ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, online first, 1–15

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2026.5666

RESEARCH PAPER

Received: 7.02.2026
Accepted: 28.08.2026
Online first: 28.09.2026

PLANT GROWTH PROMOTER ENHANCED PHOTOSYNTHESIS AND FRUIT QUALITY IN STRAWBERRY. A CULTIVAR-SPECIFIC MULTIVARIATE ANALYSIS

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ABSTRACT

Spring protected strawberry production in northern China is constrained by elevated temperatures and narrowed diurnal range, yet cultivar-specific management strategies remain undefined. To identify optimized foliar treatments for three cultivars (Benihoppe, Yuexiu, White Snow Princess), plants were sprayed with S-abscisic acid (S-ABA), amino acids, jasmonate, or 5-aminolevulinic acid (5-ALA) at 10, 50, and 100 mg · L^{-1}. Growth, photosynthetic, and fruit quality traits were analyzed using two-way ANOVA, stepwise regression, OPLS-DA, and PLS-SEM. Cultivar was dominant for most traits. Optimal responses were achieved with 50 mg · L^{-1} 5-ALA for Benihoppe, S-ABA or 10 mg · L^{-1} 5-ALA for Yuexiu, and 10 mg · L^{-1} 5-ALA for White Snow Princess. PLS-SEM identified vegetative growth as the central driver of fruit colour (0.922) and fruit quality (0.845), while photosynthesis exerted a significant direct negative effect on growth (−0.709), revealing a resource-based tradeoff under elevated spring temperatures. Stepwise regression confirmed that 5-ALA promoted total soluble solids (TSS) accumulation more strongly than cultivar effects for Benihoppe, whereas high concentration (100 mg · L^{-1}) suppressed fruit weight. These findings support cultivar-specific precision management, emphasizing growth promotion over photosynthetic enhancement to sustain fruit quality under spring stress.

Keywords: *Fragaria × ananassa*, protected cultivation, elevated temperature, foliar plant promoter, 5-aminolevulinic acid, multivariate analysis

INTRODUCTION

In the major strawberry-producing regions of northern China, a recurring production issue characterised by diminished fruit flavour, soft texture, and a sharp decline in marketable yield, typically occurs from late March to early April. This phenomenon is primarily driven by spring heatwaves, triggered by abrupt temperature increases from late March onwards, and by a concurrent narrowing of the diurnal temperature range. As climate change intensifies, high-temperature conditions and reduced diurnal temperature variation have emerged as key climatic constraints on protected strawberry quality [Park et al. 2023]. Strawberries possess shallow, temperature-sensitive root systems, making them highly vulnerable to temperatures outside the optimal range [Thokchom et al. 2022, Akhtar et al. 2025]. Exposure to 35 °C induces significant physiological damage, while 40 °C causes severe injury, including disrupted photosynthesis, hormonal imbalance, and oxidative stress [Manafi et al. 2021, Akhtar et al. 2025]. Moreover, rising nocturnal

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temperatures in spring consistently reduce the protected diurnal temperature range to below 8 °C. This condition, wherein high daytime temperatures suppress photosynthesis and elevated nighttime temperatures increase respiratory consumption, creates a compounded stress that ultimately reduces sugar content, increases acidity, impairs colour development, and decreases fruit size [Menzel 2022, Ullah et al. 2024]. Effective mitigation strategies are therefore essential for the sustainability of protected strawberry production.

Foliar application of bioactive compounds offers a rapid and targeted approach for enhancing plant stress tolerance. Beyond nutritional supplementation, biostimulants and plant growth regulators, such as melatonin, nitric oxide, and calcium, have shown promise in alleviating heat-induced damage in strawberries by activating antioxidant defences or inducing heat shock protein expression [Manafi et al. 2021, Wang et al. 2025]. Nevertheless, critical knowledge gaps persist. First, most studies have evaluated single compounds under controlled environments [Bieniasz et al. 2022], with limited systematic comparison of different signalling molecules under realistic stress conditions. Second, it remains unclear whether compounds such as S-abscisic acid (S-ABA), jasmonic acid, and 5-aminolevulinic acid (5-ALA) primarily enhance source capacity and protect photosynthetic systems, or whether they compensate for reduced diurnal temperature range by suppressing nocturnal respiration and reducing metabolic consumption. More broadly, their dominant physiological targets whether "source" (photosynthetic assimilation and partitioning), "sink" (fruit metabolism and growth), or cellular protection (antioxidant and osmotic regulation) have yet to be resolved [Garrido-Bigotes et al. 2017, Li et al. 2022, Zhong et al. 2025], impeding the development of physiologybased precision management. Third, although strawberry cultivars differ genetically in heat tolerance, the interaction between genotype and treatment remains poorly understood, limiting the transferability of management strategies across cultivars.

To address these gaps, a protected experiment was conducted from 13 March to 10 April 2024, a period encompassing the complete sequence of three thermal phases: an optimal window, a critical discomfort phase, and a fully unsuitable phase. Three representative cultivars Benihoppe, Yuexiu, and White Snow Princess were selected to compare the regulatory effects of foliar sprays of S-ABA, amino acid fertiliser, jasmonic acid, and 5-ALA. Using multivariate statistical approaches [Al-Madhagi et al. 2018], including Two-way analysis of variance, stepwise regression, orthogonal partial least squares discriminant analysis (OPLS-DA), and partial least squares structural equation modelling (PLS-SEM) we aimed to identify the primary physiological targets through which these signalling molecules compensate for combined lowdiurnalrange and hightemperature conditions, and to determine cultivar-specific optimal foliar management strategies. A PLS-SEM approach was employed to elucidate the causal relationships among photosynthesis, vegetative growth, fruit colour, and fruit quality. The findings are expected to provide a theoretical basis for improving stress resilience in spring protected strawberry production.

MATERIALS AND METHODS

Plant materials and experimental site. The experiment was conducted in March–April 2024 in strawberry production facilities under protected cultivation at the Henan Modern Agricultural Research and Development Base (35 °00'19" N, 113 °42'16"E). The trial period, spanning 13 March to 10 April (four weeks), coincided with the natural spring warming phase. According to data from the Yuanyang Meteorological Station, outdoor temperatures ranged from 0–24 °C in mid-March to 8–26 °C in early April, with 18 sunny days recorded during this interval. Internal protected temperature logs indicated that daily maximum temperatures reached or exceeded 35 °C on 11 days, while nocturnal temperatures consistently remained above 12 °C. The diurnal temperature amplitude progressively narrowed from over 10 °C to less than 8 °C over the course of the study. This period comprehensively covered three distinct thermal phases: a suitable window, a critical transition period, and a fully unsuitable phase. Three commercially important strawberry cultivars: Benihoppe, Yuexiu, and White Snow Princess were selected for this study. All cultivars were grown under the same protected cultivation conditions with identical mulching and uniform agronomic management throughout the experimental period.

Experimental design and plant promoter. A completely randomized block design with three replicates was employed. Seven foliar fertilizers were applied, including a water-sprayed control (CK) (Table 1).

Foliar sprays were applied to runoff on four dates (March 13, 20, 27, and April 3, 2024). Each application was performed at a volume sufficient to thoroughly wet both leaf surfaces to the point of imminent run-off. All other agronomic practices were kept uniform across treatments. Growth parameters were recorded on 10 April (after five weeks from first application). Fruits from the retained cohort, those that had developed to marketable maturity, were harvested on the same date for quality assessment.

