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Irrigation Cycle Optimization and Water Management in Stevia Production in Vertical Hydroponic Systems

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ABSTRACT

Vertical hydroponic systems are becoming a key component of sustainable agriculture by enabling year-round production in limited space and closed-loop water management. In this study, the effects of irrigation cycling on the growth, biomass, biochemical quality, and water use efficiency of *Stevia rebaudiana* Bertoni in vertical hydroponic systems were investigated. The experiment was conducted under four irrigation cycle conditions—each defined by a different off-time per tower (H1: 150 s, H2: 300 s, H3: 450 s, H4: 600 s) with a fixed 15-second on-time—in four independent towers each with 21 planting slots. Seedlings obtained from the same individual were distributed in a balanced short-to-long arrangement to standardize initial conditions. The 300-second off-time group (H2) achieved the highest values in dry biomass (1.90 ± 1.04 g/plant), total phenolic content (7.14 ± 0.34 mg gallic acid equivalents/g), DPPH antioxidant activity (5.90 ± 0.15 mmol Trolox equivalent/g), and yield-based water use efficiency (3.17 g/L). The group with the longest off-time (H4: 600 s) reduced water consumption but fell notably behind in biomass and quality parameters. Stem length on day 30 showed a strong Spearman correlation with dry weight ($\rho = 0.873$, $p < 0.001$), suggesting that non-destructive dimensional measurements can serve as practical biomass predictors. The findings demonstrate that a 300-second off-time simultaneously optimizes growth, biochemical quality, and water efficiency in vertical hydroponic Stevia production, and indicate that irrigation cycle design should consider product quality and biomass yield integrity alongside water savings.

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Introduction

Urbanization, climate change, and water scarcity have increased interest in systems such as controlled-environment agriculture (CEA) and vertical farming (Benke & Tomkins, 2017; Kalantari et al., 2018; Vatistas et al., 2022). Bringing production closer to consumption centers, the possibility of year-round production independent of climatic fluctuations, and resource recovery are among the key motivations behind these systems (Benke & Tomkins, 2017; Kalantari et al., 2018). Vertical hydroponic approaches eliminate the need for soil, enable high yields in limited space, and allow water to be reused in closed loops (Vatistas et al., 2022). Closed/controlled-environment agriculture also strengthens resource efficiency by enabling precise adjustment of environmental parameters and improving process traceability (Kalantari et al., 2018; Vatistas et al., 2022).

Stevia is a plant of growing economic importance in the food industry owing to its role as a source of low-calorie sweeteners (steviol glycosides) (Pal et al., 2015). The plant's growth dynamics are sensitive to light, nutrition, and water management; consequently, growth and quality outputs can change substantially under controlled-environment applications (Hastings et al., 2015; Pal et al., 2015; Ptak et al., 2024). Balanced nutrient management and environmental stability in Stevia cultivation are critical for simultaneously maintaining biomass accumulation and quality parameters (Pal et al., 2015; Tavarini et al., 2015). The combination of hydroponics and controlled environments is therefore regarded as a powerful approach supporting production sustainability for medicinal-aromatic plants such as Stevia (Vatistas et al., 2022; Pal et al., 2015).

In hydroponic production, the nutrient solution is delivered to the root zone; irrigation cycle parameters including on-time, off-time, and flow rate directly shape root-zone moisture-oxygen balance. In aeroponic systems, the nutrient solution is misted into the root zone, providing higher oxygen exposure. In both system types, cycle design plays a decisive role in growth and water use efficiency. Optimizing the balance between off-times and on-times is therefore a fundamental design requirement in vertical hydroponic systems (Diarra et al., 2023; Liu et al., 2025; Vatistas et al., 2022).

Studies jointly evaluating flow-period parameters on Stevia yield and water use efficiency in vertical hydroponic systems are limited. In this study, independent reservoirs per tower and balanced plant height distribution were used to isolate the effect of off time. The objective was to comparatively establish the effects of four irrigation cycle off times on Stevia morphological growth, biomass yield, biochemical quality, and water consumption, and to provide a practical framework for irrigation protocol optimization in vertical hydroponic production.

Vertical farming is defined as multi-story production systems with controlled environmental variables, offering year-round production, area efficiency, and water-saving advantages alongside closed-loop hydroponic approaches (Benke & Tomkins, 2017; Kalantari et al., 2018; Vatistas et al., 2022). Resource efficiency comparisons show that different soilless system configurations produce marked divergences in water use efficiency (WUE) outputs (Carotti et al., 2023; Regmi et al., 2024). WUE values depend strongly on the calculation approach used; comparative analysis based on gross volume reduction rather than net consumption is recognized as a valid practical approach (Regmi et al., 2024). The limitations of monitoring nutrient solutions solely by electrical conductivity (EC) are also noted; ion-based verification remains relevant because EC does not directly reflect individual ion concentrations (Fathidarehnijeh et al., 2024).

In hydroponic systems, irrigation cycle parameters directly affect growth and biomass formation through the root-zone moisture-oxygen balance (Diarra et al., 2023; Liu et al., 2025). Studies in nutrient film technique (NFT) systems have shown that intermittent flow can reduce root-zone stress and increase yield, while higher irrigation frequency may boost yield but reduce per-unit water productivity (Diarra et al., 2023; Liu et al., 2025). A positive relationship between improved root-zone oxygenation and photosynthetic capacity and biomass accumulation has also been documented in NFT systems (Nitu et al., 2024). Direct evidence regarding root-oxygen dynamics generated by second-scale short-pulse flows in tower-type Stevia systems remains limited.

In Stevia cultivation, nutrient parameters such as nitrogen level establish a direct balance between biomass accumulation and steviol glycoside profiles (Tavarini et al., 2015; Pal et al., 2015). Light quality and photosynthetic photon flux density (PPFD) are determinative for biomass and metabolite outputs; red light has been reported to increase fresh biomass while green light can increase total steviol glycoside content (Chou et al., 2025; Ptak et al., 2024). Growth and steviol glycoside levels vary depending on system type and growing conditions in hydroponic and aeroponic system comparisons (Hastings et al., 2015; Judičkaitė et al., 2025). FTIR-confirmed presence of inulin-type fructans in Stevia root fractions supports the applicability of FTIR-based spectral characterization for biochemical profile comparison (Lima et al., 2025). Multi-factor studies addressing light, nutrition, and irrigation cycle interactions in the same experimental set are noted to be limited, and validated integrated yield-quality indicators at tower scale are needed (Pal et al., 2015; Tavarini et al., 2015; Diarra et al., 2023). This study partially addresses this gap by presenting an integrated evaluation combining irrigation cycling with total phenolic content (TPC), DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant activity, chlorophyll content, and WUE.

