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High Temperature-Induced Suppression of Flower Bud Formation in Passion Fruit (*Passiflora edulis* f. *flavicarpa*)

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Abstract

High temperature is a major environmental stress that severely limits passionfruit (*Passiflora edulis*) productivity by impairing floral initiation. However, the physiological and molecular mechanisms underlying this process remain poorly understood. In this study, we investigated the effects of varying durations and intensities of heat stress on flower bud differentiation in passionfruit. Our results showed that prolonged exposure to temperatures above 35 °C significantly delayed or completely inhibited bud formation, accompanied by altered carbohydrate and nitrogen metabolism, accumulation of osmolytes (soluble protein and proline), and dynamic changes in antioxidant enzyme activities (SOD, POD, CAT). Notably, short-term heat stress induced a transient increase in salicylic acid (SA) levels and upregulation of SA biosynthesis genes (*PeEDS1.2*, *PeICS1*) and WRKY transcription factors (PeWRKY11/15), which were associated with sustained floral initiation. In contrast, prolonged stress suppressed SA accumulation and signaling, leading to bud abortion. Comparative transcriptomic analysis further revealed enrichment of pathways related to secondary metabolite biosynthesis, plant hormone signal transduction, and phenylpropanoid biosynthesis under heat stress. These findings highlight the critical role of SA in balancing heat tolerance and reproductive development and provide candidate gene resources for the molecular breeding of heat-resistant passionfruit varieties. This study offers new insights into the thermotolerance mechanisms of fruit crops under sustained high-temperature stress.

Keywords: passion fruit; heat stress; flower bud formation; salicylic acid

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1. Introduction

Passionfruit (*Passiflora edulis*), an economically significant perennial crop, is highly sensitive to temperature fluctuations, with optimal growth and floral development occurring between 20 and 30 °C [1]. In recent years, with the increasing frequency of extreme heat events, short-term field temperatures exceeding 40 °C have become more common during summer, posing a major threat to passionfruit production [2,3]. Understanding the mechanisms underlying heat stress responses is essential for developing strategies to sustain yield under climate change. Moreover, the reproductive phase, particularly floral initiation [1,4], represents a critical window of vulnerability to elevated temperatures in this species.

Elevated temperatures severely impair numerous reproductive processes in passionfruit, including bud differentiation, floral organ development, and pollination, leading to

bud abortion and reduced fruit set [5–7]. Under prolonged high temperatures, passionfruit vines often exhibit a sharp decline in bud number and failure of flowers to open or be successfully pollinated [2,8]. Studies demonstrate that fruit set rates can decline sharply to 25% under mean and maximum temperatures of 32 °C and 38 °C, respectively, while rates exceed 60% under milder conditions around 22/30 °C with high humidity [9]. These findings consistently highlight the severe impact of high-temperature stress on passionfruit flowering and fruit production.

Despite these observable impacts, the physiological and molecular mechanisms through which varying durations of high-temperature stress affect floral initiation in passionfruit remain poorly understood, particularly under realistic field-like temperature regimes. Furthermore, the interaction between heat duration and key metabolic and hormonal pathways regulating floral transition in passionfruit has not been systematically investigated.

Flowering in plants involves complex transitions, including floral induction, initiation, and organ development [10]. Floral induction, a critical phase transitioning from vegetative to reproductive growth, is regulated through multi-layered mechanisms including resource allocation, hormonal signaling, and activation of key flowering-time genes [11,12]. Following induction, the shoot apical meristem undergoes floral bud differentiation—comprising physiological and morphological phases—which is crucial for determining fruit yield and quality [13–15]. In passionfruit, successful floral development is specifically indicated when the longest bract of a visible bud exceeds 3 mm in length [16], yet how this delicate developmental process is coordinated under abiotic stress, particularly prolonged high temperature, remains poorly understood.

