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THE EFFECT OF EXOGENOUS SPERMIDINE ON THE GROWTH AND PHYSIOLOGICAL RESPONSES OF STRAWBERRY (*FRAGARIA* × *ANANASSA* Duch.) SEEDLINGS UNDER HIGH-TEMPERATURE STRESS

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Abstract

To test whether exogenous spermidine (SPD) alleviates heat stress in strawberry seedlings and to identify the physiological processes involved, seedlings of the heat-sensitive cultivar 'Hongyan' were foliar-sprayed with 0.5, 1 or 2 mmol·L^{-1} SPD and assessed at 1, 3, 5 and 7 d of heat stress (mean 37.5°C by day and 23.5°C at night). Growth, gas exchange, membrane injury, osmolyte contents and antioxidant enzyme activities were measured. Heat stress suppressed seedling growth and photosynthesis, aggravated membrane damage and malondialdehyde (MDA) accumulation, and disturbed osmotic and antioxidant metabolism. SPD opposed each of these responses, and its benefit rose and then fell with concentration, peaking at 1 mmol·L^{-1}. At 1 mmol·L^{-1}, net photosynthetic rate at 7 d was 2.66-fold that of the untreated heat-stressed control, and relative electrolyte leakage (REL) and MDA were 30.45% and 25.44% lower. The optimum therefore lies at an intermediate dose, and these results provide a practical SPD concentration for improving heat tolerance in strawberry seedling production.

Key words: Strawberry (*Fragaria* × *ananassa* Duch.); High-temperature stress; Spermidine; Antioxidant enzymes; Osmotic adjustment; Photosynthesis

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a perennial herb of the Rosaceae that originated in Europe from a cross between *F. chiloensis* (from South America) and *F. virginiana* (from North America), and it is adapted to temperate and subtropical cultivation. The fruit is uniform in shape, attractive in color, sweet–sour in taste, juicy and aromatic, and rich in essential nutrients (Zhao *et al.,* 2018); strawberry is therefore both widely consumed and economically important. With progressive global warming, however, high-temperature episodes increasingly constrain strawberry growth (Xu & Wang, 2020). Heat raises cell-membrane permeability and disturbs water balance, which suppresses chlorophyll biosynthesis and impairs photosynthetic performance (Giampieri *et al.,* 2015).

Persistent heat also disrupts ionic homeostasis, and excessive ion accumulation is cytotoxic, disturbs metabolism and impairs strawberry production, causing substantial economic loss (Ullah *et al.,* 2024). Field cooling in strawberry production relies on shade nets, sprinkler irrigation and pad-fan systems. Shade nets and sprinklers provide limited cooling, and their efficiency depends strongly on ambient humidity and air movement; pad-fan systems cool more effectively but require high capital and operating inputs, which restricts their adoption in commercial production (Benzzine *et al.,* 2026). Cost-effective and easily applied alternatives are therefore needed.

Polyamines are aliphatic nitrogen-containing bases that occur in plant cells mainly in free and conjugated forms. They are synthesized from amino acids and comprise mainly putrescine, spermine, spermidine, thermospermine and cadaverine, the free forms being the most abundant (Chen *et al.,* 2019). Spermidine (SPD), spermine (Spm) and putrescine (Put) are distributed throughout plant tissues, where they stabilize membranes, modulate growth, development and senescence, and mediate stress responses and acclimation (Li *et al.,* 2020; Blázquez & Miguel, 2024). In lettuce under high temperature, exogenous SPD improved nitrogen metabolism (Yu *et al.,* 2022). In tomato, exogenous SPD raises antioxidant enzyme activity and promotes reactive oxygen species (ROS) scavenging, thereby alleviating heat-induced damage (Sang, 2017). Foliar application of SPD also improves growth under heat stress in cucumber (Wang *et al.,* 2018) and jujube (Liu *et al.,* 2024), and under drought stress in grape (Gao, 2018). The effect of SPD on heat tolerance in strawberry, however, has been little studied.

We measured growth parameters, total chlorophyll content, relative electrolyte leakage, malondialdehyde, osmolyte concentrations and antioxidant enzyme activities under different exogenous SPD concentrations, in order to characterize the thermotolerance conferred by SPD and its underlying mechanisms in strawberry seedlings, and to identify the concentration that best alleviates heat stress. We hypothesized that exogenous SPD would alleviate heat-induced damage in a dose-dependent manner by stabilizing membranes, enhancing osmotic adjustment and

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strengthening ROS scavenging, and that an intermediate concentration would be the most effective. The results are intended to support both the theory and the practice of strawberry cultivation and seedling production.

Materials and Methods

Plant material. The heat-sensitive strawberry cultivar 'Hongyan' was used. Seventy-five uniform, healthy plug seedlings were transplanted in November 2025 and acclimated under control conditions for at least two weeks, until they had produced 5–6 fully expanded leaves, at which point the treatments began. The plant material had been prepared in September–October 2025, and the heat treatment, sampling and measurements were completed in January–February 2026. The 75 seedlings were divided into five treatments of 15 seedlings each; each treatment comprised three biological replicates of five seedlings, and at each sampling date the leaves of the five seedlings within a replicate were pooled into one sample.

Experimental design. The experiment was carried out once, without repetition in time, in growth chambers at Huai'an University. The chambers were set to a 14 h/10 h photoperiod (07:00–21:00, LED panels), a PPFD of 400 ± 20 μmol $m^{-2}s^{-1}$ at canopy level, 70 ± 5% relative humidity and 400 μmol mol^{-1} CO_2. Each light/dark phase was held at one constant set point, with 60-min linear transitions at 07:00 and 21:00 between the two levels, and the set points did not change over the course of the treatment. The control was set to 22°C by day and 16°C at night, the heat treatment to 37°C by day and 23°C at night. In the heat treatment, air temperature fluctuated around the set point, staying within 35–38°C by day and 20–25°C at night (7-d means 37.5 and 23.5°C). All five treatments shared one chamber in a single run. Pots were weighed daily at 17:00 and watered back to the container-capacity weight determined at transplanting, identically for every treatment throughout the stress period.

Foliar treatment. Spermidine (CAS 124-20-9; purity ≥ 99%, GC) was purchased from Macklin Biochemical Co., Ltd. (Shanghai, China) as the free base and stored sealed in the dark at 2–8°C. Working solutions of 0.5, 1 and 2 mmol·L^{-1} (all expressed as free base) were prepared in distilled water immediately before use from a 100 mmol·L^{-1} stock kept in the dark at 4°C for no more than one week. Treatments were applied at 11:00 on each of three consecutive days with a hand-held fine-nozzle sprayer, covering the adaxial and abaxial leaf surfaces evenly until the leaves were fully wetted but not dripping (approximately 20 mL per plant); control plants received an equal volume of distilled water at the same time (Table 1). No surfactant was added, so as not to interfere with the electrolyte-leakage measurements. After three consecutive days of spraying, the seedlings were transferred to the high-temperature environment (mean 37.5°C during the day and 23.5°C at night).