Materials and Methods

Experimental Setup

The experiment was conducted in four independent vertical hydroponic systems established in a closed environment. The study was carried out at the Quality Analysis Laboratory and the acclimatization room of the Nuclear Energy Research Institute, Turkish Energy, Nuclear and Mineral Research Agency (TENMAK). The vertical hydroponic tower system utilized in this research is the subject of a patent. Each tower consists of 7 modules with 3 slots per module, giving a total of 21 slots per tower (Figure 1). Each tower was operated with an independent 10-L reservoir and independent pump (12 V, 5.0 L/min nominal flow rate, 60 psi / 4.1 bar, splash-type flow). Each module contains 3 plant slots, and a distributor piece positioned every two modules redirects and slows water within the tower. Nutrient solution targets were maintained at EC = 1.1 and pH = 6.0. Lighting was provided by 2-m full-spectrum LED (44 W, 4000 K, ~180 µmol m^{-2} s^{-1} PPFD) fixtures at an average distance of 20 cm from plants, with a 16-hour photoperiod. Ambient temperature was 23–24°C and relative humidity was 60–80%.

Stevia seedlings obtained from the same individual were placed in all towers. Stem length and root length were recorded at D0 (experiment start) and on day 30 (D30). Growth coefficient (GR) was calculated as (D30−D0)/D0 per plant. Plants were distributed in the same short-to-long arrangement across all towers, with approximately 10 shorter and 11 taller seedlings per tower.

Study Variables

The single independent variable was irrigation cycle off-time (H1: 150 s, H2: 300 s, H3: 450 s, H4: 600 s); on-time was fixed at 15 s. Dependent variables were: morphological growth indicators (D0, D30, absolute growth, GR), biomass parameters (fresh and dry weight, water content), biochemical quality indicators (TPC, DPPH, chlorophyll), and tower-based water consumption with yield-based WUE (WUE_Y). Water consumption was calculated from the gross volume reduction in each tower's independent reservoir. A total of 14.5 L of water was provided to each reservoir (including initial fill) during the experiment; 21 mL Canna Aqua Vega A+B nutrient solution and 12 mL hypochlorous acid were applied periodically throughout. Pump flow rate was calibrated per tower via a 60-second volume test.

Additionally, shoot-specific WUE (WUE_{shoot}) was calculated as shoot dry biomass per gross volume reduction to provide a complementary biomass-based metric. WUE_Y and WUE_{shoot} were calculated using Equation 1 and 2:

WUE_Y = D30 total dry biomass (g) / Gross volume reduction (L) (1)
WUE_{shoot} = Shoot dry biomass (g) / Gross volume reduction (L) (2)

Sample Preparation and Extraction

Plant samples were dried at 35°C for 72 hours and ground to pass through a 500-µm sieve. The drying temperature was selected with preservation of bioactive compounds in mind (Periche et al., 2015; Lemus-Mondaca et al., 2016). Extraction was carried out with minor modifications according to Zaidan et al. (2019): 5 g per sample was mixed with 90% methanol (1:20 solid-to-liquid), macerated at 40°C for 90 min in a shaking water bath, filtered through Whatman No. 1 filter paper, and centrifuged at 9000 rpm for 10 min. Supernatants were stored at −18°C until analysis. Extraction and filtration steps are illustrated in Figure 2.

Total Phenolic Content (TPC)

TPC was determined spectrophotometrically using the Folin–Ciocalteu method (Singleton & Rossi, 1965; Singleton et al., 1999) with gallic acid as standard. Samples were diluted 1:21 (v/v) with 90% methanol and absorbance was measured at 765 nm using a Jenway 6505 UV-Vis spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE)/g dry weight (DW).

DPPH Antioxidant Activity

Antioxidant activity was determined with modifications of Brand-Williams et al. (1995). Samples were diluted 1:21 (v/v) with 90% methanol; absorbance was measured at 517 nm (Jenway 6505 UV-Vis). Results were expressed as mmol Trolox equivalent antioxidant capacity (TEAC)/g DW.

Chlorophyll Content

Chlorophyll content was determined with minor modifications according to Ucar et al. (2018). Ground samples (0.1 g) were extracted with acetone (10 mL), vortexed 1 min, and supplemented with 3 mL 80% acetone; the mixture was held in ice water in the dark for 10 min. Samples were centrifuged at 9000 rpm (4°C, 10 min); the pellet was re-extracted with 2 mL 80% acetone and supernatants were pooled to 10 mL and diluted 1:6 (v:v). Absorbances were read at 663 nm and 645 nm. Chlorophyll amounts were calculated using Equations 3–5:

Chlorophyll a (mg/g DW) = $12.7A_{663} - 2.69A_{645}$ (3)
Chlorophyll b (mg/g DW) = $22.9A_{645} - 4.68A_{663}$ (4)
Total Chlorophyll (mg/g DW) = Chlorophyll a + Chlorophyll b (5)

FTIR Analysis

FTIR spectrum was used as a complementary profile comparison tool; O–H, C–H, and glycosidic bond regions were monitored for biochemical fingerprint comparison (Eliwa & Ahmed, 2025; Martono & Rohman, 2019).

Statistical Analysis

Statistical analyses were performed using the scipy.stats and scikit-posthocs libraries in Python 3. Since the normality assumption could not be fully satisfied for growth and biomass variables, the Kruskal–Wallis test was applied; for significant results, Mann–Whitney U tests with Bonferroni correction (α = 0.0083, 6 pairs) were used for pairwise comparisons.

Figure 1. General view of the experimental setup (tower system and independent reservoirs).

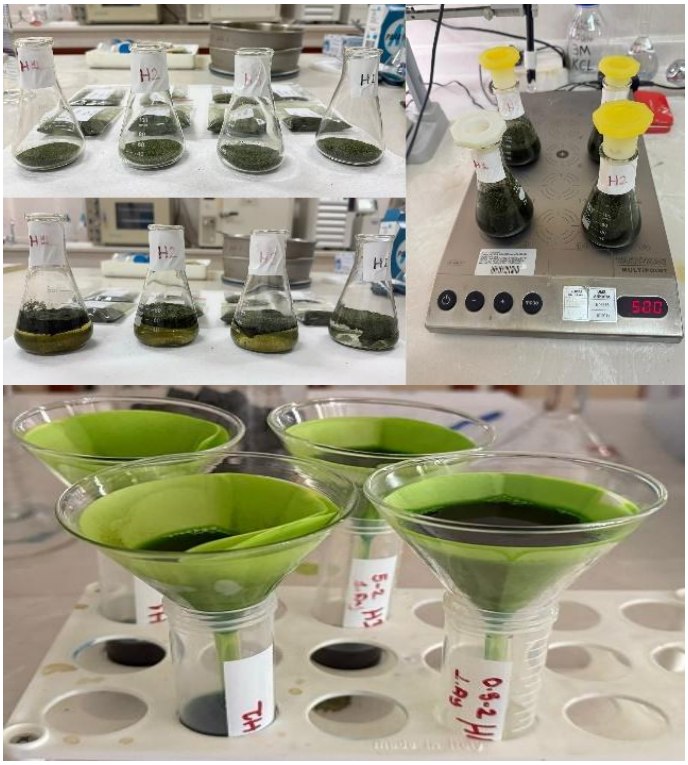

Figure 2. Extraction and filtration steps. (a) Extraction stage; (b) Filtration stage

Effect size was reported as ε^2. One-way ANOVA + Tukey HSD was applied for biochemical data (effect size η^2). Spearman rank correlation analysis was used for plant-level relationships. Principal component analysis (PCA) was applied to standardized variables at both plant and tower level. Water consumption and WUE_Y were reported descriptively only. Significance threshold was $\alpha = 0.05$.