Given that plant responses to heat often involve accumulation of osmoprotectants, modulation of antioxidant systems, and alterations in phytohormone signaling, this study combined physiological characterization with transcriptomic and hormonal analyses to investigate the response of passionfruit floral initiation to high-temperature stress. Plants were subjected to different durations of thermal treatment at 30 °C and 35 °C to simulate realistic field conditions. We quantified key indicators, including soluble sugars, starch, total nitrogen, proline, soluble protein, antioxidant enzyme activities, and endogenous hormone levels. Furthermore, RNA-Seq analysis was performed to identify critical genes and pathways involved in the heat stress response. Through investigating the effects of prolonged high-temperature stress on passionfruit floral bud formation, identifying associated physiological and metabolic changes, and elucidating the role of salicylic acid and its gene regulatory networks, this study aims to provide a multi-level understanding of the mechanisms underlying heat-induced repression of floral initiation and to propose potential strategies for enhancing thermotolerance in passionfruit.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The passionfruit (*Passiflora edulis*) cultivar 'Qinmi' (QM) was used in this study. Grafted seedlings were transplanted individually into plastic pots (14.5 cm diameter × 12.5 cm height) filled with a pre-mixed substrate of peat, vermiculite, and perlite in a 3:1:1 ($v/v/v$) ratio. Plants were cultivated in a greenhouse located in Fuzhou, Fujian Province, China (26°12′ N, 119°33′ E), under an average temperature of 25 °C and natural light, with photoperiods maintained as needed. At the pre-floral bud stage, uniformly growing plants were selected and transferred to growth chambers for temperature treatments. The chambers were set to a 14 h light/10 h dark photoperiod with 70% relative humidity. Standard horticultural practices, including drip irrigation and routine nutrient manage-

ment [17], were applied to support optimal plant growth and development throughout the cultivation period.

2.2. Quantification of Endogenous Hormone Levels

Plant hormone analysis was conducted according to the previous study [17], with small modifications. The contents of major endogenous hormones, including auxin (IAA), gibberellic acid (GA_3), cytokinin (CTK), abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA), were quantified in apical meristem tissues. After grinding the plant samples in liquid nitrogen and thoroughly mixing them, approximately 0.3 g of the sample was taken and placed in a 15 mL centrifuge tube. Then, 5 mL of the extraction solution (isopropanol: water: formic acid, 80:19:1, *v*/*v*) was added, and the mixture was homogenized for 2 min. It was then placed at 4 °C and ultrasonically extracted for 1 h. The extract was centrifuged at 10,000 r/min for 10 min to obtain the supernatant. Next, 1 mL of dichloromethane was added, and ultrasonic extraction was carried out at 4 °C for 30 min. The mixture was centrifuged at 10,000 r/min for 10 min to obtain the supernatant. This ultrasonic extraction was repeated once, and the supernatants from the three extractions were combined. The solution was then reduced to the aqueous phase under nitrogen at room temperature and made up to 1 mL. After shaking and mixing well, it was diluted twice with methanol and passed through a 0.22 μm filter for UPLC-MS/MS analysis (AB QTRAP® 6500+, AB Sciex, Foster City, CA, USA).

2.3. Analysis of Antioxidant Enzyme Activities

Plant samples were processed following the manufacturer's protocols. The activities of key antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), were quantified using commercial assay kits (YX-W-A500, YX-W-A502, and YX-W-A501; Sinobestbio, Shanghai, China). All measurements were conducted in triplicate with appropriate blank controls included.

2.4. Quantification of Carbohydrates, Total Nitrogen, and Soluble Protein

Soluble sugar content was determined using a Plant Soluble Sugar Assay Kit (YX-W-B602, Sinobestbio, Shanghai, China). Starch content was measured with a Starch Content Assay Kit (YX-W-C400, Sinobestbio, Shanghai, China), following the manufacturer's protocols. Total nitrogen content was analyzed via microwave digestion with H_2SO_4–H_2O_2, followed by colorimetric detection using Nessler's reagent. Soluble protein content was quantified using a BCA Protein Assay Kit (YX-W-C202, Sinobestbio, Shanghai, China), with absorbance measured at 562 nm.

