HortScience 61(2):444–451. 2026. https://doi.org/10.21273/HORTSCI19113-25

Interactive Effects of Maximum Daytime and Minimum Nighttime Temperatures on Spinach Growth and Physiological Characteristics

Nethone Samba
Faculty of Food and Agricultural Sciences, Fukushima University, Fukushima, 960-1296, Japan

Hisao Akasaka
The United Graduate School of Agricultural Sciences, Iwate University, Iwate, 020-8550, Japan; and Iwate Agricultural Research Center, Kenpoku Agricultural Research Institute, Iwate, 028-6222, Japan

Ken-ichiro Yasuba, Tanjuro Goto, and Minori Hikawa-Endo
Graduate School of Environmental and Life Science, Okayama University, Okayama, 700-8530, Japan

Yoko Miyama
Faculty of Food and Agricultural Sciences, Fukushima University, Fukushima, 960-1296, Japan; and The United Graduate School of Agricultural Sciences, Iwate University, Iwate, 020-8550, Japan

Keywords. photosynthesis, quantum efficiency, stomatal aperture, stomatal conductance, transpiration

Abstract. **High temperatures restrict spinach growth, and the plant's growth and physiological responses to heat remain poorly understood. It remains unclear whether high daytime or elevated nighttime temperatures have a more negative impact on spinach growth. In addition, the interaction effect of maximum daytime and minimum nighttime temperatures on spinach growth remains unknown. This study was conducted to address these issues. Spinach was grown in controlled environments under four temperature treatments: 30 and 20 °C (T30/20), 30 and 25 °C (T30/25), 35 and 20 °C (T35/20), and 35 and 25 °C (T35/25). These treatments represent the maximum daytime temperature and minimum nighttime temperature, respectively, and were maintained for 45 days. Plant growth characteristics were monitored, and the physiological responses to temperature regimes were assessed. The results show that compared with T30/20, dry matter production decreased by 15.4% with increased nighttime temperature (T30/25), decreased by 42.3% with increased daytime temperature (T35/20), and decreased by 57.7% when both daytime and nighttime temperatures were increased (T35/25). However, there was no statistically significant interaction effect (*$P > 0.05$*) between daytime maximum and nighttime minimum temperatures on plant biomass production variables. In comparison with T30/20, the T35/25 treatment increased significantly plant stomatal conductance, stomatal apertures, transpiration rate, and leaf temperature during heat waves. The T35/25 treatment also decreased the quantum efficiency in light compared with the other treatments. Plant biomass production did not improve with the T35/20 and T35/25 treatments, likely as a result of a decoupling of photosynthesis and stomatal conductance during heat waves. Overall, these results reveal that maximum daytime and minimum nighttime temperatures exert additive effects on spinach growth.**

Spinach (*Spinacia oleracea* L.) is a nutritious, green leafy vegetable commonly grown in cold regions or during cool seasons in many countries. It is a rich source of vitamins and minerals, including Fe, Na, K, and Ca (Citak and Sonmez 2009; Qin et al. 2017). In addition, spinach contains a high concentration of lutein (Tai et al. 2020), which has antioxidant properties (Krinsky et al. 2003). A 100-g serving has only 23 kcal and offers essential amino acids, vitamins, and minerals that contribute to a large portion of the recommended daily allowance (US Department of Agriculture 2019). Moreover, fresh or raw spinach contains compounds including (Z)-3-hexenal and methanethiol, which contribute to its distinctive aroma and enhance its appeal to consumers (Masanetz et al. 1998). Many consumers consider spinach a healthy vegetable, and its production has nearly doubled during the past decade (Food and Agriculture Organization of the United Nations 2015). The global market for spinach is valued at $39.6 billion, with ∼34 million t produced worldwide in 2023 (Food and Agriculture Organization of the United Nations 2025). The top spinach-producing countries are China, the United States, Turkey, and Japan (Food and Agriculture Organization of the United Nations 2025). In 2023, Japan ranked as the fourth largest producer of fresh spinach globally, with a total production of 206,800 t across 18,700 ha (∼30.1% in greenhouses) (Ministry of Agriculture, Forestry and Fisheries 2025).

Japanese farmers sow spinach in spring, autumn, and winter, and harvest it about ∼1 month later. During the summer months, spinach is cultivated in colder regions, such as high-altitude areas (Tai et al. 2020). However, the shipping volume of spinach during the summer has decreased because of recent heat waves (Ministry of Agriculture, Forestry and Fisheries 2025). For example, the extreme summer heat events in 2023 and 2024 disrupted the supply of several vegetables to urban areas (Jilatu et al. 2025). Furthermore, in Aug 2025, the price of spinach was ∼1.5 times higher than it was in March (Ministry of Agriculture, Forestry and Fisheries 2025).

In recent years, Japan has experienced an increase in the number of summer days, with daily maximum temperatures exceeding 35 °C (Japan Meteorological Agency 2025). Summer 2023 recorded the highest average temperatures in Japan in the past 126 years (Wong 2023). The average temperature from June to August was 1.76 °C warmer than the 1991 to 2020 average (Sato et al. 2024; Takemura et al. 2024). Notably, northern and eastern Japan faced unprecedented temperature increases of 3.0 and 1.7 °C above normal, respectively. The ambient temperature inside greenhouses during the summer season in Japan can reach up to 50 °C (Tai et al. 2020). In addition, climate change is increasing the average ambient temperatures and the number of warm days and nights—a trend expected to continue in the coming decades (Intergovernmental Panel on Climate Change 2022; Prasad et al. 1999).

High temperatures affect spinach negatively by reducing seed germination and stunting growth, leading to lower yield and quality (Yan et al. 2016). Previous experiments revealed that spinach grows optimally at temperatures between 15 and 25 °C (Isaza et al. 2025). Its seeds can germinate in soil temperatures ranging from 5 to 30 °C, with the greatest germination rates observed at 20 °C. Germination rates drop significantly between 25 and 30 °C (Atherton and Farooque 1983), and germination ceases entirely at 35 °C (Leskovar et al. 1999). Seedling root development requires temperatures > 18.9 °C, whereas shoot growth is limited when temperatures are < 12.3 °C or > 23.3 °C (Wilcox and Pfeiffer 1990). Moreover, when spinach is exposed to heat shock (35–50 °C) for 30 min, carbon dioxide assimilation decreases, and pigment proteins in thylakoid membranes aggregate, which slows the plant's ability to photosynthesize (Tang et al. 2007). Furthermore, the first heat-shock proteins in spinach leaf tissue are induced when the temperature reaches 28 °C, and a full range of heat-shock proteins is produced at 36 °C (Somers et al. 1989). As a result, cultivating spinach during the summer has become increasingly challenging in Japan and many other countries because of recent heat waves.

Nighttime temperatures are rising faster than daytime ones, reducing diurnal temperature differences (Davy et al. 2017; Vose et al. 2005).

Different crops respond uniquely to high daytime and high nighttime temperatures (Djanaguiraman et al. 2013; Gibson and Mullen 1996; Lobell et al. 2005). For example, soybeans showed comparable yield reductions under high daytime (39/20 °C) and nighttime temperatures (30/29 °C) (Djanaguiraman et al. 2013). In rice, high daytime temperatures had a more negative impact on seed set and grain weight than high nighttime temperatures alone (Shi et al. 2017). In a heat-susceptible wheat cultivar (*Triticum aestivum* L.), yield reductions were similar for high daytime (35/15 °C) and nighttime temperatures (25/24 °C), but were greater when both were elevated (35/24 °C) (Narayanan et al. 2015). Lettuce exhibited its most rapid growth in a controlled environment with day/night temperatures of 25/25 °C, respectively (Knight and Mitchell 1983). In addition, there was no significant interaction between daytime and nighttime temperatures affecting its growth parameters (Hicklenton and Wolynetz 1987). Hong et al. (2018) recommended using daytime/nighttime temperatures of 20/10 °C, respectively to achieve better yield and quality of violet rape (*Brassica campestris* ssp. *chinensis* L.), rather than higher temperature combinations such as 30/20 °C or 30/15 °C. Hu et al. (2004) reported that the quality of spinach could be improved by using night-cooling greenhouses during the summer. However, most studies investigating the effects of daytime and nighttime temperatures on spinach (Leskovar et al. 1999; Yamori et al. 2005) have not highlighted the distinct effects of high daytime temperatures compared with elevated nighttime temperatures or the combined effects of both. In addition, studies focusing on the effect of high daytime temperatures (Tai et al. 2020; Tang et al. 2007; Yoneda et al. 2022) often lack detailed information on spinach physiological responses throughout the day.

