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Synergistic Effects of Rhizosphere Temperature and Growing Media on Growth and Physiological Attributes of Strawberry cv. ‘Akihime’

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ABSTRACT

Strawberries are high-value crops prized for their nutritional and economic significance. This study hypothesized that root-zone temperature (RZT) and growing media (GM) influence strawberry growth and physiological responses. While individual effects of RZT or GM have been studied, little is known about their combined impact on hydroponic strawberries. To address this gap, we investigated how RZT and GM affect growth, stolon formation, and physiological changes in ‘Akihime’ strawberries. A factorial experiment was conducted using four RZT treatments (10 ± 2, 15 ± 2, 20 ± 2, and 25 ± 2 °C) and two growing media (peat moss + perlite and water culture). Greenhouse conditions were maintained at 25 ± 2 °C with 70–80% relative humidity and a photosynthetic photon flux density of 241 µmol m–2 s^{-1}. The results indicated that RZTs of 15 ± 2 °C and 20 ± 2 °C improved overall plant growth and biomass accumulation, while 25 ± 2 °C promoted stolon and runner development but hindered daughter plant formation. Substrate-based media outperformed water culture in supporting stolon and runner production. Leaf nitrogen and potassium concentrations peaked at 10 ± 2 °C and 15 ± 2 °C, respectively, while the water-culture medium enhanced nitrogen uptake. Leaf ATP concentration was highest at 20 ± 2 °C in substrate-grown plants, whereas root ATP peaked at 25 ± 2 °C in water culture. These findings demonstrated that careful management of RZT and GM can improve greenhouse strawberry productivity, and growers can tailor production strategies to meet specific cultivation goals.

Abbreviations: Anthocyanin Acyltransferase (AT), adenosine triphosphate (ATP), Chiang Mai University (CMU), deep-water culture (DWC;), growing media; (GM), Glutathione S-Transferase (GST), Hexose Transporter (HT), Pyruvate Kinase (PK), root-zone temperature (RZT), Sucrose-Phosphate Synthase (SPS), UDP-Glucose: Flavonoid 3-O-Glucosyltransferase (UFGT)

Introduction

Strawberry (*Fragaria* × *ananassa* Duchesne) is one of the most widely cultivated horticultural fruit crops worldwide, valued for its appealing nutritional profile and high economic return. Optimizing strawberry production in hydroponic greenhouses requires a nuanced understanding of how

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environmental variables influence plant physiology and development. Among these, root-zone temperature (RZT) and growing media (GM) are crucial factors in controlled-environment horticulture. Both can be strategically regulated to enhance strawberry performance (González-Fuentes et al., 2016; Sakamoto et al., 2016; Jo and Shin, 2022b). 'Akihime' is a Japanese cultivar of garden strawberry (*Fragaria* × *ananassa* Duch.), developed in Shizuoka Prefecture in the late 1980s. It was originally bred for early-spring production in the Kuno stone-wall farming region and, by 2003, had been adopted by more than 90% of local growers (Toshihiko et al., 2024). This cultivar produces large, bright red fruits with soft flesh and a high soluble solids content, averaging 10.6° Brix at full ripeness. It also exhibits a notably high sugar-to-acid ratio (~14.3), giving the fruit an exceptionally sweet flavor with mild acidity (Hong et al., 2018). These traits, high sugar content, low acidity, vibrant color, and sustained yield, make 'Akihime' a promising candidate for sustainable strawberry cultivation. RZT is a key regulator of plant growth and physiological function in strawberries, influencing factors such as oxygen availability, nutrient uptake, photosynthesis, and overall plant development. Optimal RZT enhances root and shoot growth, increases photosynthetic efficiency, and improves nutrient absorption. Conversely, extreme root temperatures can disrupt dry matter partitioning, inhibit growth, and trigger stress responses (Malcolm et al., 2006; Yasunaga, 2008; Hayashi et al., 2024). Techniques such as root-zone cooling during periods of high ambient temperature or heating during winter have been shown to mitigate stress and improve yield, sugar content, acidity, and flowering rates in strawberries (Yasunaga, 2008; Jo and Shin, 2022a). However, large diurnal fluctuations in RZT can reduce leaf area and biomass accumulation, underscoring the importance of maintaining a stable root environment (González-Fuentes et al., 2016). Although RZT is known to influence key physiological processes such as photosynthesis, respiration, and nutrient transport, detailed studies examining its specific effects on strawberry physiology remain limited (Levine et al., 2023). Notably, RZT extremes, above 30 °C or below 5 °C, can impair growth and physiological performance (Sakamoto et al., 2016; Jo and Shin, 2022b; Jiang et al., 2023), further highlighting the need for precise RZT management in controlled environments. These insights affirm that an optimal RZT is essential for maximizing growth, physiological efficiency, and yield in strawberry cultivation. Growing media provides water, air, nutrients, and mechanical support to plants. Commonly used substrates in strawberry production include peat moss, coconut coir, perlite, and vermiculite (Treadwell et al., 2007). In addition, strawberries can be successfully cultivated in deep-water culture hydroponic systems (Yafuso and Boldt, 2024). The choice of growing medium plays a crucial role in root health and nutrient dynamics, ultimately affecting plant performance. Responses to GM vary depending on cultivar and environmental conditions and can be further influenced by other factors such as light intensity and shading (Madhavi et al., 2021; Islam et al., 2023). Understanding the interactions between GM and environmental factors is therefore vital for optimizing strawberry production in hydroponic systems.

Substrate-based systems, utilizing materials such as coconut fiber, peat, and perlite, are widely adopted in strawberry cultivation to enhance growth and yield. These media improve plant biomass, height, and root volume, thereby supporting vigorous vegetative development and increased productivity (Cruz et al., 2023). Alternative substrates, including wood fiber, coir, and peat, have demonstrated comparable benefits, making them viable options for soilless cultivation (Woznicki et al., 2021b). High yields and excellent fruit quality have been reported using peat–perlite and coconut coir mixtures, with marketable yields matching or even surpassing those from traditional soil-based systems (Wang et al., 2016). In addition to yield advantages, substrate systems support environmental sustainability by reducing water and nutrient runoff, particularly when combined with automated irrigation technologies (Choi et al., 2016). Water-based hydroponic systems, such as deep-water culture (DWC), also offer unique advantages. These systems simplify root harvesting by eliminating substrate debris and, when properly managed, can support plant growth comparable to that achieved with peat-based substrates (Yafuso and Boldt, 2024). However, DWC systems require careful management of the nutrient solution's pH, which tends to decline over time. Frequent monitoring and the use of buffering agents are necessary to maintain optimal growth conditions (Yafuso and Boldt, 2024). Despite their benefits, both substrate and DWC systems present distinct challenges. Substrate systems are associated with higher material costs and demand precise irrigation and nutrient management to ensure consistent performance (Al-Raisy et al., 2010; Wang et al., 2016). On the other hand, DWC systems require constant oversight of the nutrient solution to maintain uniformity in plant growth (Yafuso and Boldt, 2024). Nonetheless, both systems are valuable tools in modern strawberry cultivation, offering pathways to increase productivity while addressing sustainability goals. Given these complexities, understanding the interaction between root-zone temperature and growing media is crucial for optimizing strawberry production in greenhouse settings. This knowledge is particularly important for developing sustainable cultivation practices.

However, to date, no studies have thoroughly examined the combined effects of RZT and GM on strawberry growth and physiology. We therefore hypothesize that specific combinations of root-zone temperatures and growing media synergistically influence the growth and physiological responses of hydroponically grown 'Akihime' strawberries, a cultivar prized for its exceptional flavor and visual appeal in Thailand and other Asian countries. Specifically, this research aimed to answer the following questions: 1.) How do changes in rooting media temperature affect the vegetative growth, stologenesis and physiological responses of 'Akihime' strawberries? 2.) What is the impact of substrate and water rooting media on the plants growth and physiology? 3.) Are there interactive effects from the root-zone temperature and rooting media on plant growth and physiological parameters?

The findings would inform strawberry production practices, thereby contributing to global food security. It would also offer practical insights for strawberry growers and researchers. This study aimed to achieve these goals, utilizing the methodological approach detailed in the next section.

Materials and Methods

Experimental design and treatments

A factorial experiment was designed to test four different RZTs (10 ± 2 °C, 15 ± 2 °C, 20 ± 2 °C, and ambient 25 ± 2 °C) and two types of growth media (peat moss + perlite and water culture) under controlled greenhouse conditions at the King's Initiative Centre for Flower and Fruit Propagation, Ban Rai, Chiang Mai 50230, Thailand. The RZT levels were maintained using an automated water-cooling and circulating system (Fig. 1). The evaporative greenhouse environment was maintained at an atmospheric temperature of 25 ± 2 °C, a relative humidity of 70-80%, and an average photosynthetic photon flux density of 241 μmol m^{-2} s^{-1}.

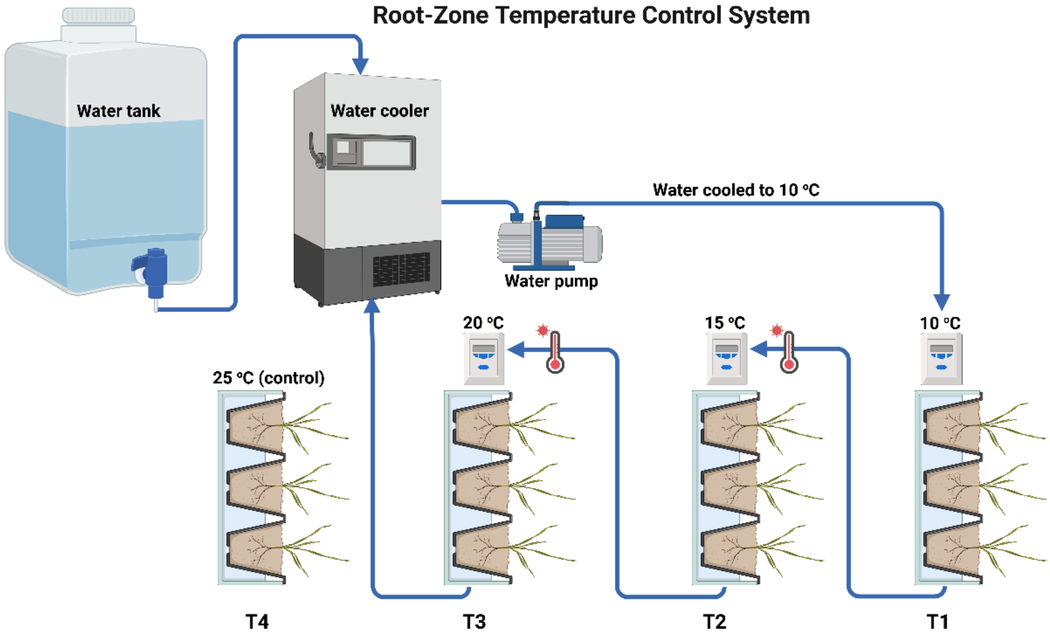

Fig. 1. Flowchart illustrating the process of root-zone temperature control. Water is first cooled to 10 °C before being directed into the experimental materials (substrate and water) and the different treatments via polyvinyl chloride (PVC) pipes with varying heating levels (15 °C and 20 °C). Treatment 4 relied on the ambient temperature of the greenhouse. Note that the cooled and heated water was not the hydroponic nutrient solution; rather, it served solely to modulate the root-zone temperature by passing through coils of PVC pipes embedded in the rooting media.

Plant materials and growth conditions

Uniform 'Akihime' strawberry daughter plants, approximately four weeks old, were obtained from the Strawberry Multiplication Unit at Mae Hia Farm, under the Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. The daughter plants were trimmed to three leaves and transplanted into polystyrene foam boxes measuring 47.5 × 30 × 26.9 cm, filled with designated growing media according to treatment combinations: either a substrate mixture of peat and perlite or a water-culture system (CMU-S2). The CMU-S2 strawberry nutrient solution (Table 1) was used both for the water culture treatment and for fertigation of the substrate-grown plants. During the juvenile stage, each plant in the substrate medium received 100 mL d^{-1} of nutrient solution, which was gradually increased to 300 mL d^{-1} as the plants matured. The nutrient solution was maintained at a pH of approximately 6.00 and an electrical conductivity (EC) of 1.5 dS m^{-1}.