Table 1. Description of foliar plant promoter applied in the experiment

Order number	Plant promoter description	Commercial source
T1	S-abscisic acid (S-ABA; 10% active ingredient), 1000× dilution	Jiangxi New Reward Biochemical Co., Ltd., China
T2	amino acid water-soluble fertilizer (amino acids ≥100 g · L^{-1}; Mn + Zn ≥ 20 g · L^{-1}), 500× dilution	Hebei Zhongbao Lvnong Crops Technology Co., Ltd., China
T3	propyl jasmonate-based foliar fertilizer (jasmonic acid propyl ester ≥100 mg · L^{-1}), 500× dilution	Hebei Zhongbao Lvnong Crops Technology Co., Ltd., China
T4	5-aminolevulinic acid (5-ALA), 10 mg · L^{-1}	Shanghai Macklin Biochemical Co., Ltd., China (purity ≥99%)
T5	5-aminolevulinic acid (5-ALA), 50 mg · L^{-1}	
T6	5-aminolevulinic acid (5-ALA), 100 mg · L^{-1}	
CK	water spray (control)	

Growth measurements. For each experimental unit, ten plants were selected. Prior to the initial application, uniform seedlings with consistent growth status and similar size were selected and labelled to minimize initial plant differences among treatments [Al-Madhagi et al. 2019]. All developed fruits were removed, and only uniform flower buds and young fruitlets (within 7 days after petal fall) were retained.

On April 10 (five weeks after the first application), leaf length, leaf width, petiole length, and petiole diameter were recorded on five randomly chosen plants per experimental unit. All leaf-related morphological parameters were measured on fully expanded functional leaves located in the middle layer of each plant, following a unified sampling standard across all treatments. Considering the short experimental period (28 days), it was difficult to accurately distinguish whether the fully expanded functional leaves emerged before or after foliar spraying; therefore, the uniform middle-layer functional leaf sampling method was adopted to ensure data comparability among different treatments. Given that all plants were selected for uniformity in size and growth status prior to treatment initiation, differences in leaf morphology among treatments after 28 days can be reasonably interpreted as treatment effects on leaf expansion. A digital calliper (Model 500-196-30, Mitutoyo, Japan) was used for petiole diameter measurements.

Leaf photosynthetic characteristics. On the same day (April 10, 09:00–12:00), net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were measured on one functional leaf per plant (three randomly chosen plants per experimental unit) using an LI-6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). The leaf chlorophyll index (SPAD value) was determined on all ten plants per experimental unit using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan).

Fruit colour indices. On the same date (April 10), fruit colour was measured using a spectrophotometric colourimeter (CR-400, Konica Minolta, Inc., Tokyo, Japan) on the same ten commercially mature fruits per experimental unit used for quality assessment. The L*, a*, b*, C*, and h° values were obtained, and the total colour difference (ΔE) relative to the control was calculated.

Fruit quality attributes. On the same date (April 10), for each unit experiment, ten commercially mature fruits were analyzed. Single-fruit weight was recorded (BSA423S-CW, Sartorius, Germany; 0.01 g pre-

cision). Firmness was measured using a penetrometer (GY-4, Landian, China) with a 3.5-mm probe. Total soluble solids (TSS) content was determined with a digital refractometer (PAL-1, Atago, Japan). Titratable acidity (TA) and Vitamin C (Vc) content were analysed by automatic potentiometric titration using a ZDJ-4B titrator (Shanghai Yidian Scientific Instrument Co., Ltd., China). The TSS/TA ratio was calculated.

Data analysis. Data were analyzed by Two-way ANOVA with cultivar (three levels: Benihoppe, Yuexiu, and White Snow Princess) and plant promoter (Pp) (seven levels: CK, T1–T6) as fixed factors, including their interaction, followed by Tukey's HSD post-hoc tests ($P < 0.05$). When a significant interaction was detected, simple effects analysis was conducted separately for each cultivar. Stepwise regression was performed to identify key predictors among cultivar dummy variables, plant promoter dummy variables, and 5-ALA concentration (entry/removal: $P < 0.05$/ $P > 0.10$). All data are presented as means ± sSEM. SPSS Statistics was used for ANOVA and regression; OPLS-DA was performed with SIMCA-P (v14.1); PLS-SEM was constructed using SmartPLS 4.0; Figures were generated with Origin 2024.

RESULTS

Table 2. Two-way ANOVA and multiple comparison results for various traits of strawberry under cultivar, plant promoter and their interaction

Indicator	Cultivar (C)	Plant promoter (Pp)	C × Pp	Cultivar of multiple comparisons	plant promoter of multiple comparisons
	F	*F*	*F*	(Y) (W)	CK T1 T2T3 T4 T5 T6
Leaf length (cm)	90.884***	7.275***	3.671**	A A B	C AB A BC AB AB BC
Leaf width (cm)	26.083***	3.671**	1.995	A A B	B AB A AB AB B B
Petiole diameter (mm)	86.186***	4.51**	3.575**	A B C	B AB A A AB A A
Petiole length (cm)	125.205***	1.975	1.802	A B C	B AB A AB AB AB AB
Pn (μmol · m^{-2} · s^{-1})	32.438***	2.858*	3.022**	A B B	B B AB AB AB AB A
Gs (mol · m^{-2} · s^{-1})	36.65***	1.756	1.967	C B A	A A A A A A A
Ci (μmol · mol^{-1})	16.261***	3.827**	2.861**	B B A	B AB A A A AB AB
Tr(mmol · m^{-2} · s^{-1})	40.145***	3.763**	4.189***	C B A	C BC ABC ABC AB ABC A
SPAD	95.88***	69.452***	47.263***	B C A	CD EF B F DE BC A
L*	6791.766***	1.373	1.790	C B A	A A A A A A A
a*	4430.718***	3.225*	1.774	A B C	AB AB B A AB AB AB
b*	865.259***	2.717*	2.385*	A B C	A A A A A A A
C*	3633.282***	3.552*	2.161*	A B C	AB ABC C A ABC BC ABC
h°	1479.192***	2.369*	1.441	B B A	AB AB A AB B AB AB
ΔE	0.57	2.457*	1.406	A A A	B AB AB AB A AB AB
TA (%)	295.841***	8.252***	6.089***	A A B	A A A B A A A
TSS (%)	798.689***	127.748***	105.793***	A B C	C CD E B D A E
TSS/TA	104.001***	16.17***	12.191***	B C A	BCD CD D A BC B CD
Firmness (kg · cm^{-2})	212.393***	10.287***	33.335***	B A B	AB CD ABC BCD A ABC D
Single fruit weight (g)	1170.587***	327.889***	31.129***	A B A	C B B A B A A
Vc (mg · 100 mL^{-1})	916.238***	6.325***	14.143***	A B C	BC C BC BC AB A BC