Sampling and substrate water management. Leaf samples were harvested for physiological assays at 1, 3, 5 and 7 d of heat stress, with three biological replicates per treatment. Substrate moisture was maintained by daily weighing as described above, so that all treatments were kept at a comparable substrate water status throughout the stress period. Collected leaf tissue was snap-frozen in liquid nitrogen and stored at −80°C; all physiological measurements were made after sampling had been completed.

Measurements of growth-related parameters. Plant height was measured with a ruler from the substrate surface to the highest point of the canopy. Crown diameter was measured with a digital vernier caliper (resolution 0.01 mm) at approximately 1 cm above the substrate surface, and two perpendicular measurements per plant were averaged (Li, 2000).

Measurements of photosynthetic parameters. Net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), intercellular CO_2 concentration (*Ci*) and transpiration rate (*Tr*) were measured with a portable photosynthesis system (LDGH80GT4SL, Shandong Laende Intelligent Technology Co., Ltd.) between 08:00 and 10:30 on each sampling date. Measurements were made on one leaf per plant (the second fully expanded leaf counted from the apex) at a leaf-chamber PPFD of 800 μmol m^{-2} s^{-1}, 400 μmol mol^{-1} CO_2, a leaf temperature of 25 °C and a flow rate of 800 mL min^{-1}. Chlorophyll was extracted by soaking 0.2 g of fresh leaf tissue in 20 mL of 95 % ethanol in the dark for 24 h, with periodic shaking, until the tissue was fully bleached; absorbance was read at 665 and 649 nm against a 95 % ethanol blank, and total chlorophyll content was expressed on a fresh-weight basis (mg g^{-1} FW) (Lichtenthaler & Wellburn, 1983).

Measurements of cell membrane permeability and membrane lipid peroxidation. Relative electrolyte leakage (REL) was obtained from the electrical conductivity of leaf discs before (C1) and after boiling (C2) and calculated as C1/C2 × 100 %. After C1 had been recorded, the tubes were sealed and boiled in a water bath for 10 min, cooled to room temperature under running cold water, equilibrated for 10 min and shaken thoroughly before C2 was measured. Malondialdehyde (MDA) content was determined by the thiobarbituric acid method. Samples were extracted with 10% (w/v) trichloroacetic acid (TCA), the extract was mixed with 0.6 % (w/v) thiobarbituric acid (TBA) solution, and the absorbance was read at 532 and 600 nm; MDA content was expressed as nmol g^{-1} FW (Li, 2000).

Measurements of osmolyte contents. Proline (Pro) content was determined by ninhydrin colorimetry and expressed as μg g^{-1} FW. L-proline standards covering 0–300 μg per reaction mixture were carried through the same coloration procedure as the samples; absorbance was read at 520 nm, and the resulting linear calibration was used to calculate Pro in each sample. Soluble sugar and soluble protein contents were measured by anthrone–sulfuric acid colorimetry and Coomassie brilliant blue G250 staining, respectively (Li, 2000), and both were expressed as mg g^{-1} FW.

Measurements of antioxidant enzyme activities. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities were determined by the NBT method, the guaiacol method (Amako *et al.,* 1994) and the

potassium permanganate titration method (Li, 2000), respectively. One unit of SOD activity was defined as the amount of enzyme that causes 50% inhibition of NBT photoreduction, and activity was expressed as U g^{-1} FW; POD activity was expressed as ΔA470 min^{-1} g^{-1} FW, and CAT activity as μmol H_2O_2 min^{-1} g^{-1} FW.

Statistical analysis

Data were processed in Microsoft Excel 2021, figures were drawn in OriginPro 2025 (OriginLab, USA) and statistical tests were run in SPSS 27.0. Normality (Shapiro–Wilk) and homogeneity of variance (Levene) were checked before analysis; data were then subjected to one-way ANOVA at each sampling date followed by Duncan's multiple range test at $p<0.05$, and values are means ± SD ($n = 3$). Pearson correlation analysis was performed on pooled observations across all treatment–time combinations ($n = 60$), and principal component analysis was applied to the 7 d data ($n = 15$) after z-score standardization, with the comprehensive score weighted by the variance contribution of each component.

Results

Effects of exogenous spermidine on growth-related parameters of strawberry seedlings under high-temperature stress: Plant height and crown diameter differed only slightly among treatments at 1 d, the first sampling date (all differences <3.50% of the control mean; Tables 2, 3). Because the seedlings had received only one day of treatment at this point, and because these differences were small and did not follow the SPD concentration gradient, they reflect the variation present among the starting seedlings rather than a treatment response, and do not affect comparisons at later dates. Both variables increased steadily in CK, whereas height growth in HW was almost arrested: at 7 d, HW plants reached only 12.94 cm, 9.51% less than CK, and their crown diameter reached 8.33 mm, 7.75% less than CK. Heat stress therefore strongly repressed vegetative growth. Plant height was significantly greater in every SPD treatment than in HW from 3 d onwards, and at 5 and 7 d the response to concentration was non-monotonic, rising from T1 to T2 and falling again at T3. For crown diameter a significant increase over HW appeared only at 7 d, and only in T2 and T3 (Table 3). Height in HW stagnated from 3 d onwards and was significantly below CK at 5 and 7 d ($p<0.05$). T2 performed best: at 5 d, plants under T2 reached 13.85 cm, significantly taller than HW and T1, and at 7 d 14.22 cm, which was 9.89 % taller than HW and not significantly different from CK (0.56% lower). At 7 d, T1 plants reached 13.98 cm, 8.04% taller than HW and not significantly different from T2, and T3 plants 13.94 cm, 2.52% below CK and 7.73% above HW, indicating a weaker effect than T2 but not significantly different from T1. Crown diameter did not differ significantly between any treatment and CK at 1 or 3 d. At 5 d it was 3.58%, 1.96%, 1.15% and 1.62% below CK in HW, T1, T2 and T3, respectively, and 1.68%, 2.51% and 2.04% above HW in T1, T2 and T3. At 7 d the corresponding reductions relative to CK were 7.75%, 5.65%, 0.55% and 3.54%, and the increases relative to HW were 2.28%, 7.80% and 4.56%. Exogenous SPD at an appropriate concentration therefore alleviated the inhibition of height and crown-diameter growth by heat stress, with T2 (1 mmol·L^{-1} SPD) the most effective.

Table 1. Treatment method.

Treatment	Method	Abbreviation
1	Normal temperature + distilled water	CK
2	High temperature + distilled water	HW
3	High temperature + 0.5 mmol·L^{-1} SPD	T1
4	High temperature + 1 mmol·L^{-1} SPD	T2
5	High temperature + 2 mmol·L^{-1} SPD	T3

CK – control; SPD – spermidine

Table 2. Effects of exogenous spermidine at different concentrations on plant height of strawberry seedlings under high-temperature stress.