Results

Initial Homogeneity and Plant Losses

According to statistical analysis, no significant difference in stem length was found among towers at the start of the experiment (Kruskal–Wallis: H = 2.550, p = 0.466, $\varepsilon^2 = -0.006$), confirming balanced initial conditions (Vatistas et al., 2022). During the experiment, one plant was lost in tower H2 and two in tower H3; these individuals were excluded from analyses. Valid observation counts were H1: 21, H2: 20, H3: 19, H4: 21.

Morphological Growth

Growth data are summarized in Table 1 and Figure 3. Day-30 stem length (D30) differed significantly among towers (H = 8.316, p = 0.040, $\varepsilon^2 = 0.069$). Groups H1 (150 s) and H2 (300 s) showed higher mean D30 than H3 and H4 (H1: 16.0 ± 3.1 cm; H2: 15.7 ± 4.0 cm; H3: 13.8 ± 2.4 cm; H4: 13.9 ± 2.6 cm); H1–H3 (p = 0.032) and H1–H4 (p = 0.035) differences were significant. Between-group differences in absolute growth were stronger (H = 11.332, p = 0.010, $\varepsilon^2 = 0.108$); H1 showed significantly higher growth than H3 (p = 0.006) and H4 (p = 0.023). No significant difference was found for growth coefficient GR (H = 1.703, p = 0.636).

Biomass Yield

Biomass data are presented in Table 2 and Figure 4. Significant differences were observed for shoot fresh weight (SFW) (H = 10.666, p = 0.014, $\varepsilon^2 = 0.100$), shoot dry weight (SDW) (H = 9.580, p = 0.023, $\varepsilon^2 = 0.086$), and total dry weight (H = 9.173, p = 0.027, $\varepsilon^2 = 0.080$). H2 achieved the highest SDW (1.49 ± 0.77 g) and total dry weight (1.90 ± 1.04 g). H2–H4 differences were significant for SDW (p = 0.003), total dry weight (p = 0.004), and water content (p = 0.003). Water content differed among groups (H = 10.603, p = 0.014); H2 had the highest (9.64 ± 4.86 g) and H4 the lowest (5.28 ± 2.01 g) value.

Biochemical Quality Parameters

Biochemical results are presented in Table 3, Figure 5, and Figure 6. Significant between-group differences were detected for all biochemical parameters. TPC showed strong differentiation (F = 89.70, p < 0.001, $\eta^2 = 0.971$); H2 reached the highest TPC (7.14 ± 0.34 mg GAE/g), significantly higher than H1 (p < 0.001), H3 (p = 0.017), and H4 (p = 0.001). DPPH antioxidant activity showed a similar pattern (F = 131.00, p < 0.001, $\eta^2 = 0.980$); H2 (5.90 ± 0.15 mmol TEAC/g) was significantly higher than H3 and H4 (p < 0.001). For total chlorophyll (a+b), H1 had the highest value (7.86 ± 0.27 mg/g DW; F = 16.13, p < 0.001, $\eta^2 = 0.858$); differences between H1 and H2 (p = 0.015), H3 (p = 0.014), and H4 (p < 0.001) were significant.

Water Consumption and Water Use Efficiency

Water consumption and WUE_Y are shown in Table 4 and Figure 7. Gross volume reduction in H1 and H2 was 11.4 L and 12.0 L respectively; H3 and H4 were lower at 9.3 L and 8.9 L. The H1–H2 mean was 22.2% higher than the H3–H4 mean. WUE_Y was highest in H2 (3.17 g/L) and lowest in H4 (2.25 g/L). As a complementary biomass-based metric, shoot-specific water use efficiency (WUE_{shoot}) followed the same pattern, with H2 recording the highest value (2.49 g/L) and H4 the lowest (1.84 g/L), further supporting the superiority of the 300-second off-time in converting water input into shoot biomass.

Table 1. Descriptive statistics of growth indicators by tower (mean ± SD).

Variable	H1 (150 s)	H2 (300 s)	H3 (450 s)	H4 (600 s)
D0 (cm)	7.26 ± 2.09	7.33 ± 2.45	6.63 ± 1.81	6.55 ± 1.52
D30 (cm)	16.02 ± 3.10	15.65 ± 4.04	13.79 ± 2.45	13.86 ± 2.64
Absolute Growth (cm)	8.76 ± 1.92	8.33 ± 1.98	7.16 ± 1.17	7.31 ± 1.74
GR	1.301 ± 0.459	1.232 ± 0.444	1.168 ± 0.444	1.164 ± 0.344

GR = (D30−D0)/D0. D0: H = 2.55, p = 0.466 (ns); D30: H = 8.32, p = 0.040*; Absolute: H = 11.33, p = 0.010*; GR: ns.

Table 2. Descriptive statistics of biomass parameters by tower (mean ± SD).

Variable	H1 (150 s)	H2 (300 s)	H3 (450 s)	H4 (600 s)
Shoot Fresh Weight (g)	9.03 ± 3.86	11.14 ± 5.62	8.74 ± 4.67	6.06 ± 2.26
Shoot Dry Weight (g)	1.21 ± 0.65	1.49 ± 0.77*	1.11 ± 0.62	0.78 ± 0.31
Total Dry Weight (g)	1.40 ± 0.78	1.90 ± 1.04*	1.31 ± 0.74	0.95 ± 0.39
Shoot Water Content (g)	7.81 ± 3.39	9.64 ± 4.86	7.63 ± 4.05	5.28 ± 2.01

Individual tare correction applied. *: highest value. SDW: H = 9.58, p = 0.023*; Total DW: H = 9.17, p = 0.027*; Shoot Water Content: H = 10.60, p = 0.014*.

Table 3. Descriptive statistics of biochemical parameters by tower (mean ± SD, n = 3).

Parameter	H1 (150 s)	H2 (300 s)	H3 (450 s)	H4 (600 s)
TPC (mg GAE/g)	4.91 ± 0.37 b	7.14 ± 0.34 a*	3.92 ± 0.24 bc	3.41 ± 0.22 c
DPPH (mmol TEAC/g)	3.01 ± 0.12 b	5.90 ± 0.15 a*	2.34 ± 0.36 c	1.90 ± 0.36 c
Chlorophyll a (mg/g DW)	5.69 ± 0.20 a*	5.26 ± 0.11 b	5.21 ± 0.03 b	4.90 ± 0.12 b
Chlorophyll b (mg/g DW)	2.18 ± 0.09 a*	1.95 ± 0.08 ab	2.00 ± 0.05 ab	1.87 ± 0.08 b
Total Chlorophyll (mg/g DW)	7.86 ± 0.27 a*	7.21 ± 0.18 b	7.21 ± 0.07 b	6.77 ± 0.20 b

One-way ANOVA + Tukey HSD. Different letters indicate significant differences (p < 0.05). *: highest value. TPC: F = 89.70, p < 0.001; DPPH: F = 131.00, p < 0.001; Chl a+b: F = 16.13, p < 0.001.