Note: Values are F-statistics; *, **, and *** indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; (H) = Benihoppe, (Y) = Yuexiu, (W) = White Snow Princess; multiple comparisons for both cultivar and plant promoter were performed using Tukey's HSD test ($P < 0.05$); in the multiple comparison columns, uppercase letters are ordered alphabetically from highest to lowest mean value within each row (A > B > C); different letters within the same row indicate significant differences, whereas the same letter indicates no significant difference; plant promoter levels codes: CK = water control, T1 = S-abscisic acid, T2 = amino acid fertilizer, T3 = propyl jasmonate, T4 = 10 mg · L^{-1} 5-ALA, T5 = 50 mg · L^{-1} 5-ALA, T6 = 100 mg · L^{-1} 5-ALA

Vegetative growth. Two-way ANOVA revealed that cultivar had highly significant effects on leaf length, leaf width, petiole diameter, and petiole length ($P < 0.001$). Plant promoter significantly affected leaf length, leaf width, and petiole diameter ($P < 0.01$), but not petiole length ($P > 0.05$). The cultivar × promoter interaction was significant for leaf length and petiole diameter ($P < 0.01$), but not for leaf width or petiole length (Table 2). In Figure 1, in Benihoppe, T2 exhibited the most pronounced growthpromoting effects, increasing leaf length, leaf width, and petiole length by 35.5%, 20.6%, and 17.7%, respectively (P < 0.05); T3 also significantly increased petiole diameter by 20.7% ($P < 0.05$). In Yuexiu, T1 significantly enhanced leaf length and petiole length (+25.7–25.9%, $P < 0.05$), while T2 significantly increased leaf width (+32.1% $P < 0.05$). In White Snow Princess, T5 and T6 promoted petiole growth, particularly increasing petiole diameter and petiole length. Overall, optimal growth-promoting treatments were cultivar-specific: T2 for Benihoppe, T1 for Yuexiu, and T5/T6 for White Snow Princess.

Photosynthetic characteristics. Two-way ANOVA revealed that cultivar had highly significant effects on Pn, Gs, Ci, Tr, and SPAD ($P < 0.001$). Plant promoter significantly affected Pn ($P < 0.05$), Ci and Tr ($P < 0.01$), and SPAD ($P < 0.001$), but not Gs ($P > 0.05$). The cultivar × promoter interaction was significant for Pn, Ci, Tr, and SPAD ($P < 0.01$ to $P < 0.001$), but not for Gs (Table 2). In Figure 2, in Benihoppe, T2–T6 increased Pn, peaking

Figure 1. Growth parameters of three strawberry cultivars under seven plant promoter levels (CK and T1–T6): a) leaf length, b) leaf width, c) petiole diameter, d) petiole length. CK, water control; T1, S-ABA; T2, amino acids; T3, propyl jasmonate; T4–T6, 10, 50, and 100 mg · L^{-1} 5-ALA, respectively. Data are means ± SE. Different lowercase letters within each cultivar indicate significant differences among plant promoter levels based on one-way ANOVA followed by Tukey's HSD test ($P < 0.05$). The same applies to subsequent figures

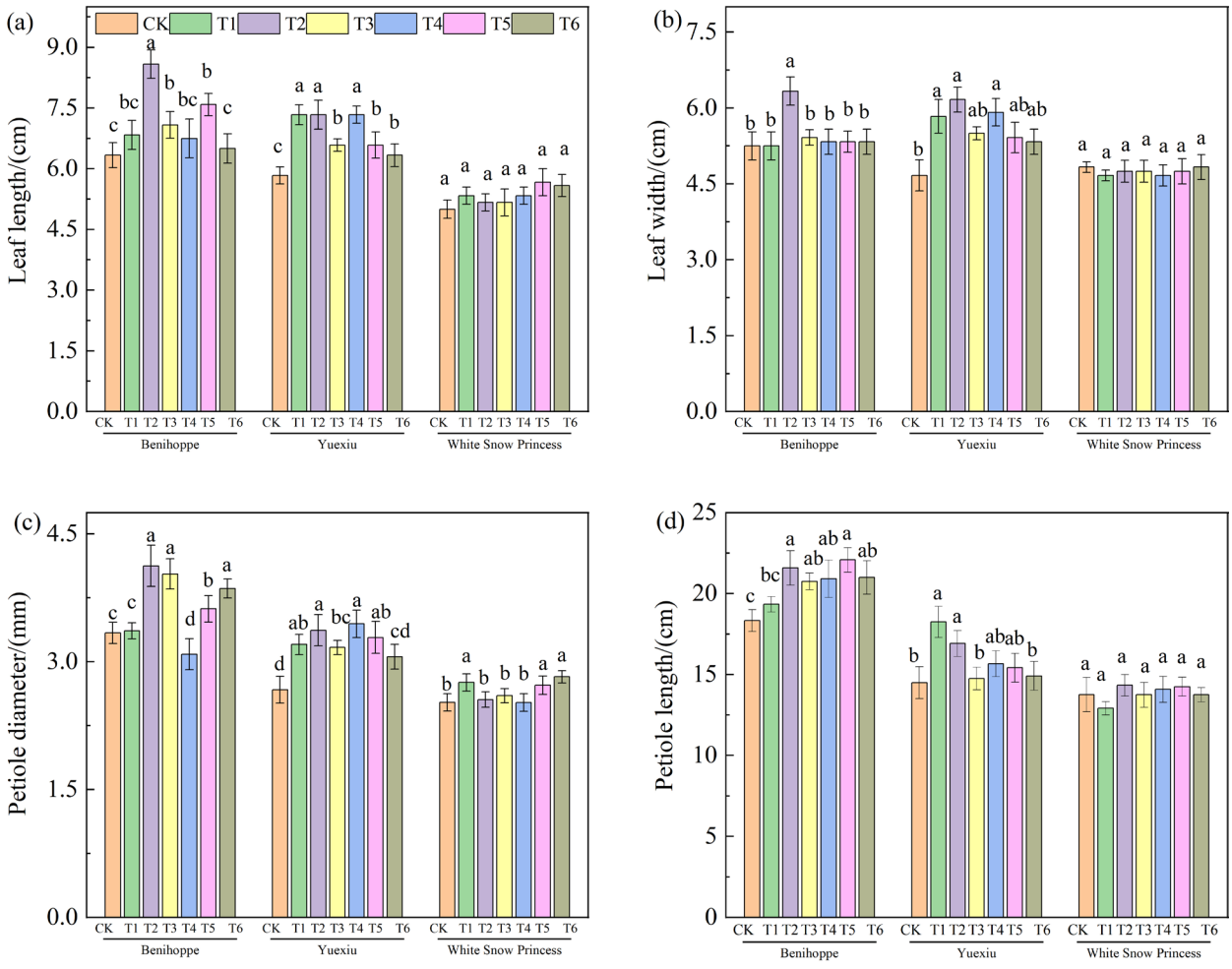


Figure 2. Photosynthetic characteristics and leaf SPAD readings of three strawberry cultivars under various foliar plant promoters (CK, T1–T6): a) net photosynthetic rate, b) intercellular CO_2 concentration, c) stomatal conductance, d) transpiration rate, e) SPAD value