Table 1. CMU-S2 strawberry nutrient stock solution formula (1:100 dilution) used for growing medium and fertigation in water and substrate systems, respectively.

Elements	Concentration (mg L^{-1})	Fertilizer source
Macronutrients		
N	147.85	$NH_4H_2PO_4$, KNO_3, $Ca(NO_3)_2$
P	39.75	$NH_4H_2PO_4$, KH_2PO_4
K	255.31	KH_2PO_4, KNO_3
Mg	28.51	$MgSO_4.7H_2O$
Ca	73.23	$Ca(NO_3)_2$
S	38.44	$MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $MnSO_4.5H_2O$
Micronutrients		
Zn	1.37	$ZnSO_4.7H_2O$
Mn	0.61	$MnSO_4.5H_2O$
B	1.05	H_3BO_3
Mo	0.18	$MoO_3.2H_2O$
Fe	3.99	Iron chelate
Cu	0.23	$CuSO_4.5H_2O$

CMU: Chiang Mai University, pH: ~6.00, Electrical conductivity: ~1.5 dS m^{-1}.

Measurement of growth and runner production parameters

Vegetative growth attributes of the strawberry plants were assessed based on foliage development, shoot and root length, and leaf area. Data on the number of leaves and plant height (cm) were collected from the mother plants during the growth period, while root length (cm) and leaf area (cm^2) were measured at harvest. Leaf area was determined by scanning the fully expanded leaves of four plants per treatment group using an EPSON 3110 tabletop scanner (Epson Co., Ltd., Bangkok, Thailand). The scanned images were analyzed with ImageJ software, and mean values were recorded. Additional growth parameters included the number of stolons per plant, number of runners per stolon, total runner yield per plant, number of leaves per runner, and runner height (cm).

Leaf greenness and leaf gas exchange measurements

Leaf greenness (SPAD) was assessed using a handheld SPAD meter (SPAD-502Plus®, Konica Minolta Inc., USA) across all leaves of the sampled plants. Gas exchange parameters of the strawberry plants were evaluated using a portable photosynthesis system (LI-6800®, LI-COR Inc., USA) during the morning hours (9:00–11:00 am) on clear and sunny days. The measured parameters included net assimilation rate (Pn), leaf transpiration rate (E), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci).

Laboratory determination of total nitrogen, phosphorus and potassium in strawberry leaves

Total nitrogen (%) and potassium (%) concentrations in strawberry leaf tissues were analyzed at the Soil Science Laboratory, Faculty of Agriculture, Chiang Mai University, Thailand. Briefly, harvested whole plants were washed first with tap water and subsequently rinsed using deionized water. They were then shade-dried and separated into roots and leaves before being oven-dried for 72 h at 60-75 °C. Nitrogen concentration was adjudged using Duma's combustion procedures. Total potassium was analyzed using atomic absorption spectrophotometry, as outlined by Mizukoshi et al. (1994).

Laboratory determination of ATP

Adenosine triphosphate (ATP) concentrations in the leaves and roots of strawberry plants were analyzed at the Plant Physiology Laboratory, Faculty of Agriculture, Chiang Mai University, Thailand. Four plants per treatment were harvested, immediately frozen at −20 °C, and later ground in liquid nitrogen. ATP extraction and quantification were performed using a high-performance liquid chromatography (HPLC) system (Agilent 1260 Infinity II, Agilent, USA), following the method described by Liu et al. (2006). Briefly, approximately 2 g of pulverized leaf and root tissue was extracted with 0.6 mol L^{-1} perchloric acid in an ice bath. The homogenate was centrifuged, and the resulting supernatant was neutralized with potassium hydroxide (KOH) to precipitate potassium perchlorate. The solution was then filtered prior to analysis. HPLC was carried out using a reverse-phase ODS column with a gradient elution of phosphate buffer (pH 7.0) and acetonitrile. ATP was detected at a wavelength of 254 nm, and quantification was performed by comparing retention times and peak areas to those of external standards. Final ATP concentrations in leaf and root tissues were expressed as micrograms of ATP per gram of fresh weight (μg g^{-1} FW).

Data analysis

A two-way analysis of variance (ANOVA) was utilized for data analysis, employing relevant Python program libraries. Treatment means that were statistically significant ($P < 0.05$) were differentiated using the Least Significant Difference (LSD) method at a 95% confidence level. Additionally, Pearson's correlation coefficient analyses ($P < 0.05$) were conducted using R program libraries to examine the relationships among the observed growth and physiological attributes of the treated plants.

Results

Number of leaves, leaf area, plant height and root length

Significant interactions ($P < 0.05$) between root-zone temperature (RZT) and growing media (GM) occurred in all measured plant growth parameters (Table 2). Strawberry plants grown at an RZT of 15 ± 2 °C exhibited the most vigorous growth, with the highest number of leaves (18.67 ± 1.57), greatest leaf area (14.68 ± 0.50 cm^2), and tallest plant height (37.42 ± 3.25 cm). In contrast, plants grown at 25 ± 2 °C showed significantly reduced growth across all parameters, with the lowest number of leaves (13.94 ± 0.80) and smallest leaf area (10.78 ± 2.75 cm^2), indicating heat stress at this elevated temperature. Regarding growing media, substrate-based systems generally promoted better above-ground growth, with greater plant height (34.98 ± 4.08 cm) and leaf area (13.97 ± 0.65 cm^2) compared to water culture. However, water culture supported superior root development, with a maximum root length of 31.56 ± 7.20 cm—approximately 8.5% longer than in the substrate medium. The interaction effects further revealed that the combination of 15 ± 2 °C RZT and substrate media resulted in the most favorable overall growth, while the combination of 25 ± 2 °C RZT and water culture led to the poorest performance, particularly in terms of leaf area and root development (Fig. 2).

Table 2. Growth of 'Akihime' strawberry in response to root-zone temperature and growing media at 12WAT (mean ± SD).

Factors	Number of leaves	Plant height (cm)	Root length (cm)	Leaf area (cm^2)
RZT (°C)				
10 ± 2	$15.94{\pm}2.67^c$	$34.09{\pm}2.52^b$	$32.64{\pm}5.73^b$	$12.43{\pm}1.87^c$
15 ± 2	$18.67{\pm}1.57^a$	$37.42{\pm}3.25^a$	$26.54{\pm}0.82^c$	$14.68{\pm}0.50^a$
20 ± 2	$17.83{\pm}4.26^b$	$33.24{\pm}2.47^b$	$35.69{\pm}3.51^a$	$13.04{\pm}1.57^b$
25 ± 2	$13.94{\pm}0.80^d$	$28.28{\pm}3.00^c$	$26.01{\pm}2.84^c$	$10.78{\pm}2.75^d$
F-Test ($P < 0.05$)	*	*	*	*
LSD	0.52	1.55	0.69	0.30
Growing media (GM)				
Substrate	$15.19{\pm}2.94^b$	$34.98{\pm}4.08^a$	$28.88{\pm}2.30^b$	$13.97{\pm}0.65^a$
Water	$18.00{\pm}2.82^a$	$31.54{\pm}4.15^b$	$31.56{\pm}7.20^a$	$11.50{\pm}2.47^b$
F-Test ($P < 0.05$)	*	*	*	*
LSD	0.37	1.09	0.49	0.21

* Significant (F-test; $P < 0.05$), and ^{abc}: treatment means that are not statistically different according to the LSD test at $P < 0.05$ are indicated by the same superscript letters in the same column, WAT: weeks after treatment, RZT: root-zone temperature.

Total fresh and dry weights

Biomass accumulation in 'Akihime' strawberries was assessed at 12 weeks after transplanting (WAT) under different RZT and growing media (GM) treatments (Table 3). The results revealed significant ($P < 0.05$) interactions between RZT and GM for both fresh and dry biomass, underscoring the crucial influence of these factors on plant productivity. Plants grown at an RZT of 20 ± 2 °C exhibited the highest fresh and dry weights, indicating that this temperature range provides optimal conditions for biomass accumulation. In contrast, plants subjected to 25 ± 2 °C RZT displayed the lowest biomass values, suggesting that elevated root-zone temperatures induce thermal stress, thereby reducing plant growth. Across media types, substrate cultivation significantly outperformed water culture in supporting biomass production. Total fresh and dry weights under substrate conditions were approximately 34% and 11.5% higher, respectively, than those observed in water culture. These findings highlight the superior ability of substrate media to support plant growth. The interaction effects further indicated that the combination of 20 ± 2 °C RZT and substrate medium yielded the highest overall biomass accumulation (Fig. 3). Conversely, the lowest biomass was recorded under the combination of 25 ± 2 °C RZT and water culture, with corresponding fresh and dry weights of 70.71 ± 11.64 g and 19.36 ± 0.52 g, respectively.

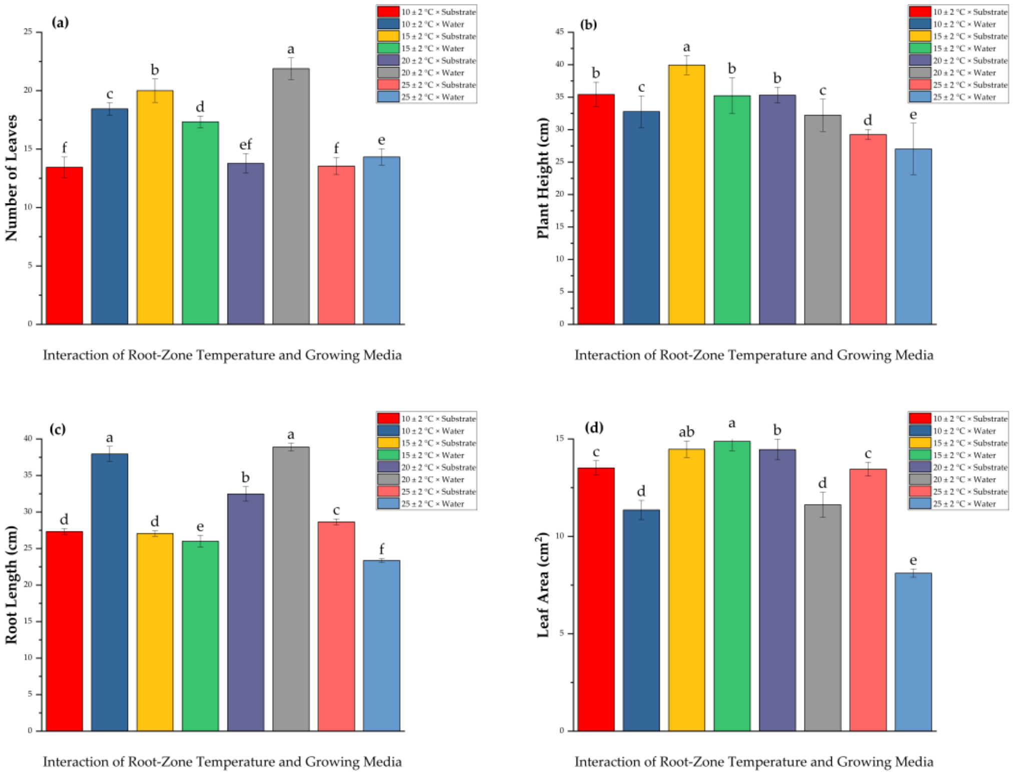

Fig. 2. Interactive effects of root-zone temperature and growing media on growth parameters of ‘Akihime’ strawberries at 12 weeks after treatment. Statistical significance was analyzed using two-way ANOVA ($P < 0.05$), with bars representing the standard deviation (SD). Different letters above the bars indicate significant differences among means based on Fisher's least significant difference (LSD) at a 95% confidence level. RZT levels: RZT1 (10 ± 2 °C), RZT2 (15 ± 2 °C), RZT3 (20 ± 2 °C), and RZT4 (25 ± 2 °C). GM types: GM1 (peat + perlite, 3:1 substrate media) and GM2 (water-culture media using CMU-S2 solution). TRT: treatment combinations Panels depict (**a**) the number of leaves; (**b**) plant height (cm); (**c**) root length (cm); and (**d**) leaf area (cm^2).

