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Effect of root-zone temperature on resource redistribution and centellosides accumulation in *Centella asiatica* under vertical farming

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ABSTRACT

Centella asiatica L. Urban (*C. asiatica*) is a stoloniferous medicinal herb valued for centelloside production. In this study, we examined how root-zone temperature (RZT) affects growth, physiology, mineral nutrient partitioning, and centelloside accumulation in *C. asiatica* grown at 25 °C, with RZT controlled at 10 °C (LRT), 20 °C (NRT), and 30 °C (WRT). LRT severely inhibited root development, water status, mineral nutrient accumulation, gas exchange, chlorophyll status, and shoot growth. However, anthocyanin, asiaticoside, and madecassoside concentrations increased under LRT. NRT maintained stable root development, water relations, and photosynthetic performance, but resulted in the lowest centelloside accumulation. Under WRT, root development was restricted, whereas shoot biomass and photosynthetic rate were maintained. Warm RZT increased the root-to-shoot mobility of Mg, Fe, and P, indicating altered nutrient partitioning rather than improved nutrient uptake. This nutrient distribution pattern was associated with sustained shoot function and increased centelloside accumulation under WRT. Although suboptimal RZT increased centelloside concentrations, only WRT enhanced total centelloside yield by maintaining shoot growth, suggesting its potential for short-term centelloside-enriched *C. asiatica* production in vertical farming systems.

Abbreviations: Ci, Intercellular CO_2 concentration; DW, Dry weight; ETR, Electron transport rate; Fv/Fm, Maximum quantum efficiency of PSII; FW, Fresh weight; g_s, Stomatal conductance; LA, Leaf area; LRT, Low root-zone temperature; NPQ, Non-photochemical quenching; NRT, Normal root-zone temperature; A, Net photosynthetic rate; MC, Moisture content; RZT, Root-zone temperature; SLA, Specific leaf area; TF, translocation factor; Tr, Transpiration rate; WRT, Warm root-zone temperature; HSD, Honestly significant difference; YII, Effective quantum yield of PSII.

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1. Introduction

Commonly known as gotu kola or tiger grass, *Centella asiatica* L. Urban (*C. asiatica*) is a stoloniferous medicinal herb belonging to the Apiaceae family (Bylka et al., 2013). It thrives in warm and humid environments, and is primarily distributed in tropical and subtropical regions (Gohil et al., 2010). The main bioactive components of *C. asiatica* are triterpenoid saponins, particularly asiaticoside and madecassoside (Alam et al., 2025), which exhibit pharmacological activities including anti-inflammatory (Saha et al., 2013), antibacterial (Harun et al., 2019), and scar improvement (Jenwitheesuk et al., 2018). Centellosides biosynthesis is highly sensitive to environmental factors, such as light quality and intensity (Song et al., 2022, Shin and Lee, 2025), temperature (Pipatsitee et al., 2023), and nutrient supply (Shawon et al., 2023). Therefore, the environmental regulation of centellosides production is essential for optimizing the yield of medicinal compounds in controlled cultivation systems.

A consistent cultivation environment is essential for the stable production of centellosides in *C. asiatica*, and vertical farming systems can provide such conditions by precisely controlling key environmental factors, including light, temperature, and nutrient management. However, maintaining these controlled conditions in vertical farms requires a high energy input, with temperature regulation comprising a substantial portion of operational costs (Cobank, 2022). Compared with whole-environment temperature regulation, root-zone temperature (RZT) control offers a more localized approach by directly regulating the root-zone environment, thereby reducing energy consumption (He et al., 2013, Song et al., 2023). This makes RZT control an energy-efficient strategy for achieving both sustainability and consistent crop quality in vertical farming. Despite these advantages, the effects of RZT control have mainly been examined in a limited range of crops, and its applicability to high-value crops, such as medicinal plants, remains insufficiently evaluated (Aji et al., 2020, Hayashi et al., 2024, Levine et al., 2023).

In this study, RZT treatments of 10, 20, and 30 °C were established under a constant air temperature of 25 °C to characterize the responses of *C. asiatica* to a broad RZT gradient rather than to determine the precise optimal RZT. The 20 °C treatment was selected as a practical reference RZT based on a previous hydroponic smart-farm study of *C. asiatica*, in which the nutrient solution temperature was maintained at 20 ± 1 °C (Lee et al., 2025). The 10 °C and 30 °C treatments were used to represent strong root-zone cooling and elevated RZT, respectively. Because RZT responses of *C. asiatica* under vertical farming conditions remain poorly understood, this experimental design was intended to identify the directional effects of contrasting RZT conditions on growth, photosynthesis, mineral nutrient uptake, and centelloside accumulation.

The RZT refers to the temperature environment surrounding the roots and is primarily determined by nutrient solution temperature in hydroponics. Since roots are primary organs responsible for the absorption and long-distance transport of water and inorganic nutrients (Rellán-Álvarez et al., 2016), suboptimal RZT can alter root cell division, cell elongation, hydraulic conductivity, and root structural development (Hasanuzzaman et al., 2013, Kawasaki et al., 2013). These changes in root function affect water absorption, nutrient transport, photosynthetic and transpiration responses (Wittayathanarattana et al., 2022, Hayashi et al., 2024, Li et al., 2023), and may also be involved in the accumulation of sugars, pigments, and secondary metabolites through stress-related hormonal signaling and metabolic reprogramming (Xia et al., 2021b, Pregitzer and King, 2005, Kang et al., 2025). Therefore, precise management of RZT can be utilized as an important strategy to simultaneously control productivity and quality in vertical farming.

C. asiatica is an important medicinal and cosmeceutical plant because its major compounds, madecassoside and asiaticoside, are associated with wound healing, skin repair, and anti-inflammatory activities. Since Prasad et al. (2012) demonstrated the feasibility of hydroponic cultivation of *C. asiatica*, various cultivation factors have been investigated, including nutrient solution EC (Shawon et al., 2023), light intensity (Shawon et al., 2025, Shin and Lee, 2025), photoperiod (Yang et al., 2026), and air temperature (Pipatsitee et al., 2023). However, temperature-related studies have primarily focused on aerial temperature, whereas the independent effects of RZT under vertical farming conditions remain poorly understood. Therefore, in this study, three RZT treatments—10, 20, and 30°C—were established under a constant aerial temperature of 25°C to broadly characterize the RZT-dependent responses of *C. asiatica*. The 20°C treatment was selected as a practical reference RZT based on a previous smart-farm hydroponic study, in which the nutrient solution temperature for *C. asiatica* was maintained at 20 ± 1°C (Lee et al., 2025). In contrast, the 10°C and 30°C treatments were set as strong low-RZT and warm-RZT conditions, respectively. This study aimed to evaluate how these contrasting RZT conditions affect growth, photosynthesis, mineral nutrient uptake, and centelloside accumulation in *C. asiatica*.

2. Materials and methods

2.1. Plant materials and cultivation conditions

The experiment was conducted in a controlled-environment growth room equipped with white LEDs (JC11003–16002A; Samsung, Seoul, Korea). *C. asiatica* seedlings with three fully developed leaves were separated from their mother plants. The seedlings were transplanted into sponges (25 × 25 × 30 mm; W × L × H), placed in a bottom-irrigation tray and irrigated with distilled water for one week to promote root establishment. Based on findings from previous studies, we determined a cultivation method for *C. asiatica* and transplanted the established seedlings into expanded polystyrene cultivation beds (35 L capacity) using a deep-flow technique system. Each cultivation bed accommodated two plates in a 3 × 4 arrangement, and three beds were prepared. We installed two airstones per bed and operated an air pump to ensure continuous circulation of the nutrient solution. The nutrient solution was refreshed weekly.

The air temperature was maintained at 25 ± 1 °C, relative humidity at 60 ± 10%, photosynthetic photon flux density (PPFD) at 150 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (white LED), and photoperiod of 16 h with a daily light integral of 8.64 $mol \cdot m^{-2} \cdot d^{-1}$. We used Hoagland's nutrient solution (pH 6.0, EC 2.0 dS m^{-1}) as the hydroponic medium and replenished small amounts every 2 d to compensate for evaporation and uptake, maintaining a volume of 35 L. The pH and EC of the nutrient solution were measured every 09:00. The pH was adjusted to 6.0 using NaOH, while the EC was maintained by replacing the nutrient solution weekly. After a one-week acclimatization period, we subjected the plants to the respective treatments for three weeks, after which we harvested the samples. The plants were maintained under the same environmental conditions at 25 ± 1 °C (ambient temperature) for two weeks before being subjected to the RZT treatment (Fig. 1).

2.2. Experimental design and RZT

RZT treatments consisted of: (1) strong low RZT (LRT, 10 °C), (2) mild low RZT (NRT, 20 °C), and (3) mild high RZT (WRT, 30 °C) (Table 1). Following Pipatsitee's (2023) report that 25 °C is optimal for *C. asiatica* growth, we maintained the air temperature at 25 °C. Plants were randomly assigned to each RZT treatment. The cultivation beds were randomly positioned and secured within the growth chamber. To minimize the non-RZT environmental effects, the growth panel positions within each treatment were rotated weekly.