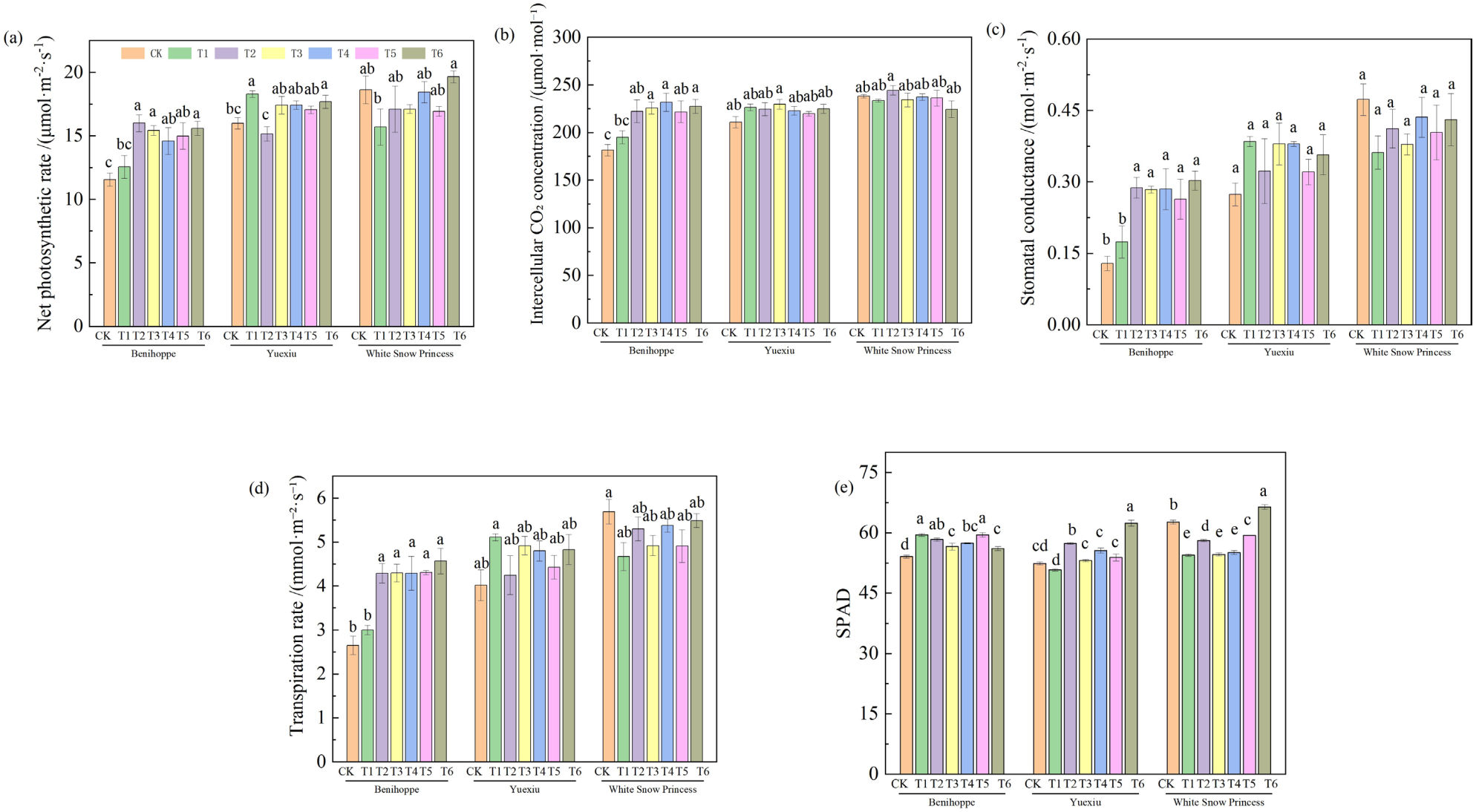
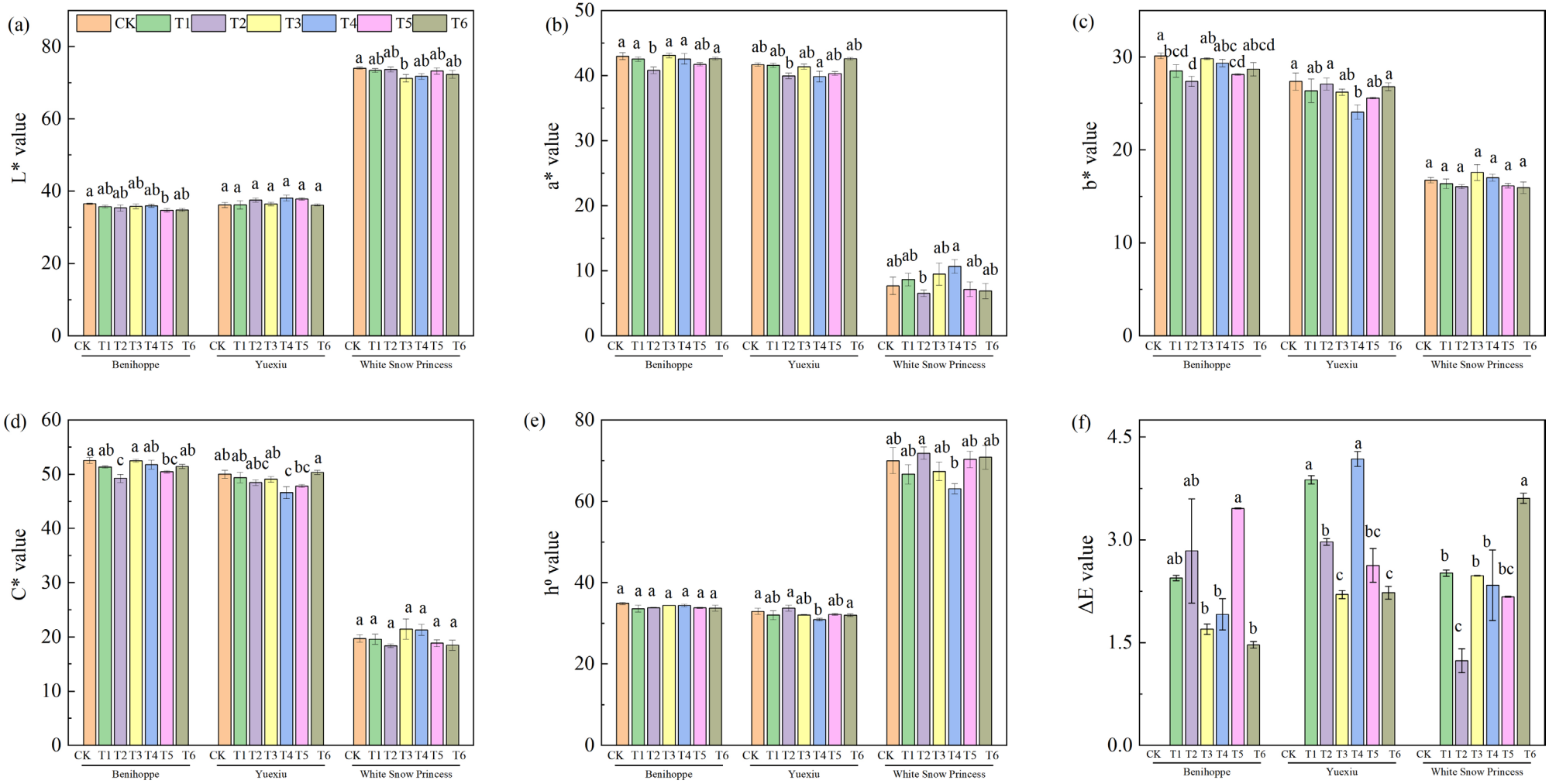