Highlighting the growth and physiological traits of spinach under high daytime and nighttime temperatures is necessary to determine whether one has a greater impact on reducing biomass production. Furthermore, understanding how lowering daytime and nighttime temperatures influences spinach growth is crucial for developing technology to control greenhouse temperatures and sustain spinach production during summer. We hypothesized that 1) spinach growth and biomass production would be affected differently by increased daytime and nighttime temperatures, with the combination of both having the most significant negative impact; and 2) the physiological responses would vary based on temperature treatments and the time of the day. Therefore, the objectives of our study were 1) to determine whether increased daytime or increased nighttime temperatures affect spinach biomass production more negatively, 2) to examine whether there is an interaction effect of maximum daytime and minimum nighttime temperatures on plant growth, and 3) to analyze the physiological responses of plants under different temperature conditions. The experiment set maximum temperatures at 35 and 30 °C, whereas minimum temperatures were set at 25 and 20 °C, covering all possible combinations of these settings.

Materials and Methods

Plant cultivation methods. The experiment was conducted using phytotrons at Fukushima University from 23 Jun to 7 Aug 2025. The inner volume of each phytotron (Nippon Medical Machinery Manufacturing Co, Ltd, Tokyo, Japan) was ~7.2 m^3 (length, 1.9 m; width, 1.9 m; height, 2 m). Spinach (*Spinacia oleracea* L., cv. Justice) seeds (Sakata Seed Corp, Yokohama, Japan) were sown (15 seeds per pot) in Wagner pots (Mizuken Co., Ltd., Sakai, Japan; depth, 20 cm; diameter, 16 cm) filled with commercial substrate (Nae Shokunin 130; Kaneko Seed Co, Ltd, Maebashi, Japan) on 23 Jun and were thinned to five plants per pot 7 d after seeding (DAS). The pots were watered manually with a nutrient solution (electrical conductivity = 0.8 $dS{\cdot}m^{-1}$) prepared according to the Otsuka-A formula (OAT Agrio Co, Ltd, Tokyo, Japan), containing 23 ppm NH_4-N, 233 ppm NO_3-N, 120 ppm P_2O_5, 405 ppm K_2O, 230 ppm CaO, 60 ppm MgO, 1.5 ppm MnO, 1.5 ppm B_2O_3, 2.7 ppm Fe, 0.03 ppm Cu, 0.09 ppm Zn, and 0.03 ppm Mo. The water retention of the substrate, maintained at < 20 kPa, and the temperature of the substrate at a depth of 10 cm were monitored using a power factor meter (Daiki Ltd, Tokyo, Japan) and a thermo recorder (model TR-50U2; T&D Corp, Tokyo, Japan), respectively. The environmental conditions, including temperature and relative humidity, in each phytotron were set to meet the requirements of each treatment.

Experimental treatments. Spinach plants were submitted to four temperature regimes to assess their growth characteristics and physiological responses. The temperature treatments for day and night were set at 30/20 °C (T30/20) (control treatment), 30/25 °C (T30/25), 35/20 °C (T35/20), and 35/25 °C (T35/25). These temperatures represent the maximum daytime/minimum nighttime values. Temperature treatments were set for 12 h during the day starting at 7:00 AM and 12 h at night starting at 7:00 PM. The temperature shift between day and night was carefully controlled to prevent sudden changes, promoting more natural plant growth. In each phytotron, spinach was grown in five pots, with each pot containing five plants exposed to sunlight. The photosynthetic photon flux density (PPFD) from natural light reaching inside the phytotrons was recorded every minute from sunrise to sunset.

A schematic diagram of the temperature treatments is presented in Fig. 1.

Plant growth and biomass evaluation. The number of seedlings that emerged was counted daily, starting 1 DAS and continuing for 7 d. The seedling emergence rate was calculated as the ratio of the number of seedlings that emerged to the number of seeds sown. Starting 2 weeks after seeding, the number of leaves per plant and the plant height were evaluated every week. In addition, the soil plant analysis development (SPAD) value was recorded on the largest leaves weekly beginning 3 weeks after seeding using a chlorophyll meter (SPAD-502 Plus; Konica Minolta Inc, Tokyo, Japan). At the end of the experiment, five plants per treatment were selected and their leaves were detached for further analysis. The lamina length, lamina width, and petiole length of the five largest leaves were measured using a ruler. The leaf area was estimated using ImageJ software (ImageJ, ver. 1.54d; National Institutes of Health, Bethesda, MD, USA) according to the method described by Martin et al. (2021). Each plant leaf was detached and placed against a high-contrast background, with a ruler included for scale calibration. A digital camera (iPhone SE; Apple, Cupertino, CA, USA) was positioned ~50 cm above to capture the images. These images were saved in jpeg format and imported into ImageJ, where they were converted to 8-bit grayscale, and contrast was adjusted for better clarity. A reference line on the ruler established a measurement scale, allowing ImageJ to report the leaf area in square centimeters.

At harvest, 45 DAS, fresh weight of the shoots was measured immediately. The harvested shoots were dried in a forced-air oven

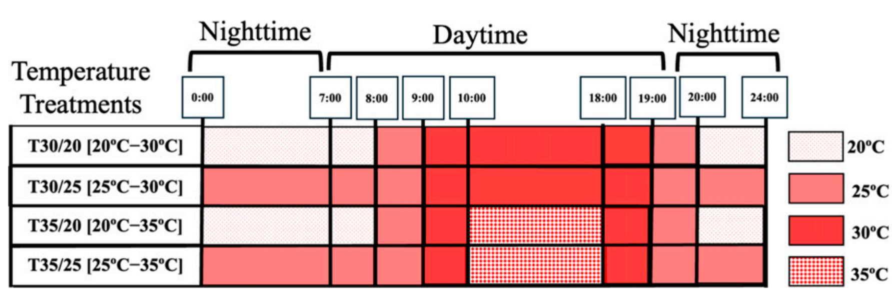

Fig. 1. Schematic diagram of the temperature treatments for 24 h.

Received for publication 2 Nov 2025. Accepted for publication 24 Dec 2025.
Published online 26 Jan 2026.
Part of this research was supported by the Fukushima Institute for Research, Education and Innovation under research grant JPFR25020104.
Data will be made available on request.
The authors declare that they have no conflicts of interest to disclose.
N.S.: writing the original draft and investigation; H.A.: investigation; Y.K.: investigation; T.G.: investigation; M.H.-E.: investigation; Y.M.: validation, supervision, investigation, and conceptualization.
Y.M. is the corresponding author. E-mail: y_miyama@agri.fukushima-u.ac.jp.

at 70 °C for 72 h to determine their dry weight.

Evaluation of plant physiology. Plant physiological responses to temperature treatments were evaluated 29 and 30 DAS. Data were collected from the largest leaf of each pot at 2 h intervals from 8:00 AM to 6:00 PM, to monitor the plant's dynamic responses more closely and to identify temporal patterns and stress events more accurately. The LI-600 porometer/fluorometer (LI-COR Biosciences, Lincoln, NE, USA) was used to measure stomatal conductance, leaf temperature, transpiration rate, and quantum efficiency in light. The quantum efficiency in light was derived from chlorophyll fluorescence parameters that were measured directly from light-adapted leaves using the following formula:

$$\text{Quantum efficiency in light} = \frac{(\text{Maximum fluorescence in light} - \text{Minimum fluorescence in light})}{\text{Maximum fluorescence in light}}.$$

In addition, a stomata scope (model HQ-1010; Happy Quality Co, Ltd, Hamamatsu, Japan) was used to assess stomatal aperture.