To regulate the RZT, we installed a cooling coil inside each polystyrene cultivation bed and connected it to a chiller unit (model HC−30; Jeotech, Daejeon, Korea). An automatic temperature controller (model DOV-882T; Daeil, Busan, Korea) was used to maintain constant nutrient solution temperatures during the day and night. RZT was controlled using submersible heaters (Aquarium Sterilization Heater, GT−500;

Aquarex, China). Cooling units were installed in each nutrient solution reservoir to achieve precise temperature regulation across treatments. We monitored the nutrient solution temperature in all treatment groups using a four-channel thermocouple data logger (HOBO UX120–014M; Onset, Bourne, MA, USA), which recorded measurements at 5-min intervals throughout the study period.

2.3. Growth characteristics

Growth traits were assessed at the end of the 3-week RZT treatment period. We separated the plants into shoots and roots and evaluated their growth. The growth characteristics included the fresh weight (FW), dry weight (DW), number of leaves (LN), total leaf area (LA), moisture content (MC), and specific leaf area (SLA). At harvest, the plants were sampled between 09:00 and 12:00 to minimize the effects of diurnal temperature. After carefully removing the surface moisture, the shoots, roots, and runners were cut off and immediately weighed to determine the fresh biomass. The shoots were used to assess LN and LA, whereas the roots were used for morphological analysis. All the shoot-related growth measurements were performed on the parent plants. LA was determined by measuring all fully expanded leaves with a width of $>$ 10 mm. The sum of these measurements was recorded as the total leaf area. DW was determined after oven drying the samples until a constant mass was reached.

Shoots were separated into leaves and petioles for the subsequent analyses. FW and DW were measured using an analytical balance (PX224KR/E; OHAUS, NJ, USA), and the biomass of both shoots and roots was calculated accordingly. LA was determined using a leaf area meter (LI−3100; LI-COR Biosciences, Lincoln, NE, USA) based on fully expanded leaves. The moisture content was calculated as follows: $(FW - DW)/FW \times 100$. SLA was calculated as LA (cm^2) per leaf dry weight (g). DW was obtained by drying the samples at 70 °C for 24 h in a drying oven (WOF−155, Daihan, Wonju, Korea). Root morphology parameters, including root surface area, average root diameter, root volume, root tip number, and root fork number were measured using a professional scanner (Expression 1000XL; Epson America Inc., Long Beach, CA, USA) connected to a WinRhizo Pro 2007a image analysis system (Regent Instruments, Sainte-Foy, QC, Canada). SPAD and anthocyanin values were measured non-destructively the day before harvest between 09:00 and 12:00, targeting the right side of the widest leaf, fully expanded leaves at a position avoiding the leaf veins. Measurements were taken three times using a chlorophyll meter (SPAD−502 Plus; Minolta Camera Co., Osaka, Japan) and an anthocyanin meter (ACM−200 Plus; Opti-Sciences, Inc., Hudson, NH, USA).

2.4. Measurement of gas exchange and chlorophyll fluorescence parameters

Gas exchange parameters and chlorophyll fluorescence variables were measured on the same day and at the same time as the SPAD and anthocyanin assessments. Gas exchange parameters were measured on the right side of the widest, fully expanded leaf, avoiding the main veins. The measured parameters included the net photosynthetic rate (A), intercellular CO_2 concentration (Ci), stomatal conductance (g_s), and transpiration rate (Tr). All the measurements were conducted using a 1 cm^2 transparent film leaf chamber attached to a portable gas exchange system (LI−6400XT; LI-COR Inc., Lincoln, USA).

Table 1
Treatment conditions based on air and RZT differences.

Treatments	RZT (°C)	Air temperature (°C)	ΔT[a](°C)
LRT	10	25	−15
NRT	20	25	−5
WRT	30	25	+ 5

[a] ΔT = RZT – Air temperature.

The Ci values were automatically calculated by using the following formula:

$$Ci \approx Cs - \frac{A}{g}$$

where C_s is the sample cell CO_2 concentration, A is the photosynthetic rate, and g is the conductance.

The leaves were clamped in the chamber and the data were recorded after confirming that the net photosynthetic rate and g_s had stabilized, which took approximately three min. Measurement conditions were controlled at a PPFD of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, chamber temperature of 25 °C, a relative humidity of 60 ± 3%, and a CO_2 influx concentration of 500 $\mu mol\ mol^{-1}$. The leaf chamber temperature was measured with reference to ambient air temperature. Meanwhile, the abaxial leaf temperature was recorded using an infrared sensor. For each treatment, nine representative plants were selected and one leaf per plant was measured once.

Chlorophyll fluorescence was measured on the same leaves that were previously used for transpiration measurements using a photosynthetic fluorometer (Mini-PAM II; Heinz Walz, Effeltrich, Germany). During measurement, the fiber-optic probe was positioned at a fixed distance of approximately 1 cm from the leaf surface and held at a 60° angle using a fixation clip. The photosynthetically active photon flux density was monitored using the internal microquantum sensor of the Mini-PAM II system.

To assess the maximum quantum efficiency of PSII (Fv/Fm), leaves were dark-adapted for 20 min prior to measurement using a dark leaf clip (DLC−8; Heinz Walz GmbH, Effeltrich, Germany) before measurement. This adaptation period was sufficient for the full relaxation of rapidly reversible non-photochemical quenching (NPQ) and stabilization of the Ft values. Fv/Fm was calculated according to the formula [Fv/Fm = (Fm − Fo)/Fm] as proposed by Genty et al. (1989). The effective quantum yield of PSII [Y(II)] was calculated as (Fm′ − Fs)/Fm′, and the electron transport rate (ETR) was estimated using the equation:

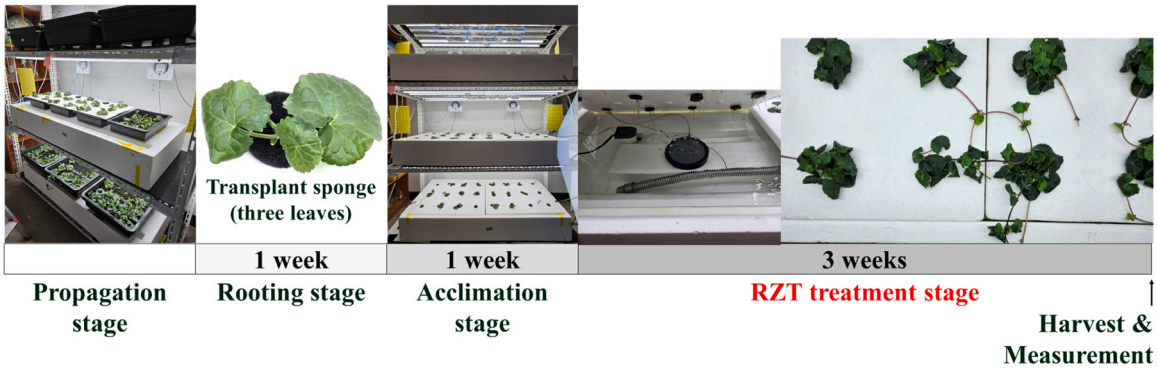


Fig. 1. Overview of the workflow, cultivation environment, and sampling timeline for *Centella asiatica* under controlled RZT treatments.

$ETR = Y(II) \times PAR \times 0.5 \times 0.84.$

2.5. Mineral nutrient analysis

The mineral nutrient accumulation in the leaves and roots of *C. asiatica* subjected to three different RZT treatments for 3 weeks was analyzed in triplicate. For the sample preparation, 0.1 g of oven-dried sample was digested with 5 mL of 70% HNO_3 and 1 mL of HCl using a microwave digestion system (Ethos EASY Advanced; Milestone, Sorisole, Italy) at 125 °C for 1–2 h. This was followed by further heating at 200 °C for 2 h. After digestion, the solution was made up to a final volume of 10 mL with deionized water, corresponding to a 100-fold dilution. An additional 10-fold dilution was performed for inductively coupled plasma analysis, resulting in a total dilution factor of 1000. The concentrations of five mineral elements, calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg), and iron (Fe), were determined by inductively coupled plasma–optical emission spectroscopy (ICP-OES; Optima 8300 DV, PerkinElmer, USA). The translocation factor (TF) was calculated to assess nutrient movement from roots to shoots using the following equation: TF = nutrient concentration in shoots / nutrient concentration in roots. A higher TF indicates greater relative translocation or accumulation of nutrients in shoots.

2.6. High-performance liquid chromatography analysis

As triterpenoid saponins predominantly accumulate in the leaves of *C. asiatica*, madecassoside and asiaticoside were quantified exclusively in the leaves (Mangas et al., 2008, Baek et al., 2022) using a modified method based on Song et al. (2022). High-performance liquid chromatography (HPLC) analysis was performed under the conditions summarized in Table S1.