Figure 3. Fruit colour attributes of three strawberry cultivars under six foliar biostimulant treatments (T1–T6) and water control (CK): a) L*, b) a*, c) b*, d) C*, e) h°, f) ΔE



under T2 (+38.5%, $P < 0.05$); T6 produced highest Gs and Tr. T3, T4, and T6 elevated Ci (+24.4–27.7%, $P < 0.05$). SPAD peaked under T1 and T5, exceeding the control; T6 (56.11) also exceeded the control ($P < 0.05$). In Yuexiu, T1 and T6 increased Pn (+14.4% and +10.6%, $P < 0.05$); T6 gave highest SPAD (62.43, +19.2%, $P < 0.05$); T1 reduced SPAD ($P < 0.05$). In White Snow Princess, T6 had the highest Pn (19.66), but not significantly; T1 suppressed Pn (−15.7%, $P < 0.05$). T6 gave highest SPAD (66.45, +6.0%, $P < 0.05$); T1, T3, and T4 reduced SPAD (−13.1% to −12.1%). Responses were cultivar-specific, with 5-ALA effects on SPAD varying by concentration.

Fruit colour attributes. Two-way ANOVA revealed that cultivar had highly significant effects on L*, a*, b*, C*, and h° ($P < 0.001$), but not on ΔE (Table 2). Plant promoter significantly affected a*, b*, C*, h°, and ΔE ($P < 0.05$), but not L* ($P > 0.05$). No significant cultivar × plant promoter interactions were detected for any colour parameter (Table 2). For a*, post-hoc comparisons revealed that a* was significantly lower under T2 than under T3 (Table 2). In Figure 3, in Benihoppe, T5 gave greatest ΔE (3.46), reducing L* (−5.0%) and b* ($P < 0.05$); T2 reduced a* and C* (−5.1% and −6.3%, $P < 0.05$). T1, T3, T4, and T6 showed smaller ΔE (1.47–2.45) without significant changes. In Yuexiu, T4 gave highest ΔE (4.18), reducing a, b, and C* (−4.5%, −12.1%, −6.8%, $P < 0.05$). T1 (ΔE = 3.88) affected no single parameter significantly. In White Snow Princess, T6 gave highest ΔE (3.61); T4 increased a* (+38.4%, $P < 0.05$), indicating reddening; T3 reduced L* (−3.8%) and T4 reduced h° (−9.9%) ($P < 0.05$). No significant changes were observed in b* or C*. Responses were cultivar-specific, with white-fruited White Snow Princess showing marked reddening against a low red baseline.

Fruit quality attributes. Two-way ANOVA revealed that cultivar had highly significant effects on TA, TSS, TSS/TA, firmness, single fruit weight, and Vc ($P < 0.001$). Plant promoter also significantly affected all these traits ($P < 0.001$ to $P < 0.01$). The cultivar × promoter interaction was significant for all quality traits ($P < 0.001$) (Table 2). For traits with significant interactions, simple effects analysis was performed to examine promoter effects within each cultivar. In Figure 4, in Benihoppe, T5 performed best, increasing Vc, firmness, single fruit weight, and TSS by 3.8%, 18.8%, 26.2%, and 13.4%, respectively ($P < 0.05$). T3 improved TSS/TA ratio (+19.6%) and fruit weight (+31.7%, $P < 0.05$), with TSS slightly below T5. T6 had the highest Vc and single fruit weight but reduced TSS by 11.9% ($P < 0.05$). In Yuexiu, T3 increased TSS/TA (+7.8%, $P < 0.05$); T5/T6 raised TSS (+2.1%, $P < 0.05$); T2/T4 enhanced firmness (+24.7%, $P < 0.05$) but reduced TSS; T1 maximised fruit weight (+29.4%, $P < 0.05$). In White Snow Princess, T4 gave highest TSS/TA (20.07) and lowest TA (0.42%, $P < 0.05$); T3 and T6 maximised fruit weight (+31.7%, $P < 0.05$); T2 had highest Vc (+7.1%, $P < 0.05$); all treatments reduced firmness, with T1 lowest (−21.9%, $P < 0.05$).

OPLS-DA using all measured variables. showed excellent predictive ability, with $R^2Y = 0.845$ and $Q^2 = 0.805$ after 200 permutation tests (Figure 5). The first two principal components explained 73.79% of total variance. The 95% confidence ellipses of the three cultivars were completely separated, with no overlap, and replicates clustered tightly, confirming stable cultivar-specific differences.

Using VIP > 1 as a threshold, the top discriminative variables were petiole length (2.235), single fruit weight (2.120), intercellular CO_2 concentration (Ci) (1.725), Vc (1.607), and Pn (1.478). Variables with VIP < 0.5 were considered to have negligible contribution to cultivar separation, including TA (0.004), leaf width (0.058), a* (0.251), Gs (0.281), fruit firmness (0.313), leaf length (0.315), L* (0.322), TSS (0.353), fruit hue angle h° (0.376), TSS/TA (0.397), and total color difference ΔE (0.499).

Path analysis via structural equation modelling (SEM). PLS-SEM analysis indicated that the constructed "photosynthesis–growth–fruit colour–fruit quality" model exhibited strong explanatory power, with R^2 values of 0.303, 0.908, and 0.811 for growth, fruit colour, and fruit quality, respectively (Figure 6). Path analysis revealed that photosynthesis exerted a significant direct negative effect on growth (standardised path coefficient = −0.709, $P < 0.05$), suggesting a resource-based trade-off between these two processes. Growth had a strong positive direct effect on fruit colour (0.922, $P < 0.05$) and emerged as the primary driver of colour development. By contrast, photosynthesis showed no significant direct effect on fruit colour (−0.086), exerting its influence only indirectly through growth. Fruit quality was regulated through multiple synergistic pathways.

Figure 4. Fruit quality attributes of three strawberry cultivars treated with six foliar biostimulants (T1–T6) and water control (CK): a) titratable acidity, b) total soluble solids, c) vitamin C, d) TSS/TA ratio, e) fruit firmness, f) single fruit weight