Spearman Rank Correlations

Spearman analysis revealed that D30 showed strong positive correlation with dry weight ($\rho = 0.873$, $p < 0.001$), fresh weight ($\rho = 0.822$, $p < 0.001$), and water content ($\rho = 0.794$, $p < 0.001$) (Table 5; Figure 8). Absolute growth correlated strongly with dry weight ($\rho = 0.759$, $p < 0.001$). GR showed no significant correlation with dry weight ($\rho = -0.082$, $p > 0.05$).

FTIR Spectral Analysis

FTIR spectra are shown in Figure 9. All four groups exhibited profiles consistent with characteristic Stevia leaf extract band regions (Eliwa & Ahmed, 2025; Lima et al., 2025): ~3280 cm^{-1} O–H/N–H stretching; ~2916 cm^{-1} aliphatic C–H stretching; ~1606 cm^{-1} C=O/amide I; 1000–1250 cm^{-1} cellulose/polysaccharide C–O–C stretching bands.

Table 4. Gross water consumption, yield-based WUE_Y and biomass-based WUE_{shoot} by tower.

Tower	Off-time (s)	Gross Vol. Reduction (L)	Total Dry Biomass (g)	WUE_{shoot} (g/L)	WUE_Y (g/L)
H1	150	11.4	29.40	2.23	2.58
H2	300	12.0	38.05*	2.49*	3.17*
H3	450	9.3	24.91	2.27	2.68
H4	600	8.9	20.02	1.84	2.25

WUE_Y = Total dry biomass (g) / Gross volume reduction (L). WUE_{shoot} = Shoot dry biomass (g) / Gross volume reduction (L). Gross-based; n = 1 tower/cycle.

Table 5. Spearman rank correlation coefficients at plant level (n = 77).

	D30	GR	Abs. Growth	Shoot FW	Shoot DW	Shoot WC
D30	—	0.020	0.837***	0.822***	0.873***	0.794***
GR		—	0.357**	−0.017	−0.082	−0.004
Abs. Growth			—	0.705***	0.759***	0.685***
Shoot FW				—	0.942***	0.996***
Shoot DW					—	0.916***
Shoot WC						—

**p < 0.01; ***p < 0.001. FW: fresh weight; DW: dry weight; WC: water content; Abs. Growth: absolute growth; GR: growth coefficient.

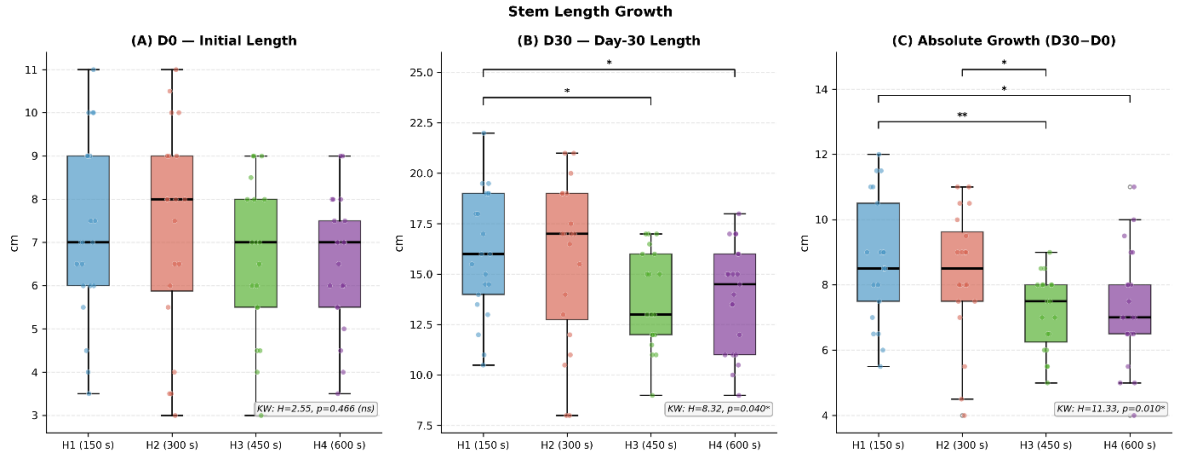

Figure 3. Stem length growth by tower

(A) Initial length (D0); (B) Day-30 length (D30); (C) Absolute growth (D30−D0). * p < 0.05; ** p < 0.01 (Kruskal–Wallis + Mann–Whitney U, Bonferroni).

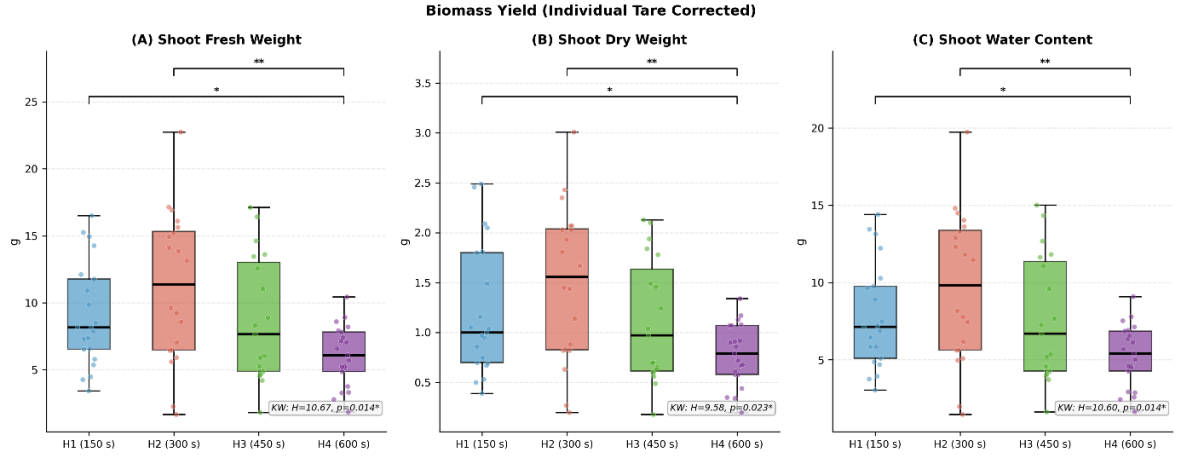

Figure 4. Biomass yield by tower

(A) Fresh weight – shoot; (B) Dry weight – shoot; (C) Water content. * p < 0.05; ** p < 0.01.