Table 3. Fresh and dry biomass of 'Akihime' strawberry at 12 weeks after treatment under different root-zone temperatures and growing media (mean ± SD).

Factors	**Fresh weight (g)**	**Dry weight (g)**
RZT (°C)		
10 ± 2	104.38±17.64^{c}	23.40±1.10^{c}
15 ± 2	133.67±19.50^{b}	24.24±1.46^{b}
20 ± 2	171.16±47.85^{a}	28.98±5.51^{a}
25 ± 2	94.51±26.56^{d}	19.84±0.62^{d}
F-Test ($P < 0.05$)	*	*
LSD	4.33	0.55
Growing media (GM)		
Substrate	151.75±40.63^{a}	25.58±5.44^{a}
Water	100.12±23.35^{b}	22.65±2.08^{b}
F-Test ($P < 0.05$)	*	*
LSD	3.06	0.39

* Significant (F-test; $P < 0.05$), and ^{abc}: treatment means that are not statistically different according to the LSD test at $P < 0.05$ are indicated by the same superscript letters in the same column, RZT: root-zone temperature.

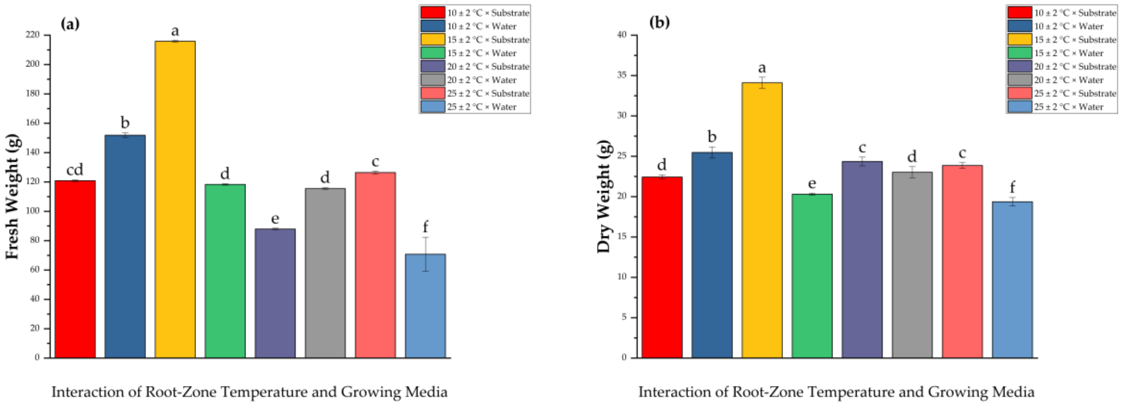

Fig. 3. Biomass accumulation of 'Akihime' strawberries at 12 weeks after treatment in response to the interactive effects of root zone temperatures (RZT) and growing media (GM). Statistical significance was analyzed using ANOVA ($P < 0.05$), with bars representing the standard deviation (SD). Different letters above the bars indicate significant differences among means based on Fisher's least significant difference (LSD) at a 95% confidence level. RZT levels: RZT1 (10 ± 2 °C), RZT2 (15 ± 2 °C), RZT3 (20 ± 2 °C), and RZT4 (25 ± 2 °C). GM types: GM1 (peat + perlite, 3:1 substrate media) and GM2 (water-culture media using CMU-S2 solution). TRT: treatment combinations. Panels depict (**a**) fresh weight (g) and (**b**) dry weight (g).

Runner production

The experiment also evaluated the effects of root-zone temperature (RZT) and growing media (GM) on runner development in 'Akihime' strawberries at 12 weeks after transplanting (WAT) (Table 4). Both RZT and GM significantly ($P < 0.05$) influenced stolon and daughter plant production, along with associated vegetative traits, with notable interactions observed between the two factors. The highest number of stolons per plant was recorded at an RZT of 15 ± 2 °C, whereas the maximum number of daughter plants per stolon and per plant occurred at 20 ± 2 °C and 25 ± 2 °C, respectively. In contrast, the tallest daughter plants were observed at 10 ± 2 °C, with plant height progressively decreasing as RZT increased (Fig. 4). These results indicate that RZTs in the range of 15–20 °C can be optimal for runner initiation, while elevated temperatures, particularly 25 ± 2 °C, promote the development of daughter plants. Among the media types, substrate cultivation supported superior runner performance, with higher stolon production (5.89 ± 2.20), greater daughter plant output per stolon (12.11 ± 5.79), and total daughter plants per plant (69.78 ± 42.50) compared to water culture. The combination of 25 ± 2 °C RZT and substrate yielded the highest number of daughter plants per plant and per stolon, suggesting a synergistic effect of elevated temperature and substrate media on runnering capacity. Conversely, the lowest daughter plant production was observed under the combination of 10 ± 2 °C RZT and water culture, highlighting the compounded inhibitory effect of low temperature and suboptimal growing media.

Chlorophyll index and photosynthetic attributes

The study assessed the effects of root-zone temperature (RZT) and growing media (GM) on leaf greenness and gas exchange parameters in 'Akihime' strawberries at 12 weeks after transplanting (WAT) (Table 5). The results revealed no statistically significant differences ($P > 0.05$) attributable to RZT, GM, or their interaction across any of the measured parameters. Leaf greenness, assessed by SPAD values, ranged from 43.70 ± 1.94 at 20 ± 2 °C RZT to 46.61 ± 2.73 at 10 ± 2 °C RZT. Gas exchange parameters followed a similar pattern, showing only minor variations that were not statistically significant. The net photosynthetic rate (Pn) was highest at 10 ± 2 °C RZT and lowest at 25 ± 2 °C, although these differences were not significant. Transpiration (E) and stomatal conductance (Gs) exhibited only slight fluctuations across RZT treatments. Similarly, growing media had no significant effect on any measured parameter. Mean SPAD values were 44.72 ± 3.59 for substrate and 45.65 ± 3.13 for water media. Net photosynthesis was marginally higher in substrate compared to water media, while transpiration and stomatal conductance remained relatively stable between the two. The interaction between RZT and GM also did not yield significant effects. The highest SPAD value (47.94 ± 3.01) was recorded under 10 ± 2 °C RZT in water culture, while the lowest Pn (5.95 ± 2.95 μmol CO_2 m^{-2} s^{-2}) occurred at 25 ± 2 °C RZT in water media. Transpiration and stomatal conductance similarly exhibited only negligible variation across treatment combinations.

Table 4. Interactive effects of root-zone temperature and growing media on stolon, runner, and daughter plant production in ‘Akihime’ strawberry (Mean ± SD).

Factors	Stolon/plant	Daughter plants/stolon	Daughter plants /plant	Leaves	Height (cm)
RZT (°C)					
10 ± 2	3.78±1.60^{d}	4.11±2.68^{d}	19.11±16.25^{d}	5.60±0.52^{b}	26.40±2.77^{a}
15 ± 2	6.50±2.41^{a}	5.44±2.45^{c}	40.67±28.62^{c}	6.90±1.45^{a}	25.40±2.59^{a}
20 ± 2	5.11±2.22^{c}	15.00±6.59^{a}	56.22±18.84^{b}	6.50±0.71^{a}	21.97±4.30^{b}
25 ± 2	5.78±1.44^{b}	11.61±1.69^{b}	90.17±50.31^{a}	5.90±0.57^{b}	19.32±2.23^{c}
F-Test (p<0.05)	*	*	*	*	*
LSD	0.52	0.95	5.85	0.50	2.56
Growing media					
Substrate	5.89±2.20^{a}	12.11±5.79^{a}	69.78±42.50^{a}	6.10±0.64	24.10±4.28
Water	4.58±1.98^{b}	5.97±4.10^{b}	33.06±28.59^{b}	6.35±1.27	22.55±3.95
F-Test ($P < 0.05$)	*	*	*	ns	ns
LSD	0.36	0.67	4.14	0.35	1.81
RZT × GM					
10 ± 2 °C × Substrate	5.22±0.67^{c}	6.67±0.50^{e}	34.67±3.94^{d}	5.80±0.45^{cd}	26.40±3.73^{a}
10 ± 2 °C ×Water	2.33±0.50^{e}	1.56±0.53^{g}	3.56±1.24^{f}	5.40±0.55^{d}	26.80±1.79^{a}
15 ± 2 °C ×Substrate	8.80±0.67^{a}	7.78±0.67^{de}	68.11±6.15^{b}	5.60±0.55^{cd}	25.60±3.78^{b}
15 ± 2 °C × Water	4.22±0.44^{d}	3.11±0.33^{f}	13.22±2.73^{e}	8.20±0.45^{a}	25.20±0.84^{a}
20 ± 2 °C ×Substrate	3.00±0.50^{e}	13.11±0.60^{b}	39.33±6.75^{cd}	6.80±0.45^{b}	24.90±4.05^{a}
20 ± 2 °C × Water	7.22±0.44^{b}	10.11±0.78^{c}	73.11±8.16^{b}	6.20±0.84^{bc}	19.04±1.91^{b}
25 ± 2 °C × Substrate	6.56±0.53^{b}	20.89±1.76^{a}	137.00±16.96^{a}	6.20±0.45^{bc}	19.50±2.42^{b}
25 ± 2 °C × Water	5.00±1.66^{c}	9.11±0.33^{cd}	43.33±12.49^{c}	5.60±0.55^{cd}	19.14±2.30^{b}
F-Test ($P < 0.05$)	*	*	*	*	*
LSD	0.73	1.34	8.28	0.71	3.62
CV (%)	14.65	15.78	17.06	8.8	12.06

*Significant, ^{ns}: not significant (F-test; $P < 0.05$), and ^{abc}: treatment means that are not statistically different according to the LSD test at $P < 0.05$ are indicated by the same superscript letters in the same column, RZT: root-zone temperature.

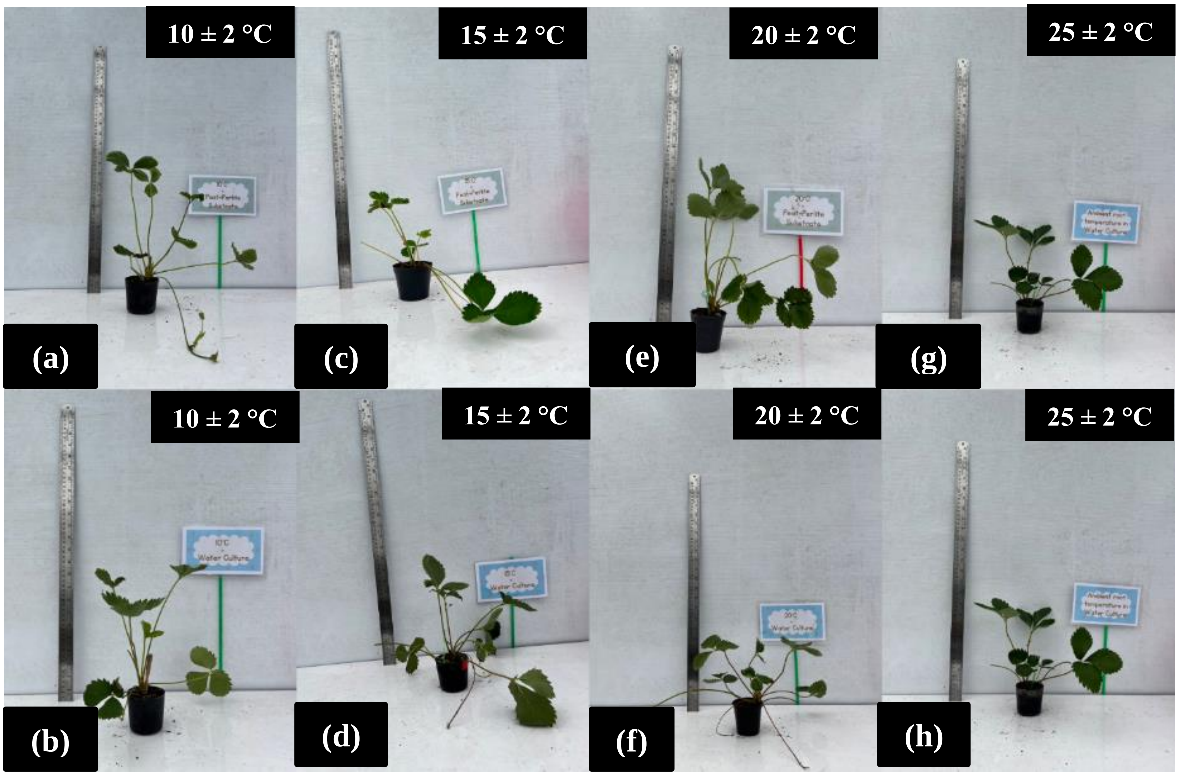

Fig. 4. Daughter plants of 'Akihime' strawberry under different root zone temperatures (RZT) and growing media (GM) treatments. Plants in the upper and lower panel were grown in substrate and water medium, respectively. (**a**) 10 ± 2 °C × substrate; (**b**) 10 ± 2 °C × water; (**c**) 15 ± 2 °C × substrate; (**d**) 15 ± 2 °C × water; (**e**) 20 ± 2 °C × substrate, (**f**) 20 ± 2 °C × water; (**g**) 25 ± 2 °C × substrate; and (**h**) 25 ± 2 °C × water.