Data analysis. The collected data were tested for normality using the Shapiro-Wilk test before undergoing analysis through a two-way analysis of variance with a general linear model procedure. To differentiate the means for each significant variable measured, Tukey's multiple comparison tests were used at a significance level of $P < 0.05$. The statistical analysis was performed using XLSTAT software (ver. 2024.4.2; XLSTAT statistical and data analysis solution, Paris, France).

Results

Changes in environmental parameters. During the experiment, the relative humidity in each phytotron was maintained at ~60% ± 10% from 8:00 AM to 7:00 PM and at 70% ± 10% from 7:00 PM to 8:00 AM. On average, each treatment received a PPFD of 283.6 ± 132.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ every day, with the daily PPFD ranging from a minimum of 39.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to a maximum of 467.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 2B).

In phytotrons with daytime temperatures of 35 °C, daytime average root-zone temperatures were 30.6 °C with T35/25 and 29.1 °C with T35/20. In contrast, at 30 °C, they measured 26.7 °C with T30/20 and 27.7 °C with T30/25. At night, with temperatures of 25 °C, root-zone temperatures were 25.0 °C with T30/25 and 24.8 °C with T35/25, whereas at 20 °C, they were 20.3 °C with T30/20 and 20.1 °C with T35/20. Figure 2 shows the changes in the root-zone temperature throughout the day in the four treatments (Fig. 2A) and the average daily PPFD received in each phytotron (Fig. 2B). PPFD data were recorded every minute. Root-zone temperature was measured at a depth of 10 cm and recorded every 10 min.

Seedling emergence rate. At 7 DAS, the seedling emergence rate showed no significant differences among the treatments. However, we noticed that seedlings emerged more quickly with T30/25 and T35/25. By 4 DAS, emergence rates reached 49.3% with T35/25 and 54.7% with T30/25, compared with 41.3% with T35/20 and 36% with T30/20 (Table 1).

Plant growth and biomass production. The plant height varied by treatment. After 6 weeks, the tallest plants were seen with T30/20, followed by T30/25, T35/20, and T35/25, with T35/25 showing a 41.6% height reduction compared with T30/20 (Fig. 3A). No significant height differences were observed between T30/25 and T35/20. The number of leaves was significantly greater with T30/20 (Fig. 3B). However, the SPAD values were greater with T35/25 and T35/20, and the lowest was with T30/20 (Fig. 3C).

The temperature treatments had a significant effect on plant biomass production and the growth characteristics of leaf petioles. T35/25 and T35/20 decreased the fresh weight by 35.2% and 23.3%, respectively, in comparison with T30/20 (Table 2). Increasing the nighttime temperature to 25 °C in T30/25 did not affect fresh weight significantly; however, it reduced dry matter production significantly, by 15.4% than that of T30/20. However, lowering the nighttime temperature to 20 °C in the T35/20 treatment increased fresh matter production by 18.4% and dry matter production by 36.4% compare with T35/25.

The petiole length of mature leaves was reduced significantly by high-temperature treatments, at night and during the day. In addition, the leaf area was also diminished as a result of the high-temperature treatments. No significant interaction effect of daytime maximum temperatures × nighttime minimum temperatures was observed on the evaluated variables.

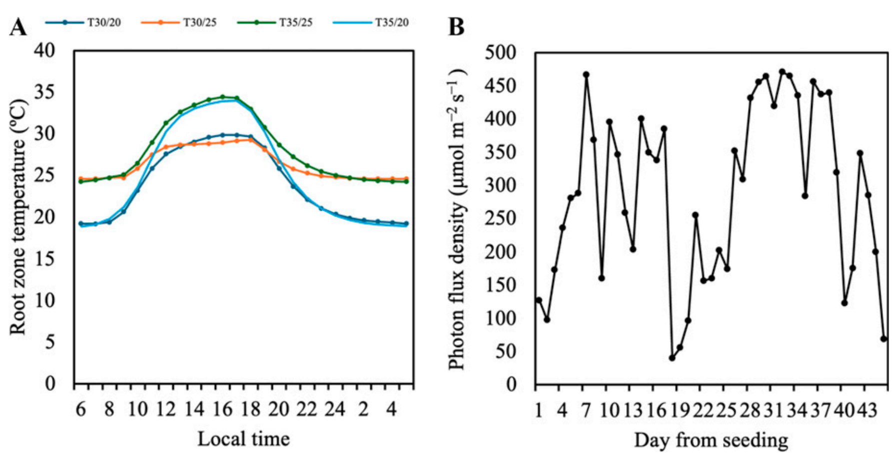

Fig. 2. Average root zone temperature (**A**) and average daily photosynthetic photon flux density in the phytotrons (**B**). T30/20 = 30 and 20 °C daytime/nighttime temperature, respectively; T30/25 = 30 and 25 °C daytime/nighttime temperature, respectively; T35/25 = 35 and 25 °C daytime/nighttime temperature, respectively; T35/20 = 35 and 20 °C daytime/nighttime temperature, respectively.

Table 1. Seedling emergence rate from 0 to 7 d after seeding (DAS).[i]

	Seedling emergence rate (%)				
Treatment	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
T30/20	12.0 ± 11.9 ab[ii]	36.0 ± 3.7 a	61.3 ± 11.9 a	66.7 ± 12.5 a	66.7 ± 12.5 a
T30/25	25.3 ± 11.9 b	54.7 ± 5.6 b	72.0 ± 7.3 a	77.3 ± 6.0 a	78.7 ± 5.6 a
T35/25	22.7 ± 12.1 ab	49.3 ± 18.0 ab	64.0 ± 19.3 a	65.3 ± 19.7 a	68.0 ± 20.8 a
T35/20	5.3 ± 5.6 a	41.3 ± 7.3 ab	60.0 ± 10.5 a	65.3 ± 11.0 a	65.3 ± 11.0 a
Tmax	NS	NS	NS	NS	NS
Tmin	*	*	NS	NS	NS
Tmax × Tmin	NS	NS	NS	NS	NS

[i] Data are presented as the mean values of five replications.

[ii] Different letters within a date indicate significant differences at $P < 0.05$ by Tukey's test. Treatment effects were significant at the 5% probability level or were not significant.

NS, * Nonsignificant or significant at $P = 0.05$, respectively.

T30/20 = 30 and 20 °C daytime/nighttime temperature, respectively; T30/25 = 30 and 25 °C daytime/nighttime temperature, respectively; T35/25 = 35 and 25 °C daytime/nighttime temperature, respectively; T35/20 = 35 and 20 °C daytime/nighttime temperature, respectively; Tmax = maximum temperature; Tmin = minimum temperature.

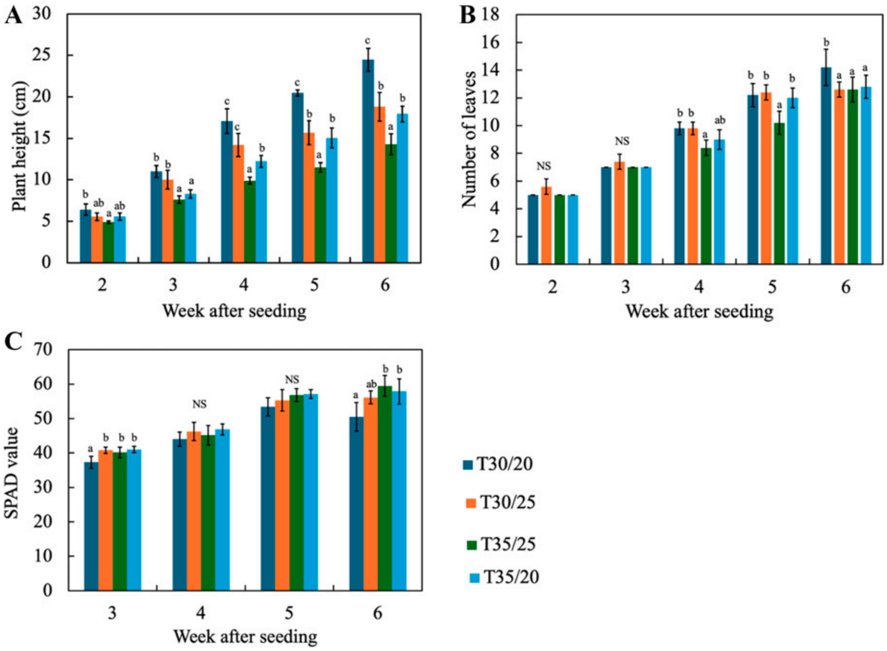

Fig. 3. Changes in plant height (**A**), leaf number (**B**) and leaf soil plant analysis development (SPAD) value (**C**). Data represent the means of five selected plants. Error bars indicate the standard deviation. Different letters within a week indicate significant differences at $P < 0.05$ by Tukey's test. T30/20 = 30 and 20 °C daytime/nighttime temperature, respectively; T30/25 = 30 and 25 °C daytime/nighttime temperature, respectively; T35/25 = 35 and 25 °C daytime/nighttime temperature, respectively; T35/20 = 35 and 20 °C daytime/nighttime temperature, respectively. NS = Nonsignificant.