A total of 50 mg of dried *C. asiatica* leaf powder was extracted using 80% methanol for 1 h in the dark. The resulting extract was centrifuged, and the supernatant was filtered through a 0.45 μm polyvinylidene fluoride syringe filter before injection into the HPLC system. Calibration curves were prepared using a series of known concentrations of each standard. In this study, the individual yields of asiaticoside and madecassoside were calculated by multiplying their respective tissue concentrations by the shoot dry weight. Additionally, the quality index of the phytochemical profile was assessed by calculating the ratio of asiaticoside concentration to madecassoside concentration.

2.7. Statistical analysis

All the physiological and morphological parameters, except root characteristics and mineral and metabolite analyses, were measured once for each of the nine individual *C. asiatica* plants per treatment. Root characteristics were measured once in five biological replicates per treatment. For mineral and metabolite analyses, the leaves and roots were harvested separately from nine individual plants per treatment (biological replicates), ground into powder, and each sample was analyzed in triplicate (technical replicates). To ensure statistical validity and prevent pseudoreplication, the independent cultivation bed (n = 3 beds per treatment), rather than individual plants, was defined as the true biological replicate (experimental unit) for the analysis of variance (ANOVA). Prior to statistical analysis, the data obtained from individual plants within the same bed were averaged to represent that single biological replicate. Mineral concentration was analyzed in both leaves and roots. Meanwhile, centellosides metabolite analysis was performed only for the leaf samples. All statistical analyses were performed using R software (version 4.1.2, R Project for Statistical Computing, Vienna, Austria). Treatment effects were assessed by one-way analysis of variance, and mean comparisons were conducted using Tukey's honestly significant difference (HSD) test with the emmeans and multcomp packages at a significance level of $P < 0.05$. Figures were generated using SigmaPlot (version 12.5; Systat Software Inc., USA).

3. Results

3.1. Growth characteristics

The growth response of *C. asiatica* varied under different RZT treatments (Fig. 2). The growth parameters were the highest under NRT and lowest under LRT. However, we observed no significant differences in shoot fresh and dry weights between the NRT and WRT treatments. Shoot fresh and dry weights under LRT were 50.42% and 32.08% lower, respectively, than those under WRT (Fig. 2A). Root FW decreased by 56.19% in LRT and 41.90% in WRT, while root DW decreased by 35.29% and 23.53%, respectively (Fig. 2B). However, root dry weight did not differ between NRT and WRT. Leaf number decreased by 50.32% in the LRT and 13.79% in the WRT compared to that in the NRT. The total LA decreased by 40.09% and 7.12%, respectively (Table 2). The shoot MC decreased by 12.53% in the LRT compared to the NRT. However, there was no significant difference between the NRT and WRT. Root MC decreased by 12.53% in the LRT and by 5.44% in the WRT. SLA was 15.06% lower in the LRT and 14.59% lower in the WRT than in the NRT.

3.2. Root morphological traits

The root morphological traits differed significantly among the RZT treatments (Fig. 3). The root surface area decreased under both treatments, showing a 62.9% reduction under the LRT and a 67.6% reduction under the WRT relative to the NRT (Fig. 3A). The average root diameter increased under both conditions, with a 60.0% increase under LRT and an 87.5% increase under WRT compared to that under NRT (Fig. 3B). The root volume decreased by 40.5% under the LRT and 39.2% in the WRT treatment, indicating a consistent reduction relative to the NRT (Fig. 3C). The number of root tips showed a substantial decline, decreasing by 66.0% under the LRT and 61.6% In the WRT treatment compared with the NRT (Fig. 3D). The number of root forks was reduced by 75.7% under the LRT and 73.7% under the WRT, representing the largest proportional decrease among the measured traits (Fig. 3E). Root division and elongation were both highest under the NRT, with no significant difference between the LRT and WRT (Fig. 3A, B, and D). In contrast, the average root thickness was greater in the LRT and WRT groups than in the NRT group (Fig. 3E).

3.3. Chlorophyll and anthocyanin accumulation

Changes in leaf pigmentation were observed depending on the RZT treatment (Fig. 4). Pronounced leaf chlorosis was observed after the LRT (Fig. 4A). The SPAD values were significantly lower under LRT than under NRT and WRT, and no significant differences were observed between NRT and WRT (Fig. 4B). SPAD decreased by 57.44% under the LRT compared to that under the NRT. Meanwhile, the WRT caused only a minor reduction of 13.12%. In contrast, anthocyanin was significantly higher under the LRT than under the NRT and WRT, with no difference between the NRT and WRT (Fig. 4C). Anthocyanin increased by 24.09% under the LRT relative to that under the WRT.

3.4. Gas exchange parameters

RZT significantly affected the gas exchange parameters in *C. asiatica* (Fig. 5). The g_s and Tr were highest under the NRT, followed by the WRT and LRT (Fig. 5A and B). The g_s decreased substantially under both treatments, with a 62.07% reduction under LRT and a 44.83% reduction under WRT relative to NRT. Tr similarly declined, decreasing by 58.00% under LRT and 39.33% under WRT compared to NRT. The Ci showed a slight decrease of 5.15% under the LRT and 5.33% under WRT compared with the NRT, but there was no difference between the treatment groups (Fig. 5C). The A was lowest under LRT. Meanwhile, NRT and WRT did not differ significantly (Fig. 5D). A exhibited a

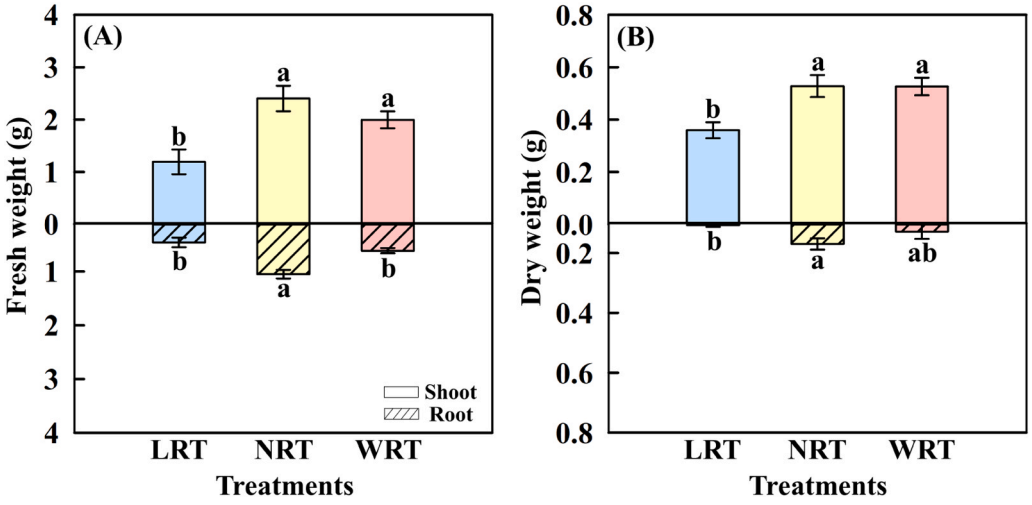


Fig. 2. Effects of RZT treatments (10 °C, 20 °C, and 30 °C) on the growth of *Centella asiatica*. Shown are (A) fresh weight and (B) dry weight of the shoot and root after 3 weeks of treatment under constant air temperature (25 °C). Data represent means ± standard error. One-way ANOVA showed a significant effect of RZT treatment, and different letters indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test.

Table 2
Growth characteristics of the *Centella asiatica* that was subjected to 3-week RZT.

Treatments	Number of leaves	Total leaf area (cm^2)	Moisture content (%)		Specific leaf area ($cm^2\ g^{-1}$)
			Shoot	Root	
LRT	9.22 ± 3.27 b[a]	36.95 ± 6.95 b	66.82 ± 1.86c	73.17 ± 0.93c	160.51 ± 5.23 b
NRT	18.56 ± 2.67 a	61.67 ± 5.69 a	77.51 ± 1.08 a	83.65 ± 0.6 a	188.97 ± 5.79 a
WRT	16.00 ± 2.03 a	57.28 ± 6.25 a	73.26 ± 0.84 b	79.1 ± 1.22 b	161.40 ± 0.19 b
P value	0.0137	0.0065	0.0002	0.0001	0.0042

[a] Values represent the mean ± standard error. Different letters within the same column indicate significant differences ($P < 0.05$) according to Tukey's HSD test.

31.05% reduction under the LRT, whereas the WRT caused a decrease of 3.97% compared with the NRT.

3.5. Chlorophyll fluorescence responses

The chlorophyll fluorescence parameters were significantly affected by the RZT treatment (Fig. 6). The Y(II) and ETR of PSII were significantly higher under the LRT than under the NRT, while WRT was not different from NRT (Fig. 6A and B). Y(II) increased by 37.50% under the LRT and 12.50% under the WRT compared with the NRT. The ETR showed a 34.85% increase under the LRT and a 10.00% increase under the WRT relative to the NRT. In contrast, the maximum quantum efficiency and NPQ of PSII did not differ significantly among the treatments (Fig. 6C and D).