Table 5. Leaf greenness and gas exchange of ‘Akihime’ strawberry in response to differing root-zone temperature and growing media (mean ± SD).

Factors	SPAD	Pn (µmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	Gs (mol m^{-2} s^{-1})
RZT (°C)				
10 ± 2	46.61±2.73	8.94±1.35	3.08±0.23	0.28±0.03
15 ± 2	45.23±5.38	7.65±2.47	2.74±0.56	0.22±0.07
20 ± 2	43.70±1.94	8.20±0.88	2.83±0.46	0.23±0.06
25 ± 2	44.68±1.66	7.20±2.15	3.06±0.65	0.25±0.08
F-Test (*P* < 0.05)	^{ns}	^{ns}	^{ns}	^{ns}
LSD	2.18	1.84	0.52	0.07
Growing media				
Substrate	44.72±3.59	8.29±1.96	2.77±0.51	0.23±0.07
Water	45.65±3.13	7.71±1.77	3.09±0.44	0.24±0.06
F-Test (*P* < 0.05)	^{ns}	^{ns}	^{ns}	^{ns}
LSD	1.54	1.30	0.37	0.0500
RZT × GM				
10 ± 2 °C × Substrate	45.27±1.65	8.90±1.96	3.00±0.16	0.2775±0.02
10 ± 2 °C × Water	47.94±3.01	9.00±0.63	3.17±0.28	0.2825±0.05
15 ± 2 °C × Substrate	44.71±6.85	7.10±3.42	2.56±0.74	0.2400±0.10
15 ± 2 °C × Water	45.74±3.73	8.21±1.32	2.90±0.32	0.1975±0.04
20 ± 2 °C × Substrate	43.97±1.96	8.72±0.79	2.60±0.49	0.2450±0.08
20 ± 2 °C × Water	43.41±2.00	7.67±0.66	3.06±0.32	0.2150±0.03
25 ± 2 °C × Substrate	44.92±1.40	8.45±0.56	2.90±0.56	0.2350±0.08
25 ± 2 °C × Water	44.44±1.94	5.95±2.95	3.22±0.78	0.2675±0.05
F-Test (*P* < 0.05)	^{ns}	^{ns}	^{ns}	^{ns}
LSD	3.10	2.60	0.73	0.1000
CV (%)	7.25	22.27	17.15	27.7600

^{ns}: not significant (F-test; *P* < 0.05), RZT: root-zone temperature.

Total leaf nitrogen and potassium concentration

The study examined the effects of varying root-zone temperature (RZT) and growing media (GM) on leaf nitrogen and potassium concentrations in 'Akihime' strawberries at 12 weeks after transplanting (WAT) (Table 6). The results revealed significant effects ($P < 0.05$) of RZT on both nitrogen and potassium concentrations, while GM had a significant impact on nitrogen concentration only. Additionally, the interaction between RZT and GM was significant for both nutrients. Leaf nitrogen concentration declined progressively with increasing RZT, with the highest value recorded at 10 ± 2 °C RZT (2.46 ± 0.20%). These findings suggest that lower root-zone temperatures may enhance nitrogen assimilation or retention in foliage. Across media types, water-based cultivation resulted in higher nitrogen concentrations than substrate, implying improved nitrogen availability or uptake efficiency in the aqueous system. Potassium concentration was significantly influenced by RZT, with no significant effect from GM. The highest potassium concentration (2.50 ± 0.06%) was observed at 15 ± 2 °C RZT, indicating that this temperature range may be optimal for potassium accumulation in strawberry leaves. Interaction effects between RZT and GM revealed distinct nutrient uptake patterns. The highest nitrogen concentration (2.65 ± 0.03%) was recorded under the combination of 10 ± 2 °C RZT and water media, whereas the lowest (1.59 ± 0.03%) was observed at 25 ± 2 °C RZT in water media. Substrate consistently supported lower nitrogen levels across all RZT treatments. For potassium, the highest concentration (2.51 ± 0.05%) occurred at 15 ± 2 °C RZT in substrate media, while the lowest (1.50 ± 0.12%) was recorded at 25 ± 2 °C RZT in water media (Fig. 5).

Table 6. Total leaf nitrogen and potassium concentrations in 'Akihime' strawberry at 12 weeks after treatment under different root-zone temperatures and growing media (mean ± SD).

Factors	Total N (% g^{-1} DW)	Total K (% g^{-1} DW)
RZT (°C)		
10 ± 2	2.46±0.20^{a}	2.16±0.36^{b}
15 ± 2	2.31±0.04^{b}	2.50±0.06^{a}
20 ± 2	1.97±0.10^{c}	2.36±0.15^{a}
25 ± 2	1.62±0.05^{d}	1.58±0.12^{c}
F-Test ($P < 0.05$)	*	*
LSD	0.04	0.16
Growing media (GM)		
Substrate	2.02±0.28^{b}	2.20±0.37
Water	2.16±0.40^{a}	2.10±0.44
F-Test ($P < 0.05$)	*	^{ns}
LSD	0.03	0.11
CV (%)	1.66	7.28

*Significant (F-test; $P < 0.05$), and ^{abc}: treatment means that are not statistically different according to the LSD test at $P < 0.05$ are indicated by the same superscript letters in the same column, RZT: root-zone temperature.

Adenosine triphosphate (ATP) concentration in leaves and roots

The study evaluated the synergistic effects of root-zone temperature (RZT) and growing media (GM) on adenosine triphosphate (ATP) concentrations in the leaves and roots of 'Akihime' strawberries at 12 weeks after transplanting (WAT). The findings revealed that both RZT and GM significantly influenced ATP accumulation in both tissues, with pronounced interaction effects between the two factors (Table 7). Leaf ATP concentrations were highest at moderate RZTs (15–20 °C), indicating that these temperatures favor optimal metabolic activity. Additionally, water-based cultivation systems supported consistently higher ATP levels in leaves compared to substrate-based systems, suggesting enhanced nutrient or oxygen availability in the aqueous medium. The interaction between RZT and GM further emphasized the conditions most conducive to ATP biosynthesis in leaves, with the highest concentrations observed at 20 ± 2 °C RZT in substrate media. In contrast, root ATP concentrations were more responsive to elevated temperatures, peaking at 25 ± 2 °C RZT. Unlike the leaf tissue, substrate media supported greater ATP accumulation in roots across most temperature treatments, likely due to improved root aeration and mechanical support. Overall, the interaction between RZT and GM revealed a complex tissue-specific pattern of ATP regulation. The highest ATP levels in leaves and roots were observed under distinct conditions: 20 ± 2 °C RZT in substrate media for leaves and 25 ± 2 °C RZT in water media for roots. Conversely, the lowest ATP concentrations in both tissues were associated with extreme RZT conditions, regardless of the media type (Fig. 6). These results highlight the crucial role of optimized environmental and cultural conditions in modulating energy metabolism in hydroponically grown strawberries.

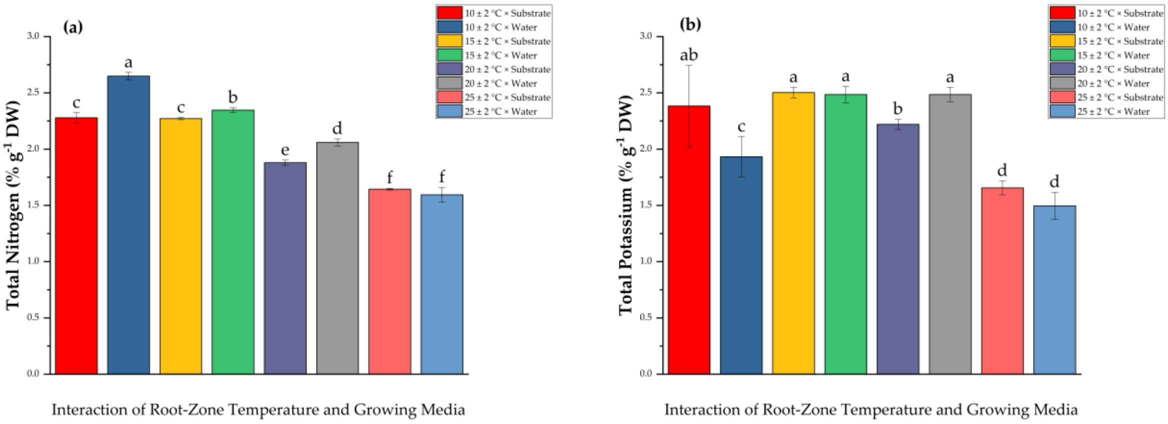

Fig. 5. Total leaf nitrogen and potassium concentrations of 'Akihime' strawberries in response to combined effects of different root zone temperatures (RZT) and growing media (GM). Statistical significance was analyzed using ANOVA ($P < 0.05$), with bars representing the standard error of the mean (SEM). Different letters above the bars indicate significant differences among means based on Fisher's least significant difference (LSD) at a 95% confidence level. RZT levels: RZT1 (10 ± 2 °C), RZT2 (15 ± 2 °C), RZT3 (20 ± 2 °C), and RZT4 (25 ± 2 °C). GM types: GM1 (peat + perlite, 3:1 substrate media) and GM2 (water-culture media using CMU-S2 solution). TRT: treatment combinations. Panels depict (**a**) Total nitrogen and (**b**) Total potassium.

Table 7. Synergistic effects of root-zone temperature and growing media on leaf and root ATP concentrations in 'Akihime' strawberry after 12 weeks of treatment (mean ± SD).

Factors	ATP in Leaves (μg g^{-1} FW)	ATP in Roots (μg g^{-1} FW)
RZT (°C)		
10 ± 2	63.94±39.63^{d}	48.34±16.44^{c}
15 ± 2	121.52±94.45^{b}	25.83±8.65^{d}
20 ± 2	149.61±68.50^{a}	80.80±58.45^{b}
25 ± 2	100.74±8.97^{c}	112.60±63.69^{a}
F-Test ($P < 0.05$)	*	*
LSD ($P < 0.05$)	4.84	4.11
Growing Media		
Substrate	95.55±77.84^{b}	71.33±39.98^{a}
Water	122.35±52.57^{a}	62.45±65.63^{b}
F-Test ($P < 0.05$)	*	*
LSD ($P < 0.05$)	3.42	2.91
RZT × GM		
10 ± 2 °C × Substrate	26.90±1.89	63.00±7.43
10 ± 2 °C × Water	101.00±3.33	33.70±2.18
15 ± 2 °C × Substrate	33.30±2.92	33.80±2.15
15 ± 2 °C × Water	210.00±8.75	17.90±1.15
20 ± 2 °C × Substrate	214.00±5.71	135.00±4.35
20 ± 2 °C × Water	85.70±3.21	26.20±1.38
25 ± 2 °C × Substrate	108.00±5.02	53.20±5.40
25 ± 2 °C × Water	93.10±2.88	172.00±3.35
F-Test ($P < 0.05$)	*	*
LSD ($P < 0.05$)	6.85	5.82
CV (%)	4.31	5.96

*Significant (F-test; $P < 0.05$), and ^{abc}: treatment means that are not statistically different according to the LSD test at $P < 0.05$ are indicated by the same superscript letters in the same column, RZT: root-zone temperature.