Plant physiological responses to different temperature treatments. The average stomatal aperture showed significant differences between treatments at 2:00 PM and 4:00 PM. However, for the rest of the day, there were no significant differences among the treatments (Fig. 4A). The largest average stomatal apertures were recorded with T35/25 and T35/20 at 2:00 PM and 4:00 PM. In addition, stomatal conductance and transpiration rate were significantly greater with T30/25 and T35/25 at 8:00 AM and 10:00 AM (Fig. 4B and 4C). After 10:00 AM, these values showed no significant differences until 4:00 PM, when T35/25 exhibited greater levels of stomatal conductance and transpiration rate. By 6:00 PM, no significant differences were observed among the treatments. Moreover, the quantum efficiency in light was reduced significantly with T35/25 at 4:00 PM (Fig. 4D). At other times of the day, no significant differences were observed among the treatments for quantum efficiency. Last, leaf temperature did not differ significantly between treatments at 8:00 AM and 6:00 PM; however, it was significantly higher with T35/25 and T35/20 at other times of the day than with T30/20 and T30/25 (Fig. 4E).

Discussion

Spinach seedling emergence rate under heat stress. Seedlings at 7 DAS exhibited similar final emergence rates across treatments; however, the speed of emergence differed markedly. Higher nighttime temperatures accelerated seedling emergence (Table 1). This contrasts with earlier reports indicating reduced spinach germination at > 25 °C (Chitwood et al. 2016; Yan et al. 2016). The discrepancy may be explained by genotypic differences, because spinach cultivars are known to respond differently to temperature regimes during germination (Chitwood et al. 2016). Moreover, the diurnal temperature fluctuations provided by the phytotrons may have contributed to improved seedling emergence, consistent with observations made by Liu et al. (2013). Similarly, Leskovar et al. (1999) found that alternating temperature regimes (25/15 °C, 30/15 °C, and 35/20 °C) enhanced spinach germination relative to a constant 30 °C. These findings suggest that temperature variation rather than absolute temperature likely promote the spinach seedling emergence rate under elevated temperature conditions.

Growth and biomass production of spinach under heat stress. Results regarding plant height, leaf number, and leaf area show that increasing temperatures, whether during the day, at night, or both, affect spinach shoot growth negatively. This finding aligns with several previous studies that have reported the detrimental impact of high temperatures on plant growth (Haghighi et al. 2014). However, it contradicts the findings of Ohtaka et al. (2020), who reported that a daytime/nighttime temperature of 30/25 °C increased tomato stem length and thickness compared with a control treatment of 25/20 °C. In addition, other studies have argued that a positive difference between daytime and nighttime temperatures (daytime temperature > nighttime temperature) leads to an increase in plant height, internodal length, and the number of leaves (Fraszczak 2012; Inthichack et al. 2013; Pollet et al. 2009). Our results indicate that the positive difference between daytime and nighttime temperatures (T30/25, T35/20, T35/25) did not enhance plant height, leaf number, or leaf area because the plants were under stress.

Dry matter production decreased by 15.4% in response to an increased nighttime temperature (T30/25), whereas it decreased by 42.3% in response to an increased daytime temperature (T35/20) and by 57.7% in response to combined increased daytime and nighttime temperatures (T35/25) (Table 2). These results suggest that spinach biomass production was affected more adversely by the combination of high daytime and nighttime temperatures than by high daytime temperatures alone or high nighttime temperatures alone. Similar to our findings, Uz Zaman et al. (2022) reported reductions in spinach biomass production

Table 2. Effect of temperature treatments on shoot biomass production and leaf growth characteristics.[i]

Treatment	Shoot FW/plant (g)	Shoot DW/plant (g)	Lamina length (cm)	Lamina width (cm)	Petiole length (cm)	Total leaf area/plant (cm^2)
T30/20	19.3 ± 1.6 c[ii]	2.6 ± 0.1 d	10.3 ± 0.8 a	8.5 ± 1.2 a	8.8 ± 1.1 c	438.8 ± 126.9 b
T30/25	17.6 ± 0.5 c	2.2 ± 0.1 c	10.5 ± 0.8 a	8.1 ± 1.3 a	7.2 ± 1.0 b	368.4 ± 57.1 ab
T35/25	12.5 ± 0.4 a	1.1 ± 0.1 a	9.5 ± 1.4 a	7.3 ± 0.6 a	5.2 ± 0.3 a	232.7 ± 67.2 a
T35/20	14.8 ± 1.0 b	1.5 ± 0.2 b	11.3 ± 1.2 a	7.3 ± 1.2 a	6.1 ± 0.7 ab	279.0 ± 91.7 ab
Tmax	***	***	NS	NS	***	**
Tmin	***	***	*	NS	**	*
Tmax × Tmin	NS	NS	NS	NS	NS	NS

[i] Data are presented as mean values ± the standard deviation of five replications.

[ii] Different letters within a column indicate significant differences at $P < 0.05$ by Tukey's test.

NS, *, **, *** Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively.

DW = dry weight; FW = fresh weight; T30/20 = 30 and 20 °C daytime/nighttime temperature, respectively; T30/25 = 30 and 25 °C daytime/nighttime temperature, respectively; T35/25 = 35 and 25 °C daytime/nighttime temperature, respectively; T35/20 = 35 and 20 °C daytime/nighttime temperature, respectively; Tmax = maximum temperature; Tmin = minimum temperature.