Treatment	Plant height/cm			
	1 d	3 d	5 d	7 d
CK	12.45±0.05^{c}	12.84±0.02^{c}	13.28±0.07^{c}	14.30±0.13^{a}
HW	12.78±0.05^{ab}	12.93±0.12^{c}	13.03±0.03^{d}	12.94±0.14^{d}
T1	12.61±0.11^{bc}	13.10±0.02^{b}	13.63±0.08^{b}	13.98±0.19^{bc}
T2	12.87±0.16^{a}	13.28±0.07^{a}	13.85±0.03^{a}	14.22±0.12^{ab}
T3	12.71±0.06^{ab}	13.36±0.02^{a}	13.69±0.12^{b}	13.94±0.04^{c}

CK – control; SPD – spermidine; values within a column followed by different lowercase letters are significantly different ($p<0.05$)

Table 3. Effects of exogenous spermidine at different concentrations on plant crown diameter of strawberry seedlings under high-temperature stress.

Treatment	Crown diameter/mm			
	1 d	3 d	5 d	7 d
CK	8.26±0.10^{a}	8.46±0.04^{a}	8.66±0.17^{a}	9.03±0.23^{a}
HW	8.32±0.33^{a}	8.34±0.14^{a}	8.35±0.21^{a}	8.33±0.09^{c}
T1	8.29±0.07^{a}	8.37±0.13^{a}	8.49±0.19^{a}	8.52±0.22^{bc}
T2	8.29±0.17^{a}	8.41±0.27^{a}	8.56±0.14^{a}	8.98±0.08^{a}
T3	8.32±0.13^{a}	8.46±0.23^{a}	8.52±0.06^{a}	8.71±0.24^{ab}

CK – control; SPD – spermidine; values within a column followed by different lowercase letters are significantly different ($p<0.05$)

Effects of exogenous spermidine on gas-exchange parameters of strawberry seedlings under high-temperature stress: Sustained heat stress severely inhibited photosynthetic carbon assimilation (Fig. 1A–D). *Pn*, *Gs* and *Tr* in HW declined progressively with stress duration while *Ci* rose, and the magnitude of both changes increased as the stress continued; foliar SPD attenuated all four responses at 3, 5 and 7 d. At 7 d, *Pn*, *Gs* and *Tr* in HW were 81.79%, 85.94% and 84.13% below CK, and *Ci* was 36.76% above it. T2 was the most effective concentration throughout: at 7 d it raised *Pn*, *Gs* and *Tr* by 166.46%, 148.77% and 167.44% and lowered *Ci* by 18.75% relative to HW, and the three concentrations ranked T2 > T1 > T3 for all four parameters. Because *Pn* and *Gs* fell from 3 d onwards while *Ci* rose, the inhibition of photosynthesis was governed mainly by non-stomatal rather than stomatal limitation. Had stomatal closure been the primary constraint, the intercellular CO_2 concentration would have fallen together with stomatal conductance; instead it lay 24.66%, 32.40% and 36.76% above CK at 3, 5 and 7 d and kept rising, so that CO_2 supply exceeded the carboxylation capacity of the mesophyll. Foliar SPD therefore mitigated the heat-induced impairment of photosynthetic performance, most effectively at T2 (1 mmol·L^{-1} SPD).

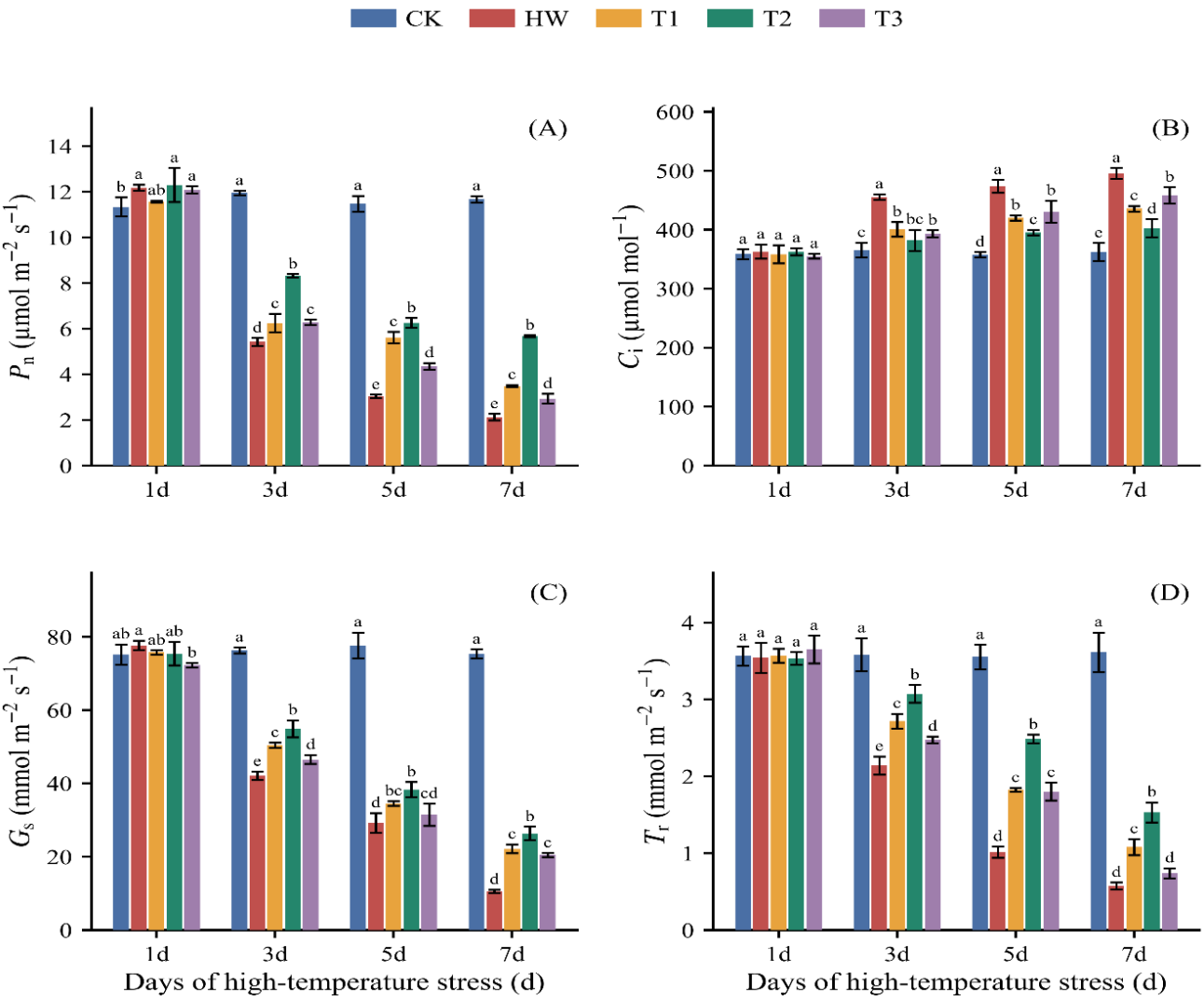


Fig. 1. Effects of exogenous spermidine at different concentrations on the photosynthetic parameters of strawberry seedlings under high-temperature stress. (A) Net photosynthetic rate (*Pn*, μmol m^{-2} s^{-1}); (B) intercellular CO_2 concentration (*Ci*, μmol mol^{-1}); (C) stomatal conductance (*Gs*, mmol m^{-2} s^{-1}); (D) transpiration rate (*Tr*, mmol m^{-2} s^{-1}). Bars are means ± SD (n = 3). Different lowercase letters above bars indicate significant differences among treatments at the same sampling time ($p<0.05$, one-way ANOVA followed by Duncan's multiple range test).