2.5. RNA Extraction, RNA Sequencing and qRT-PCR

Total RNA from each sample was extracted with a TRIzol reagent kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The stand-specific RNA library was constructed by using the NEB#E7530 Ultra RNA library prep kit from Illumina (NEB, Ipswich, MA, USA). Then, the cDNA fragments were purified with a QiaQuick PCR extraction kit (Qiagen, Venlo, The Netherlands) and ligated into Illumina sequencing adapters. After agarose gel electrophoresis, suitable fragments were selected as templates for PCR amplification, and sequencing was carried out on an Illumina HiSeq2500 system (paired-end reads were generated) [18]. The total data output obtained from RNA sequencing is summarized in Table S1. Real-time quantitative PCR (qRT-PCR) was performed according to the previous study [19]. The primer sequences used for qRT-PCR in this study are shown in Table S11.

2.6. Statistical Analysis

Data processing was performed using Microsoft Excel 2021, and all graphical presentations were generated with GraphPad Prism version v8.0. The results are expressed as the mean ± standard deviation (Mean ± SD) from three independent biological replicates. Each treatment consisted of three individual seedlings. Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Significant differences ($p < 0.05$) are indicated with lowercase letters or asterisks in the figures and tables.

3. Results

3.1. High Temperature Inhibits Flower Bud Formation in Passionfruit

The flower bud differentiation process in passionfruit (*P. edulis*) exhibits high sensitivity to temperature conditions. Investigating the specific effects of elevated temperatures and their duration on passionfruit flower bud differentiation provides a foundation for understanding the impact of summer heat stress on passionfruit flowering. Under controlled conditions of 25 °C/20 °C (16 h day/8 h night), passionfruit seedlings demonstrated normal growth and development, with visible floral buds emerging at shoot apices within 10 days (Figure 1). All temperature treatments were applied under a controlled 16 h light/8 h dark photoperiod. In contrast, at 30 °C/25 °C, flower bud formation was delayed by 13 days compared to the control group, requiring 23 days for initiation (Table 1). Notably, at 35 °C/30 °C, no flower bud formation was observed throughout the experimental period, despite maintained plant viability and vigorous vegetative growth. The most extreme treatment at 40 °C/35 °C conditions initially showed no abnormalities during the first 14 days, but subsequently induced progressive growth inhibition. Apical meristem wilting became apparent after 15 days of treatment, culminating in complete plant mortality by day 22 (Figure 1). These findings demonstrate that elevated temperatures significantly affect passionfruit flower bud differentiation, with increasing temperatures correlating with prolonged differentiation timelines. Notably, temperatures exceeding 35 °C completely inhibited the flower bud differentiation process.

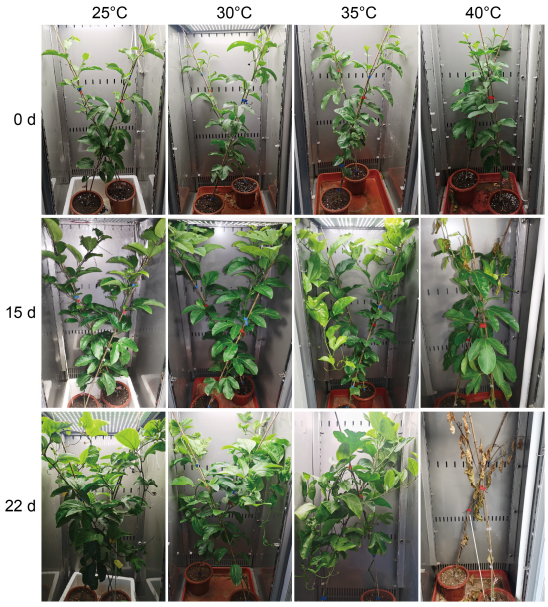


Figure 1. Progression of heat stress symptoms in passionfruit plants under different temperatures. Phenotypes were recorded at 0, 15, and 22 days (d) during exposure to 25 °C, 30 °C, 35 °C, or 40 °C. The series of images demonstrates the incremental stress response, from mild growth delay at 35 °C to complete mortality at 40 °C by 22 d.

Table 1. Effect of different temperatures on flower bud formation.

