/Fm values of all treatments remained consistently above 0.8 after three weeks of transplanting, indicating that manipulation of the L/D cycle did not induce severe photoinhibition or structural damage to PSII. These results suggest that the differences in plant performance among the treatments were not attributable to the impairment of the photosynthetic apparatus itself. Chlorophyll can absorb light energy via three pathways, which can be used for photosynthetic reactions, or in some cases, the excess energy is dissipated as heat or reemitted as fluorescence (see Maxwell and Johnson, 2000). Importantly, an increase in the efficiency of one pathway is generally accompanied by a decrease in the other two pathways, reflecting dynamic energy allocation within the photosynthetic system (Misra et al., 2012). In addition, the effective quantum yield of PSII (φPSII) and electron transport rate (ETR) are widely used indicators of photochemical performance (Kelly et al., 2020). Previous studies have reported a strong linear relationship between φPSII and ETR under varying light intensities, indicating coordinated regulation of photochemical processes (Fu et al., 2012). Furthermore, Mao et al. (2019) demonstrated that lettuce grown under short periods of high-intensity light achieved comparable growth to control plants receiving the same daily light integral (DLI), as a greater proportion of the absorbed energy was directed toward photochemical processes rather than dissipated as heat through NPQ. In this study, C2L8 and C4L4, which were characterized by relatively short L/D cycles, exhibited lower φPSII and ETR values at five weeks than other treatments. In addition, these treatments showed slightly higher NPQ values during the early growth period than the control. These results indicate that under short L/D cycles, a larger fraction of the absorbed light energy may have been allocated to non-photochemical quenching rather than photochemical reactions. Previous studies have shown that light environments, including photoperiod and light intensity, regulate secondary metabolite biosynthesis by influencing photosynthetic efficiency and stress-related signaling (Wu et al., 2025). Accordingly, although direct molecular evidence is lacking, these photochemical responses provide a physiological basis for hypothesizing that altered energy partitioning under short L/D cycles may contribute not only to reduced carbon assimilation but also to shifts in downstream metabolic allocation. Taken together, although no direct molecular or biochemical evidence was obtained in this study, the observed photochemical responses support a hypothetical mechanism in which altered energy partitioning under short L/D cycles reduces the efficiency of carbon assimilation, potentially contributing to lower biomass accumulation in C2L8 and C4L4 groups. This interpretation is consistent with our earlier observation that the photosynthetic rate alone did not fully explain the differences in biomass production among the treatments. Moreover, such shifts in photochemical and non-photochemical energy allocation may influence downstream carbon partitioning, thereby affecting the balance between primary growth and secondary metabolite biosynthesis, as discussed in the next section.