Effects of exogenous spermidine on total chlorophyll content in strawberry seedling leaves under high-temperature stress: Total chlorophyll content in HW declined gradually with stress duration and was 56.46% below CK at 7 d (Fig. 2). Foliar SPD raised chlorophyll content at every concentration, most strongly under T2 and T3: relative to HW at 3, 5 and 7 d, T1 increased it by 12.61%, 40.72% and 62.64%, T2 by 14.26%, 55.70% and 87.10%, and T3 by 14.86%, 47.26% and 66.21%. At 7 d, chlorophyll content under T2 was significantly higher than under the other heat treatments. Exogenous SPD therefore alleviated the loss of chlorophyll under heat stress, with T2 (1 mmol·L^{-1} SPD) the most effective and T3 next.

Effects of exogenous spermidine on relative electrolyte leakage and MDA content in strawberry seedling leaves under high-temperature stress: Relative electrolyte leakage and MDA content in HW both rose steadily with stress duration, reaching 48.68% and 12.84 nmol g^{-1} FW at 7 d, the latter 170.89% above CK, so the two indicators of membrane damage advanced together (Fig. 3A, B). Foliar SPD reduced both at every concentration: relative to HW at 3, 5 and 7 d, leakage was 21.09%, 12.49% and 13.81% lower under T1, 34.31%, 28.91% and 30.45% lower under T2, and 23.48%, 19.64% and 18.61% lower under T3, and MDA was 11.45%, 4.94% and 9.42% lower under T1, 21.32%, 21.30% and 25.44% lower under T2, and 15.56%, 11.11% and 18.72% lower under T3. At 7 d both were lowest under T2, MDA significantly so, and T2 was also the most effective concentration for leakage throughout, at 34.31%, 28.91% and 30.45% below HW. The two responses therefore tracked each other across all treatments, and exogenous SPD limited heat-induced membrane damage most effectively at 1 mmol·L^{-1} (T2), maintaining membrane integrity both by restraining lipid peroxidation and by conserving the selective permeability of the membrane.

Effects of exogenous spermidine on Pro content in strawberry seedling leaves under high-temperature stress: Pro content in HW rose and then fell as the stress continued (Fig. 4). Foliar SPD increased it further at every concentration: relative to HW at 3, 5 and 7 d, Pro was 12.76%, 13.18% and 9.55% higher under T1, 28.77%,

51.58% and 57.01% higher under T2, and 33.30%, 43.49% and 35.23% higher under T3. At 7 d, Pro under T2 was significantly higher than under the other treatments. Appropriate concentrations of exogenous SPD therefore strengthened osmotic adjustment by promoting Pro accumulation, thereby alleviating heat-induced damage.

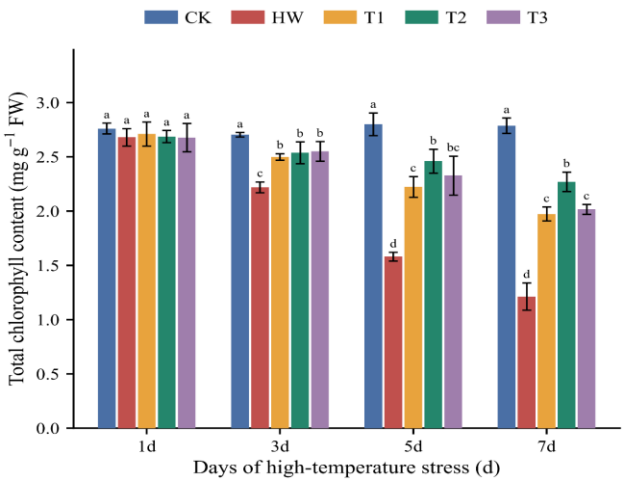


Fig. 2. Effects of exogenous spermidine at different concentrations on the total chlorophyll content (mg g^{-1} FW) of strawberry seedlings under high-temperature stress. Bars are means ± SD (n = 3). Different lowercase letters above bars indicate significant differences among treatments at the same sampling time ($p<0.05$, one-way ANOVA followed by Duncan's multiple range test).

Effects of exogenous spermidine on soluble sugar and soluble protein contents in strawberry seedling leaves under high-temperature stress: Both osmolytes rose and then fell in HW as the stress continued, and soluble protein stayed low in CK, near 6 mg g^{-1} FW at every sampling time (Fig. 5A, B). Foliar SPD raised both at every concentration: relative to HW at 3, 5 and 7 d, soluble sugar was 3.71%, 7.74% and 12.79% higher under T1, 20.97%, 48.23% and 86.45% higher under T2, and 25.69%, 42.46% and 54.50% higher under T3, and the corresponding gains in soluble protein were 4.25%, 14.13% and 6.79% under T1, 14.31%, 43.69% and 53.66% under T2, and 19.73%, 39.04% and 31.11% under T3. The advantage of T2 (1 mmol·L^{-1} SPD) widened as the stress persisted: it produced the largest increases over HW at both 5 and 7 d for the two compounds, and by 7 d the sugar and protein contents under T2 were 86.45% and 53.66% above HW, against 54.50% and 31.11% under T3, whereas at 3 d the three concentrations differed far less (T3 highest for both, T2 intermediate), reflecting the transient accumulation that all SPD treatments shared early in the stress rather than a concentration effect. T2 also held the highest content of the three SPD treatments at 5 and 7 d for both compounds.

Effects of exogenous spermidine on antioxidant enzyme activities in strawberry seedling leaves under high-temperature stress: Antioxidant enzyme activities in strawberry seedling leaves generally rose and then fell under heat stress (Fig. 6). SOD and POD activities in HW both peaked at 3 d and then declined, whereas CAT activity (Fig. 6C) peaked at 5 d; foliar SPD significantly increased the activity of all three enzymes. At 7 d, relative to HW, SOD activity was 11.99%, 29.19% and 22.09% higher under T1, T2 and T3, POD activity (Fig. 6B) 87.20%, 125.77% and 94.57% higher, and CAT activity 13.15%, 36.78% and 23.90% higher; the ranking was T2 > T3 > T1 for all three enzymes, and T2 gave the largest increase at 3, 5 and 7 d. Exogenous SPD at an appropriate concentration therefore enhanced the scavenging of superoxide anions and H_2O_2 most effectively at 1 mmol·L^{-1}, mitigating heat-induced oxidative damage. The size of this effect depended on concentration, being greatest at T2 (1 mmol·L^{-1} SPD).