3.6. Mineral nutrient absorption pattern in shoot and root tissues

The RZT treatment altered the total mineral nutrient concentration and redistribution in *C. asiatica* (Fig. 7 and Fig. S1). Under LRT and WRT, whole-plant concentrations of all five measured mineral nutrients were lower than those under NRT. Ca, Mg, and Fe concentrations decreased significantly in both shoots and roots, with the greatest Fe reduction observed in roots under WRT. (Fig. 7A–7 C). On the other hand, K decreased compared to NRT in the shoot, but showed an increasing pattern in the roots under LRT (22.93 ± 0.07 mg g^{-1} DW) and WRT (19.21 ± 0.11 mg g^{-1} DW) condition (Fig. 7D and Fig. S1). Root P concentration decreased under both LRT (−58.81%) and WRT (−52.46%), whereas shoot P concentration increased under WRT (3.65 ± 0.01 mg g^{-1} DW, +35.69% vs. NRT; Fig. 7E). LRT had no significant effect on shoot P concentration (2.67 ± 0.01 mg g^{-1} DW) compared to NRT (2.69 ± 0.01 mg g^{-1} DW). The TF for Ca decreased with increasing RZT (Fig. S1), whereas the TF for Mg increased under LRT (1.70) and WRT (1.63) compared with NRT (1.50). The TF for Fe markedly increased under WRT to 0.65, representing a 10.8-fold increase relative to NRT. In contrast, the TF for K decreased substantially under LRT (0.65) and WRT (1.12). However, the TF for P was higher in WRT (0.65) and LRT (0.55) than in NRT (0.22).

3.7. Centelloside accumulation

The concentrations and total major centelloside yield of major triterpenoid saponins in *C. asiatica* leaves were significantly affected by RZT (Fig. 8). The concentration of asiaticoside increased by 32.9% under the LRT and 69.8% under the WRT compared with that under the NRT. Meanwhile, that of madecassoside increased by 41.4% and 76.4%, respectively (Fig. 8A and B). However, the total madecassoside yield and total asiaticoside yield were significantly higher under WRT than under NRT, increasing by 91.9% and 84.6%, respectively (Fig. 8C and D). There were no significant differences in total leaf saponin yield between the LRT and NRT groups. Correlation analysis between dry biomass and total centellosides yield in *C. asiatica* leaves showed that WRT produced high biomass and centellosides levels (Fig. 8E). *C. asiatica* grown in vertical farm had an asiaticoside 3–4 times higher than madecassoside under all treatments. The AS/MS ratio was significantly higher under NRT than under LRT (Fig. 8F).

4. Discussion

4.1. Effects of RZT on root-system development

Under identical air temperature conditions, differential RZT treatment for 3 weeks after transplanting resulted in distinct shoot and root growth responses in *C. asiatica*.

Low RZT (LRT) markedly inhibited both shoot and root growth in *C. asiatica*, consistent with previous findings in Arabidopsis and lettuce showing that low RZT suppresses shoot and root growth (Lee et al., 2012, Sakamoto and Suzuki, 2015, Levine et al., 2023). The pronounced reduction in root growth under LRT may be explained, at least in part, by low-temperature disruption of auxin-mediated root development. Cold stress has been shown to inhibit basipetal auxin transport and impair

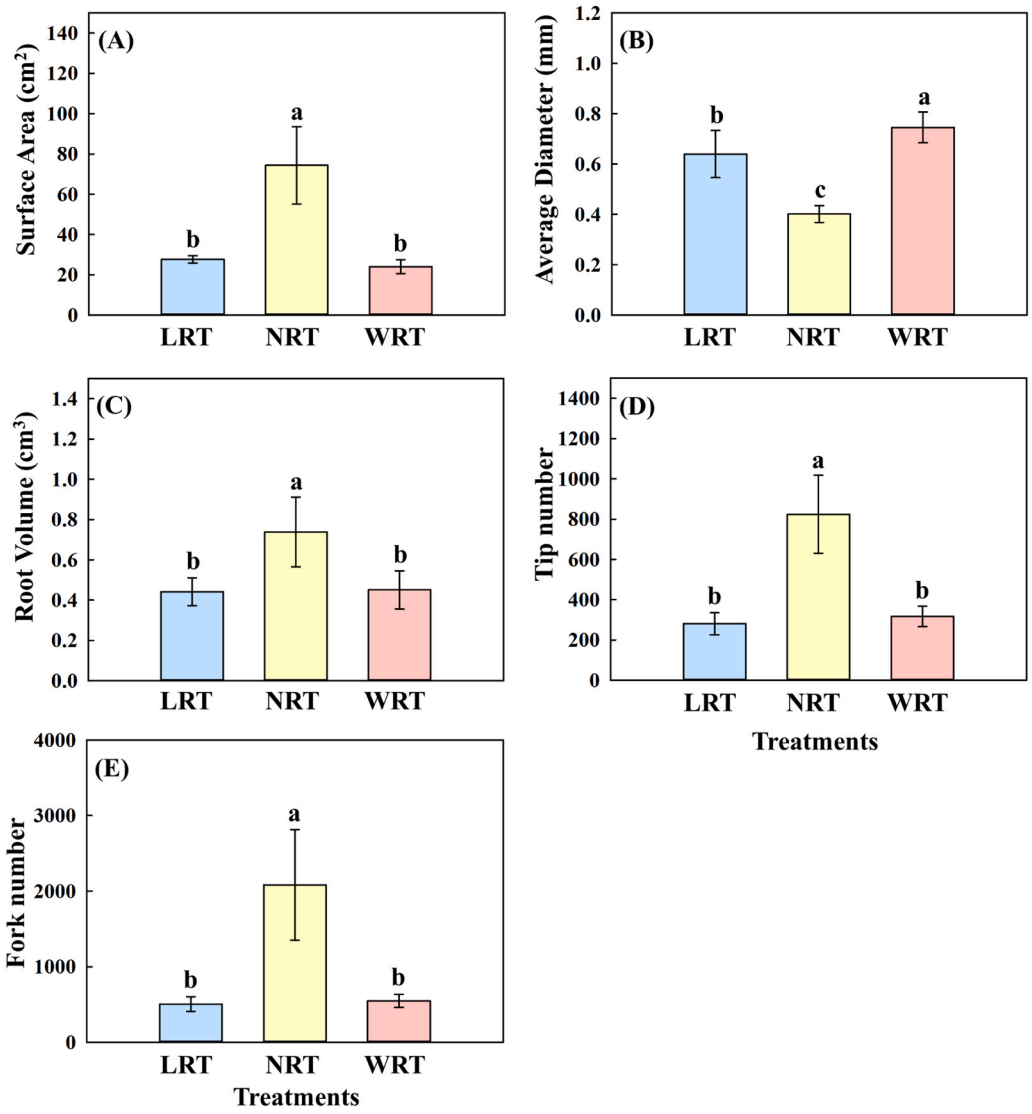


Fig. 3. Effects of RZT (10 °C, 20 °C, and 30 °C) on the root morphology of *Centella asiatica* after 3 weeks of treatment under a constant air temperature of 25 °C. Shown are (A) surface area, (B) average diameter, (C) root volume, (D) tip number, and (E) fork number. One-way ANOVA showed a significant effect of RZT treatment, and different letters indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test.

PIN2/PIN3-mediated auxin redistribution in Arabidopsis (Shibasaki et al., 2009), while low temperature can also reduce auxin accumulation, decrease root meristem size and cell number, and thereby suppress root growth through ARR1/12-mediated regulation (Zhu et al., 2015). Although auxin distribution was not measured in the present study, the marked decreases in root surface area, root volume, root tip number, and root fork number under 10°C RZT indicate that LRT restricted root-system expansion and branching in *C. asiatica*. These morphological constraints suggest that LRT delayed root development, which may have reduced the capacity for water and mineral nutrient acquisition and consequently contributed to suppressed whole-plant growth.

WRT markedly suppressed root development in *C. asiatica* to a level comparable to that observed under LRT. This response is consistent with previous studies showing that supraoptimal or ambient high RZT can restrict root growth and plant productivity in hydroponic crops. In lettuce, 35°C RZT reduced root dry weight, total root length, root volume, and shoot dry weight compared with 25°C RZT, whereas ambient high RZT under tropical cultivation reduced biomass and photosynthetic performance relative to cooled RZT conditions (Tan Laypheng et al., 2002, He et al., 2013, Levine et al., 2023). Mechanistically, supraoptimal RZT has been associated with reduced root hydraulic conductivity, shoot water potential, g_s, leaf area, and dry weight in *Capsicum annuum* (Dodd et al., 2000), and high RZT in lettuce inhibited root elongation and reduced total root length, root tip number, and root surface area partly through ethylene-mediated responses (Qin et al., 2007). Consistent with these findings, the reduced root surface area, root volume, root tip number, and root fork number under WRT indicate that warm RZT constrained root-system expansion and branching in *C. asiatica*. However, the maintenance of shoot biomass under WRT suggests that this inhibitory effect was expressed more strongly in the root system than in the shoot, implying different sensitivity thresholds between root and shoot tissues under sustained RZT changes.