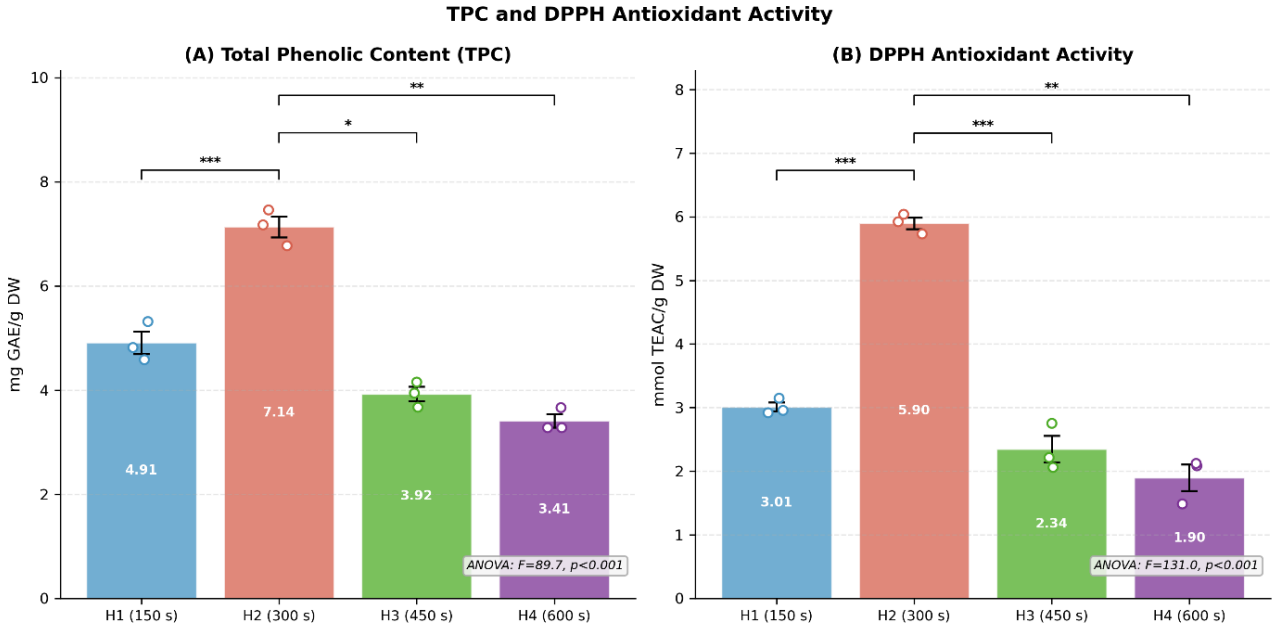

Figure 5. TPC and DPPH antioxidant activity by tower (n = 3/tower). Different letters indicate significant groups by Tukey HSD ($p < 0.05$).

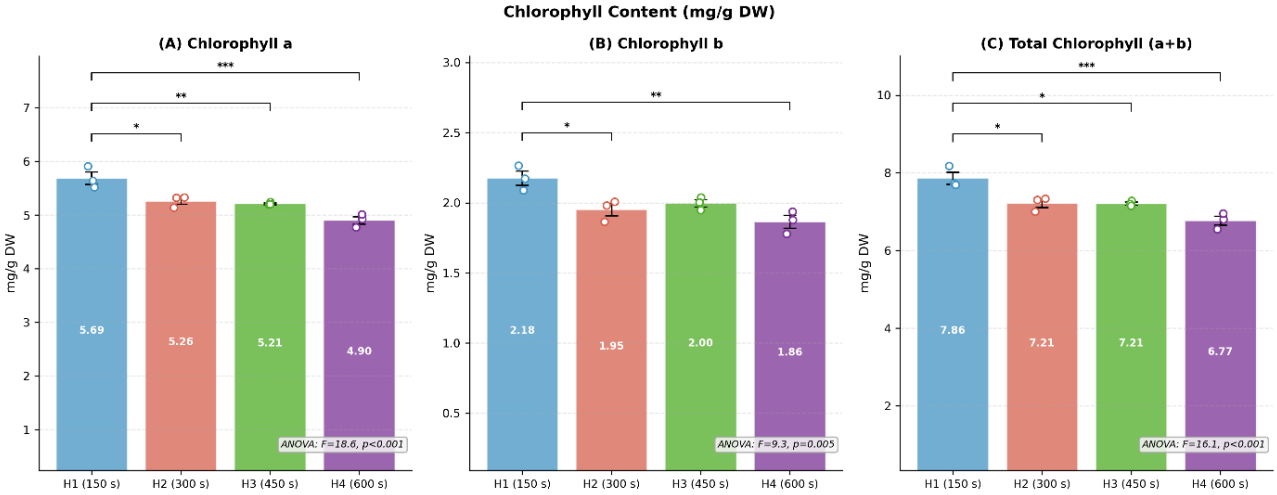

Figure 6. Chlorophyll content by tower (n = 3/tower).
(A) Chlorophyll a; (B) Chlorophyll b; (C) Total chlorophyll (a+b).

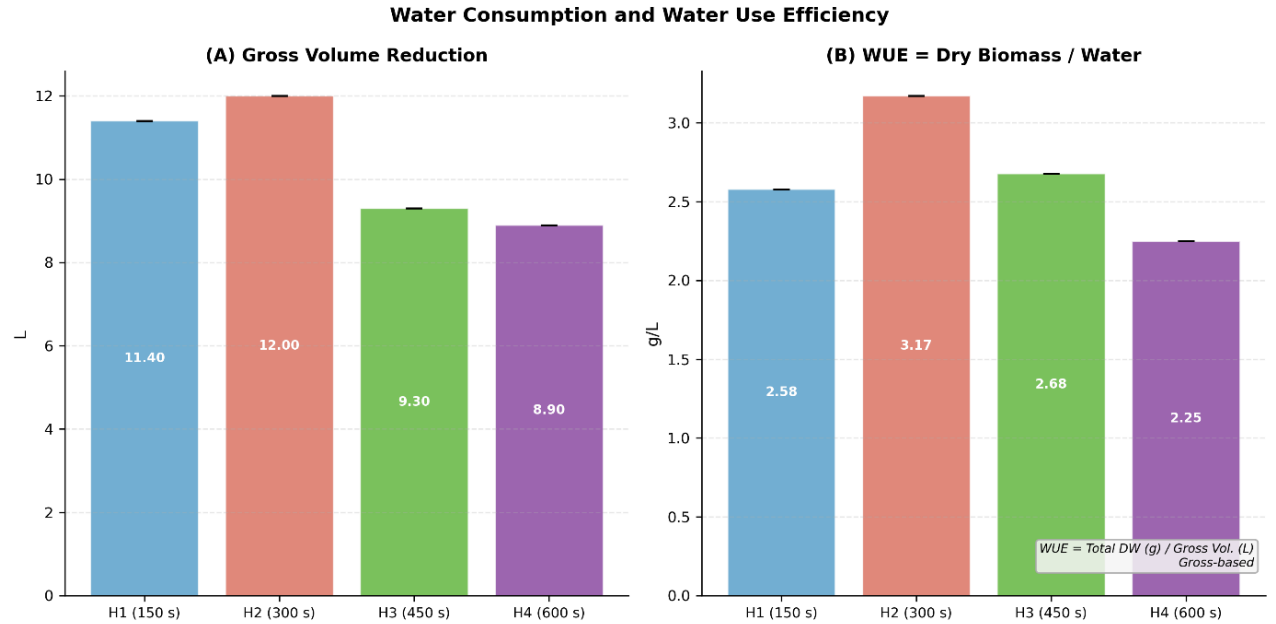

Figure 7. Gross water consumption and WUE_Y.