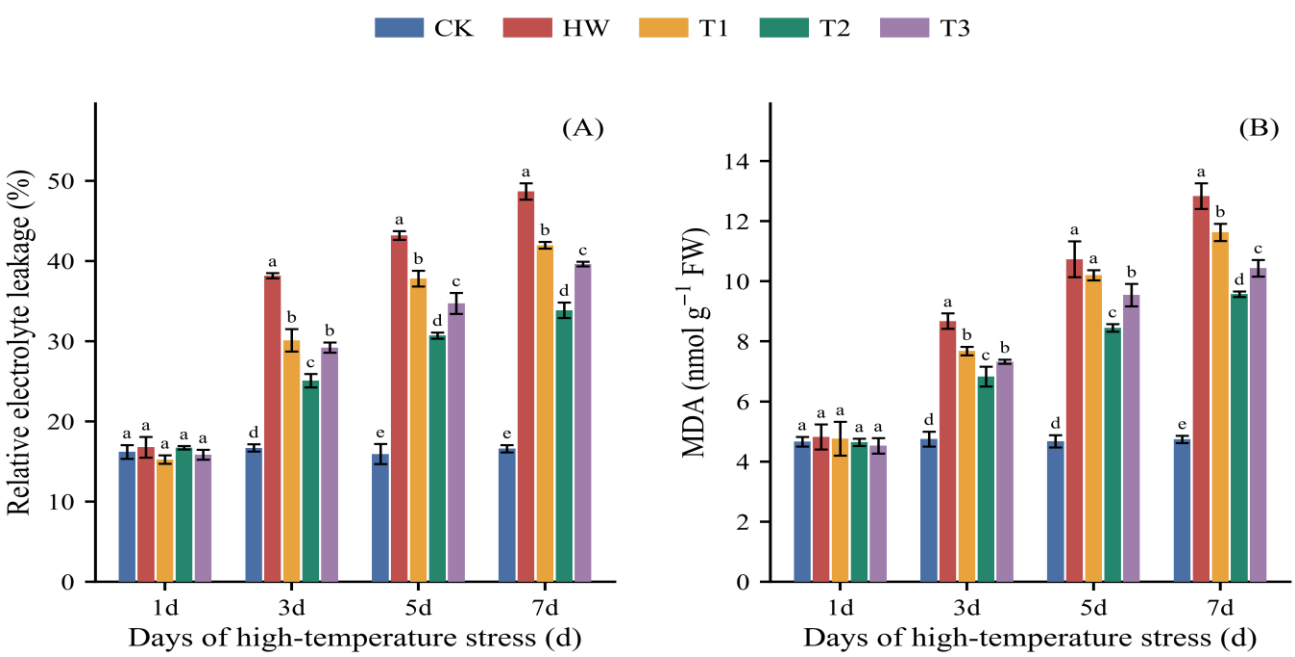


Fig. 3. Effects of exogenous spermidine at different concentrations on cell-membrane damage in strawberry seedling leaves under high-temperature stress: (A) relative electrolyte leakage (REL, %), an index of membrane permeability; (B) malondialdehyde (MDA) content (nmol g^{-1} FW), the end product of membrane lipid peroxidation, under high-temperature stress. Bars are means ± SD (n = 3). Different lowercase letters above bars indicate significant differences among treatments at the same sampling time ($p<0.05$, one-way ANOVA followed by Duncan's multiple range test).

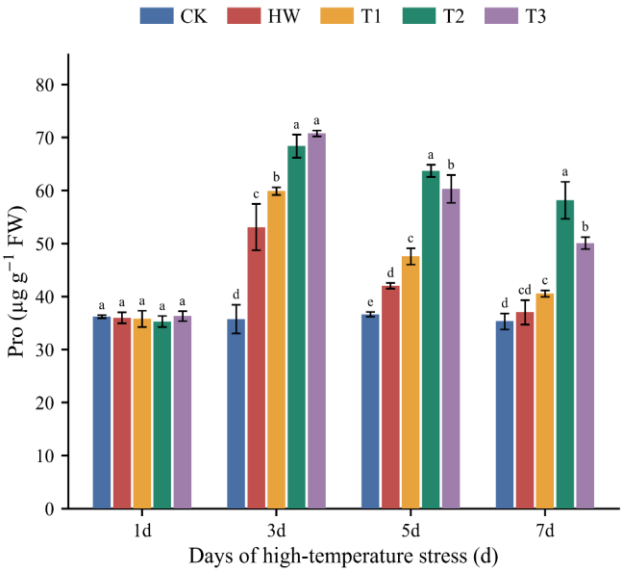


Fig. 4. Effects of exogenous spermidine at different concentrations on the proline (Pro) content (μg g^{-1} FW) of strawberry seedling leaves under high-temperature stress. Bars are means ± SD (n = 3). Different lowercase letters above bars indicate significant differences among treatments at the same sampling time ($p<0.05$, one-way ANOVA followed by Duncan's multiple range test).

Correlation analysis, principal component analysis and comprehensive evaluation of the physiological indices: Pearson correlations were computed for the 15 indices on pooled observations across all treatment–time combinations (n = 60; Fig. 7). Because the same plants were measured at every sampling time, these values are repeated measures, so part of the correlation structure reflects the sampling design rather than a physiological relationship, and the significance levels in Fig. 7, which treat them as independent, are indicative only. The analysis is descriptive, and no physiological inference is drawn from the correlations involving growth parameters, whose values rise with time in all treatments. As a check on the pooling, the analysis was repeated on the 20 treatment means and on day 7 alone (n = 15). All 37 coefficients with $|r| \geq 0.60$ in Fig. 7 kept their sign in both checks (day 7: $|r|$ = 0.77–0.99; treatment means: mean absolute change in r across all 105 pairs = 0.01), and the 15 samples at 7 d are independent, so the P values for that subset are unaffected by the repeated-measures structure; every coefficient cited below reached $|r| \geq 0.86$ at day 7 ($p<0.001$). Of the 105 coefficients, 23 (22%) changed sign, all of which were weak in the pooled matrix ($|r| \leq 0.48$); 13 of them involved the two growth parameters, whose coefficients reverse once the between-date trend is removed (plant height against *Pn*: r = −0.42 with dates pooled, +0.64 at day 7). *Pn* was positively correlated with *Gs* (r = 0.98), *Tr* (r = 0.96) and total chlorophyll content (r = 0.85) and negatively with *Ci* (r = −0.90; all $p<0.001$), following the gas-exchange pattern in Fig. 1A–C. REL was closely correlated with MDA (r = 0.98), and both were negatively correlated with *Pn* (r = −0.97 and −0.96), indicating that membrane damage was coupled to the decline in photosynthesis. Among the defense-related indices, SOD was correlated with POD (r = 0.96), POD with CAT (r = 0.91) and Pro with soluble sugar (r = 0.96; all $p<0.001$). The variables in these three groups therefore shifted together across treatments rather than independently.