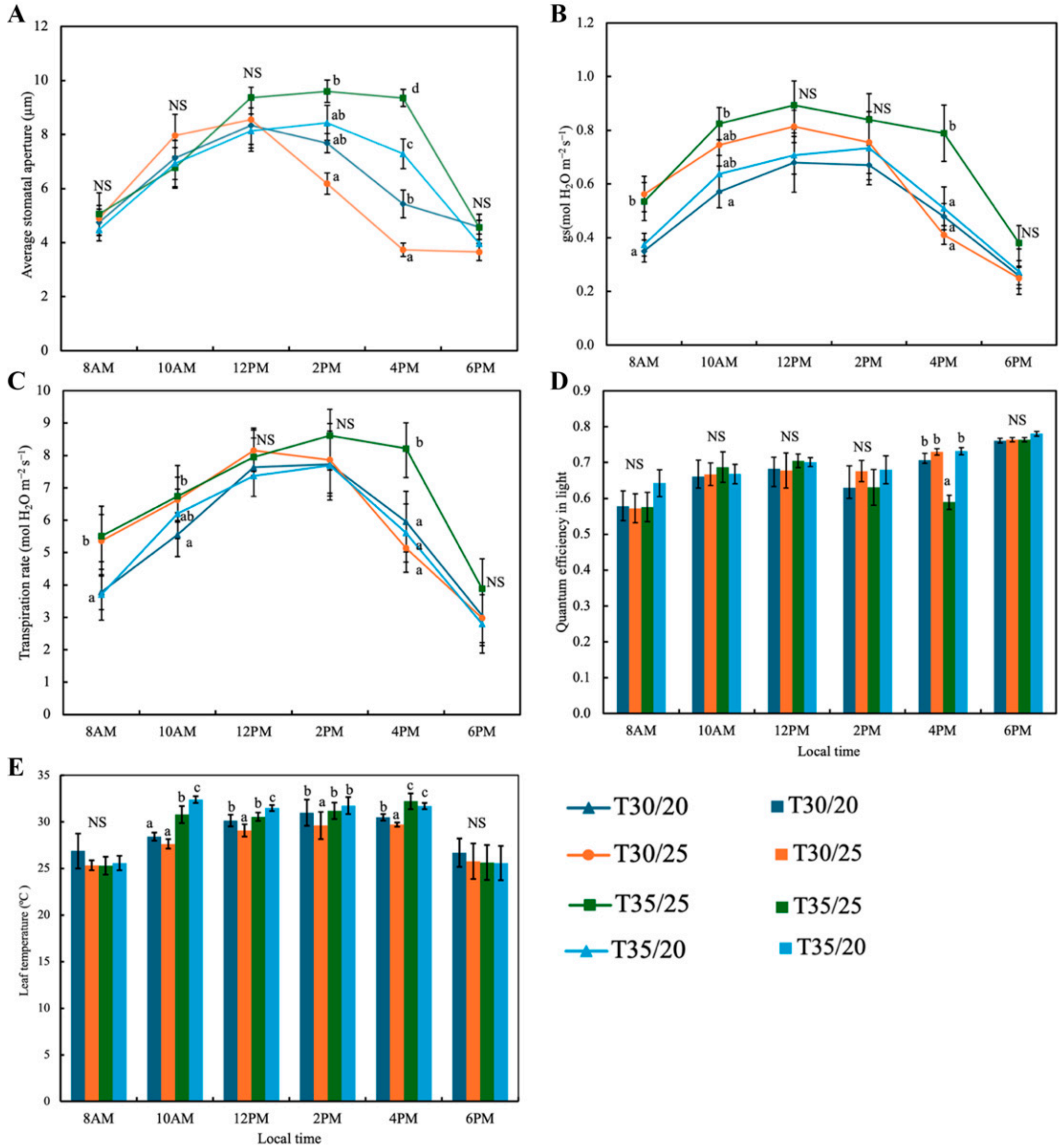

Fig. 4. Plant physiological response to different temperature treatments. (**A**) Average stomatal aperture. (**B**) Stomatal conductance (gs). (**C**) Transpiration rate. (**D**) Quantum light efficiency. (**E**) Leaf temperature. Data represent the means of five selected plants. Error bars indicate the standard deviation. Different letters within a week indicate significant differences at $P < 0.05$ by Tukey's test. T30/20 = 30 and 20 °C daytime/nighttime temperature, respectively; T30/25 = 30 and 25 °C daytime/nighttime temperature, respectively; T35/25 = 35 and 25 °C daytime/nighttime temperature, respectively; T35/20 = 35 and 20 °C daytime/nighttime temperature, respectively. NS = Nonsignificant.

in response to elevated daytime/nighttime temperatures (35/25 °C). The results of our study suggest that merely lowering the daytime temperature, as recommended previously by Tai et al. (2020) and Yoneda et al. (2022), will not allow the spinach plants to reach their full production potential. To maximize spinach biomass production, it is essential to lower temperatures during both the day and the night to optimal levels. In addition, we observed no interaction effect between the maximum temperatures during the day and the minimum temperatures at night. This finding is significant, because it differs from the interactions observed in several other crops (Narayanan et al. 2015; Parkash et al. 2024). It should be considered when developing technologies for controlling spinach production in protected cultivation.

Spinach physiological responses to heat stress. At the end of the experiment, the SPAD values were significantly greater with T35/25, T35/20, and T30/25 compared with T30/20. This finding indicates that chlorophyll content in the leaves increased significantly with rising temperatures. In a previous study, Zhao et al. (2022) found that the chlorophyll a + b content increased under heat stress (35/25 °C) in lettuce leaves. The reasons for increased leaf chlorophyll content under heat stress are diverse. Some researchers reported that it is the result of water loss from the leaves, whereas others suggest that plants dissipate excess photosynthetic energy by increasing the chlorophyll a + b content under heat stress (Dong et al. 2013). It is also speculated that an increased transpiration rate under heat stress facilitates the uptake of certain nutrients, such as NO_3^- (Frantz et al. 2004). In our experiment, the differences in transpiration rates observed across various temperature treatments (Fig. 4C) may have affected nutrient uptake, leading to variations in SPAD values. Higher temperature treatments could have enhanced nutrient absorption as a result of increased transpiration rates associated with open stomata (Inthichack et al. 2012).

Stomata regulate gas exchange in leaves by controlling CO_2 uptake, water loss, and evaporative cooling. Plant leaves adjust their stomatal aperture continuously based on external environmental cues, water status (Lin et al. 2015), and internal signals such as hormones (Haworth et al. 2018), ensuring an appropriate balance. In our experiment, the stomatal aperture was adjusted differently in

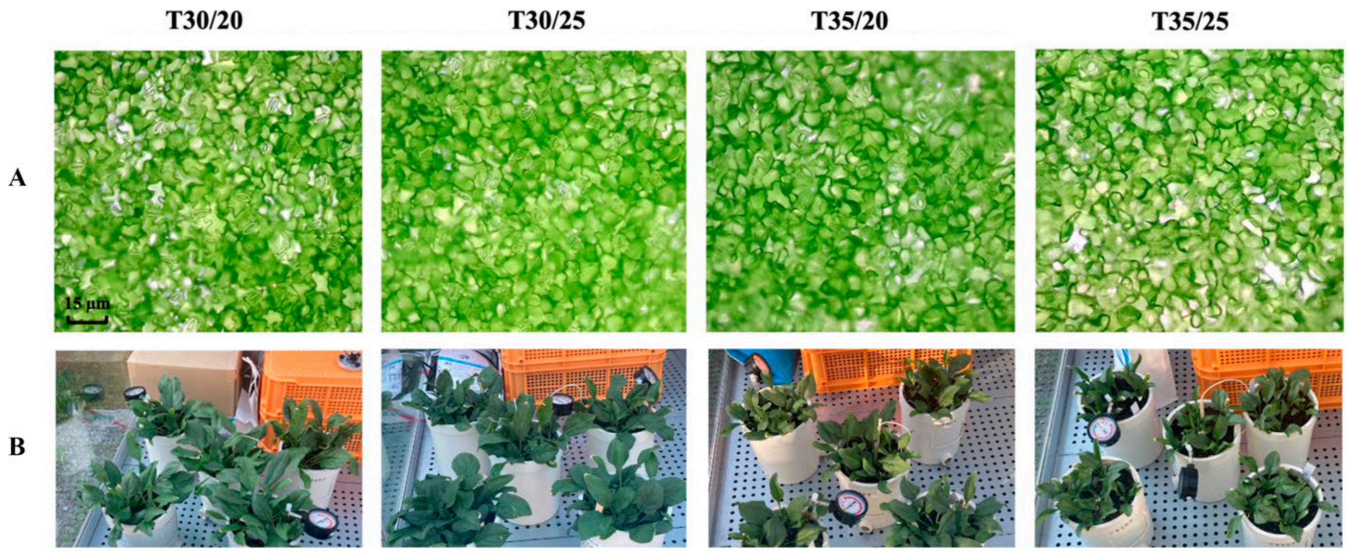

Fig. 5. Stomatal opening at 4 PM with different temperature treatments (**A**) and plants at 30 d after seeding (**B**).

response to temperature variations across different treatments. The higher nighttime temperature treatments (T30/25 and T35/25) resulted in increased stomatal conductance and transpiration rates during the early morning hours (8:00 AM and 10:00 AM). A similar finding was reported by Pinto et al. (2025) in a study on wheat crops, in which genotypes exposed to high nighttime temperatures exhibited greater stomatal conductance at 9:00 AM and 10:00 AM. Pinto et al. (2025) suggest that the circadian clock, which regulates metabolic processes and prepares the plant for predictable events such as dawn, may explain the effect of elevated nighttime temperatures on stomatal conductance in the morning.