Together, these findings suggest that LRT and WRT did not suppress root development through identical physiological constraints. Low RZT may have limited root development by disrupting auxin-mediated meristem activity and root hydraulic function, whereas warm RZT may have involved impaired root hydraulic capacity and ethylene-related inhibition of root elongation. However, further analyses of plant hormone dynamics and root hydraulic conductivity are required to clarify how low and warm RZT differentially regulate root development and root-to-shoot growth responses in *C. asiatica*. The maintenance of shoot biomass under WRT, despite marked inhibition of root

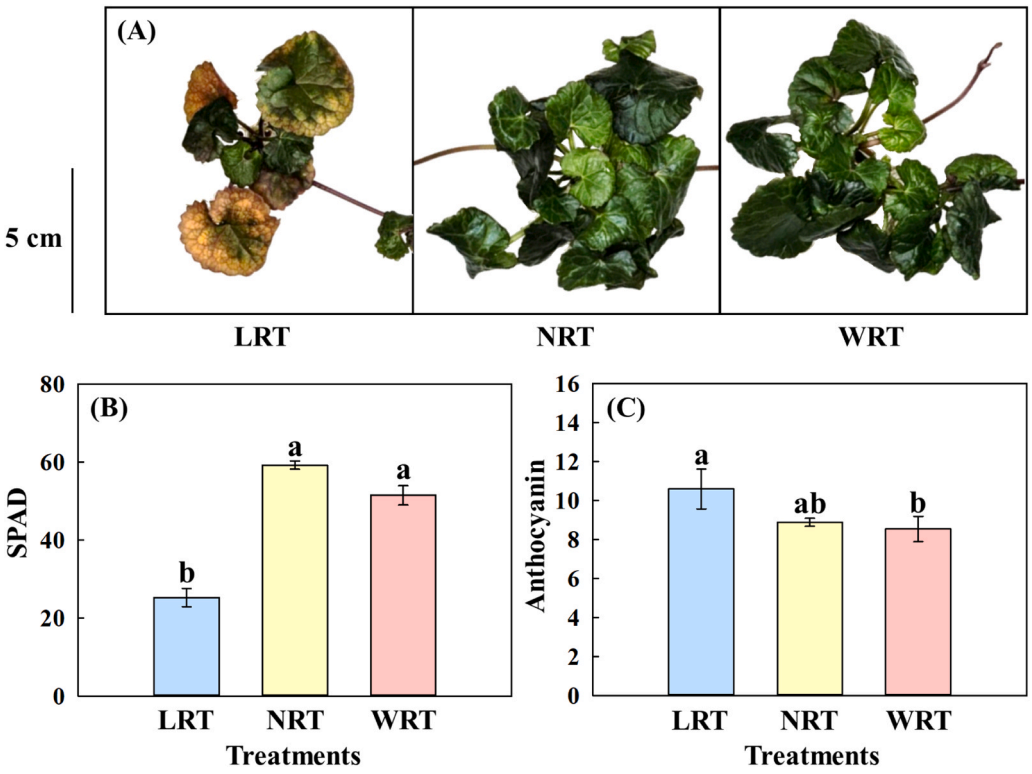


Fig. 4. Effects of RZT treatments (10 °C, 20 °C, and 30 °C) on the chlorophyll pigments of *Centella asiatica*. Shown are the (A) growth, (B) SPAD, and (C) anthocyanin after 3 weeks of treatment under constant air temperature (25 °C). Data represent means ± standard error. One-way ANOVA showed a significant effect of RZT treatment, and different letters indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test.

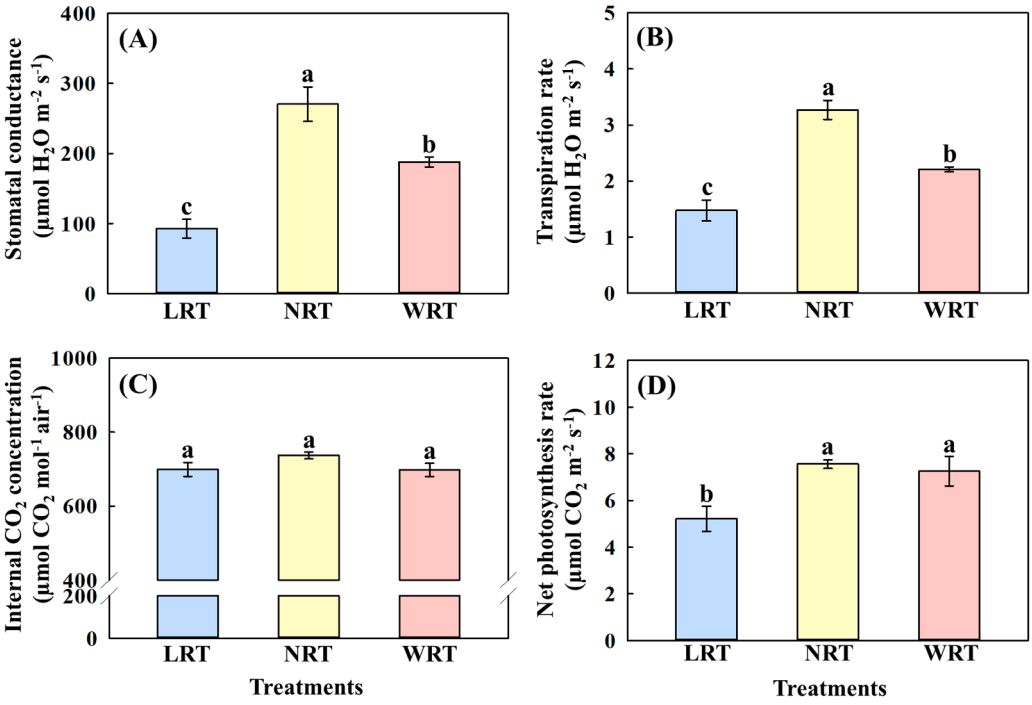


Fig. 5. Effects of RZT treatments (10 °C, 20 °C, and 30 °C) on the gas exchange parameter of *Centella asiatica*. Shown are the (A) stomatal conductance, (B) transpiration rate, (C) intercellular CO_2 concentration, and (D) net photosynthesis rate after 3 weeks of treatment under constant air temperature (25 °C). Data represent means ± standard error. One-way ANOVA showed a significant effect of RZT treatment, and different letters indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test.

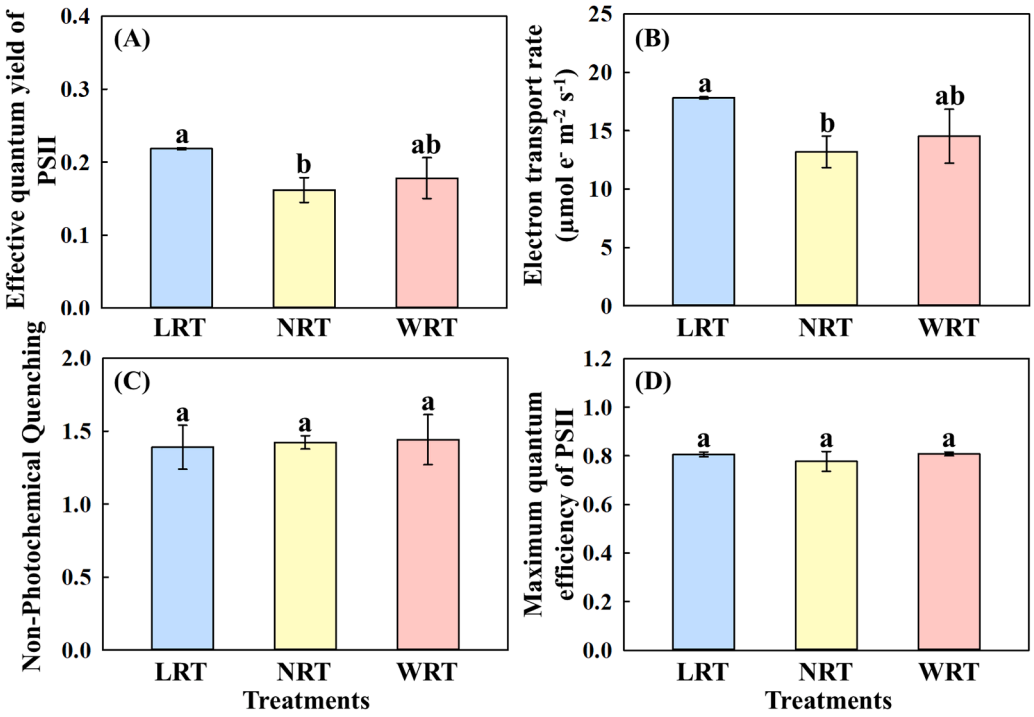


Fig. 6. Effects of RZT treatments (10 °C, 20 °C, and 30 °C) on the chlorophyll fluorescence parameter of *Centella asiatica*. Shown are the (A) effective quantum yield of photosystem II [Y(II)], (B) electron transport rate (ETR), (C) non-photochemical quenching of chlorophyll fluorescence (NPQ), and (D) maximum quantum yield of photosystem II (Fv/Fm) after 3 weeks of treatment under constant air temperature (25 °C). Data represent means ± standard error ($n = 9$). One-way ANOVA showed a significant effect of RZT treatment, and different letters indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test.

development, further suggests that root and shoot tissues may differ in their sensitivity thresholds to sustained RZT changes.