4.5. *Antioxidant capacity*

Medicinal plants often contain polyphenols, which act as antioxidants and possess valuable pharmacological effects (Ghasemzadeh et al., 2010; Seevaratnam et al., 2012). Antioxidant systems play a central role in photoprotective and stress response mechanisms in plants exposed to fluctuating light environments (Smirnoff, 1998). Previous studies on spinach have also reported that TPC accumulation is more strongly induced by light than by darkness, primarily through TPC biosynthesis via the phenylalanine ammonia pathway, which is regulated by a PAL core rate-controlling enzyme (Kang and Saltveit, 2003; Zhan et al., 2020). Another study reported that the shorter the photoperiod, the higher the TP/TF antioxidant activity, which was similar to the results observed in this study (Song et al., 2020). However, Bian et al., (2016) reported that CL actually enhanced TP and antioxidant activity in lettuce, while another found that antioxidant activity in lettuce is rhythmically regulated the day as a result of its circadian rhythm (Soengas et al., 2018). Taken together, these studies suggest that antioxidant responses are modulated not only by light duration but also by the interactions between photoperiod and endogenous circadian regulation. In this study, the antioxidant activity was significantly higher in the C4L4 group than in the CON group, with C4L4 exhibiting the highest overall activity, suggesting that short L/D cycles may enhance antioxidant metabolism through altered photophysiological states. From a physiological perspective, these metabolic responses may be linked to changes in photochemical efficiency and carbon utilization observed under short L/D cycles. Previous studies have shown that carbon imbalance, such as excessive sugar accumulation due to incomplete utilization, can disrupt reactive oxygen species (ROS) homeostasis via HXK1-dependent sugar signaling within the SnRK1–TOR metabolic network (Asad et al., 2024). This disruption can alter redox metabolism in chloroplasts and mitochondria, affect electron transport, and interfere with NADPH-dependent antioxidant pathways, ultimately promoting ROS accumulation (Keunen et al., 2013). Based on these findings, we hypothesized that shortened dark periods may limit respiratory carbon turnover, leading to metabolic imbalance and increased ROS generation, which in turn stimulates the biosynthesis of phenolic compounds and other antioxidants as an adaptive stress response. Ascorbic acid is an essential plant metabolite involved in environmental stress processes, such as photosynthesis and photoprotection. It functions as a non-enzymatic antioxidant that scavenges ROS (Smirnoff and Wheeler, 2000). According to Goss and Jakob (2010), ascorbic acid promotes the formation of epoxidase in the xanthophyll cycle and acts more quickly and effectively in photoprotection than NPQ, which dissipates as heat (Xu et al., 2000). In this study, NPQ values and ascorbic acid levels were elevated in C2L8 and C4L4, suggesting that short cycles enhance photoprotective mechanisms, potentially through rapid ROS scavenging by ascorbic acid (Fig. 6D, Table 1). Notably, biomass accumulation exhibited an inverse relationship with phenolic content and antioxidant capacity across the L/D treatments, indicating a trade-off between primary growth and secondary metabolite production. This pattern aligns with previous findings in lettuce, where increased phenolic accumulation and antioxidant capacity were associated with reduced growth (Son and Oh, 2013). Although this study did not directly quantify sugars, ROS levels, or gene expression related to antioxidant metabolism, the observed coordination between photochemical responses and metabolite accumulation supports a hypothetical mechanism linking L/D cycle manipulation to stress-related metabolic reprogramming. Further studies integrating metabolomic and transcriptomic analyses are therefore required to validate these mechanisms and clarify the downstream physiological and molecular consequences of L/D cycle variations.

4.6. Centellosides concentration

Triterpenes, secondary metabolites, are produced in most plant species via interactions between the plant and its environment. Moreover, triterpenes are a major indicator of *C. asiatica* and are known as saponin-based substances (Prasad et al., 2019). In general, triterpene biosynthesis proceeds via two pathways: the mevalonate and methylerythritol phosphate pathways (Augustin et al., 2011). Moreover, saponin-based substances (i.e., madecassoside and asiaticoside) are produced via the glycosylation of sapogenin-based substances (i.e., madecassic acid and asiatic acid). Glycosylation is a crucial process that plays a key role in the biological activities of various saponins. However, experimental data on the mechanism of triterpene glycosylation remain lacking (Lambert et al., 2011). As ROS production in plants plays a common role in signal transduction for all environmental stress responses, it has been reported that the metabolism of ginsenoside, a saponin, is regulated by oxidative stress (Yendo et al., 2010). Furthermore, Müller et al. (2013) reported that high levels of PAR enhanced the saponin formation of *C. asiatica* centellosides, indicating that sapogenins were biosynthesized into saponins via glycosylation under oxidative stress. In this study, the centellosides content were highest under C4L4, implying sensitivity to stress from altered L/D cycles. Furthermore, previous studies have reported that stress increases the activity of antioxidant enzymes, leading to a corresponding increase in centelloside content (Biswas et al., 2020). Furthermore, increased transcription of genes involved in centellosides biosynthesis, such as squalene synthase, farnesyl pyrophosphate synthase, and β-amyrin synthase, has been observed during this process. Although gene expression was not assessed in this study, these findings collectively support the possibility that L/D cycle manipulation induces stress-related metabolic reprogramming that favors centellosides production. Recent transcriptomic analyses have identified candidate genes correlated with centelloside accumulation and demonstrated that the major triterpenes in *C. asiatica* exhibit antioxidant activity that contributes to ROS scavenging (Wan et al., 2024; Lim et al., 2024). Moreover, studies using abiotic elicitors in hairy root cultures have consistently reported increased centellosides production, reinforcing the concept that stress-induced signaling enhances secondary metabolite biosynthesis (Hassanabad et al., 2025). Taken together, although ROS levels, glycosylation activity, and triterpenoid gene expression were not directly measured in the present study, the observed increase in centellosides content under short L/D cycles is consistent with a hypothetical mechanism in which L/D cycle–induced physiological stress alters ROS-related metabolism, thereby promoting centellosides biosynthesis. Thus, we observed that centellosides production by *C. asiatica* was efficiently induced under a variety of L/D cycles. However, despite the high and steady demand for *C. asiatica* due to its novel therapeutic benefits, like many other medicinal plants, more than 90 % of commercial products are sourced via wild harvesting before being supplied to the industry. This presents important limitations in terms of sustainability and quality control during production (Prasad et al., 2019). The results of this study suggest that manipulation of the L/D cycle represents a promising environmental lever to modulate centellosides accumulation, providing a physiological basis for optimizing *C. asiatica* production in controlled environment agriculture.