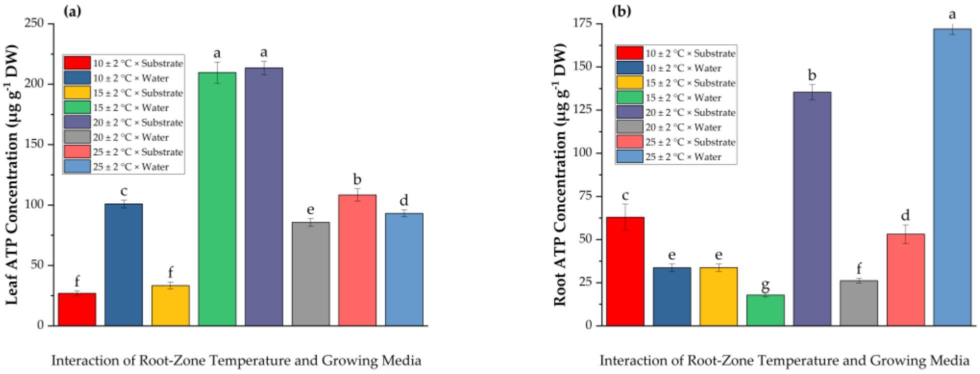

Fig. 6. Adenosine Triphosphate (ATP) concentration in the leaves and roots of 'Akihime' strawberry plants in response to the interactive effects of four root-zone temperature (RZT) levels and two types of growing media. (**a**) ATP concentration in leaves and (**b**) ATP concentration in roots. RZT treatments include R1: 10 ± 2 °C, R2: 15 ± 2 °C, R3: 20 ± 2 °C, and R4: 25 ± 2 °C. Growing media are designated as M1: substrate-based medium (peat + perlite in a 3:1 ratio) and M2: deep water culture. TRT: treatment combinations. Data were analyzed using ANOVA at a significance level of $P < 0.05$. Bars with different letters indicate significant differences determined by Fisher's LSD test ($P < 0.05$). Error bars represent the standard deviation of the mean (SD).

Relationship among growth and physiological traits of 'Akihime' strawberry plants

Figure 7 illustrates Pearson's correlation coefficients among 17 key traits related to plant morphology, physiology, and nutrition in 'Akihime' strawberries, assessed at 12 weeks after treatment under varying root-zone temperatures (RZT) and growing media (GM). The corresponding p-values are presented in Table 8. Notably, total dry weight exhibited a strong positive correlation with total fresh weight (r = 0.89), as well as a moderate correlation with the number of daughter plants (r = 0.65), indicating a close relationship between biomass accumulation and reproductive output. Additionally, total dry weight correlated positively with the number of leaves (r = 0.42), root length (r = 0.47), and leaf area (r = 0.43), suggesting these morphological attributes contribute to overall plant vigor. Among the physiological parameters, the net photosynthetic rate (Pn) showed a strong positive association with total nitrogen content (r = 0.79) and stomatal conductance (gs) (r = 0.61), reflecting the interdependence of nutrient availability and gas exchange in photosynthetic performance. Furthermore, stomatal conductance was highly correlated with transpiration rate (E) (r = 0.75), reinforcing the intrinsic link between water vapor loss and CO_2 uptake. From a nutritional perspective, both total potassium and total nitrogen concentrations were positively correlated with key growth traits. Significant associations were observed with the number of leaves (r = 0.49 and r = 0.48, respectively) and total dry weight (r = 0.60 for both nutrients), emphasizing their essential roles in promoting vegetative development. Conversely, several negative correlations suggest potential trade-offs in resource allocation. The SPAD index, an indicator of leaf chlorophyll content, demonstrated negative correlations with root length (r = -0.35) and root ATP concentration (r = -0.28), implying an inverse relationship between chlorophyll density and root activity under certain conditions. Additionally, a moderate negative correlation was observed between leaf area and SPAD values (r = -0.33), indicating that increased leaf expansion does not necessarily equate to higher chlorophyll content per unit area.

Discussion

The present study investigated the interactive effects of root-zone temperature (RZT) and growing media (GM) on the growth and physiological responses of 'Akihime' strawberries cultivated under greenhouse conditions. The results demonstrated that both RZT and GM significantly influenced plant growth, development, and runnering behavior. Mother plants exhibited enhanced vegetative growth and biomass accumulation under moderate RZT treatments of 15 ± 2 °C and 20 ± 2 °C, suggesting that an optimal RZT range for canopy development lies between 13 °C and 22 °C. This finding aligns with prior research indicating that moderate RZTs enhance water-use efficiency and nutrient uptake, contributing to improved plant vigor (Fan et al., 2024). Interestingly, elevated RZT (25 ± 2 °C) stimulated root elongation, potentially through auxin-mediated cell division, a mechanism similarly reported in Arabidopsis thaliana (Ai et al., 2023). However, this observation contrasts with findings by Sakamoto et al. (2016), who reported greater root biomass at 10 °C in hydroponically grown strawberries. This discrepancy may be attributable to differences in cultivar sensitivity, environmental settings, or hydroponic system design. Moreover, a substantial increase in stolon and daughter plant production was observed at 25 ± 2 °C RZT, suggesting a possible thermomorphogenic shift favoring clonal propagation under high root-zone temperatures. This divergence in organ-specific responses could reflect a stress-induced reallocation of resources, where elevated RZT reduces root oxygen availability and cell viability (Sakamoto et al., 2016), thereby limiting root biomass while accelerating vegetative structure formation. Supporting this notion, He et al. (2009) reported that increased RZT in lettuce led to a shift in photoassimilate allocation, favoring root thickening and reproductive effort over elongation and vegetative growth.

Under stress conditions, suboptimal RZTs can trigger metabolic adjustments that influence assimilate partitioning. Heat stress, for instance, enhances carbon loss via respiration and alters carbohydrate dynamics by increasing leaf soluble sugars while decreasing starch reserves (Gavrichkova et al., 2019). These alterations may contribute to the observed shift toward stolon formation at elevated RZT. Furthermore, RZT modulates root metabolic activity and hormonal balances. Low RZT has been shown to elevate levels of proline and glutamine in roots, both of which are associated with stress adaptation and growth regulation (Kang et al., 2025). Hormonal signaling pathways also underpin the observed trade-offs between vegetative growth and stolon development. Gibberellins are known to promote stolon formation, while strigolactones (SLs) play key roles in shoot branching and nutrient reallocation (Guo et al., 2021). The interplay between these hormones and environmental cues such as RZT likely governs the allocation of assimilates and organ development in strawberry plants. The choice of growing medium further modulated plant performance. Peat moss combined with perlite supported superior shoot growth and biomass accumulation, whereas water-based media favored enhanced root development and leaf proliferation.

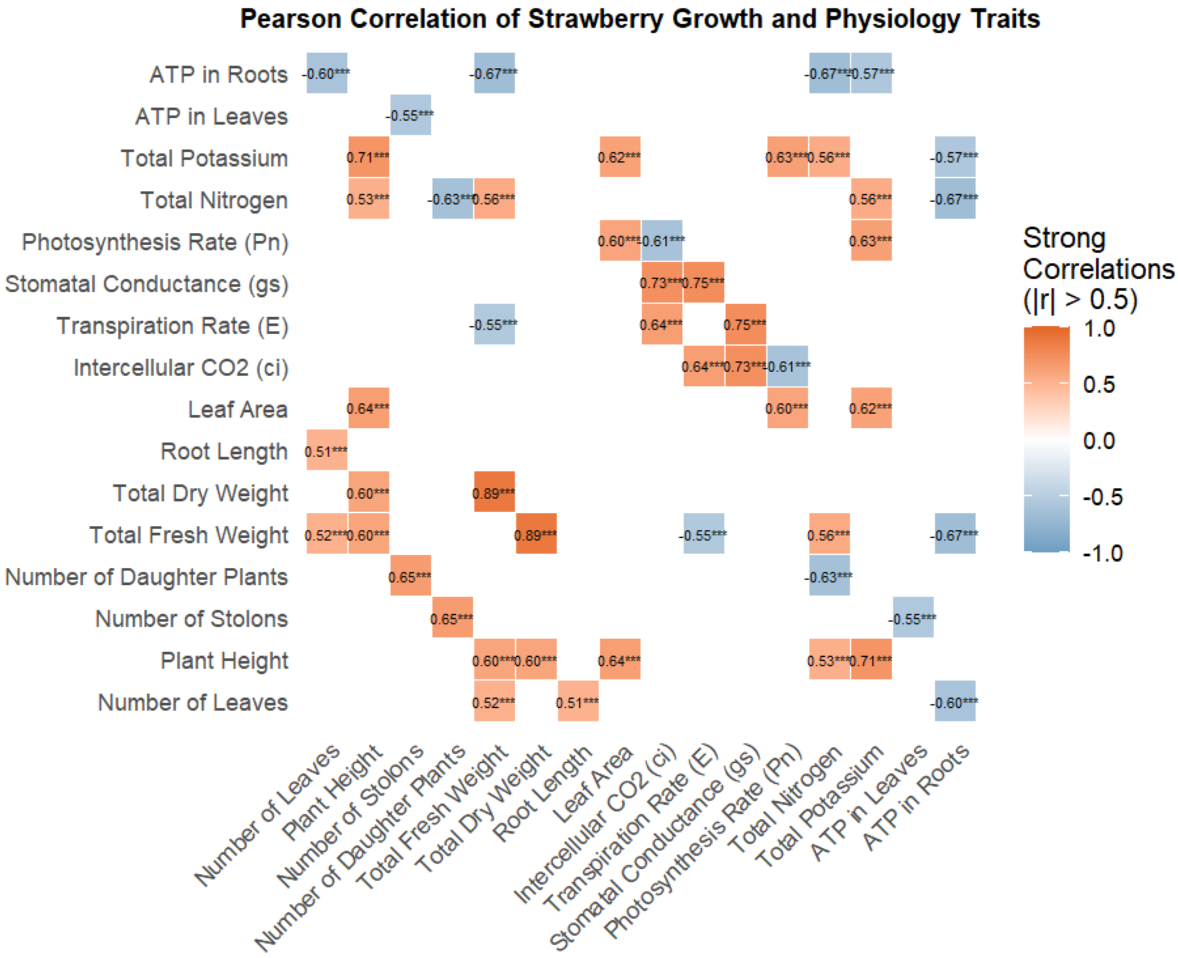

Fig. 7. Pearson correlation heat map depicting relationships among morphological, physiological, and nutrient-associated traits in 'Akihime' strawberry plants grown under variable root-zone temperature and growing media conditions. The color scale ranges from -1 (blue, strong negative correlation) to +1 (red, strong positive correlation), with only significant correlations ($\alpha = 0.05$) displayed, * = significant at $P < 0.05$, ** = significant at $P < 0.01$, *** = significant at $P < 0.001$.

Table 8. Pairwise p-values for Pearson's correlations among 'Akihime' strawberry growth and physiological parameters.