Treatment (°C)	25	30	35	40
Days to first flower bud formation (d)	10 ± 0.5	23 ± 1.0	Healthy, without flower buds	The stem tip withered
Number of flower buds in 26 d	14 ± 1.0	3 ± 0.6	0	0

3.2. Prolonged High-Temperature Exposure Severely Suppresses Passionfruit Flower Bud Initiation

To further investigate the effect of high-temperature duration on floral bud initiation in *P. edulis*, plants were exposed to 30 °C under controlled photoperiodic conditions for defined intervals (1, 6, 11, and 16 h/day). In the control group (0 h), flower bud differentiation occurred as early as 8 days after treatment, whereas the treated groups exhibited delayed initiation, with floral buds first observed at 10, 12, 22, and 28 days, respectively (Figure 2, Table 2). Specifically, 1 h and 6 h exposures delayed the first flower bud appearance by 2 and 4 days, respectively, whereas longer exposures (11 h and 16 h) resulted in more pronounced delays of 14 and 20 days. The results demonstrated that high-temperature treatments consistently delayed flower bud initiation compared to the control, confirming that under 30 °C/25 °C conditions, exposure duration directly governs initiation timing, with prolonged heat stress progressively extending the developmental timeline.

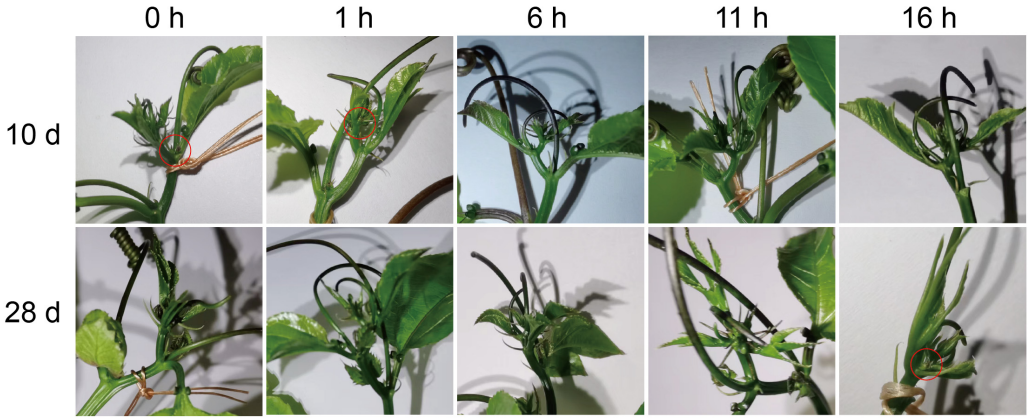


Figure 2. Dynamics of flower bud formation in passionfruit under 30 °C treatments with varying durations. Passionfruit plants were exposed to 30 °C for 0, 1, 6, 11, or 16 h per day. Photographs of representative apical meristems were taken at 10 and 28 days after the initiation of treatments. The visible floral buds are indicated by red circles.

Table 2. Effect of treatment duration at 30 °C on flower buds of 'QM'.

Exposure Time at 30 °C (h)	0	1	6	11	16
Days to first flower bud formation (d)	8 ± 0.6	10 ± 0.6	12 ± 1.0	22 ± 1.0	28 ± 0.6
Number of flower buds in 10 d	6 ± 0.6	2 ± 1.0	0	0	0
Number of flower buds in 28 d	25 ± 1.5	22 ± 1.0	17 ± 2.0	7 ± 0.6	1 ± 0.6

Note: QM, 'Qinmi' cultivar.

Subsequently, utilizing an identical experimental framework, treatments at 35 °C were implemented to evaluate critical temperature thresholds governing flower bud formation. After 8 days of treatment, flower buds formed in the 1 h, 3 h, and 6 h groups but not in the 11 h and 16 h groups (Table 3). By day 20, flower buds had also developed in the 11 h treatment, whereas the 16 h exposure completely inhibited flower bud formation. Notably, short-term exposures (1 h, 3 h, and 6 h) exhibited similar flower bud initiation

timing and numbers compared to the optimal temperature conditions, whereas the 11 h treatment delayed bud formation, and the 16 h treatment completely suppressed it (Table 3). These results demonstrate that sustained 35 °C heat stress significantly inhibits flower bud initiation, with the inhibitory effect becoming increasingly severe with prolonged exposure. This suggests that passionfruit flowering is highly sensitive not only to temperature intensity but also to the duration of heat stress, with critical thresholds determining reproductive success.