4.2. Effects of RZT on shoot growth responses

RZT markedly regulated not only root-system development but also shoot growth in *C. asiatica*. LN and LA were reduced under LRT but maintained under WRT, indicating treatment-dependent shoot growth responses to altered RZT. This pattern is broadly consistent with previous studies in lettuce and pepper showing that altered RZT can restrict root development and modify shoot growth (Tan Laypheng et al., 2002, He et al., 2013). In contrast, leaf compactness tended to increase under both LRT and WRT. Previous studies have reported that stress-induced expansion of the leaf epidermal layer and increased mesophyll cell density can contribute to the maintenance of water balance and photosynthetic stability (Enstone et al., 2002, Yao et al., 2023). Thus, the increased leaf thickness under LRT and WRT may reflect an adaptive structural adjustment that helps maintain leaf function under RZT-induced stress.

Taken together, the chlorophyll fluorescence, SPAD, gas-exchange, P redistribution, and anthocyanin results suggest that RZT stress may have affected carbon assimilation primarily through changes in stomatal regulation, transpiration, chlorophyll status, and resource supply, rather than through severe direct damage to PSII photochemistry.

4.3. Impacts of RZT on water and mineral nutrient uptake and translocation

During the 3-week period after transplanting, RZT stress reduced water and mineral nutrient uptake and altered mineral nutrient translocation in *C. asiatica*. The root system is the plant organ directly exposed to changes in RZT; therefore, inhibition of root development can be considered a primary response to suboptimal RZT. In the present study, root surface area, root volume, tip number, and fork number were reduced under both LRT and WRT conditions, indicating that both low and warm RZT restricted fine-root development. Under WRT, root compactness was observed, but this was accompanied by reductions in root surface area, root volume, tip number, and fork number, suggesting that root morphological development was still restricted. Because fine roots, root tips, and lateral branches are major sites for water and mineral nutrient acquisition, these morphological changes suggest a reduced capacity for resource uptake (Zobel et al., 2007, Lynch, 2013).

Previous studies have reported that low RZT can suppress aquaporin-related water transport and reduce root hydraulic conductivity (Maurel et al., 2008, Murai-Hatano et al., 2008, Lee et al., 2012). Although these hydraulic traits were not directly measured in the present study, the observed reductions in transpiration, water content, and mineral nutrient accumulation are consistent with limited root water and nutrient supply under RZT stress.

Transpiration is the primary driving force for the long-distance transport of water and inorganic nutrients from the roots to the shoots (Huang et al., 2024). Therefore, changes in transpiration under RZT stress provide an important basis for interpreting the altered water and nutrient status of *C. asiatica*. Under LRT, the reduction in g_s and transpiration suggests that water flux from roots to shoots was restricted. This reduced transpiration-driven flow may have contributed to lower mineral nutrient accumulation in both shoots and roots, because nutrient transport through the xylem is closely associated with water movement (Brodribb, Mcadam, 2017). However, the nutrient responses under RZT stress cannot be explained solely by reduced transpiration. Although total mineral nutrient accumulation decreased under RZT stress compared with NRT, the root-to-shoot mobility of Mg, Fe, and P increased. This suggests that these mineral nutrients may have been relatively more allocated to or retained in the shoots rather than the roots in response to RZT stress. Under WRT, total mineral nutrient accumulation decreased compared with NRT, whereas root-to-shoot P redistribution was enhanced. This indicates that the increased shoot P concentration was more closely associated with redistribution than with

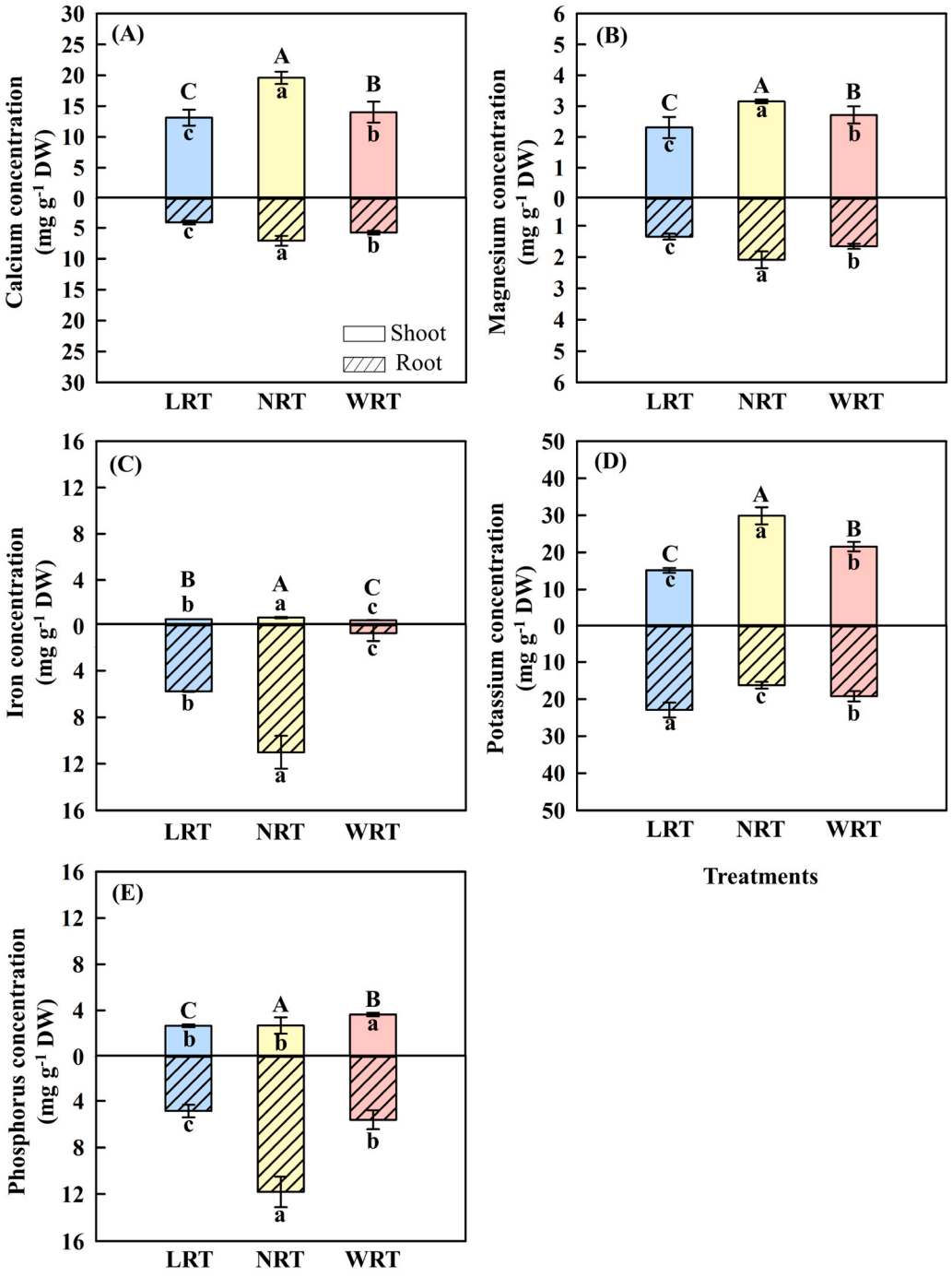


Fig. 7. Concentration of five mineral nutrients in *Centella asiatica* grown under three different RZT treatments (10 °C, 20 °C, and 30 °C) for 3 weeks. (A) Calcium, (B) magnesium, (C) iron, (D) potassium, and (E) phosphorus concentrations (mg g^{-1} DW) in shoot and root tissues. Data represent means ± standard error. One-way ANOVA showed a significant effect of RZT treatment, and different letters indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test. Lowercase letters indicate significant differences among parts, whereas uppercase letters indicate significant differences in total concentration ($P < 0.05$).

increased total uptake. Previous studies in tomato, lettuce, and maize have also reported that RZT can influence P uptake or redistribution, supporting the possibility that P allocation is sensitive to root-zone thermal conditions (Kawasaki et al., 2014, Levine et al., 2023, Xia et al., 2021a). K showed a different translocation pattern from Mg, Fe, and P. Although K accumulation in the roots was relatively high under suboptimal RZT, K mobility from the roots to the shoots was inhibited, suggesting that K distribution between roots and shoots was altered. The reduced translocation of K to the shoots under suboptimal RZT may be associated with the decreases in g_s and transpiration observed in this study.