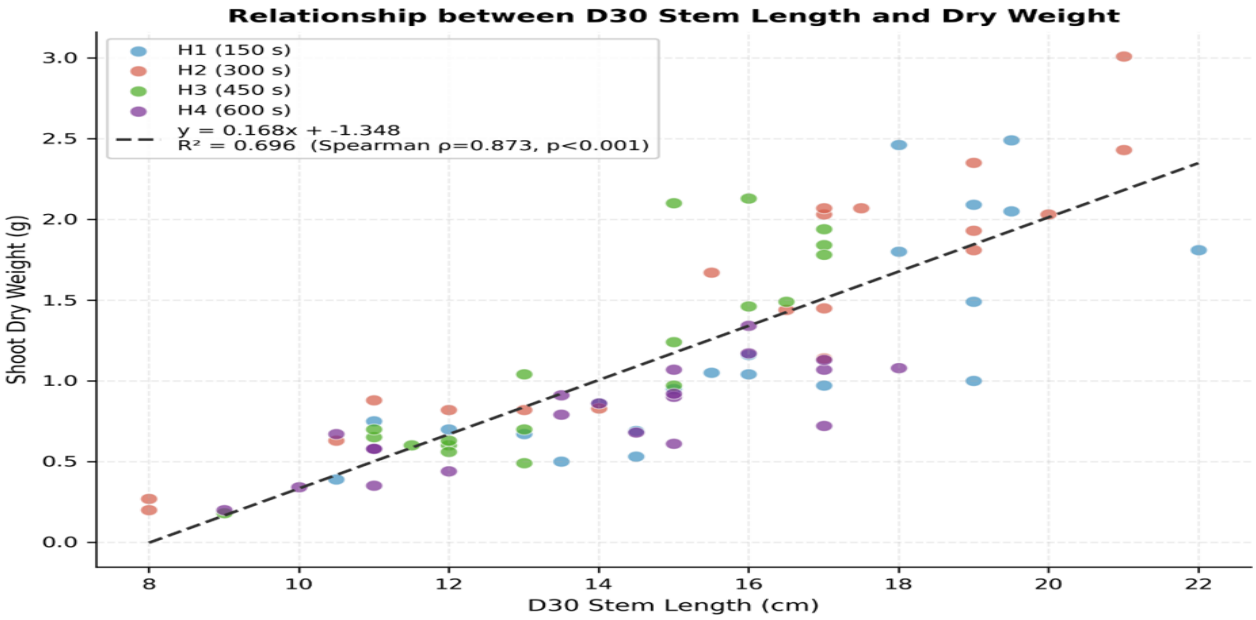

Figure 8. Relationship between D30 stem length and dry weight

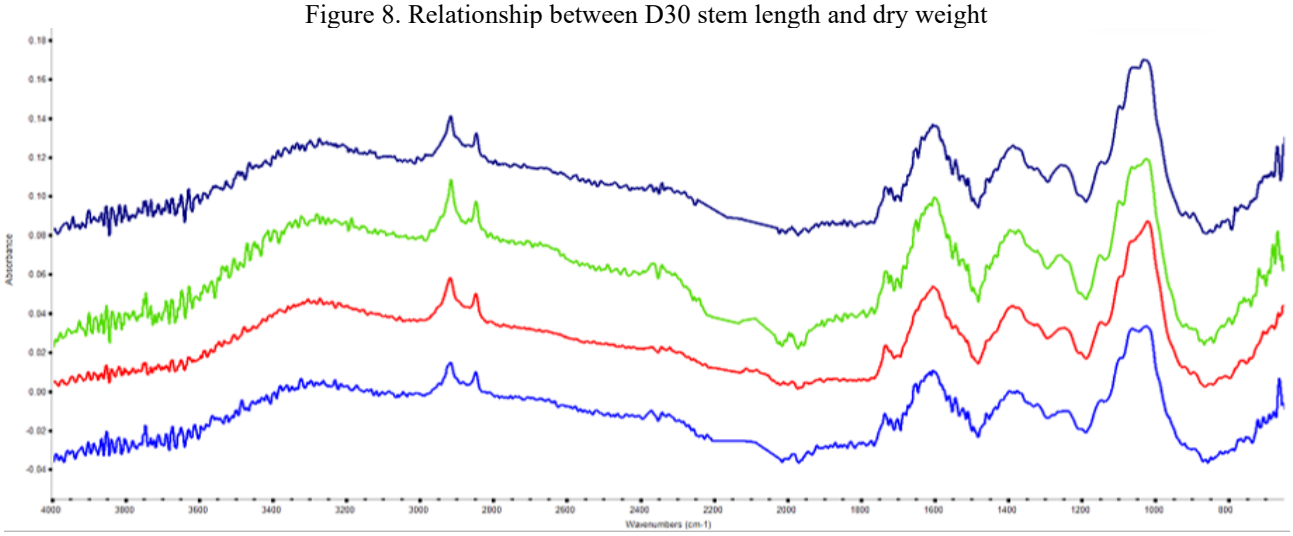

Figure 9. FTIR spectral profiles of irrigation cycle groups. (From top to bottom: H1, H2, H3, H4)

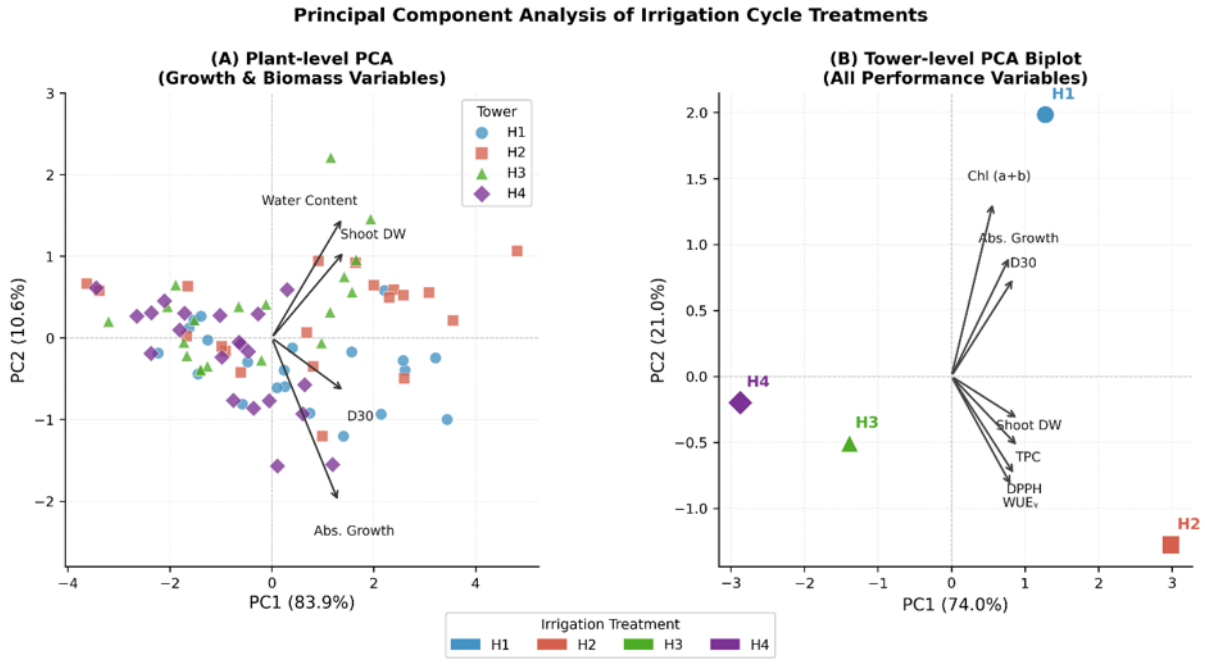

Figure 10. Principal component analysis (PCA) of irrigation cycle treatments

(A) Plant-level PCA based on growth and biomass variables (PC1: 83.9%, PC2: 10.6%); shoot fresh weight was excluded due to near-perfect correlation with shoot dry weight (ρ = 0.942). (B) Tower-level PCA biplot based on all performance variables (PC1: 74.0%, PC2: 21.0%).