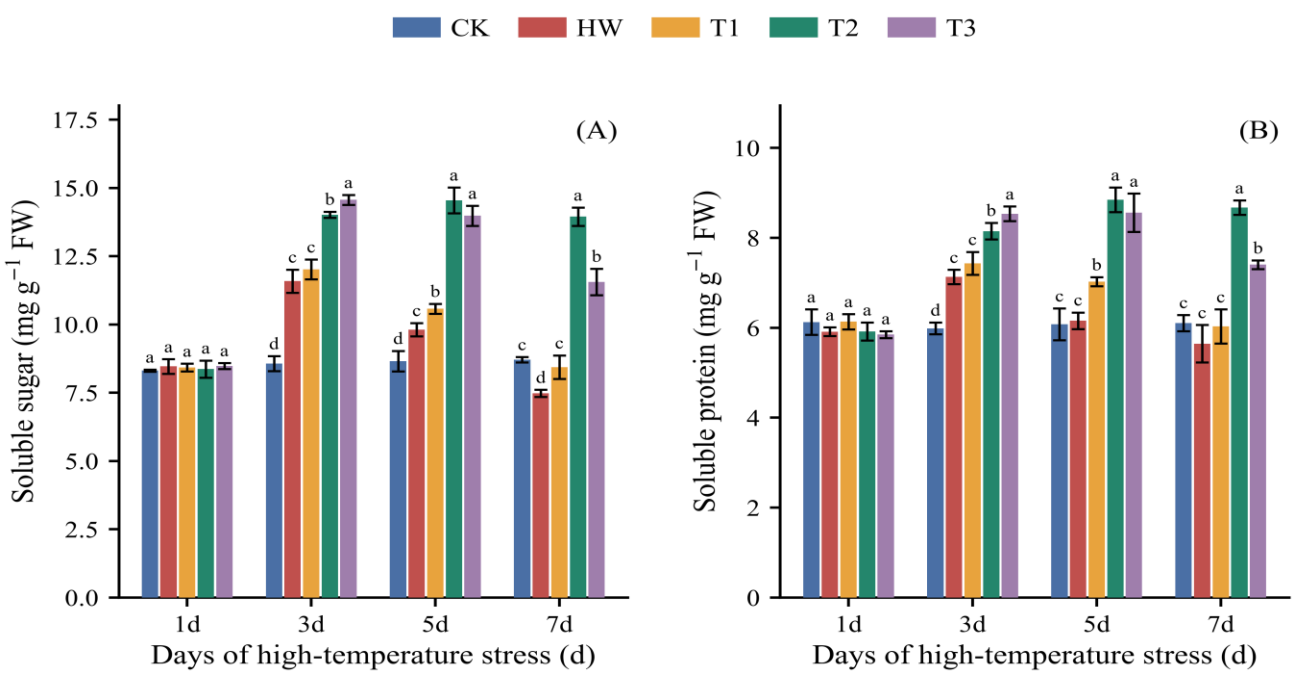


Fig. 5. Effects of exogenous spermidine at different concentrations on the soluble sugar (A) and soluble protein (B) contents (mg g^{-1} FW) of strawberry seedling leaves under high-temperature stress. Bars are means ± SD (n = 3). Different lowercase letters above bars indicate significant differences among treatments at the same sampling time ($p<0.05$, one-way ANOVA followed by Duncan's multiple range test).

Table 4. Principal component analysis and contribution rates.

Index	Principal component 1	Principal component 2
Plant height	0.72	0.60
Crown diameter	0.74	0.54
Total chlorophyll	0.92	0.35
Net photosynthetic rate (Pn)	0.99	−0.07
Stomatal conductance (Gs)	0.99	−0.16
Intercellular CO_2 concentration (Ci)	−0.91	−0.21
Transpiration rate (Tr)	0.98	−0.12
Relative electrolyte leakage (REL)	−0.99	−0.07
MDA	−0.98	−0.02
Pro	−0.19	0.97
Soluble sugar content	0.06	0.97
Soluble protein content	0.04	0.97
SOD	−0.37	0.92
POD	−0.35	0.88
CAT	−0.39	0.91
Eigenvalue	8.04	6.11
Variance contribution rate (%)	53.59	40.74
Cumulative contribution (%)	53.59	94.34

n = 15 observations (five treatments × three replicates), z-score standardized before analysis; values are the loadings of each index on each principal component

Principal component analysis was performed on the indices measured at 7 d, using five treatments × three replicates (n = 15 observations; Table 4). After standardization in SPSS, two principal components with eigenvalues greater than 1 were extracted, accounting for 53.59% and 40.74% of the variance (94.34% cumulative). Because the 15 variables were measured on only 15 observations, the loadings are best read as a descriptive summary of this data set rather than as stable estimates of the underlying physiological structure. The first component was dominated by the photosynthetic parameters (net photosynthetic rate and stomatal conductance 0.99 each; transpiration rate 0.98; total chlorophyll content 0.92) and, in the opposite direction, by REL (−0.99) and MDA (−0.98), and therefore contrasts photosynthetic capacity with cell-membrane injury. The second was loaded by soluble sugar, soluble protein and Pro (0.97 each) and by SOD (0.92), CAT (0.91) and POD (0.88), and thus represents osmotic adjustment and antioxidant capacity, the main defenses against heat-induced oxidative and osmotic stress.

The variance contribution of each principal component, as a proportion of the cumulative variance, was used as its weight to calculate and rank the comprehensive scores of the five treatments (Table 5). A higher score indicates a better overall physiological status of the seedlings under that treatment and a stronger alleviating effect of SPD.

The comprehensive scores of the three SPD treatments ranked T2 (0.69) > T3 (0.01) > T1 (−0.31); T2 was close to CK (0.72), whereas T1 and T3 were markedly lower, a pattern consistent with the non-monotonic concentration response seen in the growth and gas-exchange data. HW scored lowest of the five treatments (−1.11), well below CK and the three SPD treatments, consistent with its poor growth under heat stress. T2 (1 mmol·L⁻¹ SPD) therefore achieved the highest score among the SPD treatments and came close to the normal-temperature control, and is regarded as the most effective of the concentrations tested for alleviating high-temperature stress in strawberry seedlings under the conditions of this study.