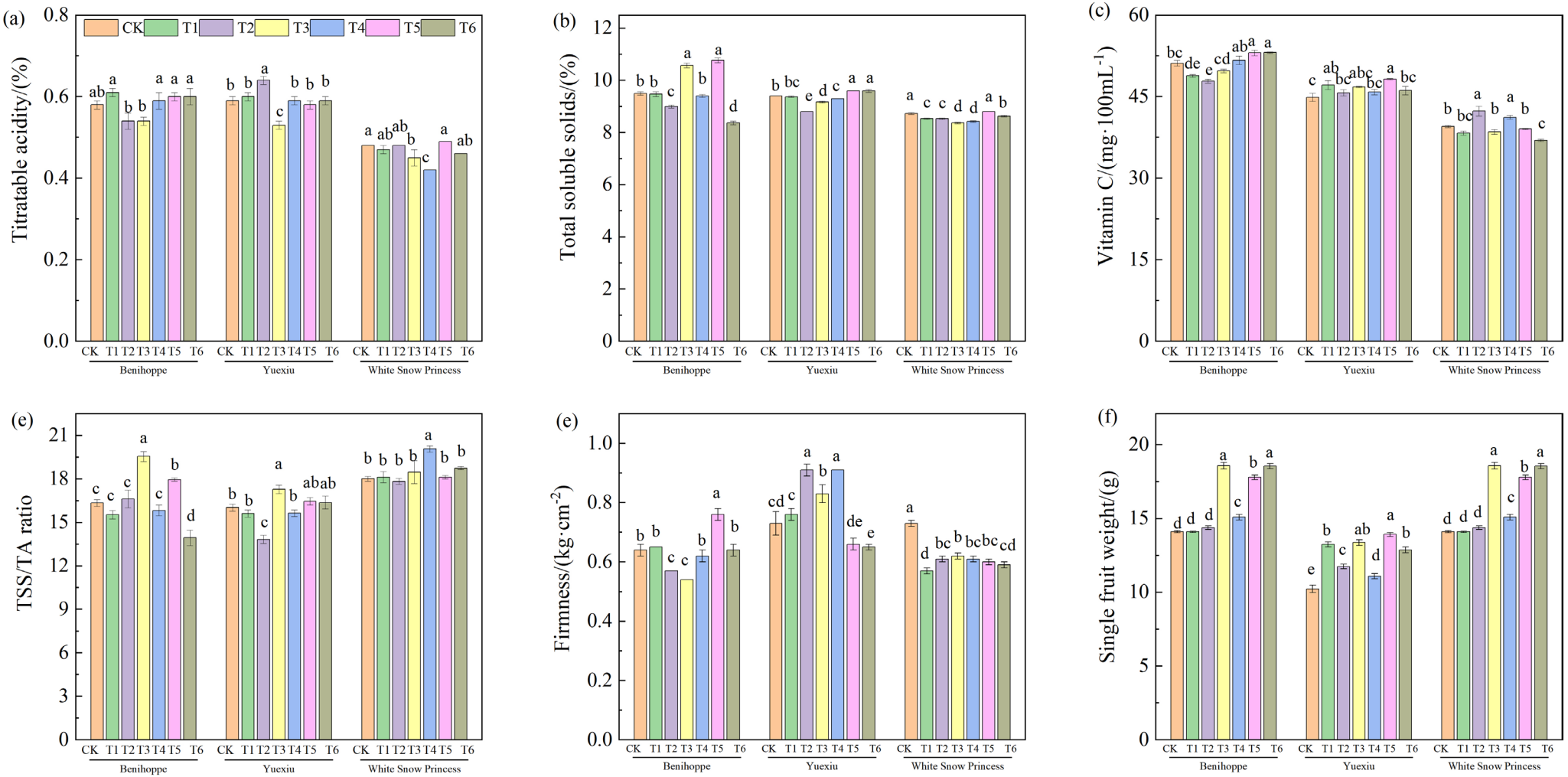


Figure 5. Orthogonal partial least squares discriminant analysis (OPLS-DA) of integrated vegetative, photosynthetic, and fruit quality traits for three strawberry cultivars (Benihoppe, Yuexiu, and White Snow Princess): a) score plot with 95% confidence ellipses showing distinct clustering of each cultivar; b) permutation test (200 random permutations) indicating no overfitting; c) S-plot combined with VIP scores identifying variables with VIP > 1 as key discriminative traits. Variables with VIP > 1 are highlighted in red

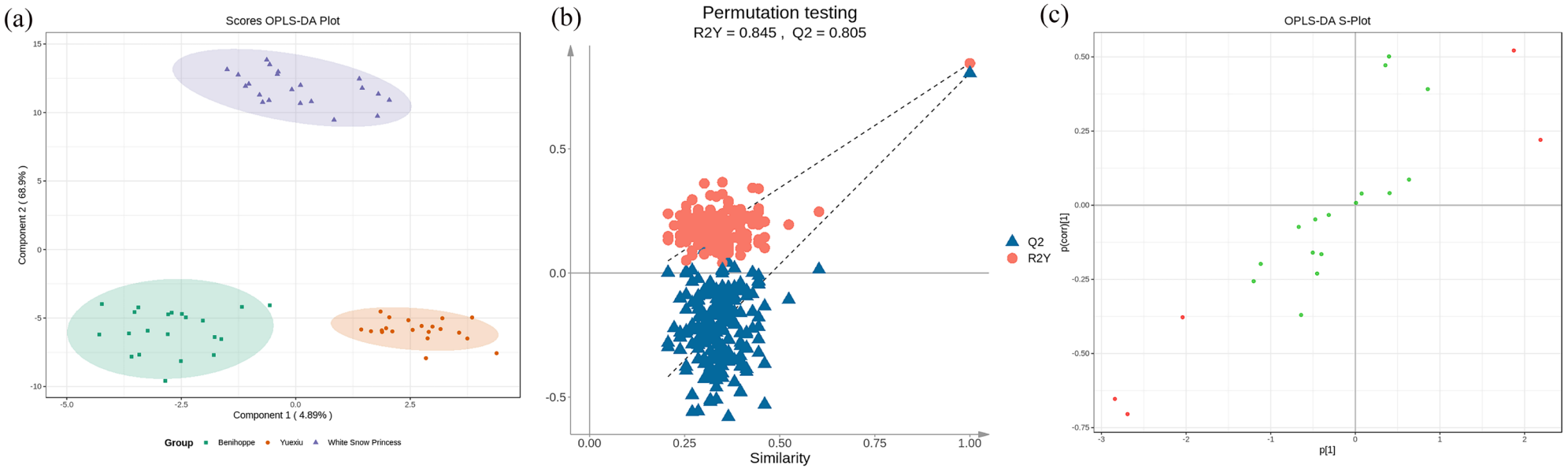


Figure 6. Path diagram of the partial least squares structural equation model (PLS-SEM) illustrating relationships among photosynthesis, vegetative growth, fruit colour, and fruit quality under elevated spring temperatures. Standardised path coefficients are shown; solid lines indicate significant effects ($P < 0.05$), and dashed lines indicate nonsignificant effects. R^2 values for endogenous variables are displayed in parentheses

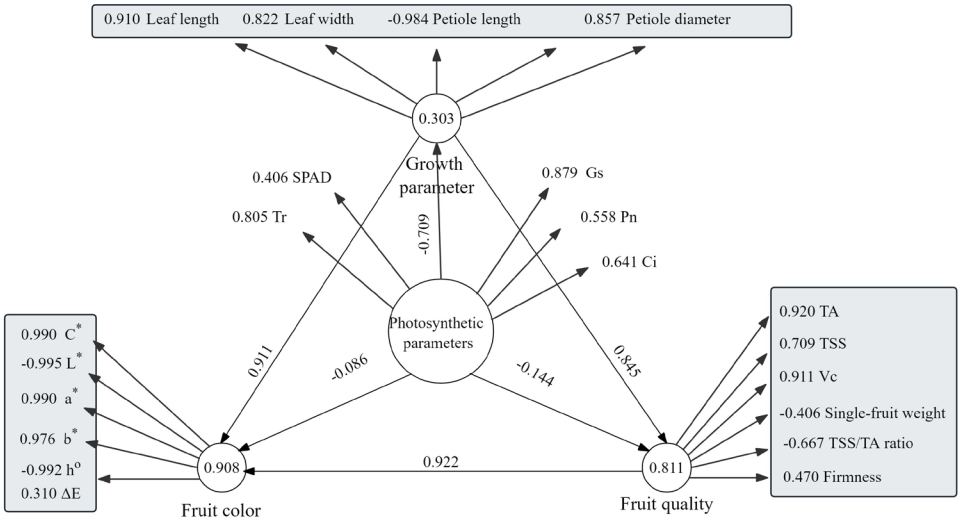


Table 3. Stepwise regression analysis of vegetative, photosynthetic, and fruit quality traits in response to cultivar, foliar treatments, and 5-aminolevulinic acid (5-ALA) concentration