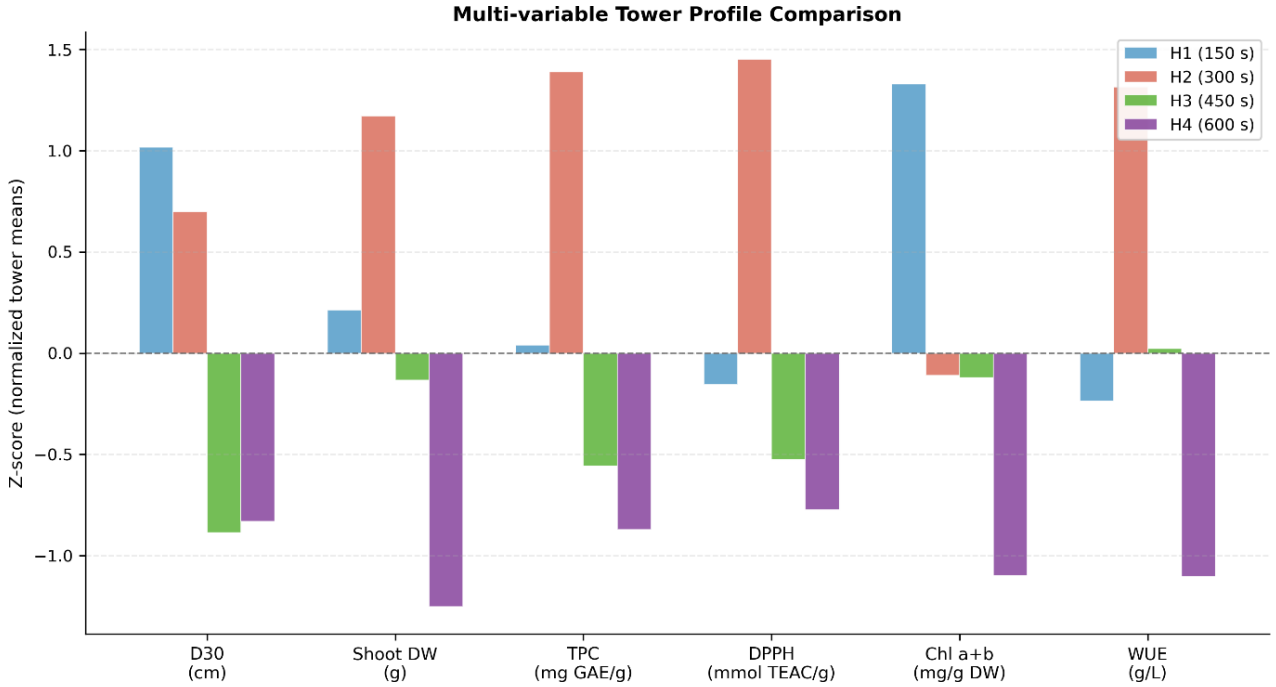

Figure 11. Multi-variable tower profile comparison (Z-score normalized). Each variable is standardized by its own mean and standard deviation.

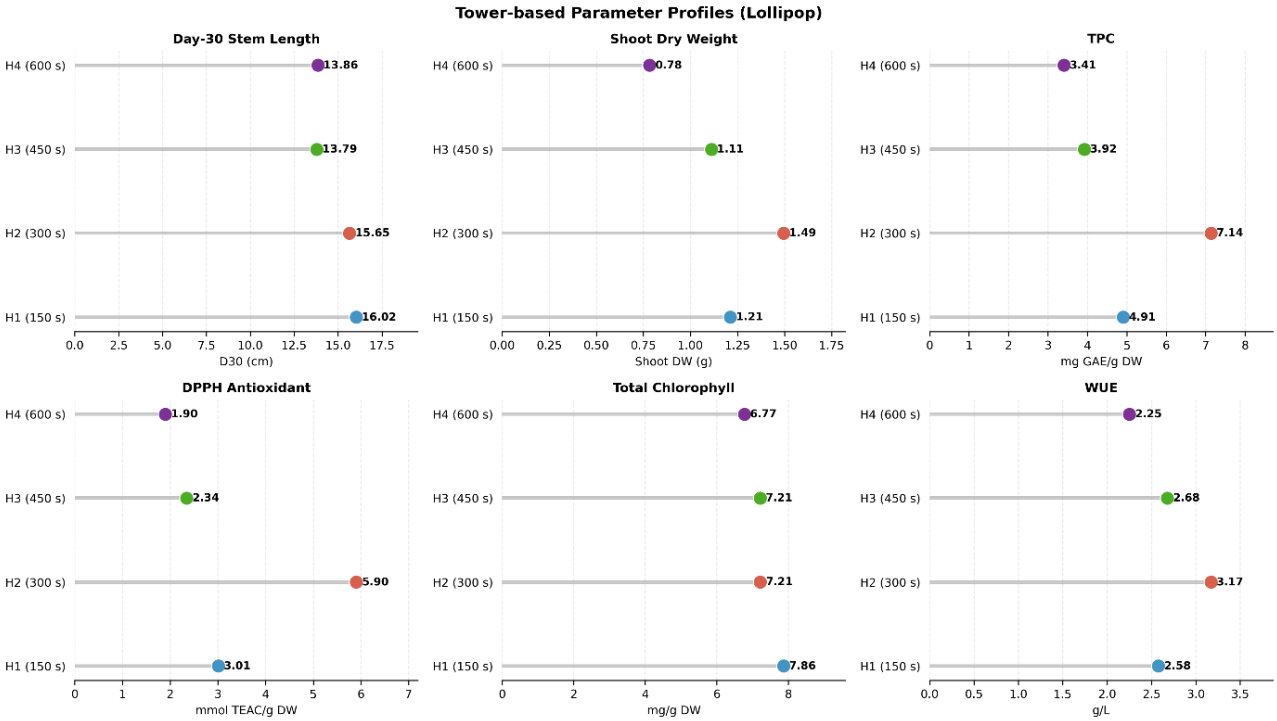

Figure 12. Tower-based parameter profiles – absolute mean values

Tower group means are presented comparatively for each parameter (D30: day-30 stem length; Shoot Dry Weight: shoot dry weight; WUE: Water Use Efficiency).

Discussion

The superiority of short off-time groups (H1–H2) in D30 and absolute growth is consistent with literature reporting that more frequent irrigation improves root-zone moisture-oxygen balance and growth performance in hydroponic systems (Diarra et al., 2023; Liu et al., 2025). The similar GR across groups, however, suggests that cycling affects the absolute growth achievable by plants rather than their proportional growth capacity.