A daytime temperature of 35 °C, particularly when combined with a warm night (T35/25), resulted in the largest stomatal apertures in the afternoon (2:00 PM and 4:00 PM) (Fig. 5). This led to increased stomatal conductance and transpiration rates at 4:00 PM with T35/25. The relationship between increased stomatal aperture, stomatal conductance, transpiration rate, and warmer air temperature has been documented in various species, including bean, corn, wheat, sunflower (Hofstra and Hesketh 1969), soybean, and tobacco (*Nicotiana tabacum*) (Sinha et al. 2022). High temperatures increase stomatal opening, conductance, and transpiration rates in spinach plants, allowing them to survive heat waves through evaporative cooling (Urban et al. 2017). As temperatures rise, water viscosity decreases and mesophyll conductance improves, enhancing water supply to evaporation sites. This raises turgor pressure in guard cells, expanding the stomatal aperture (Cochard et al. 2000; Von Caemmerer and Evans 2015).

Although stomata were more open and transpiration was greater with T35/25, the quantum efficiency of photosystem II (PSII) declined in the late afternoon (4:00 PM) with that treatment (Fig. 4D). This finding confirms that high-temperature stress affects the functionality of the photosynthetic machinery negatively (Mathur et al. 2014). The reduced quantum efficiency of PSII with T35/25 at 4:00 PM may be attributed to the high nighttime temperature of 25 °C. This elevated temperature likely increased respiratory C losses, resulting in a depletion of carbohydrate reserves needed for the following day's photosynthesis (Abbas et al. 2024). In addition, the limited availability of photosynthetic sinks may have interrupted or damaged the light reactions (light harvesting, electron transport, and photochemical processes) under the conditions of high irradiance and temperature in the afternoon (Abbas et al. 2024).

Physiological mechanisms underlying spinach dry matter reduction under heat stress. Spinach grown with T35/25 showed significantly greater stomatal conductance but recorded the lowest dry matter production. Previous studies (Leuning 1995; Mirfenderesgi et al. 2016; Verhoef and Egea 2014; Xu et al. 2016) have associated high stomatal conductance with increased photosynthesis rates and, consequently, greater dry matter production. This contradicts our findings. The discrepancy may be attributed to the elevated temperatures of our experiment, which could decouple net photosynthesis from stomatal conductance, leading to their independence. This phenomenon has been observed by Urban et al. (2017) in poplar (*Populus deltoides* × *nigra*) and loblolly pine (*Pinus taeda*), as well as by Bisbis et al. (2023) in tomato during heat waves. According to Bisbis et al. (2023), at high temperatures, tissue cooling may be more important for stress management than limiting transpiration. Consequently, stomatal conductance decouples from photosynthesis, allowing plants to mitigate stress-related damage temporarily, as long as water uptake and intact xylem vessels are maintained.

Reduced biomass production resulting from the increased daytime temperature with T35/20 can be attributed to the disruption of the plant's photosynthetic machinery, which is affected negatively by high temperatures, as reported by several authors (Ashraf and Harris 2013; Centritto et al. 2011). Conversely, the decreased biomass production resulting from an increased nighttime temperature with T30/25 can be linked to heightened night respiration, which degrades the carbohydrates stored during the day (Mohammed and Tarpley 2009; Peng et al. 2013; Wen et al. 2018). The combination of elevated daytime and nighttime temperatures with T35/25 further increased night respiration while simultaneously impacting the photosynthetic machinery during the day, leading to a significant biomass loss (Deryabin and Popov 2024; Narayanan et al. 2015).

Conclusion

We examined how different temperature conditions affect the growth and physiological responses of spinach. Our findings reveal that alternating temperatures throughout the day enhanced germination and seedling emergence rates. However, the temperature treatments of T30/25, T35/20, and T35/25 reduced biomass production in spinach. This indicates that high daytime and high nighttime temperatures affect spinach growth negatively, with high daytime temperatures having a more detrimental impact. Furthermore, our research showed that daytime and nighttime temperatures influence spinach growth independently. During the day, heat waves disrupt the relationship between stomatal conductance and photosynthesis, resulting in reduced carbohydrate storage. At night, elevated temperatures increase respiration rates, leading to C loss.

References Cited

Abbas A, Rossi S, Huang B. 2024. Plant metabolic responses and adaptation mechanisms to elevated night temperature associated with global warming. Grass Res. 4:e015. https://doi.org/10.48130/grares-0024-0013.

Ashraf M, Harris PJ. 2013. Photosynthesis under stressful environments: An overview.