4.7. Correlation analysis

This study aimed to analyze the changes in the physiological and metabolic responses of *C. asiatica* when light and dark conditions were altered while maintaining the same DLI. Although the influence of DLI has been reported in previous studies, the effects of varying light and dark durations at a constant DLI have not been systematically investigated yet. Biomass was highest in C0L24 with a long light period, likely because the extended photosynthetic period, along with increased leaf number and leaf area, directly contributed to the accumulated biomass. Lanoue et al. (2022) confirmed that amaranth and green basil had greater fresh biomass when grown at CL compared with 16 h when DLI was the same, which was consistent with the results of this study. Another study reported that leaf characteristics were enhanced under CL compared with 12 h, but photosynthetic performance was similar, which contributed to maintaining photosynthesis without excessive starch and sucrose accumulation in the leaves under CL conditions (Lanoue et al., 2019). However, further investigation of carbohydrate dynamics is required to fully elucidate the underlying mechanisms. In contrast, C4L4, characterized by a shorter light period and higher L/D cycle frequency, exhibited relatively suppressed growth but the highest accumulation of antioxidant compounds, including centellosides. This pattern suggests a trade-off between biomass accumulation and secondary metabolite synthesis, consistent with the growth–differentiation balance hypothesis, which proposes that environmental constraints, such as light-induced stress, can redirect carbon allocation from primary growth toward secondary metabolism (Herms and Mattson, 1992). From a physiological perspective, this trade-off appears to be closely associated with changes in the photochemical performance. In the present study, shorter L/D cycles were associated with reduced photochemical efficiency and increased non-photochemical quenching, indicating enhanced excitation energy dissipation, rather than photochemical energy use. Importantly, the maintenance of Fv/Fm values and chlorophyll content within non-stress ranges suggests that L/D cycle manipulation imposed moderate regulatory physiological stress rather than irreversible photodamage to the photosynthetic apparatus. These alterations in photochemical efficiency may influence carbon assimilation efficiency and redox homeostasis, thereby modifying carbon partitioning between primary growth and secondary metabolite biosynthesis. Specifically, longer photoperiods favor sustained photochemical activity and carbon assimilation, thereby promoting primary growth, whereas shorter photoperiods increase excitation pressure and energy dissipation, conditions that are commonly associated with stress-responsive metabolic reprogramming. Under such conditions, light-induced stress has been reported to activate ROS-mediated signaling pathways, promoting the accumulation of secondary metabolites, including phenolics and terpenoids, as part of adaptive defense responses (Sharma et al., 2019). Accordingly, the elevated centellosides accumulation observed under short L/D cycles may reflect a stress-related redirection of carbon flux rather than a direct consequence of reduced photosynthetic capacity. It should be noted that this study did not directly quantify molecular indicators such as ROS levels, carbohydrate status, or gene expression related to secondary metabolism, which represents a limitation in fully elucidating the underlying regulatory mechanisms. Therefore, the interpretations presented here should be regarded as a hypothetical mechanism derived from coordinated physiological and metabolic observations rather than definitive causal evidence. While Fayezizadeh et al. (2024) reported higher antioxidant accumulation under longer light periods in microgreen basil, particularly under continuous light, the underlying antioxidant-related stress response mechanisms appear to be comparable. These contrasting results further highlight that the interaction between DLI and photoperiod, rather than either factor alone, determines whether assimilated carbon is preferentially invested in primary growth or diverted to secondary metabolism. However, additional experiments are needed to clarify whether growth suppression under short photoperiods in this study primarily reflects reduced photosynthetic carbon gain or insufficient respiratory carbon turnover during dark phases. Previous studies have demonstrated that integrated metabolomic and transcriptomic analyses are effective tools for elucidating stress-induced metabolic reprogramming and identifying key regulatory nodes controlling secondary metabolite biosynthesis (Nakabayashi and Saito, 2015). Thus, future multi-omics-based investigations are essential to validate the proposed hypothetical mechanism and to elucidate how DLI–photoperiod interactions regulate carbon allocation between primary metabolism and centellosides accumulation in *C. asiatica*. Such mechanistic insights would provide a robust foundation for optimizing light management strategies tailored to specific production goals in controlled environment and vertical farming systems.