Taken together, RZT stress appears to affect mineral nutrition through two linked processes: first, by reducing root water and mineral nutrient acquisition, and second, by altering transpiration-associated nutrient transport and root-to-shoot nutrient partitioning. Thus, the effects of suboptimal RZT should be interpreted not only as reduced mineral nutrient acquisition but also as altered nutrient translocation from roots to shoots, which is closely linked to transpiration-driven water flow.

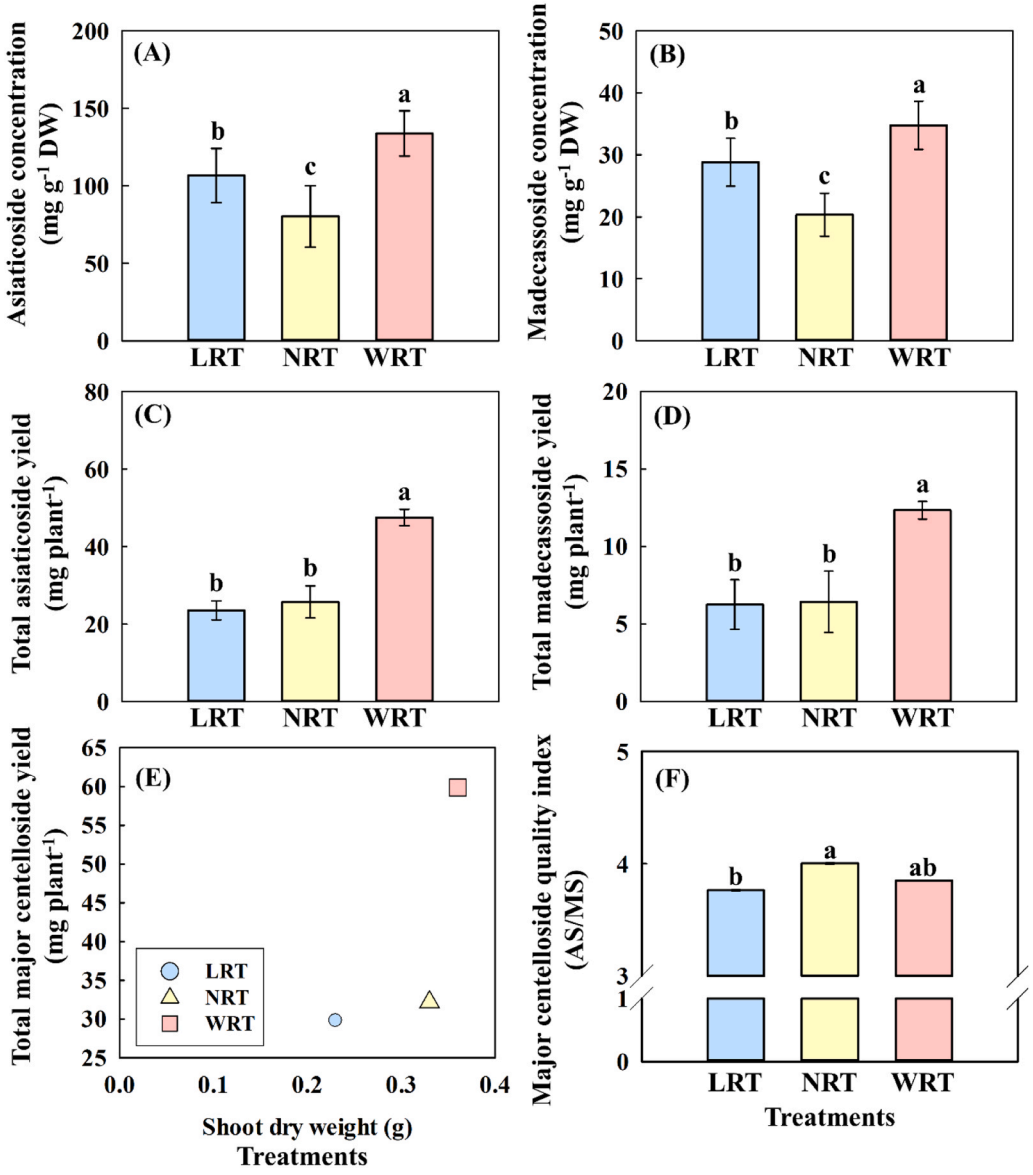


Fig. 8. Effects of RZT treatments (10 °C, 20 °C, and 30 °C) on the centellosides accumulation of *Centella asiatica*. Shown are the (A) asiaticoside concentration, (B) madecassoside concentration, (C) total asiaticoside yield, (D) total madecassoside yield, (E) correlation analysis between shoot DW and total centelloside yield, and (F) major centelloside quality index after 3 weeks of treatment under constant air temperature (25 °C). Data represent means ± standard error. One-way ANOVA showed a significant effect of RZT treatment, and different letters indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference (HSD) test.

4.4. *Effect of RZT on photosynthetic responses*

RZT-induced changes in water and mineral nutrient status were closely associated with chlorophyll status and photosynthetic responses in *C. asiatica*. Under LRT, g_s, transpiration rate, SPAD, and photosynthetic rate decreased, indicating that low RZT weakened both gas exchange and chlorophyll-related photosynthetic capacity. The reduction in g_s and transpiration suggests that water movement from roots to shoots was restricted, which may have limited leaf expansion and carbon assimilation. In addition, the decrease in SPAD indicates that low RZT negatively affected chlorophyll accumulation or maintenance. Because chlorophylls are essential pigments in the photosynthetic apparatus, reduced chlorophyll status can lower light absorption capacity and photosynthetic performance (Lichtenthaler, 1987).

However, the reduction in photosynthetic rate under LRT cannot be explained solely by stomatal limitation. In the present study, Ci did not differ significantly among RZT treatments, suggesting that the decline in photosynthetic rate was not caused only by restricted CO_2 diffusion through stomata. Therefore, the decrease in photosynthesis under LRT was likely associated with the combined effects of reduced g_s, lower transpiration, limited water and mineral nutrient supply, and decreased chlorophyll status. These responses are consistent with the strong inhibition of shoot growth under LRT, as shown by decreases in leaf number, leaf area, fresh weight, dry weight, and SPAD.

The mineral nutrient responses further support this interpretation. K is involved in stomatal regulation and transpiration control, and reduced K translocation to the shoots may be associated with the lower g_s and transpiration observed under suboptimal RZT (Alvim et al., 2025). Moreover, low shoot K levels under suboptimal RZT may have longer-term physiological consequences because K contributes not only to stomatal regulation but also to osmotic adjustment and turgor maintenance. Therefore, although WRT maintained shoot biomass

during the 3-week single-harvest cycle, reduced K translocation may become a limiting factor in longer or repeated-harvest vertical farming systems that require sustained leaf expansion and regrowth. Mg is closely associated with chlorophyll synthesis and photosynthetic enzyme activation (Hauer-Jákli and Tränkner, 2019), Fe is required for chloroplast function and photosynthetic electron transport (Kroh and Pilon, 2020), P supports photosynthetic energy metabolism and carbon assimilation (Carstensen et al., 2018), and Ca contributes to cell wall and membrane stability as well as cellular signaling (White and Broadley, 2003).

In contrast, WRT restricted root development but maintained photosynthetic rate more effectively than LRT during the 3-week treatment period. Although total mineral nutrient accumulation decreased under WRT compared with NRT, the increased root-to-shoot mobility of Mg, Fe, and P suggests that these nutrients were relatively more allocated to or retained in the shoots. This nutrient distribution pattern may have contributed to the maintenance of chlorophyll status and photosynthetic function under WRT. Therefore, WRT can be interpreted as a compensatory root-zone condition in which root development was inhibited, but shoot physiological function was partly maintained through altered nutrient partitioning.

4.5. Effect of chlorophyll fluorescence responses

Chlorophyll fluorescence responses provided additional evidence that the decrease in photosynthetic rate under LRT was not primarily caused by severe PSII damage. Under LRT, Y(II) and ETR were higher than under NRT, whereas Fv/Fm and NPQ remained stable across RZT treatments. Because Y(II) reflects the effective quantum yield of PSII photochemistry and is closely related to photosynthetic electron transport, these fluorescence parameters provide useful information on photochemical performance (Genty et al., 1989). The maintenance of Fv/Fm suggests that the maximum photochemical efficiency of PSII was not markedly impaired, and the stable NPQ indicates that non-photochemical energy dissipation did not differ substantially among treatments. Therefore, although LRT reduced g_s, transpiration, SPAD, and photosynthetic rate, the chlorophyll fluorescence results suggest that PSII structural integrity and basic photoprotective capacity were largely maintained. This interpretation is consistent with previous studies showing that photosynthetic responses are strongly influenced by RZT and its interaction with shoot environmental conditions (He et al., 2013, Yamori et al., 2022).