	NL	**PH**	**NS**	**SPAD**	**ND**	**FW**	**DW**	**RL**	**LA**	**Ci**	**E**	**Gs**	**Pn**	**N**	**K**
NL	0.00^{***}	0.01^{*}	0.02^{**}	0.95^{ns}	0.42^{ns}	0.00^{***}	0.00^{***}	0.00^{***}	0.94^{ns}	0.01^{**}	0.00^{***}	0.00^{**}	0.76^{ns}	0.00^{***}	0.00^{***}
PH		0.00^{***}	0.08^{ns}	0.95^{ns}	0.10^{ns}	0.00^{***}	0.00^{***}	0.79^{ns}	0.00^{***}	0.07^{ns}	0.00^{**}	0.68^{ns}	0.00^{***}	0.00^{***}	0.00^{***}
NS			0.00^{***}	0.15^{ns}	0.00^{***}	0.00^{***}	0.00^{***}	0.11^{ns}	0.39^{ns}	0.28^{ns}	0.75^{ns}	0.18^{ns}	0.43^{ns}	0.07^{ns}	0.04^{*}
SPAD				0.00^{***}	0.08^{ns}	0.34^{ns}	0.85^{ns}	0.68^{ns}	1.00^{ns}	0.30^{ns}	0.97^{ns}	0.14^{ns}	0.68^{ns}	0.02^{*}	0.73^{ns}
ND					0.00^{***}	0.37^{ns}	0.10^{ns}	0.52^{ns}	0.51^{ns}	0.31^{ns}	0.25^{ns}	0.03^{*}	0.99^{ns}	0.00^{***}	0.03^{*}
FW						0.00^{***}	0.00^{***}	0.40^{ns}	0.00^{***}	0.03^{*}	0.00^{***}	0.17^{ns}	0.06^{ns}	0.00^{***}	0.00^{***}
DW							0.00^{***}	0.27^{ns}	0.00^{***}	0.08^{ns}	0.00^{***}	0.87^{ns}	0.03^{*}	0.01^{**}	0.00^{**}
RL								0.00^{***}	0.78^{ns}	0.02^{*}	0.06^{ns}	0.02^{*}	0.58^{ns}	0.00^{**}	0.08^{ns}
LA									0.00^{***}	0.01^{**}	0.21^{ns}	0.94^{ns}	0.00^{***}	0.01^{**}	0.00^{***}
Ci										0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.15^{ns}	0.00^{**}
E											0.00^{***}	0.00^{***}	0.41^{ns}	0.00^{***}	0.06^{ns}
Gs												0.00^{***}	0.88^{ns}	0.91^{ns}	0.89^{ns}
Pn													0.00^{***}	0.07^{ns}	0.00^{***}
N														0.00^{***}	0.00^{***}
K															0.00^{***}

NL = Number of Leaves, PH = Plant Height, NS = Number of Stolons, ND = Number of Daughter Plants, FW = Fresh Weight, DW = Dry Weight, RL = Root Length, Ci = Intercellular CO2, E = Transpiration Rate, Gs = Stomatal Conductance, Pn = Photosynthetic Rate, N = Total Nitrogen, K = Total Potassium, *** = Significant at $P < 0.001$, ** = Significant at $P < 0.01$, * = Significant at $P < 0.05$, ^{ns} = not significant.

These differences in plant responses may stem from the contrasting physicochemical properties of the two media types, such as nutrient availability, aeration, and water-holding capacity (Ameri et al., 2020; Renfiyeni et al., 2020; Madhavi et al., 2021; Woznicki et al., 2021a). The observed variation in stolonogenesis under different media reflects the medium's influence on nutrient dynamics and root-zone microenvironments.

Furthermore, a significant interaction between RZT and growing medium was observed, highlighting their combined influence on plant development. The combination of 20 ± 2 °C RZT and water-based media resulted in enhanced leaf and root growth, yet it concurrently suppressed runner formation. In contrast, the pairing of 25 ± 2 °C RZT with substrate media yielded the highest runner production, indicating a favorable condition for clonal propagation. Interestingly, the same elevated RZT (25 ± 2 °C) in water-based media significantly inhibited shoot development, which may be attributed to stress-induced metabolic alterations that impair vegetative growth. However, the substrate medium appeared to alleviate this stress, likely through improved aeration, buffering capacity, or nutrient retention, thereby supporting shoot development under warmer conditions. This observation is consistent with previous studies reporting the beneficial effects of substrate media on shoot growth in strawberries and other horticultural crops (Yadav, 2022; Thakur et al., 2025).

At 12 weeks after treatment (WAT), both root-zone temperature (RZT) and growing medium significantly influenced the concentrations of nitrogen (N) and potassium (K) in the leaves of 'Akihime' strawberries. Notably, leaf nitrogen levels were highest at the coolest RZT (10 ± 2 °C), whereas potassium concentrations peaked at 15 ± 2 °C. This divergence suggests that N and K have distinct optimal temperature ranges for uptake and assimilation within the plant. Additionally, the water culture system promoted higher leaf nitrogen concentrations compared to substrate media, likely due to increased nutrient availability and mobility in the aqueous environment. Significant interactions between RZT and growing medium for both nutrients further indicate that the effect of temperature on nutrient uptake is modulated by the type of growing medium. These findings align with prior studies demonstrating that optimal RZT enhances root development, photosynthetic capacity, and enzymatic activity, thereby facilitating efficient nutrient uptake and assimilation (Malcolm et al., 2008; Sheibanirad et al., 2023). For example, moderate increases in temperature within the optimal range can improve photosynthesis and water-use efficiency, processes crucial for nitrogen assimilation (Malcolm et al., 2008). Conversely, suboptimal RZT limits these physiological functions, resulting in reduced nutrient uptake. At the molecular level, elevated RZT has been shown to upregulate genes encoding nitrogen transporters and key enzymes involved in nitrogen metabolism (He et al., 2013; Xia et al., 2024). Furthermore, RZT affects the rhizosphere microbial community composition and activity, which plays a pivotal role in nutrient cycling and can enhance nitrogen uptake efficiency via symbiotic interactions (Du et al., 2023).

Potassium uptake is similarly influenced by root-zone temperature (RZT), with warmer temperatures enhancing the activity of potassium transporters (Yang et al., 2020; Huimin et al., 2021). The physical and chemical properties of the growing medium, such as nutrient retention capacity and porosity, also play crucial roles in nutrient availability for plant absorption and assimilation. For instance, cocopeat has been reported to improve nitrogen uptake due to its superior nutrient retention capacity (Adak and Pekmezci, 2014). Our findings further reveal a significant interaction between RZT and growing medium on ATP concentrations in both leaves and roots of 'Akihime' strawberries. Leaf ATP levels were highest at 20 ± 2 °C RZT, reflecting enhanced metabolic and photosynthetic activity under these conditions. In contrast, root ATP concentrations peaked at 25 ± 2 °C, likely driven by increased root respiration rates. Both excessively low RZT and high RZT adversely affected ATP synthesis: cold stress inhibited ATP production at low temperatures, while elevated RZT disrupted photosynthetic processes and membrane integrity, thereby reducing ATP generation (Goraya et al., 2017; Moore et al., 2021). Regarding media effects, water-based systems supported higher ATP concentrations in leaves, potentially due to improved nutrient uptake and photosynthetic efficiency. Conversely, substrate media enhanced root ATP levels, possibly owing to better aeration and root development. Notably, the combination of 20 ± 2 °C RZT with substrate media resulted in the highest leaf ATP concentration, while 25 ± 2 °C RZT in water media yielded the highest root ATP levels. These results emphasize the importance of optimizing the interaction between RZT and growing media to maximize ATP production and improve overall plant productivity.

Both positive and negative correlations were observed among various growth and physiological attributes of the experimental strawberry plants. The strong positive association between the growth of mother plants and the production of daughter plants suggests an interconnectedness between biomass accumulation and clonal propagation processes in strawberries. This relationship may be mediated by stolon retention, which facilitates resource sharing between mother and daughter plants. Supporting this, Shi et al. (2021) reported that strawberry mother plants subjected to less frequent stolon removal produced more daughter plants and allocated a higher proportion of total biomass to daughter plants and stolons compared to plants experiencing frequent stolon removal. Runner and daughter plant production are closely linked to the carbohydrate status of the mother plant. Elevated levels of soluble sugars, particularly sucrose, in shoot tips and leaves correlate with increased runner formation (Li et al., 2020), suggesting that mother plants actively allocate carbohydrates to support runner and daughter plant development. Physiologically, the pronounced positive correlation between total nitrogen content and stomatal conductance underscores nitrogen's crucial role in modulating stomatal behavior to optimize carbon assimilation. Higher leaf nitrogen concentrations enhance photosynthetic capacity by upregulating key enzymes and promoting stomatal opening, which collectively improve water-use efficiency (WUE) and net carbon gain (Yamori et al., 2011; Song et al., 2022).

In mechanical terms, improved nitrogen nutrition drives increases in carboxylation efficiency and biomass accumulation by facilitating greater stomatal conductance while curbing excessive transpiration (Belane and Dakora, 2011; Song et al., 2022; Yan et al., 2022). Moreover, a strong positive relationship between stomatal conductance and transpiration rate in strawberry plants highlights the intrinsic link between gas exchange and water loss. Stomatal density significantly influences transpiration, with higher density generally enhancing stomatal conductance and consequently increasing transpiration rates (Blanke and Cooke, 2004). However, the stomatal density–conductance relationship can be modulated by stomatal sensitivity and environmental factors (Vráblová et al., 2017; Tulva et al., 2024), indicating a complex regulatory network governing water and carbon dynamics in strawberry leaves.

Both leaf total potassium and total nitrogen were positively associated with biomass accumulation, emphasizing their crucial roles in supporting vegetative growth. Nitrogen is essential for strawberry growth and yield, with adequate nitrogen supply enhancing plant development, fruit quality, and nutritional value (Andriolo et al., 2011; Marcellini et al., 2023; Jibia et al., 2025). It significantly affects key growth parameters such as plant height, leaf number, and overall biomass accumulation (Andriolo et al., 2011; Marcellini et al., 2023; Jibia et al., 2025). Moreover, nitrogen distribution within the plant, particularly in the leaves, correlates closely with biomass production (Andriolo et al., 2011; Jia et al., 2024; Li et al., 2024). Potassium also plays a vital role in plant growth and development by participating in enzyme activation, photosynthesis, and water regulation (Wang et al., 2018; Johnson et al., 2022). It is crucial for maintaining photosynthetic leaf area and supporting biomass accumulation. Additionally, potassium enhances the efficient utilization of nitrogen, further promoting growth and yield (Xu et al., 2020; Zhang et al., 2021). At the molecular level, potassium promotes the upregulation of genes involved in sugar and anthocyanin metabolism, including GST, AT, UFGT, SPS, HT, and PK (Huang et al., 2023). These genes are crucial for the synthesis and transport of sugars, which are essential for biomass accumulation. The expression of transporter proteins related to potassium and sugar transport is closely correlated, suggesting transcriptional coregulation that facilitates the simultaneous translocation and storage of these nutrients within plant cells (Coetzee et al., 2019). This synergy between leaf nitrogen and potassium optimizes strawberry growth and biomass accumulation (Yadav et al., 2023; Fang et al., 2024a). Our findings also highlight potential trade-offs in resource allocation, evidenced by negative correlations among key vegetative and physiological traits. Specifically, the SPAD index exhibited inverse relationships with both root length and ATP content in the roots, suggesting that higher chlorophyll concentration may coincide with reduced investment in belowground growth and root energy metabolism. This pattern may reflect adaptive resource partitioning under stress or nutrient limitations, where diminished root length could compromise water or nutrient uptake, and lower root ATP might reduce stress resilience. Similar trade-offs have been documented in other species. For example, in cotton, seed yield negatively correlated with leaf potassium concentration in some environments, indicating that elevated potassium levels do not always translate into higher yield (Tsialtas et al., 2008).

Similarly, in New Zealand woody species, intraspecific analyses have shown a decline in leaf area with increasing plant height (Burns and Beaumont, 2009). In *Pinus koraiensis*, $\delta^{13}C$, i.e., used as a proxy for water-use efficiency, was negatively correlated with leaf phosphorus content in smaller plants (Fang et al., 2024b). Additionally, long-term grazing induced a significant negative relationship between specific leaf area and leaf dry matter content (Zhang et al., 2017). Studies on tropical plants have also demonstrated that root respiration rates decrease with increasing root diameter and tissue density (Deng et al., 2024). In Quercus species, specific leaf area declines as whole-leaf vein density (VLA_{all}) increases, reflecting a trade-off between vascular redundancy and leaf area efficiency (Resco de Dios et al., 2021). Collectively, these findings illustrate complex allocation dynamics, where gains in one trait may come at the expense of others related to growth or metabolic function, though causal mechanisms require further investigation. Thus, Pearson's correlation analysis offers valuable insight into the interrelationships among strawberry growth and physiological traits under varying root zone temperatures and growing media. While strong positive correlations reaffirm established links between biomass components and nutrient status, significant negative associations reveal underlying trade-offs that could affect strawberry performance across diverse environmental conditions.