Photosynthetica. 51(2):163–190. https://doi.org/10.1007/s11099-013-0021-6.
Atherton JG, Farooque AM. 1983. High temperature and germination in spinach: I. The role of the pericarp. Sci Hortic. 19(1–2):25–32. https://doi.org/10.1016/0304-4238(83)90040-7.
Bisbis M, Cammarisano L, Heuvelink E, Marcelis LFM, Körner O. 2023. Modelling stomatal responses to high temperatures under a range of different vapor pressure deficits. Acta Hortic. 1382:171–178. https://doi.org/10.17660/ActaHortic.2023.1382.22.
Centritto M, Brilli F, Fodale R, Loreto F. 2011. Different sensitivity of isoprene emission, respiration and photosynthesis to high growth temperature coupled with drought stress in black poplar (*Populus nigra*) saplings. Tree Physiol. 31(3):275–286. https://doi.org/10.1093/treephys/tpq112.
Chitwood J, Shi A, Evans M, Rom C, Gbur EE, Motes D, Chen P, Hensley D. 2016. Effect of temperature on seed germination in spinach (*Spinacia oleracea*). HortScience. 51(12):1475–1478. https://doi.org/10.21273/HORTSCI11414-16.
Citak S, Sonmez S. 2009. Mineral contents of organically and conventionally grown spinach (*Spinacia oleracea* L.) during two successive seasons. J Agric Food Chem. 57(17):7892–7898. https://doi.org/10.1021/jf900660k.
Cochard H, Martin R, Gross P, Bogeat-Triboulot MB. 2000. Temperature effects on hydraulic conductance and water relations of *Quercus robur* L. J Exp Bot. 51(348):1255–1259. https://doi.org/10.1093/jexbot/51.348.1255.
Davy R, Esau I, Chernokulsky A, Outten S, Zilitinkevich S. 2017. Diurnal asymmetry to the observed global warming. Int J Climatol. 37(1):79–93. https://doi.org/10.1002/joc.4688.
Deryabin A, Popov V. 2024. Morphophysiological and biochemical responses of *Amaranthus caudatus* to elevated cultivation temperatures. J Plant Growth Regul. 43(7):2197–2208. https://doi.org/10.1007/s00344-024-11255-5.
Djanaguiraman M, Prasad PV, Schapaugh WT. 2013. High day- or nighttime temperature alters leaf assimilation, reproductive success, and phosphatidic acid of pollen grain in soybean [*Glycine max* (L.) Merr.]. Crop Sci. 53(4):1594–1604. https://doi.org/10.2135/cropsci2012.07.0441.
Dong Y, Tian K, Guo XH, Xiao DR, Guo XL. 2013. Impacts of regional climate change on chlorophyll fluorescence characteristics of plateau wetland plants in Napahai, Yunnan China. Ecol Environ Sci. 22:588–594. (in Chinese with an English abstract).
Food and Agriculture Organization of the United Nations. 2015. FAOSTAT Statistical Database. https://www.fao.org/faostat/en/#data/QCL. [accessed 31 Oct 2025].
Food and Agriculture Organization of the United Nations. 2025. FAOSTAT Statistical Database. https://www.fao.org/faostat/en/#data/QCL. [accessed 21 Nov 2025].
Frantz JM, Cometti NN, Bugbee B. 2004. Night temperature has a minimal effect on respiration and growth in rapidly growing plants. Ann Bot. 94(1):155–166. https://doi.org/10.1093/aob/mch122.
Fraszczak B. 2012. The effect of changes in diurnal temperature and photoperiod on growth and yielding of garden dill grown in pots. Acta Sci Polonorum Hortorum Cultus. 11:217–228.
Gibson LR, Mullen RE. 1996. Influence of day and night temperature on soybean seed yield. Crop Sci. 36(1):98–104. https://doi.org/10.2135/cropsci1996.0011183X003600010018x.
Haghighi M, Abolghasemi R, da Silva JAT. 2014. Low and high temperature stress affect the growth characteristics of tomato in hydroponic culture with Se and nano-Se amendment. Sci Hortic. 178:231–240. https://doi.org/10.1016/j.scienta.2014.09.006.
Haworth M, Marino G, Cosentino SL, Brunetti C, De Carlo A, Avola G, Riggi E, Loreto F, Centritto M. 2018. Increased free abscisic acid during drought enhances stomatal sensitivity and modifies stomatal behaviour in fast growing giant reed (*Arundo donax* L.). Environ Exp Bot. 147:116–124. https://doi.org/10.1016/j.envexpbot.2017.11.002.
Hicklenton PR, Wolynetz MS. 1987. Influence of light and dark period air temperatures and root temperatures on growth of lettuce in nutrient flow systems. J Am Soc Hortic Sci. 112(6):932–935. https://doi.org/10.21273/JASHS.112.6.932.
Hofstra G, Hesketh JD. 1969. The effect of temperature on stomatal aperture in different species. Can J Bot. 47(8):1307–1310. https://doi.org/10.1139/b69-184.
Hong Z, Khalid M, Bilal M, Juan J, Huang D, Dongqin T. 2018. Differential effect of day and night temperature regimes on the growth and biochemical attributes of violet rape (*Brassica campestris* ssp. *chinensis* L.). Pol J Environ Stud. 27(6):2553–2560. https://doi.org/10.15244/pjoes/81205.
Hu W, Tanaka SI, Genkawa T, Hori Y. 2004. Respiration rate and quality of spinach cultivated in night-cooling greenhouse in summer. J Faculty Agric Kyushu Univ. 49(2):409–417. https://doi.org/10.5109/4602.
Intergovernmental Panel on Climate Change. 2022. Food, fibre, and other ecosystem products, p 713–906. In Pörtner H-O, Roberts DC, Tignor M, Poloczanska ES, Mintenbeck K, Alegría A, Craig M, Langsdorf S, Löschke S, Möller V, Okem A, Rama B (eds). Climate change 2022: Impacts, adaptation and vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK. https://doi.org/10.1017/9781009325844.007.
Inthichack P, Nishimura Y, Fukumoto Y. 2012. Diurnal temperature alternations affect plant growth, yield and mineral composition in cabbage, lettuce and celery. J Japan Soc Agric Technol Manag. 19:37–45. https://doi.org/10.20809/seisan.19.2_37.
Inthichack P, Nishimura Y, Fukumoto Y. 2013. Diurnal temperature alternations on plant growth and mineral absorption in eggplant, sweet pepper, and tomato. Hortic Environ Biotechnol. 54(1):37–43. https://doi.org/10.1007/s13580-013-0106-y.
Isaza C, Aleman-Trejo AM, Ramirez-Gutierrez CF, Zavala de Paz JP, Rizzo-Sierra JA, Anaya K. 2025. Spinach (*Spinacia oleracea* L.) growth model in indoor controlled environment using agriculture 4.0. Sensors (Basel). 25(6):1684. https://doi.org/10.3390/s25061684.
Japan Meteorological Agency. 2025. Monitoring of climate change 2018. https://www.data.jma.go.jp/cpdinfo/monitor/index.html. [accessed 31 Oct 2025].
Jilatu C, Iizumi T, Takimoto T. 2025. Vegetable supply to Tokyo disrupted by 2023 and 2024 summer heatwaves. J Agric Meteorol. 81(4):184–193. https://doi.org/10.2480/agrmet.D-25-00020.
Knight SL, Mitchell CA. 1983. Stimulation of lettuce productivity by manipulation of diurnal temperature and light. HortScience. 18(4):462–463. https://doi.org/10.21273/HORTSCI.18.4.462.
Krinsky NI, Landrum JT, Bone RA. 2003. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr. 23:171–201. https://doi.org/10.1146/annurev.nutr.23.011702.073307.
Leskovar DI, Esensee V, Belefant-Miller H. 1999. Pericarp, leachate, and carbohydrate involvement in thermoinhibition of germinating spinach seeds. J Am Soc Hortic Sci. 124(3):301–306. https://doi.org/10.21273/JASHS.124.3.301.
Leuning R. 1995. A critical appraisal of a combined stomatal–photosynthesis model for C3 plants. Plant Cell Environ. 18(4):339–355. https://doi.org/10.1111/j.1365-3040.1995.tb00370.x.
Lin Y-S, Medlyn BE, Duursma RA, Prentice IC, Wang H, Baig S, Eamus D, de Dios VR, Mitchell P, Ellsworth DS, de Beeck MO, Wallin G, Uddling J, Tarvainen L, Linderson M-L, Cernusak LA, Nippert JB, Ocheltree TW, Tissue DT, Martin-St Paul NK, Rogers A, Warren JM, De Angelis P, Hikosaka K, Han Q, Onoda Y, Gimeno TE, Barton CVM, Bennie J, Bonal D, Bosc A, Löw M, Macinins-Ng C, Rey A, Rowland L, Setterfield SA, Tausz-Posch S, Zaragoza-Castells J, Broadmeadow MSJ, Drake JE, Freeman M, Ghannoum O, Hutley LB, Kelly JW, Kikuzawa K, Kolari P, Koyama K, Limousin J-M, Meir P, Lola da Costa AC, Mikkelsen TN, Salinas N, Sun W, Wingate L. 2015. Optimal stomatal behaviour around the world. Nat Clim Change. 5(5):459–464. https://doi.org/10.1038/nclimate2550.
Liu K, Baskin JM, Baskin CC, Bu H, Du G, Ma M. 2013. Effect of diurnal fluctuating versus constant temperatures on germination of 445 species from the eastern Tibet Plateau. PLoS One. 8(7):e69364. https://doi.org/10.1371/journal.pone.0069364.
Lobell DB, Ortiz-Monasterio JI, Asner GP, Matson PA, Naylor RL, Falcon WP. 2005. Analysis of wheat yield and climatic trends in Mexico. Field Crops Res. 94(2–3):250–256. https://doi.org/10.1016/j.fcr.2005.01.007.
Martin TN, Fipke GM, Winck JEM, Marchese JA. 2021. ImageJ software as an alternative method for estimating leaf area in oats. Acta Agron. 69:69401. https://doi.org/10.15446/acag.v69n3.69401.
Masanetz C, Guth H, Grosch W. 1998. Fishy and hay-like off-flavours of dry spinach. Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 206(2):108–113. https://doi.org/10.1007/s002170050224.
Mathur S, Agrawal D, Jajoo A. 2014. Photosynthesis: Response to high temperature stress. J Photochem Photobiol B. 137:116–126. https://doi.org/10.1016/j.jphotobiol.2014.01.010.
Ministry of Agriculture, Forestry and Fisheries. 2025. MAFF Statistical Database. https://www.maff.go.jp/e/data/stat/index.html. [accessed 21 Nov 2025].
Mirfenderesgi G, Bohrer G, Matheny AM, Fatichi S, de Moraes Frasson RP, Schäfer KV. 2016. Tree level hydrodynamic approach for resolving aboveground water storage and stomatal conductance and modeling the effects of tree hydraulic strategy. JGR Biogeosci. 121(7):1792–1813. https://doi.org/10.1002/2016JG003467.
Mohammed AR, Tarpley L. 2009. Impact of high nighttime temperature on respiration, membrane stability, antioxidant capacity, and yield of rice plants. Crop Sci. 49(1):313–322. https://doi.org/10.2135/cropsci2008.03.0161.
Narayanan S, Prasad PVV, Fritz AK, Boyle DL, Gill BS. 2015. Impact of high night-time and high daytime temperature stress on winter wheat. J Agron Crop Sci. 201(3):206–218. https://doi.org/10.1111/jac.12101.