The higher Y(II) and ETR under LRT should not be interpreted as enhanced carbon assimilation. In the present study, photosynthetic rate decreased under LRT despite the increase in photochemical fluorescence parameters. This discrepancy indicates that photochemical electron transport and CO_2 assimilation were not fully coupled under low RZT. However, because PSI activity, cyclic electron flow, and photoinhibition-related markers were not measured in this study, the increased Y(II) and ETR under LRT cannot be attributed to a specific alternative electron transport pathway. Alternative explanations, including altered excitation balance between photosystems, possible chlorophyll fluorescence measurement artifacts under stress conditions, and reduced carbon sink activity caused by growth inhibition, should also be considered. Temperature stress can differentially affect electron transport and downstream carbon assimilation processes, leading to an imbalance between photochemical activity and photosynthetic carbon fixation (Yamori et al., 2014). Moreover, Ci did not differ significantly among RZT treatments, suggesting that the decline in photosynthetic rate was not explained solely by reduced CO_2 diffusion through stomata. Thus, the reduction in photosynthesis under LRT was more likely associated with the combined effects of reduced g_s, restricted transpiration-driven water flow, decreased SPAD, and limited resource supply rather than direct photoinhibition of PSII. This interpretation is also supported by the central role of chlorophylls in photosynthetic membranes and light harvesting (Lichtenthaler, 1987).

P status may also be relevant to this interpretation, because P deficiency can impair the photosynthetic electron transport chain and alter energy metabolism, thereby affecting the balance between photochemical activity and carbon assimilation (Carstensen et al., 2018). However, because ATP production, RuBP regeneration, and chloroplast-level P status were not directly measured, the role of P should be interpreted only as a possible contributing factor.

Anthocyanin accumulation showed a different response pattern from chlorophyll fluorescence parameters. Anthocyanin content was higher under LRT than under WRT, although neither LRT nor WRT differed significantly from NRT. Previous work in cucumber has shown that low RZT stress can be associated with changes in cold-resistance metabolic pathways and anthocyanin-related responses (Sun et al., 2024). However, in the present study, this response should be interpreted cautiously because anthocyanin did not differ significantly from NRT. Therefore, the higher anthocyanin level under LRT than under WRT is better interpreted as a relative pigment response rather than direct evidence that anthocyanins maintained PSII stability.

Under WRT, Y(II), ETR, NPQ, and Fv/Fm did not indicate marked impairment of photochemical performance. This supports the interpretation that WRT restricted root development but did not immediately suppress shoot photosynthetic function during the 3-week treatment period. Taken together, the chlorophyll fluorescence, SPAD, gas-exchange, P redistribution, and anthocyanin results suggest that RZT stress affected carbon assimilation more strongly through stomatal regulation, transpiration, chlorophyll status, and resource supply than through direct damage to PSII photochemistry.

4.6. Effects of RZT on centelloside accumulation and yield

RZT strongly affected centelloside accumulation in *C. asiatica*. Asiaticoside and madecassoside concentrations increased under suboptimal RZT conditions, with the highest concentrations observed under WRT. However, centelloside concentration alone does not fully represent production performance because total yield depends on both metabolite concentration and shoot biomass. In the present study, LRT increased centelloside concentration but markedly reduced shoot growth, resulting in a limited total centelloside yield. In contrast, WRT maintained shoot biomass while increasing asiaticoside and madecassoside concentrations, thereby increasing total centelloside yield during the 3-week treatment period.

This response suggests that WRT created a condition favorable for short-term centelloside-enriched production in *C. asiatica*. Previous studies have shown that centelloside accumulation in *C. asiatica* can be altered by cultivation conditions, including hydroponic nutrient management and temperature environment (Shawon et al., 2023, Pipatsitee et al., 2023). In the present study, the increase in centelloside concentration under WRT occurred despite reduced root development, indicating that shoot-level metabolite accumulation was maintained or enhanced even when root morphological growth was restricted. Therefore, WRT may be suitable for short-term production systems targeting high centelloside yield, whereas its suitability for longer cultivation or repeated-harvest systems requires further evaluation because root development was inhibited.

The composition of individual centellosides also provides useful information for evaluating *C. asiatica* quality. Although centelloside profiles vary among cultivars, asiaticoside is generally reported to

occur at the highest concentration among centellosides (Alam et al., 2025). Consistently, asiaticoside was the dominant centelloside compared with madecassoside across all RZT treatments in the present study. Although NRT showed a relatively high AS/MS ratio, WRT produced the highest absolute asiaticoside accumulation. Therefore, NRT may be more favorable when relative asiaticoside enrichment is prioritized, whereas WRT appears more advantageous when total asiaticoside and total centelloside yield are the main production targets.

The increase in centelloside accumulation under WRT may also be related to altered mineral nutrient partitioning. In particular, WRT increased root-to-shoot P redistribution, and previous work reported that P availability and P uptake were associated with asiaticoside accumulation in *C. asiatica* (Riyanto et al., 2022). However, total P accumulation decreased under RZT stress in the present study, indicating that the increased shoot P concentration under WRT was more closely associated with redistribution than with increased total uptake. Thus, the relationship between P redistribution and centelloside accumulation should be interpreted as a possible association rather than a confirmed causal mechanism.

Overall, LRT and WRT increased centelloside concentration, but their production implications differed. LRT represented a strong stress condition in which increased centelloside concentration was offset by reduced shoot biomass. By contrast, WRT maintained shoot biomass and increased centelloside concentration, resulting in improved total centelloside yield under the 3-week cultivation period. These findings suggest that WRT can be considered a potential short-term RZT strategy for producing centelloside-enriched *C. asiatica*, while further studies are needed to determine whether this response is maintained under longer or repeated-harvest cultivation.

4.7. Limitations and future perspectives

Several limitations should be considered when interpreting the effects of RZT on growth and quality traits in *C. asiatica*. Although this study showed that RZT altered root development, water and mineral nutrient status, photosynthetic responses, and centelloside accumulation, the underlying mechanisms were not directly verified. Plant hormones, centelloside pathway-related genes and enzymes, antioxidant responses, carbohydrate-use efficiency, root hydraulic conductivity, and root respiration were not measured. Therefore, the proposed links among root growth inhibition, altered nutrient translocation, photosynthetic responses, and centelloside accumulation should be interpreted as physiological associations rather than confirmed causal mechanisms.

Another limitation is that plants were exposed to fixed RZT treatments for 3 weeks after transplanting. This continuous exposure may have imposed relatively strong root-zone stress under hydroponic vertical-farm conditions, particularly at 10°C and 30°C. Thus, the present results are useful for identifying RZT-induced stress and quality responses, but they should not be interpreted as defining the optimal RZT for commercial *C. asiatica* production. Although WRT increased centelloside concentration and total centelloside yield during the 3-week period, root development was clearly restricted, suggesting that its suitability may depend on the production objective, such as short-term centelloside-enriched harvest versus long-term or repeated-harvest cultivation requiring stable root growth.

Future studies should use milder and narrower RZT gradients to determine the optimal RZT range for hydroponically grown *C. asiatica* in vertical farms. Shorter exposure periods, recovery treatments, day–night RZT regimes, and state-based RZT control strategies should also be tested to reduce excessive root-zone stress while maintaining shoot growth and centelloside productivity. In addition, because *C. asiatica* forms clonal structures such as mother plants, runners, and daughter plants, future work should evaluate whole clonal units and integrate physiological, biochemical, and molecular analyses to clarify how RZT regulates biomass production and medicinal quality.

5. Conclusion

This study demonstrated that *C. asiatica* exhibited distinct growth, physiological, and metabolic responses to different RZTs under a constant air temperature of 25 °C. LRT strongly inhibited root development, water and mineral nutrient acquisition, gas exchange, chlorophyll status, and shoot growth, indicating that low RZT imposed severe root-zone stress. In contrast, WRT restricted root development but maintained shoot growth and photosynthetic function, which was associated with altered root-to-shoot nutrient partitioning, particularly the increased mobility of Mg, Fe, and P. WRT also increased asiaticoside and madecassoside concentrations and total centelloside yield during the 3-week cultivation period, suggesting its potential for short-term centelloside-enriched production in vertical farming systems. However, because root development was clearly reduced under WRT, future studies should evaluate milder RZT gradients, shorter or dynamic RZT treatments, and underlying physiological and molecular mechanisms to optimize RZT management for sustainable *C. asiatica* production.

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CRediT authorship contribution statement

Seong-Nam Jang: Writing – original draft, Visualization, Methodology, Formal analysis. **Ki-Ho Son:** Writing – review & editing, Supervision, Project administration. **Min-Ji Kang:** Methodology. **Gyu-Sik Yang:** Investigation. **In-Je Kang:** Investigation. **Han-Sol Sim:** Investigation. **Ga Oun Lee:** Investigation. **Uk-Hyeon Yeo:** Data curation. **Sunwoo Park:** Data curation. **Yuxin Tong:** Writing – review & editing, Methodology. **Eiji Goto:** Writing – review & editing, Methodology. **Kye Man Cho:** Writing – review & editing, Methodology.

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.2026.123679.

Data availability

Data will be made available on request.

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