Dependent variable	Variables entered	Final *β*	R^2	Model *P*
Leaf length	H, Y, T5, T4	0.782, 0.630, 0.350**, 0.279*	0.606	< 0.001
Leaf width	H, Y	0.845, 0.476	0.510	< 0.001
Petiole diameter	H, Y, 5-ALA	0.845, 0.476, 0.311**	0.601	< 0.001
Petiole length	Y	0.876***	0.760	< 0.001
SPAD	T6, H, Y	0.523, −0.549, −0.468**	0.494	< 0.001
Pn	H, 5-ALA, Y, T4	−0.864, 0.385**, −0.281, 0.236*	0.669	< 0.001
Gs	H, Y	−0.852*, −0.460**	0.518	< 0.001
Ci	—	—	—	>0.05
Tr	H, Y	−0.770*, −0.462**	0.418	< 0.001
L*	H, Y	−1.018, −0.975**	0.995	< 0.001
a*	H, Y	1.015, 0.974**	0.990	< 0.001
b*	H, Y	1.084, 0.816**	0.954	< 0.001
C*	H, Y, T5	1.038, 0.944**, −0.036*	0.989	< 0.001
h°	Y, H, T4	−1.015, −0.954, −0.073	0.976	< 0.001
ΔE	T4	0.408*	0.142	0.013
TA	H, Y	0.963, 0.916**	0.878	< 0.001
TSS	T5, H, Y	0.458, 0.628, 0.603*	0.550	< 0.001
TSS/TA	H, Y	−0.782, −0.751**	0.564	< 0.001
Firmness	Y, 5-ALA	0.451, −0.351*	0.285	< 0.001
Single fruit weight	Y, 5-ALA, T6	−0.763, 1.059, −0.561***	0.970	< 0.001
Vc	H, Y, T5	1.115, 0.605, 0.093*	0.939	< 0.001

Note: Values are standardized regression coefficients (β). (H) = Benihoppe, (Y) = Yuexiu; T4, 10 mg · L^{-1} 5-ALA; T5, 50 mg · L^{-1} 5-ALA; T6, 100 mg · L^{-1} 5-ALA; 5-ALA, 5-aminolevulinic acid concentration as a continuous variable (0, 10, 50, 100 mg · L^{-1}). Only variables that met the entry criterion ($P < 0.05$) are shown; "—" indicates no variable entered the model. Reference levels: White Snow Princess for cultivar; CK (water control) for plant promoter levels. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Adjusted R^2 values are reported

Stepwise regression. confirmed cultivar was the predominant factor for most traits (Table 3), with cultivar dummy variables entering all final models, predominantly at the first step. Colour parameters (L*, a*, b*, C*, h°) showed adjusted $R^2 > 0.95$, indicating near-complete cultivar dependence for these traits. For ΔE, only T4 was retained ($\beta = 0.408$, $P = 0.013$, $R^2 = 0.142$).

5-ALA effects were trait-specific. For TSS, T5 entered first ($\beta = 0.458$), preceding cultivar, confirming promoter effect exceeded cultivar effect. In single fruit weight, Yuexiu ($\beta = -0.763$), 5-ALA concentration ($\beta = 1.059$), and T6 ($\beta = -0.561$) were retained ($R^2 = 0.970$), but 5-ALA concentration and T6 were negatively correlated ($r = -0.88$), suggesting high concentration suppressed fruit weight. SPAD: T6 entered first ($\beta = 0.523$), T5 not retained. Firmness: only Yuexiu (β=0.451) and 5-ALA concentration ($\beta = -0.351$) were retained ($R^2 = 0.285$). T5 was retained in Vc , but effects were smaller than cultivar effects.

For Gs, Tr, TA, TSS/TA, leaf width, petiole length, and most colour parameters (L*, a*, b*), only cultivar variables were retained. Petiole diameter additionally included 5-ALA concentration. No predictors entered the Ci model.

DISCUSSION

This study examined cultivar-specific responses to foliar biostimulants under spring protected conditions of reduced diurnal temperature range (< 8 °C) and episodic daytime high temperatures (≥35 °C). The key findings, cultivar dominance, broad-spectrum 5-ALA effects with concentration dependency, and a photosynthesis–growth trade-off are discussed below in relation to their physiological mechanisms and implications for precision management.

Cultivar effect. Cultivar was the primary determinant of trait variation, with colour and quality traits almost entirely cultivar-dependent (adjusted $R^2 > 0.95$). This aligns with Kesici et al. [2020], who linked heat tolerance differences to genetic variation in heat shock proteins, antioxidant enzymes, and hormone signalling. The broad response of Benihoppe to multiple treatments suggests greater photosynthetic flexibility, whereas the specific response of Yuexiu to S-ABA may reflect an efficient ABA signalling network, consistent with the observations of Fagherazzi et al. [2021] that initial crown diameter – a proxy for plant vigour – strongly influences yield and quality in strawberry. OPLS-DA completely separated the three cultivars, with VIP analysis identifying traits spanning morphology, fruit quality, and photosynthesis as core discriminators. Notably, T4 entered the stepwise model, indicating colour difference between white-fleshed White Snow Princess and red-fruited cultivars is primarily treatment-induced. This is consistent with Ullah et al. [2025], who reported that anthocyanin synthesis is limited in white strawberries and colour regulation depends mainly on environment and cultivation practices. Cultivar dominance thus requires cultivar-specific, not universal, foliar strategies.

Broad-spectrum effects of 5-ALA, concentration dependency, and compensation for reduced diurnal temperature range. 5-ALA exhibited positive effects in all three cultivars, indicating broad-spectrum efficacy, but its mode of action varied with cultivar and concentration. For Benihoppe, T5 (50 mg · L^{-1}) was optimal for quality improvement, while T6 (100 mg · L^{-1}) most enhanced photosynthesis. For Yuexiu, T6 increased sweetness, and T5 and T6 optimised flavour. For White Snow Princess, T4 (10 mg · L^{-1}) synergistically improved both photosynthesis and quality. Stepwise regression quantified this concentration-effect relationship: T5 entered the TSS model first ($\beta = 0.458$, $P < 0.001$), preceding cultivar variables ($\beta = 0.628$ for Benihoppe, $\beta = 0.603$ for Yuexiu). This indicates that 50 mg · L^{-1} 5-ALA promotes TSS accumulation more strongly than cultivar effects, providing statistical support for its use as a chemical compensator for sugar accumulation under reduced diurnal temperature range.