Ohtaka K, Yoshida A, Kakei Y, Fukui K, Kojima M, Takebayashi Y, Yano K, Imanishi S, Sakakibara H. 2020. Difference between day and night temperatures affects stem elongation in tomato (*Solanum lycopersicum*) seedlings via regulation of gibberellin and auxin synthesis. Front Plant Sci. 11:577235. https://doi.org/10.3389/fpls.2020.577235.

Parkash V, Snider J, Awori KJ, Katta JR, Pilon C, Tishchenko V. 2024. Distinguishing high daytime from nighttime temperature effects during early vegetative growth in cotton. J Agron Crop Sci. 210(5):e12757. https://doi.org/10.1111/jac.12757.

Peng S, Piao S, Ciais P, Myneni RB, Chen A, Chevallier F, Dolman AJ, Janssens IA, Peñuelas J, Zhang G, Vicca S, Wan S, Wang S, Zeng H. 2013. Asymmetric effects of daytime and nighttime warming on northern hemisphere vegetation. Nature. 501(7465):88–92. https://doi.org/10.1038/nature12434.

Pinto RS, Garatuza-Payán J, Murchie EH, Reynolds MP, Yepez EA. 2025. Wheat genotypes selected for their high early daytime stomatal conductance under elevated nocturnal temperatures maintain high yield and biomass. AoB Plants. 17(4):plae072. https://doi.org/10.1093/aobpla/plae072.

Pollet B, Steppe K, Dambre P, van Labeke MC, Lemeur R. 2009. Temperature integration of *Hedera helix* L.: Quality aspects and growth response. Sci Hortic. 120(1):89–95. https://doi.org/10.1016/j.scienta.2008.10.001.

Prasad PVV, Craufurd PQ, Summerfield RJ. 1999. Sensitivity of peanut to timing of heat stress during reproductive development. Crop Sci. 39(5):1352–1357. https://doi.org/10.2135/cropsci1999.3951352x.

Qin J, Shi A, Mou B, Grusak MA, Weng Y, Ravelombola W, Bhattarai G, Dong L, Yang W. 2017. Genetic diversity and association mapping of mineral element concentrations in spinach leaves. BMC Genomics. 18(1):941. https://doi.org/10.1186/s12864-017-4297-y.

Sato H, Takemura K, Ito A, Umeda T, Maeda S, Tanimoto Y, Nonaka M, Nakamura H. 2024. Impact of an unprecedented marine heatwave on extremely hot summer over northern Japan in 2023. Sci Rep. 14(1):16100. https://doi.org/10.1038/s41598-024-65291-y.

Shi W, Yin X, Struik PC, Solis C, Xie F, Schmidt RC, Huang M, Zou Y, Ye C, Jagadish SK. 2017. High day- and night-time temperatures affect grain growth dynamics in contrasting rice genotypes. J Exp Bot. 68(18):5233–5245. https://doi.org/10.1093/jxb/erx344.

Sinha R, Zandalinas SI, Fichman Y, Sen S, Zeng S, Gómez-Cadenas A, Joshi T, Fritschi FB, Mittler R. 2022. Differential regulation of flower transpiration during abiotic stress in plants. New Phytol. 235(2):611–629. https://doi.org/10.1111/nph.18162.

Somers DJ, Cummins WR, Filion WG. 1989. Characterization of the heat-shock response in spinach (*Spinacia oleracea* L.). Biochem Cell Biol. 67(2–3):113–120. https://doi.org/10.1139/o89-018.

Tai C, Sawada Y, Masuda J, Daimon H, Fukao Y. 2020. Cultivation of spinach in hot seasons using a micro-mist-based temperature-control system. Sci Hortic. 273:109603. https://ui.adsabs.harvard.edu/link_gateway/2020ScHor.27309603T/doi:10.1016/j.scienta.2020.109603. https://doi.org/10.1016/j.scienta.2020.109603.

Takemura K, Sato H, Ito A, Umeda T, Maeda S, Hirai M, Tamaki Y, Murai H, Nakamigawa H, Takayabu YN, Ueda H, Kawamura R, Tanimoto Y, Naoe H, Nonaka M, Hirooka T, Mukougawa H, Watanabe M, Nakamura H. 2024. Preliminary diagnosis of primary factors for an unprecedented heatwave over Japan in 2023 summer. SOLA. 20:69–78. https://doi.org/10.2151/sola.2024-010.

Tang Y, Wen X, Lu Q, Yang Z, Cheng Z, Lu C. 2007. Heat stress induces an aggregation of the light-harvesting complex of photosystem II in spinach plants. Plant Physiol. 143(2):629–638. https://doi.org/10.1104/pp.106.090712.

Urban J, Ingwers M, McGuire MA, Teskey RO. 2017. Stomatal conductance increases with rising temperature. Plant Signal Behav. 12(8):e1356534. https://doi.org/10.1080/15592324.2017.1356534.

US Department of Agriculture. 2019. USDA national nutrient database for standard reference, legacy release. US Department of Agriculture, Agricultural Research Service, Washington, DC, USA.

Uz Zaman Q, Abbasi A, Tabassum S, Ashraf K, Ahmad Z, Siddiqui MH, Alamri S, Maqsood S, Sultan K. 2022. Calcium induced growth, physio-biochemical, antioxidant, osmolyte adjustments and phytoconstituent status in spinach under heat stress. South Afr J Bot. 149:701–711. https://doi.org/10.1016/j.sajb.2022.06.065.

Verhoef A, Egea G. 2014. Modeling plant transpiration under limited soil water: Comparison of different plant and soil hydraulic parameterizations and preliminary implications for their use in land surface models. Agric For Meteorol. 191:22–32. https://doi.org/10.1016/j.agrformet.2014.02.009.

Von Caemmerer S, Evans JR. 2015. Temperature responses of mesophyll conductance differ greatly between species. Plant Cell Environ. 38(4):629–637. https://doi.org/10.1111/pce.12449.

Vose RS, Easterling DR, Gleason B. 2005. Maximum and minimum temperature trends for the globe: An update through 2004. Geophys Res Lett. 32(23):L23822. https://doi.org/10.1029/2005GL024379.

Wen Y, Liu X, Pei F, Li X, Du G. 2018. Non-uniform time-lag effects of terrestrial vegetation responses to asymmetric warming. Agric For Meteorol. 252:130–143. https://doi.org/10.1016/j.agrformet.2018.01.016.

Wilcox GE, Pfeiffer CL. 1990. Temperature effect on seed germination, seedling root development and growth of several vegetables. J Plant Nutr. 13(11):1393–1403. https://doi.org/10.1080/01904169009364161.

Wong C. 2023. Earth just had its hottest year on record: Climate change is to blame. Nature. 623(7988):674–675. https://doi.org/10.1038/d41586-023-03523-3.

Xu X, Medvigy D, Powers JS, Becknell JM, Guan K. 2016. Diversity in plant hydraulic traits explains seasonal and inter-annual variations of vegetation dynamics in seasonally dry tropical forests. New Phytol. 212(1):80–95. https://doi.org/10.1111/nph.14009.

Yamori W, Noguchi KO, Terashima I. 2005. Temperature acclimation of photosynthesis in spinach leaves: Analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant Cell Environ. 28(4):536–547. https://doi.org/10.1111/j.1365-3040.2004.01299.x.

Yan J, Yu L, Xuan J, Lu Y, Lu S, Zhu W. 2016. De novo transcriptome sequencing and gene expression profiling of spinach (*Spinacia oleracea* L.) leaves under heat stress. Sci Rep. 6:19473. https://doi.org/10.1038/srep19473.

Yoneda Y, Kawasaki Y, Kawashima H. 2022. Control of transmitted solar radiation using photoselective covering materials improves spinach (*Spinacia oleracea* L.) yield during summer. JARQ. 56(1):67–75. https://doi.org/10.6090/jarq.56.67.

Zhao X, Sui X, Zhao L, Gao X, Wang J, Wen X, Li Y. 2022. Morphological and physiological response mechanism of lettuce (*Lactuca sativa* L.) to consecutive heat stress. Sci Hortic. 301:111112. https://doi.org/10.1016/j.scienta.2022.111112.