Conclusion

The current study provided essential insights into how root-zone temperature (RZT) and growing medium influence growth, runner production, and energy metabolism in 'Akihime' strawberry plants. We identified that an RZT range of 15–20 °C optimizes overall growth and biomass accumulation, whereas a warmer RZT of 25 °C preferentially stimulates runner production. These findings highlight the importance of tailoring RZT management according to specific production goals, enabling strawberry growers to strategically manipulate RZT to favor either vegetative growth or clonal propagation via runners. Additionally, our results demonstrate the distinct effects of growing media: the peat moss and perlite substrate enhanced shoot growth, likely owing to its favorable physical characteristics and nutrient retention, while water culture promoted root expansion, probably due to improved root zone oxygenation and nutrient availability. Consequently, growers should carefully select their growing medium based on desired growth outcomes. Moreover, our study revealed a significant interaction between RZT and growing medium, underscoring their combined influence on strawberry plant performance and emphasizing the need for integrated management of these factors.

For instance, we observed that strawberry plants grown under 20 °C RZT combined with substrate media exhibited the highest leaf ATP concentrations, demonstrating that aligning the appropriate root-zone temperature with a suitable growing medium can synergistically enhance plant productivity in hydroponic systems. The correlation analysis highlights the delicate balance between vegetative growth, clonal propagation, nutrient status, and physiological performance in strawberries under varying root conditions. Strong positive associations between mother-plant biomass and daughter-plant production underscore the crucial role of carbohydrate allocation in stolon development. Elevated leaf nitrogen and potassium levels emerged as key determinants of biomass accumulation, with nitrogen notably enhancing water-use efficiency and carbon gain through improved photosynthetic capacity. Conversely, negative correlations between the SPAD index and both root length and ATP concentration suggest resource allocation trade-offs, indicating a preferential investment in photosynthesis over root growth under stress conditions. These insights deepen our understanding of resource distribution among growth and physiological processes in strawberries. By identifying these trait interdependencies, this study provides a valuable framework for optimizing productivity in controlled-environment cultivation. Future research should investigate these trade-offs at the molecular level and evaluate their implications across different production systems. Finally, our findings carry important practical implications for hydroponic 'Akihime' strawberry production. The demonstrated superiority of substrate media for maximizing growth and runner productivity indicates its strong potential for commercial application. However, additional studies are necessary to assess the responses of other strawberry genotypes to varying root-zone temperatures and growing media. Exploring the molecular mechanisms underlying the interactive effects of RZT and growing media on strawberry growth and development will be crucial for advancing more efficient and sustainable hydroponic strawberry cultivation.

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Author Contributions

Conceptualization: SR and CI; Methodology: SR, CI, and KP; Software: SSJ; Validation: SR, CI, and KP; Formal analysis: SSJ and NO; Investigation: SSJ; Resources: SR; Data curation: SSJ; Writing—original draft preparation: SSJ; Writing—review and editing: SR, CI, NO, and KP; Visualization: SSJ; Supervision: SR and NO; Project administration: SR; Funding acquisition: SR. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Adak N, Pekmezci M. 2014. Various plant development parameters for strawberries grown in soilless culture. Journal of Food, Agriculture and Environment 12, 692-696.

Ai H, Bellstaedt J, Bartusch KS, Eschen-Lippold L, Babben S, Balcke GU, Tissier A, Hause B, Andersen TG, Delker C, Quint M. 2023. Auxin-dependent regulation of cell division rates governs root thermomorphogenesis. EMBO Journal 42.https://doi.org/10.15252/embj.2022111926.

Al-Raisy FS, Al-Said FA, Al-Rawahi MS, Khan IA, Al-Makhmari SM, Khan MM. 2010. Effects of column sizes and media on yield and fruit quality of strawberry under hydroponic vertical system. European Journal of Scientific Research 43, 48-60.

Ameri A, Tehranifar A, Davarynejad G, Shoor M. 2020. Flowering times and some growth indicators of strawberry were affected by physical properties of the growing media. Scientia Horticulturae 272.https://doi.org/10.1016/j.scienta.2020.109601.

Andriolo JL, Erpen L, Cardoso FL, Cocco C, Casagrande GS, Jänisch DI. 2011. Nitrogen levels in the cultivation of strawberries in soilless culture. Horticultura Brasileira 29, 516-519.https://doi.org/10.1590/S0102-05362011000400012.

Belane AK, Dakora FD. 2011. Photosynthesis, symbiotic N and C accumulation in leaves of 30 nodulated cowpea genotypes grown in the field at Wa in the Guinea savanna of Ghana. Field Crops Research 124, 279-287.https://doi.org/10.1016/j.fcr.2011.07.014.

Blanke MM, Cooke DT. 2004. Effects of flooding and drought on stomatal activity, transpiration, photosynthesis, water potential and water channel activity in strawberry stolons and leaves. Plant Growth Regulation 42, 153-160.https://doi.org/10.1023/B:GROW.0000017489.21970.d4.

Burns KC, Beaumont S. 2009. Scale-dependent trait correlations in a temperate tree community. Austral Ecology 34, 670-677.https://doi.org/10.1111/j.14429993.2009.01973.x.

Choi KY, Choi EY, Kim IS, Lee YB. 2016. Improving water and fertilizer use efficiency during the production of strawberry in coir substrate hydroponics using a FDR sensor-automated irrigation system. Horticulture Environment and Biotechnology 57, 431-439. doi.org/https://doi.org/10.1007/s13580-016-0072-2.

Coetzee ZA, Walker RR, Liao S, Barril C, Deloire AJ, Clarke SJ, Tyerman SD, Rogiers SY. 2019. Expression Patterns of Genes Encoding Sugar and Potassium Transport Proteins Are Simultaneously Upregulated or Downregulated When Carbon and Potassium Availability Is Modified in Shiraz (*Vitis vinifera* L.) Berries. Plant and Cell Physiology 60, 2331-2342.https://doi.org/10.1093/pcp/pcz130.

Cruz LC, Velasco VAV, Villa MS, Luna JR, del Valle JRE. 2023. Strawberry crop (*Fragaria ananassa* Duch.) in hydroponic systems with substrate mixtures. Acta Agronomica 72, 70-77.https://doi.org/10.15446/acag.v72n1.110426.

Deng D, Sun Y, Yang M. 2024. Root Respiration–Trait Relationships Are Influenced by Leaf Habit in Tropical Plants. Forests 15.https://doi.org/10.3390/f15050806.

Du C, Xu R, Zhao X, Liu Y, Zhou X, Zhang W, Zhou X, Hu N, Zhang Y, Sun Z, Wang Z. 2023. Association between host nitrogen absorption and root-associated microbial community in field-grown wheat. Applied Microbiology and Biotechnology 107, 7347-7364.https://doi.org/10.1007/s00253-023-12787-8.

Fan X, Chen X, Jin J, Cai W, Wang Q, Zhao M, Mao X, Ren N. 2024. Regulating strawberry growth by combining root-zone temperature and fertilizer concentration. Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering 40, 109-116.https://doi.org/10.11975/j.issn.1002-6819.202401130.

Fang J, Chen B, Wang F, Li W, Zhang H, Fang J, Liu S, Zheng Z, Guo M, Niu S. 2024a. Nitrogen, phosphorus, and potassium co-limitation in terrestrial ecosystems: A global meta-analysis. Plants People Planet 6, 1329-1340.https://doi.org/10.1002/ppp3.10524.

Fang T, Jin G, Liu Z. 2024b. Isotope-Based Techniques to Investigate Factors Influencing Water Use Efficiency in Pinus koraiensis Leaves during Plant Growth. Plants 13.https://doi.org/10.3390/plants13131771.

Gavrichkova O, Scartazza A, Guidolotti G, Kuzyakov Y, Leonardi L, Mattioni M, Nawrocka J, Pallozzi E, Skwarek M, Tomczyska M, Calfapietra C. 2019. When the Mediterranean becomes harsh: Heat pulses strongly affect C allocation in plant-soil-atmosphere continuum in Eucalyptus camaldulensis. Environmental and Experimental Botany 162, 181-191.https://doi.org/10.1016/j.envexpbot.2019.02.019.

Gonzalez-Fuentes JA, Shackel K, Heinrich Lieth J, Albornoz F, Benavides-Mendoza A, Evans RY. 2016. Diurnal root zone temperature variations affect strawberry water relations, growth, and fruit quality. Scientia Horticulturae 203, 169-177.https://doi.org/10.1016/j.scienta.2016.03.039.

González-Fuentes JA, Shackel KA, Lieth JH, Albornoz F, Benavides-Mendoza A, Evans RY. 2016. Diurnal Root Zone Temperature Variations Affect Strawberry Water Relations, Growth, and Fruit Quality. Scientia Horticulturae.https://doi.org/10.1016/j.scienta.2016.03.039.

Goraya GK, Kaur B, Asthir B, Bala S, Kaur G, Farooq M. 2017. Rapid injuries of high temperature in plants. Journal of Plant Biology 60, 298-305.https://doi.org/10.1007/s12374-016-0365-0.

Guo L, Plunkert M, Luo X, Liu Z. 2021. Developmental regulation of stolon and rhizome. Current Opinion in Plant Biology 59.https://doi.org/10.1016/j.pbi.2020.10.003.

Hayashi S, Levine CP, Yu W, Usui M, Yukawa A, Ohmori Y, Kusano M, Kobayashi M, Nishizawa T, Kurimoto I, Kawabata S, Yamori W. 2024. Raising root zone temperature improves plant productivity and metabolites in hydroponic lettuce production. Frontiers in Plant Science 15.https://doi.org/10.3389/fpls.2024.1352331.

He J, Qin L, Lee SK. 2013. Root-zone CO2 and root-zone temperature effects on photosynthesis and nitrogen metabolism of aeroponically grown lettuce (*Lactuca sativa* L.) in the tropics. Photosynthetica 51, 330-340.https://doi.org/10.1007/s11099-013-0030-5.

He J, Tan LP, Lee SK. 2009. Root-zone temperature effects on photosynthesis, 14C- photoassimilate partitioning and growth of temperate lettuce (*Lactuca sativa* cv. 'Panama') in the tropics. Photosynthetica 47, 95-103.https://doi.org/10.1007/s11099-009-0015-6.

Hong SJ, Yeoung YR, Eum HL. 2018. Phytochemical composition of everbearing strawberries and storage quality of strawberry fruit treated by precooling. Food Sci Biotechnol 27, 1675-1683.https://doi.org/10.1007/s10068-018-0401-6.

Huang H, Zhao X, Xiao Q, Hu W, Wang P, Luo Y, Xia H, Lin L, Lv X, Liang D, Wang J. 2023. Identification of Key Genes Induced by Different Potassium Levels Provides Insight into the Formation of Fruit Quality in Grapes. International Journal of Molecular Sciences 24.https://doi.org/10.3390/ijms24021218.

Huimin R, Hussain J, Wenjie L, Fenyong Y, Junjun G, Youhan K, Shenkui L, Guoning Q. 2021. The expression of constitutively active CPK3 impairs potassium uptake and transport in Arabidopsis under low K+ stress. Cell Calcium 98.https://doi.org/10.1016/j.ceca.2021.102447.

Islam N, Hossain I, Choudhury S. 2023. Impact of Different Shed Houses and Growing Media on Growth, Yield and Quality of Strawberry. Journal of Agricultural Production 4, 30-38.https://doi.org/10.56430/japro.1250832.

Jia Z, Zhang J, Jiang W, Wei M, Zhao L, Li G. 2024. Different nitrogen concentrations affect strawberry seedlings nitrogen form preferences through nitrogen assimilation and metabolic pathways. Sci. Hortic. 332.https://https://doi.org/10.1016/j.scienta.2024.113236.

Jiang N, Yang Z, Zhang H, Xu J, Li C. 2023. Effect of Low Temperature on Photosynthetic Physiological Activity of Different Photoperiod Types of Strawberry Seedlings and Stress Diagnosis. Agronomy 13.https://doi.org/10.3390/agronomy13051321.

Jibia SS, Panjama K, Inkham C, Sato T, Ohtake N, Ruamrungsri S. 2025. Effects of Source on the Nitrogen Uptake, Allocation Patterns, and Performance of Strawberry (*Fragaria* × *ananassa* Duch.): A ^{15}N-Tracer Study. Plants 14, 265. https://doi.org/10.3390/plants14020265.

Jo WJ, Shin JH. 2022a. Effect of root-zone heating using positive temperature coefficient film on growth and quality of strawberry (*Fragaria x ananassa*) in greenhouses. Horticulture, Environment, and Biotechnology 63, 89-100.https://doi.org/10.1007/s13580-021-00384-5.