The mechanism of 5-ALA is multifaceted. Long et al. [2022] found that 5-ALA enhances stress tolerance by regulating chloroplast ultrastructure, promoting chlorophyll synthesis, and boosting antioxidant capacity. In this study, SPAD responses to 5-ALA were concentration-dependent: Benihoppe responded best at low to moderate concentrations (10–50 mg · L^{-1}), whereas Yuexiu and White Snow Princess required high concentrations (100 mg · L^{-1}) for maximal chlorophyll response, possibly due to differences in uptake efficiency or signal transduction thresholds [Ullah et al. 2024]. Notably, T6 enhanced gas exchange in Benihoppe without significantly increasing SPAD, suggesting that 5-ALA promotes gas exchange inde-

pendently of chlorophyll content, possibly through stomatal regulation or Rubisco activity [Wang et al. 2025], thereby compensating for reduced source supply under reduced diurnal temperature range. Salwa et al. [2025] similarly confirmed that exogenous hormones and chemicals alleviate heat-induced damage by modulating stomatal behaviour and antioxidant systems. However, excessive 5-ALA can be detrimental: in the single fruit weight model, 5-ALA concentration correlated negatively with T6 ($r = -0.88$), indicating that 100 mg · L^{-1} suppresses fruit weight, revealing an optimal concentration range. This hormesis effect implies that 5-ALA application must be precisely dosed to avoid metabolic costs, especially when nocturnal respiration is already elevated under reduced diurnal temperature range.

Photosynthesis-growth trade-off under high temperature and its implications for reduced diurnal temperature range. PLS-SEM path analysis revealed a significant photosynthesis-growth trade-off: photosynthetic parameters exerted a direct negative effect on growth (standardised path coefficient = −0.709, $P < 0.05$), while growth strongly positively affected fruit colour (0.922) and fruit quality (0.845). This challenges the conventional view, articulated by Akram [2024], that enhanced photosynthesis inevitably promotes both growth and quality, highlighting the need to reconsider resource allocation under stress.

The physiological basis of this trade-off lies in resource competition. Manafi et al. [2021] and Ullah et al. [2024] noted that under high temperature, plants increase transpiration to cool leaves, but this adaptive response simultaneously elevates the risk of photoinhibition and reactive oxygen species (ROS) production, triggering costly defence mechanisms. To counter oxidative stress, plants activate energy-intensive defences (e.g., heat shock proteins, antioxidants, osmoprotectants), diverting assimilates from growth to stress responses [Akhtar et al. 2025]. In this study, T5 (50 mg · L^{-1} 5-ALA) induced only a moderate photosynthetic increase (+13.4%) but achieved synergistic gains in fruit weight (+26.2%), Vc (+3.8%), and TSS (+13.4%). This represents a more economical "moderate enhancement" strategy, contrasting with the "aggressive promotion" of T6. This finding aligns with Menzel [2024], who reported that temperatures above 30 °C suppress strawberry leaf growth, and with Bieniasz et al. [2022], who found that titanium organic complexes improve yield under heat by enhancing pollination and fruit development, not merely by increasing photosynthesis. Stepwise regression further supports this network for: Gs, Tr, TA, TSS/TA, leaf width, petiole diameter, and most colour parameters, only cultivar variables entered the model; treatment variables did not meet entry criteria. This indicates that these traits are primarily genetically regulated, with limited scope for foliar intervention. Thus, under reduced diurnal temperature range with elevated night respiration, overemphasising single photosynthetic indicators may be counterproductive; instead, synergistic optimisation of the entire photosynthesis-growth-quality pathway through moderate physiological activation is required.

Cultivar-specific responses and implications for precision fertilisation. The practical value of this study lies in revealing cultivar-specific response patterns, providing a framework for precision fertilisation under reduced diurnal temperature range and high temperature in spring protected production. The significant cultivar × treatment interactions observed for multiple traits demonstrate that the "optimal treatment" is not universal but results from the synergistic matching of cultivar, treatment type, and concentration. This finding underscores the necessity of genotype-aware management strategies in protected strawberry cultivation.

Based on the comprehensive statistical results, recommended cultivar–treatment combinations are: (1) Benihoppe – T5 (50 mg · L^{-1} 5-ALA), which performed best for TSS, fruit weight, and Vc, balancing quality and growth through moderate photosynthetic enhancement; (2) Yuexiu – T1 (S-ABA) for growth, T3 (jasmonic acid) for flavour balance (TSS/TA +7.8%), or T5/T6 for sweetness (TSS +2.1%); (3) White Snow Princess – T4 (10 mg · L^{-1} 5-ALA), which optimises flavour quality with low metabolic cost. Although jasmonic acid improved TSS/TA and fruit weight in Benihoppe, it did not enter any growth model in stepwise regression, suggesting limited promotive effects on vegetative growth. This is consistent with Ullah et al. [2024], who reported that jasmonic acid signalling promotes fruit development while inhibiting vegetative growth, and with Zhang et al. [2024], who demonstrated that plants under high temperature reallocate assimilates from growth to stress defence – a tradeoff that appears to be amplified by jasmonate application. Fruit firmness in White Snow Princess decreased under all treatments, with T1 showing the greatest reduction (−21.9% vs control). This may relate to the unique cell wall polysaccharide composition and

metabolism of its white flesh [Ullah et al. 2025], suggesting that postharvest performance must be considered alongside quality improvement. In summary, this study quantified cultivar, treatment, and interaction effects, revealed a photosynthesis-growth trade-off under spring stress conditions, and established a decision chain for cultivar-specific precision management. Future multiomics studies should elucidate the molecular networks underlying differential cultivar responses.

CONCLUSION

This study established cultivar-specific foliar strategies to mitigate elevated spring temperatures and narrowed diurnal range in protected strawberry cultivation. Cultivar was the predominant factor for most traits, with colour and quality parameters almost entirely cultivar-dependent (adjusted $R^2 > 0.95$). Optimal responses were achieved with 50 mg · L^{-1} 5-ALA for Benihoppe, S-ABA or 10 mg · L^{-1} 5-ALA for Yuexiu, and 10 mg · L^{-1} 5-ALA for White Snow Princess. PLS-SEM revealed vegetative growth as the central driver of fruit colour (0.922) and fruit quality (0.845), while photosynthesis exerted a significant direct negative effect on growth (−0.709, $P < 0.05$), indicating a resourcebased tradeoff under elevated spring temperatures. Stepwise regression confirmed that 5-ALA promoted TSS more strongly than cultivar effects in Benihoppe, whereas the highest concentration (100 mg · L^{-1}) suppressed fruit weight. Intercellular CO_2 concentration showed no significant predictors. These findings support cultivar-specific precision management, emphasizing growth promotion over photosynthetic enhancement to sustain fruit quality under spring stress conditions.

AUTHORS CONTRIBUTIONS

Si-Pu Zhang and Jia-Jia Niu contributed equally to this work and share first authorship. Conceptualization, methodology, investigation, formal analysis, writing – original draft, funding acquisition. Wei Cui: data curation, validation, visualization. Ke Zhang: resources, project administration. Yun-Feng Lu: supervision, writing – review & editing. All authors have read and approved the final manuscript.

DECLARATION OF THE USE OF GENERATIVE AI IN THE PROCESS OF PREPARING THE PAPER

The authors declare that no generative AI tools were used in the preparation of this manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

SOURCE OF FUNDING

This work was financially supported by the Modern Agricultural Industry Technology of Henan Province (HARS-22-09-G5).

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