5. Conclusion

This study demonstrated that manipulation of the light/dark (L/D) cycle significantly influences the growth, photochemical performance, and secondary metabolite accumulation in *C. asiatica*. Biomass and leaf expansion increased as the cycle length shortened and the light period was extended, whereas longer L/D cycles reduced photochemical efficiency, increased non-photochemical quenching, and ultimately limited biomass accumulation. In contrast, extended cycle lengths were associated with higher concentrations of TP, TF, ascorbic acid, and centellosides, likely reflecting stress-related protective responses to shorter light durations. Notably, continuous light (C0L24) effectively promoted biomass production without inducing apparent stress, whereas shorter L/D cycles, such as C4L4, favored secondary metabolite accumulation, potentially through oxidative stress–related mechanisms.

From an industrial vertical farming perspective, these findings provide practical insights into balancing productivity and phytochemical quality during *C. asiatica* cultivation. In particular, since lighting accounts for approximately 30–40 % of total operating costs in controlled environment farming, selecting an appropriate photoperiod can be an important management strategy to secure stable centellosides yields while improving energy efficiency. Optimizing the L/D cycle represents a viable strategy to improve energy use efficiency while maintaining target centellosides yields, with the CON treatment exhibiting a favorable trade-off between biomass production and secondary metabolite accumulation under comparable DLI conditions.

Future studies should focus on elucidating the underlying physiological and molecular mechanisms through integrated metabolomic and transcriptomic analyses to validate stress-response pathways and identify key regulatory genes involved in centellosides biosynthesis. In addition, the development of DLI-based optimization models incorporating energy consumption, together with multi-tier cultivation and LED spectrum optimization studies, will be essential for enhancing the scalability and economic feasibility of sustainable *C. asiatica* production in vertical farming systems.

CRediT authorship contribution statement

Jae Gil Yun: Resources. **Ga Oun Lee:** Investigation. **Juhwan Lee:** Writing – review & editing, Methodology. **Jun Gu Lee:** Resources. **Ki-Ho Son:** Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Myung-Min Oh:** Writing – review & editing. **Moon-Sun Yeom:** Writing – review & editing, Writing – original draft. **Gyu-Sik Yang:** Writing – original draft. **In-Je Kang:** Investigation. **Seong-Nam Jang:** Methodology. **Han-Sol Sim:** Methodology.

Funding

This research was financially supported by the Ministry of Trade, Industry, and Energy (MOTIE), Korea, under the “World Class Plus Project” (Project No. P0024386) was supervised by the Korea Institute for Advancement of Technology (KIAT).

Declaration of Generative AI and AI-assisted technologies in the writing process

The AI tool did not contribute to the development of scientific content, data interpretation, or conclusions. The authors assume full responsibility for the accuracy and integrity of the work presented in this manuscript.

Declaration of 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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2026.122732.

Data availability

Data will be made available on request.

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