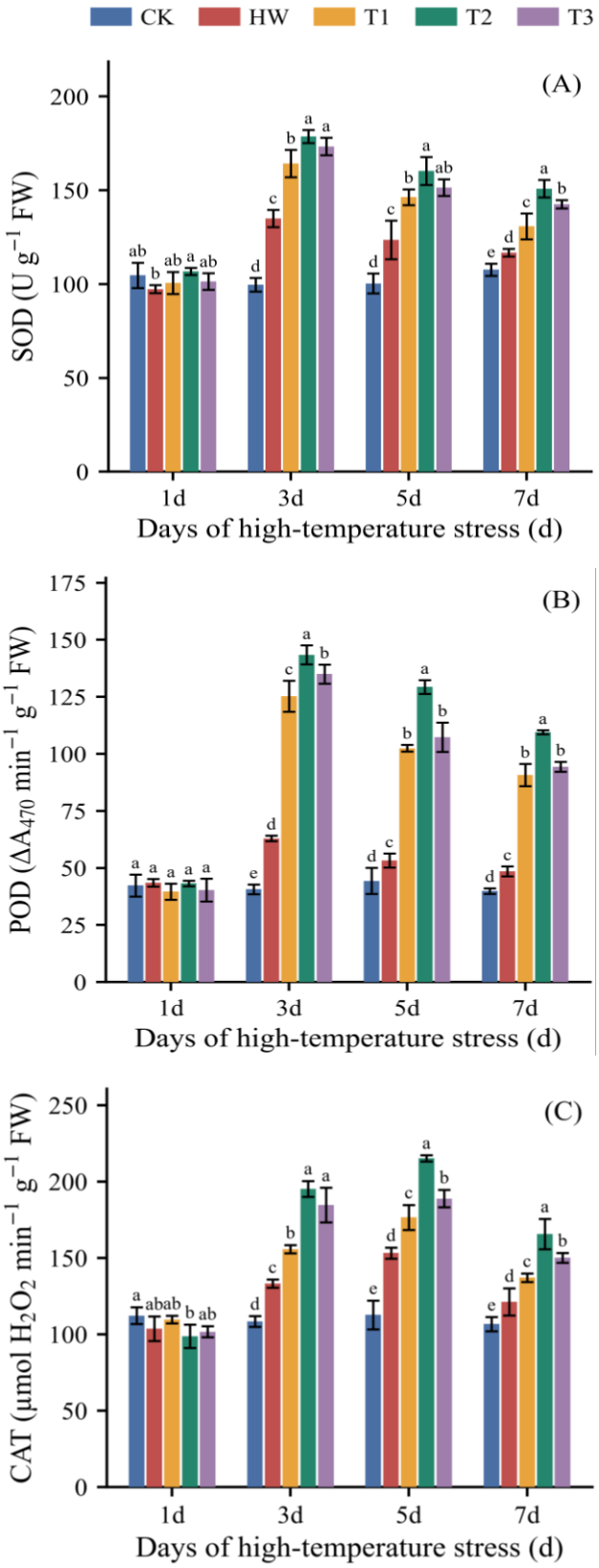


Fig. 6. Effects of exogenous spermidine at different concentrations on the antioxidant enzyme activities of strawberry seedling leaves under high-temperature stress. (A) Superoxide dismutase activity (SOD, U g^{-1} FW); (B) peroxidase activity (POD, ΔA470 min^{-1} g^{-1} FW); (C) catalase activity (CAT, μmol H_2O_2 min^{-1} g^{-1} FW). Bars are means ± SD (n = 3). Different lowercase letters above bars indicate significant differences among treatments at the same sampling time ($p<0.05$, one-way ANOVA followed by Duncan's multiple range test).

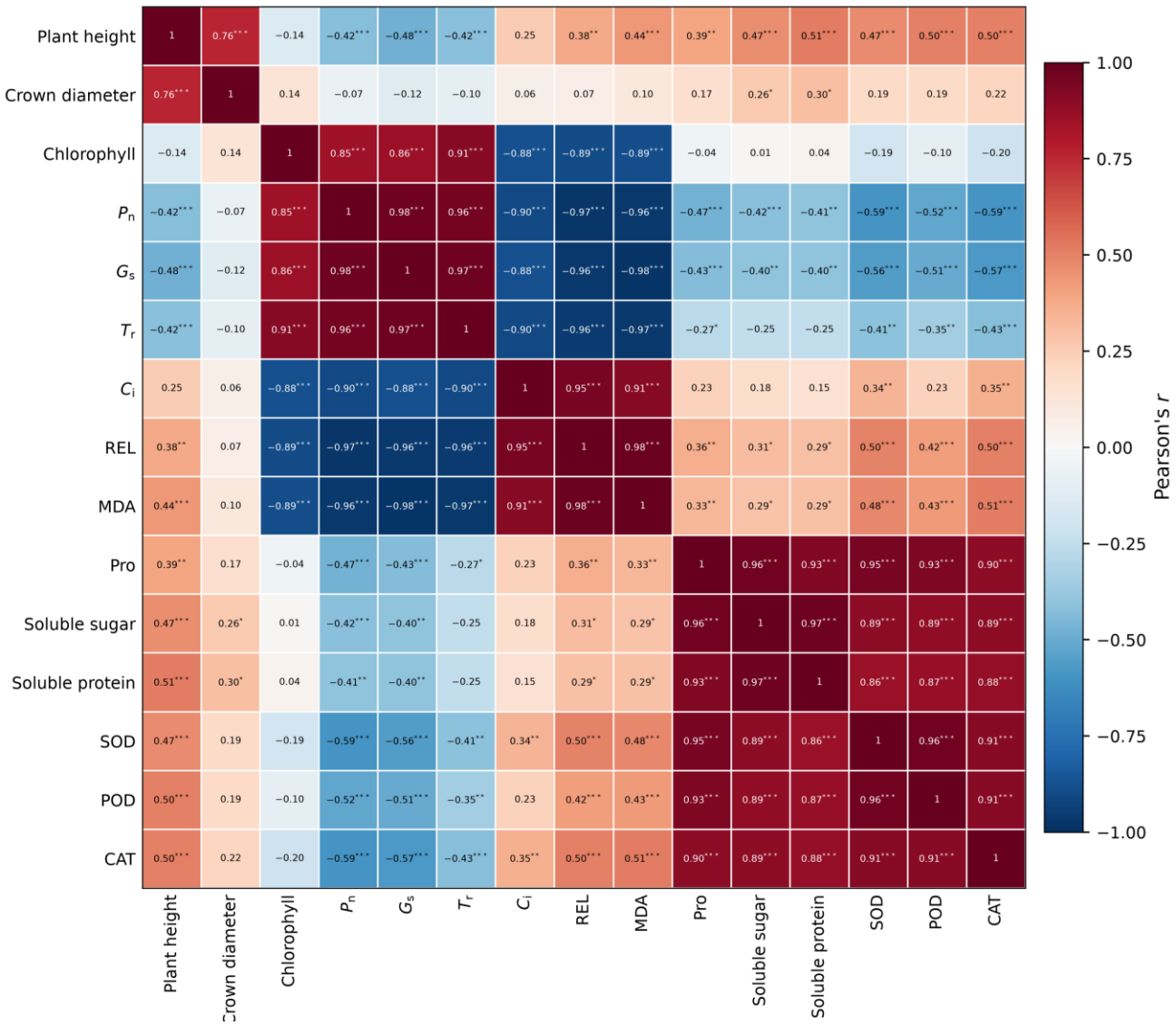


Fig. 7. Pearson correlation heatmap of 15 physiological indicators of strawberry seedlings under high-temperature stress (n = 60: 5 treatments × 4 sampling times × 3 biological replicates). Numbers in each cell are Pearson correlation coefficients (r), and asterisks indicate the significance of the correlation: * p<0.05, ** p<0.01, *** p<0.001. The 60 values are repeated measurements on the same plants, so these P values treat the observations as independent and are indicative only (see Results).

Table 5. Comprehensive scores and ranks of the five treatments.

Treatment	y_1	y_2	Comprehensive score	Rank
CK	1.79	−0.69	0.72	1
T2	0.09	1.49	0.69	2
T3	−0.44	0.60	0.01	3
T1	−0.38	−0.21	−0.31	4
HW	−1.06	−1.18	−1.11	5

y_1 and y_2 are the scores of the first and second principal components, and the comprehensive score = (53.59 y_1 + 40.74 y_2)/94.34, where the coefficients are the variance contribution rates of the first and second components and 94.34 is their sum (Table 4). A higher score indicates a better overall physiological status of the seedlings

Discussion

Strawberry is a cool-season crop with a narrow thermal optimum, and seedling-stage heat stress impairs establishment and yield (Ledesma *et al.,* 2004; Giampieri *et al.,* 2015). Foliar 1 mmol·L⁻¹ SPD alleviated this inhibition, 0.5 and 2 mmol·L⁻¹ being less effective (Tables 2-5), as in cucumber, lettuce and citrus (Wang *et al.,* 2018; Li *et al.,* 2020; Xu *et al.,* 2022) — a general feature of the SPD response.