H2's superiority in biomass accumulation demonstrates that short-to-medium off-times support both fresh and dry biomass. The contribution of irrigation cycle optimization to biomass accumulation in controlled-environment systems is highlighted in the literature (Diarra et al., 2023;

Liu et al., 2025; Vatistas et al., 2022). Dry weight-based measurement is recognized as a more stable yield indicator in Stevia research (Pal et al., 2015; Tavarini et al., 2015).

Phenolic compounds in Stevia leaves are fundamental components of antioxidant defense capacity (Pal et al., 2015; Tavarini et al., 2015).

The high TPC and DPPH values in H2 indicate that a 300-second off-time creates a particularly suitable root-zone environment in terms of water-oxygen balance (Liu et al., 2025). This is supported by studies documenting the positive effect of root-zone oxygenation on biochemical metabolism in NFT systems (Nitu et al., 2024). The finding that H1, despite highest chlorophyll content, fell behind H2 in biomass is notable; chlorophyll content alone does not determine biomass accumulation, as root-zone physiology and nutrient uptake also play decisive roles (Nitu et al., 2024; Ptak et al., 2024).

H2 had both the highest water consumption (12.0 L) and the highest WUE_Y (3.17 g/L), demonstrating that the increased water consumption of the 300-second cycle was proportionally offset by biomass accumulation (Carotti et al., 2023; Regmi et al., 2024). H4's lower water consumption but inferior biomass and quality performance suggests that long off-times may impair root-zone moisture-oxygen balance and limit metabolite accumulation (Diarra et al., 2023; Liu et al., 2025). Both WUE metrics (WUE_Y and WUE_{shoot}) consistently identified H2 as the most water-efficient treatment, confirming that the 300-second off-time maximizes biomass yield per unit water input regardless of the calculation basis. Direct evapotranspiration-based WUE could not be determined in this study due to the absence of net ET measurements; the gross volume reduction approach was therefore adopted as a practical and reproducible alternative (Regmi et al., 2024).

The strong Spearman correlation between D30 and dry weight ($\rho = 0.873$, $p < 0.001$) demonstrates that stem length is a strong biomass predictor; this supports the integration of non-destructive dimensional measurements as practical tools in vertical hydroponic systems (Pal et al., 2015; Tavarini et al., 2015).

In FTIR comparison, H2's stronger absorbance in the 950–1200 cm^{-1} fingerprint region and the prominence of glycosidic C–O (~1000–1025 cm^{-1}) and phenolic C–O (~1200–1280 cm^{-1}) bands are consistent with its high TPC and DPPH values (Pal et al., 2015; Tavarini et al., 2015; Martono & Rohman, 2019). The ~1735 cm^{-1} band in H3 indicates lipid/organic acid ester bonds (Eliwa & Ahmed, 2025); its absence in H2 confirms this accumulation was not prominent in H2. FTIR was used as a complementary profile tool, not for quantitative analysis (Martono & Rohman, 2019; Lima et al., 2025).

Principal component analysis (PCA) results are presented in Figure 10. Plant-level PCA explained 94.5% of total variance (PC1: 83.9%, PC2: 10.6%), with H2 clustering toward higher biomass and growth scores along PC1. Tower-level biplot further confirmed treatment differentiation, with H2 positioned in the direction of TPC, DPPH, and WUE_Y loading vectors (PC1: 74.0%, PC2: 21.0%).

It should be noted that each irrigation cycle treatment was represented by a single tower in this study, which constitutes the primary methodological limitation. Since treatment and tower are confounded, the observed differences cannot be attributed exclusively to irrigation cycle effects, and pseudo-replication risk must be acknowledged (Carotti et al., 2023; Yang et al., 2024). Accordingly, the reported statistical differences should be interpreted as indicative rather than conclusive, and the findings are best regarded as preliminary evidence warranting confirmation through replicated experimental designs.

The prediction that short off-times would improve growth indicators was partially supported; H1–H2 superiority was observed in D30 and absolute growth, but GR showed no significant difference. The prediction that long off-times would reduce water consumption was supported; H3 and H4 showed notably lower gross volume reduction. The prediction that an intermediate off-time would optimize growth–water efficiency balance was largely supported; H2 (300 s) outperformed all groups in biomass, phenolic content, and WUE_Y. Multi-variable tower profiles are shown in Figure 11 and Figure 12. Taken together, the 300-second off-time is a cycle parameter that simultaneously optimizes growth performance, biochemical quality, and water use efficiency (Diarra et al., 2023; Liu et al., 2025; Vatistas et al., 2022).

Conclusion

In this study, the effects of four irrigation cycle off-times on Stevia morphological growth, biomass yield, biochemical quality, and water use efficiency in vertical hydroponic tower systems were comparatively examined. The 300-second off-time group (H2) achieved the highest values for dry biomass (1.90 ± 1.04 g/plant), TPC (7.14 ± 0.34 mg GAE/g), DPPH antioxidant activity (5.90 ± 0.15 mmol TEAC/g), WUE_Y (3.17 g/L), and WUE_{shoot} (2.49 g/L). A strong Spearman correlation between D30 stem length and dry weight ($\rho = 0.873$, $p < 0.001$) was found, indicating the potential of non-destructive measurements for biomass estimation. The group with the longest off-time (H4: 600 s) reduced water consumption but was notably inferior in biomass and quality, suggesting that long off-times may impair root-zone moisture-oxygen balance and limit biomass and metabolite accumulation (Diarra et al., 2023; Liu et al., 2025).

These findings suggest that irrigation cycle design must account for product quality and biomass yield integrity, not only water savings, and that the 300-second off-time is a promising parameter for vertical hydroponic Stevia production (Liu et al., 2025; Vatistas et al., 2022; Pal et al., 2015).

As the primary methodological limitation, each irrigation cycle level was represented by a single tower, meaning that treatment effects and tower-specific structural variation cannot be fully disentangled. This pseudo-replication constraint limits the generalizability of the findings, and results should therefore be interpreted as exploratory rather than definitive (Carotti et al., 2023; Yang et al., 2024). Future studies should incorporate independent tower replication per treatment level, tower position rotation, and ion-based EC verification to strengthen causal inference (Fathidarehnijeh et al., 2024).

Declarations

Ethical Approval

This study did not involve animal subjects or human participants and did not require ethical committee approval.

Author Contribution Statement

Aydın Ozan Çetintaş: Conceptualization, methodology, data collection, formal analysis, writing the original draft, review and editing.

Halit Apaydın: Project administration, supervision, conceptualization, methodology, review and editing.

Burak Kunter: Project administration, data collection, review and editing.

Özlem Turan: Conceptualization, methodology, data collection, investigation, review and editing.

Irmak Çakın: Data collection, investigation.

Kadriye Yaprak Kantoğlu: Project administration, supervision, conceptualization, review and editing.

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Conflict of Interest

The authors declare no conflict of interest.

AI Disclosure Statement

No artificial intelligence (AI) tools were used during the preparation of this manuscript. All data collection, analysis, interpretation, and writing were performed by the authors.

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