Jo WJ, Shin JH. 2022b. Effect of root-zone heating using positive temperature coefficient film on growth and quality of strawberry (*Fragaria x ananassa*) in greenhouses. Horticulture Environment and Biotechnology 63, 89-100.https://doi.org/10.1007/s13580-021-00384-5.

Johnson R, Vishwakarma K, Hossen MS, Kumar V, Shackira AM, Puthur JT, Abdi G, Sarraf M, Hasanuzzaman M. 2022. Potassium in plants: Growth regulation, signaling, and environmental stress tolerance. Plant Physiology and Biochemistry 172, 56-69.https://doi.org/10.1016/j.plaphy.2022.01.001.

Kang MJ, Jang SN, Kang IJ, Yang GS, Son YG, Kim JY, Goto E, Son KH. 2025. Root-Zone Cooling Effects on Growth, Physiological, and Biochemical Responses of Taraxacum coreanum Under Hydroponics. Journal of Plant Growth Regulation.https://doi.org/10.1007/s00344-024-11621-3.

Levine CP, Hayashi S, Ohmori Y, Kusano M, Kobayashi M, Nishizawa T, Kurimoto I, Kawabata S, Yamori W. 2023. Controlling root zone temperature improves plant growth and pigments in hydroponic lettuce. Annals of Botany 132, 455-470.https://doi.org/10.1093/aob/mcad127.

Li S, Jiang T, Ahmed W, Yang Y, Yang L, Zhang T, Mei F, Alharbi SA, Shan Q, Guo C, Zhao Z. 2024. Deciphering the impact of nitrogen morphologies distribution on nitrogen and biomass accumulation in tobacco plants. Frontiers in Plant Science 15.https://doi.org/10.3389/fpls.2024.1377364.

Li Y, Hu J, Wei H, Jeong BR. 2020. A long-day photoperiod and 6-benzyladenine promote runner formation through upregulation of soluble sugar content in strawberry. International Journal of Molecular Sciences 21, 1-16.https://doi.org/10.3390/ijms21144917.

Liu H, Jiang Y, Luo Y, Jiang W. 2006. A simple and rapid determination of ATP, ADP and AMP concentrations in pericarp tissue of litchi fruit by high performance liquid chromatography. Food Technology & Biotechnology 44.https://doi.org/hrcak.srce.hr/file/162431.

Madhavi BGK, Khan F, Bhujel A, Jaihuni M, Kim NE, Moon BE, Kim HT. 2021. Influence of different growing media on the growth and development of strawberry plants. Heliyon 7.https://doi.org/10.1016/j.heliyon.2021.e07170.

Malcolm P, Holford P, McGlasson B, Barchia I. 2008. Leaf development, net assimilation and leaf nitrogen concentrations of five Prunus rootstocks in response to root temperature. Scientia Horticulturae 115, 285-291.https://doi.org/10.1016/j.scienta.2007.10.010.

Malcolm P, Holford P, McGlasson B, Conroy J. 2006. Root zone temperature influences growth, partitioning, leaf morphology and physiology of the peach rootstock, Green Leaf Nemaguard. Australian Journal of Experimental Agriculture 46, 689-696.https://doi.org/10.1071/EA05006.

Marcellini M, Raffaelli D, Pergolotti V, Balducci F, Marcellini M, Capocasa F, Mezzetti B, Mazzoni L. 2023. Growth and Yield of Strawberry Cultivars under Low Nitrogen Supply in Italy. Horticulturae 9.https://doi.org/10.3390/horticulturae9111165.

Mizukoshi K, Nishiwaki T, Ohtake N, Minagawa R, Kobayashi K, Ikarashi T, Ohyama T. 1994. Determination of tungstate concentration in plant materials by HNO3-HC1O4 digestion and colorimetric method using thiocyanate. Bulletin of the Faculty of Agriculture-Niigata University (Japan).

Moore CE, Meacham-Hensold K, Lemonnier P, Slattery RA, Benjamin C, Bernacchi CJ, Lawson T, Cavanagh AP. 2021. The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. Journal of Experimental Botany 72, 2822-2844.https://doi.org/10.1093/jxb/erab090.

Renfiyeni R, Andraini H, Iswaldi L. 2020. Growth and yield of Fragaria sp. in mixed and volume of plant media. *IOP Conference Series: Earth and Environmental Science*, Vol. 497: Institute of Physics Publishing.https://doi.org/10.1088/1755-1315/497/1/012007.

Resco de Dios V, Alonso-Forn D, Peguero-Pina JJ, Sancho-Knapik D, Gil-Pelegrín E, Aspinwall MJ, Blackman C, Williams DG, Granda E. 2021. Leaf vein density enhances vascular redundancy instead of carbon uptake at the expense of increasing water leaks in oaks. Environmental and Experimental Botany 188.https://doi.org/10.1016/j.envexpbot.2021.104527.

Sakamoto M, Uenishi M, Miyamoto K, Suzuki T, others. 2016. Effect of root-zone temperature on the growth and fruit quality of hydroponically grown strawberry plants. Journal of Agricultural Science 8, 122–131.https://doi.org/10.5539/jas.v8n5p122.

Sheibanirad A, Haghighi M, Pessarakli M. 2023. The effect of root zone temperature at low nitrogen level of nutrient solution on sweet pepper. Journal of Plant Nutrition 46, 3983-4000.https://doi.org/10.1080/01904167.2023.2220725.

Shi X, Hernandez R, Hoffmann M. 2021. Timing of stolon removal alters daughter plant production and quality in the ever-bearing strawberry 'Albion'. HortScience 56, 650-656.https://doi.org/10.21273/HORTSCI15624-20.

Song W, Loik ME, Cui H, Fan M, Sun W. 2022. Effect of nitrogen addition on leaf photosynthesis and water use efficiency of the dominant species *Leymus chinensis* (Trin.) Tzvelev in a semi-arid meadow steppe. Plant Growth Regulation 98, 91-102.https://doi.org/10.1007/s10725-022-00835-8.

Thakur P, Paul Sharma D, Singh Rana V, Rana N. 2025. Different Rooting Media and Planting Time Influence the Performance of Hardwood Cuttings of Kiwifruit Under Polyhouse Conditions. Applied Fruit Science 67, 76.https://doi.org/10.1007/s10341-025-01297-8.

Toshihiko N, Shinji K, Alice F. 2024. Stone wall strawberries. In: Toshihiko N, Shinji K, Alice F, eds. *Oregon State University Extension Service*, Vol. 2025. Oregon, USA: Oregon State University Extension Service.

Treadwell DD, Hochmuth GJ, Hochmuth RC, Simonne EH, Davis LL, Laughlin WL, Li Y, Olczyk

T, Sprenkel RK, Osborne LS. 2007. Nutrient management in organic greenhouse herb production: where are we now? HortTechnology 17, 461-466.https://doi.org/10.21273/HORTTECH.17.4.461.

Tsialtas JT, Tokatlidis IS, Tsikrikoni C, Lithourgidis AS. 2008. Leaf carbon isotope discrimination, ash content and K relationships with seedcotton yield and lint quality in lines of Gossypium hirsutum L. Field Crops Research 107, 70-77.https://doi.org/10.1016/j.fcr.2007.12.017.

Tulva I, Koolmeister K, Hõrak H. 2024. Low relative air humidity and increased stomatal density independently hamper growth in young Arabidopsis. Plant Journal 119, 2718-2736.https://doi.org/10.1111/tpj.16944.

Vráblová M, Vrábl D, Hronková M, Kubásek J, Šantrůček J. 2017. Stomatal function, density and pattern, and CO_2 assimilation in Arabidopsis thaliana tmm1 and sdd1-1 mutants. Plant Biology 19, 689-701.https://doi.org/10.1111/plb.12577.

Wang D, Gabriel MZ, Legard D, Sjulin T. 2016. Characteristics of growing media mixes and application for open-field production of strawberry (*Fragaria ananassa*). Scientia Horticulturae 198, 294-303.https://doi.org/10.1016/j.scienta.2015.11.023.

Wang S, Song M, Guo J, Huang Y, Zhang F, Xu C, Xiao Y, Zhang L. 2018. The potassium channel FaTPK1 plays a critical role in fruit quality formation in strawberry (*Fragaria* × *ananassa*). Plant Biotechnology Journal 16, 737-748.https://doi.org/10.1111/pbi.12824.

Woznicki T, Kusnierek K, Roos UM, Andersen S, Zimmer K, Sønsteby A. 2021a. Exploration of alternative growing media in strawberry production with focus on wood fiber from Norway spruce. *Acta Horticulturae,* Vol. 1305: International Society for Horticultural Science, 15-21.https://doi.org/10.17660/ActaHortic.2021.1305.3.

Woznicki T, Kusnierek K, Sønsteby A. 2021b. Performance of wood fibre as a substrate in hydroponic strawberry production under different fertigation strategies. Acta Horticulturae 1309, 289-296.https://doi.org/10.17660/ActaHortic.2021.1309.42.

Xia Z, Gong Y, Yang Y, Wu M, Bai J, Zhang S, Lu H. 2024. Effects of root-zone warming, nitrogen supply and their interactions on root-shoot growth, nitrogen uptake and photosynthetic physiological characteristics of maize. Plant Physiology and Biochemistry 214.https://doi.org/10.1016/j.plaphy.2024.108887.

Xu X, Du X, Wang F, Sha J, Chen Q, Tian G, Zhu Z, Ge S, Jiang Y. 2020. Effects of Potassium Levels on Plant Growth, Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple Dwarf Rootstock Seedlings. Frontiers in Plant Science 11.https://doi.org/10.3389/fpls.2020.00904.

Yadav P. 2022. Effect of Different Substrates on Growth Parameters of Zinnia spp. Journal of Agriculture Research and Technology 47, 10-14.

Yadav RK, Analin B, Panda MK, Ranjan A, Singh AP. 2023. Brassinosteroids-regulated nitrogen metabolism fine-tunes growth physiology and low nitrogen response in tomato. Environmental and Experimental Botany 216.https://doi.org/10.1016/j.envexpbot.2023.105528.

Yafuso EJ, Boldt JK. 2024. Development of a Hydroponic Growing Protocol for Vegetative Strawberry Production. HortScience 59, 384-393.https://doi.org/10.21273/HORTSCI17523-23.

Yamori W, Nagai T, Makino A. 2011. The rate-limiting step for CO_2 assimilation at different temperatures is influenced by the leaf nitrogen content in several C3 crop species. Plant, Cell and Environment 34, 764-777.https://doi.org/10.1111/j.1365-3040.2011.02280.x.

Yan T, Wang L, Zhong T, Fu C. 2022. Decadal nitrogen addition increases divergence in intrinsic water-use efficiency between sapling and mature larch plantations. Forest Ecology and Management 523.https://doi.org/10.1016/j.foreco.2022.120494.

Yang H, Li Y, Jin Y, Kan L, Shen C, Malladi A, Nambeesan S, Xu Y, Dong C. 2020. Transcriptome analysis of pyrus betulaefolia seedling root responses to short-term potassium deficiency. International Journal of Molecular Sciences 21, 1-17.https://doi.org/10.3390/ijms21228857.

Yasunaga I. 2008. Root zone aeration improves growth and yields of coir-cultured strawberry (*Fragaria x ananassa* Duch.) during summer. *Acta Horticulturae*, Vol. 779, 251-254. https://doi.org/10.17660/ActaHortic.2008.779.30.

Zhang J, Jiao X, Du Q, Song X, Ding J, Li J. 2021. Effects of Vapor Pressure Deficit and Potassium Supply on Root Morphology, Potassium Uptake, and Biomass Allocation of Tomato Seedlings. Journal of Plant Growth Regulation 40, 509-518.https://doi.org/10.1007/s00344-020-10115-2.

Zhang J, Zuo X, Yang Y, Yue X, Lü P, Zhou X, Lian J, Liu L, He Z. 2017. Response of plant community functional traits in different grasslands to enclosure and grazing in Horqin Sandy Land. Nongye Gongcheng Xuebao/Transactions of the Chinese

Society of Agricultural Engineering 33, 261-268.https://doi.org/10.11975/j.issn.1002-6819.2017.24.034.