Height growth was strongly reduced in HW, yet by 7 d HW plants were only 9.51% shorter than CK although *Pn* had fallen by 81.79% and chlorophyll by 56.46%. SPD restored both at 1 mmol·L⁻¹ (T2 9.89% taller than HW, not different from CK), consistent with the role of polyamines in stress tolerance (Shao *et al.,* 2022).

The gas-exchange data show that this inhibition was not imposed by stomatal closure: *Gs* fell from 3 d onwards, yet *Ci* never fell below CK and rose steadily (Fig. 1B), excluding CO_2 supply and pointing to the carboxylation capacity of the mesophyll. Three thermolabile steps could produce it: photosystem II and the electron-transport chain (Takahashi & Murata, 2008), the activation state of Rubisco (Crafts-Brandner & Salvucci, 2000), and peroxidation of thylakoid and envelope membranes, indicated by the parallel rises in REL and MDA, which uncouples electron transport from carbon fixation. SPD acted on this non-stomatal

component as well, raising *Pn* and *Gs* while lowering *Ci* and limiting membrane damage, as reported in drought-stressed faba bean (Abid *et al.,* 2022).

MDA, the end product of membrane lipid peroxidation, indicates cellular damage (Chen *et al.,* 2017); SPD slowed its accumulation most strongly at 1 mmol·L^{-1} and raised Pro, soluble sugar and soluble protein in parallel, so membrane protection and osmotic adjustment converged on the same concentration by 7 d.

The three osmolytes peaked at 3 d and then declined, while *Pn* and *Gs* fell further and *Ci* rose (Fig. 1). Two processes probably act together: compatible solutes are synthesized from photoassimilates, so once *Pn* collapses their substrates are exhausted; and the loss of soluble protein at 7 d, coinciding with a 170.89% rise in MDA, points to stress-induced proteolysis rather than a regulated decrease. The three behaved unequally: at 7 d soluble sugar had fallen 14.09% below CK and soluble protein 7.48%, whereas Pro remained 4.88% above it (Figs. 4, 5A, B). Because sugar is the direct product of photosynthesis and needs neither nitrogen nor extra energy to accumulate, whereas Pro and protein are derived from it at a cost of both, a sugar pool below the unstressed controls indicates a limited assimilate supply rather than a saturated capacity for solute synthesis. In T2 the three remained 57.01%, 86.45% and 53.66% above HW at 7 d, with the lowest REL and MDA, because net CO_2 assimilation was still 2.66 times that of HW and soluble protein never fell below its 3 d value, whereas in HW it fell below CK. Osmolyte accumulation in HW was therefore transient, and the optimum was distinguished by sustaining the pools rather than raising their peak: 2 mmol·L^{-1} peaked highest at 3 d and lost it thereafter, whereas 1 mmol·L^{-1} held the largest pools from 5 to 7 d, all above CK.

The SOD–POD–CAT system is the first line of defense against heat-generated reactive oxygen species (Jing *et al.,* 2020; Collado-González *et al.,* 2021), and foliar SPD raised all three, most strongly at 1 mmol·L^{-1} (at 7 d +29.19%, +125.77% and +36.78% over HW for SOD, POD and CAT). POD more than doubled while SOD rose by less than a third, an asymmetry expected when the superoxide flux is lower, since SOD acts upstream of POD and CAT (Shao *et al*., 2022). The enhancement agrees with rice (Tang *et al.,* 2018), citrus (Xu *et al.,* 2022) and lettuce (Li *et al.,* 2020).

Read against these studies, the effect sizes obtained here are of similar order but expressed in different variables. At 7 d, 1 mmol·L^{-1} SPD reduced the REL of HW by 30.45% and its MDA by 25.44%, offsetting roughly 40% of the heat-induced rise in lipid peroxidation, and raised *Pn* by 166.46%, more than the chlorophyll gain of 87.10%, so the carboxylation step recovered as well as the pigment pool. The mechanisms reported elsewhere differ: thylakoid stacking in cucumber (Wang *et al.,* 2018), glyoxalase and AsA–GSH systems in lettuce (Li *et al.,* 2020; Yu *et al.,* 2022), relieved growth inhibition in wheat and jujube (Jing *et al.,* 2020; Liu *et al.,* 2024). The thermal optimum of the crop is 15–25°C, and the mean day temperature here (37.5°C) lay 12.5°C above it while the night temperature (23.5°C) stayed within it, so exposure was interrupted rather than sustained — hence severe leaf-level injury with little height loss, whereas species held continuously above their limit lose biomass first, as in lettuce, where fresh weight fell by 82% (Li *et al.,* 2020).

The weaker performance of 2 mmol·L^{-1} SPD deserves comment. Exogenous polyamines are oxidatively deaminated by apoplastic polyamine oxidase, and the H_2O_2 produced can shift the balance from signalling towards oxidative stress at high dose (Chen *et al.,* 2019; Tyagi *et al.,* 2023); a high external concentration may also disturb endogenous polyamine homeostasis. Either mechanism predicts a non-monotonic response with an optimum, the shape observed: T3 lay between T1 and T2 for crown diameter, chlorophyll, all three enzymes and the comprehensive score. Endogenous polyamine contents were not determined, so this rests on the shape of the dose–response. These conclusions are bounded by a single heat-sensitive cultivar in one growth-chamber run, a continuous heat episode without recovery, and gravimetric rather than continuous control of water status; field validation with heat-tolerant cultivars and a recovery period is required before the dose can be recommended.

Conclusion

In conclusion, foliar spraying of exogenous SPD alleviated heat-induced growth inhibition in strawberry seedlings at all three concentrations tested, but the three differed in degree rather than in kind: the benefit increased from 0.5 to 1 mmol·L^{-1} and was smaller again at 2 mmol·L^{-1}, so that 1 mmol·L^{-1} is identified as the optimal concentration among those tested. At 1 mmol·L^{-1} this advantage was consistent across the three functional categories examined here — membrane integrity, osmotic adjustment and antioxidant defense — rather than restricted to any single indicator, and together these responses improved the heat tolerance of strawberry seedlings. The dose identified here is nevertheless specific to a single heat-sensitive cultivar under one continuous heat episode in a growth chamber, so confirmation under field or greenhouse conditions is required before it is recommended to growers.

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Author's Contributions: XINRAN SHEN conducted all the experiments, performed the data analysis and wrote the manuscript; CHUNLI LIU, LIN WANG and RENHUI ZUO provided the materials required for the experiments; QIUYANG LI and PENG CAO assisted with the preliminary data processing; MIAO GAO and JIZHONG WANG provided the project funding and supervised the study. All authors read and approved the final manuscript.

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