Table 3. Effect of treatment duration at 35 °C on flower buds of 'QM'.

Exposure Time at 35 °C (h)	0	1	3	6	11	16
Days to first flower bud formation (d)	8	8	8 ± 0.6	8 ± 1.5	20 ± 1.0	/
Number of flower buds in 10 d	6 ± 0.6	7 ± 1.5	7 ± 0.6	5 ± 1.0	0	/

Note: QM, 'Qinmi' cultivar. "/" indicates that no flower buds formed in the seedlings; therefore, no data are available for statistical analysis.

3.3. Metabolites and Enzymatic Responses to Heat Stress in Passionfruit Apical Meristems

Comparative analysis between control groups revealed significant metabolic differences associated with floral bud initiation. Apical meristems collected after 8 days at 25 °C without floral buds (CK0) and those with floral buds (CK1) showed markedly different metabolic profiles. Soluble sugar, starch, total nitrogen content, and carbon/nitrogen (C/N) ratio were all significantly higher in CK1 than in CK0—by 937.64%, 1104.13%, 72.04%, and 544.80%, respectively (Figure 3A–D). These results indicate that the accumulation of carbohydrates and nitrogenous compounds is closely correlated with floral bud formation in passionfruit.

Under 35 °C treatments (35-T1: 1 h/day, 35-T3: 3 h/day, 35-T6: 6 h/day, and 35-T11: 11 h/day), metabolite levels varied significantly with exposure duration. Soluble sugar content was highest in the 35-T11 (35 °C, 11 h/day) group (118.05 $mg \cdot g^{-1}$), representing a 951.92% increase compared to 35-T1 (35 °C, 1 h/day) (Figure 3A). Starch content and C/N ratio did not differ significantly between 35-T1 and 35-T3 but were significantly lower than in 35-T6 (35 °C, 6 h/day). The 35-T11 group showed the highest starch levels among all groups (Figure 3B). Total nitrogen was comparable between 35-T6 and 35-T11, and both were significantly higher than 35-T1 and 35-T3. Furthermore, 35-T3 (35 °C, 3 h/day) exhibited higher nitrogen content than 35-T1 (Figure 3C). Although variation was observed among treatment durations, all carbohydrate and nitrogen metrics showed an overall increasing trend under prolonged heat stress (Figure 3D), indicating that soluble sugar and starch accumulation in apical meristems is duration-dependent under high-temperature conditions.

Soluble protein and proline (Pro) levels also displayed distinct patterns. CK1 (control with floral bud) had 21.22% higher soluble protein content but 70.10% lower Pro content than CK0 (control without floral bud) (Figure 3E–F), suggesting that soluble protein accumulation supports floral initiation, whereas proline metabolism may be prioritized for synthesis and incorporation into proteins during this phase. Under 35 °C stress, both metabolites peaked in 35-T3 (110.50 $mg \cdot g^{-1}$ and 450.55 $\mu g \cdot g^{-1}$ FW, respectively) and were lowest in 35-T6. Overall, soluble protein and Pro levels increased initially, declined, then slightly rose again from 6 h to 11 h, indicating that short-term heat exposure induces accumulation, whereas prolonged stress leads to a subsequent reduction (Figure 3E,F).

Antioxidant enzyme activities were significantly lower in CK1 than in CK0. Specifically, CAT, POD, and SOD activities were reduced by 10.56%, 78.72%, and 45.13%, respectively (Figure 4A–C), implying that successful bud formation may be associated with reduced oxidative stress and lower demand for enzymatic scavenging. Under heat stress, CAT activity

first decreased, increased in 35-T6 (35 °C, 6 h/day) (peak value 233.27 nmol·min^{-1}·g^{-1} FW), and decreased again thereafter. In contrast, both POD and SOD activities generally decreased with extended exposure. The highest POD and SOD activities were observed in 35-T1 (35 °C, 1 h/day) (41,254.45 μg·g^{-1} FW and 759.00 μg·g^{-1} FW, respectively; Figure 4A–C). These trends suggest that prolonged thermal stress attenuates the antioxidant capacity in passionfruit apical meristems.

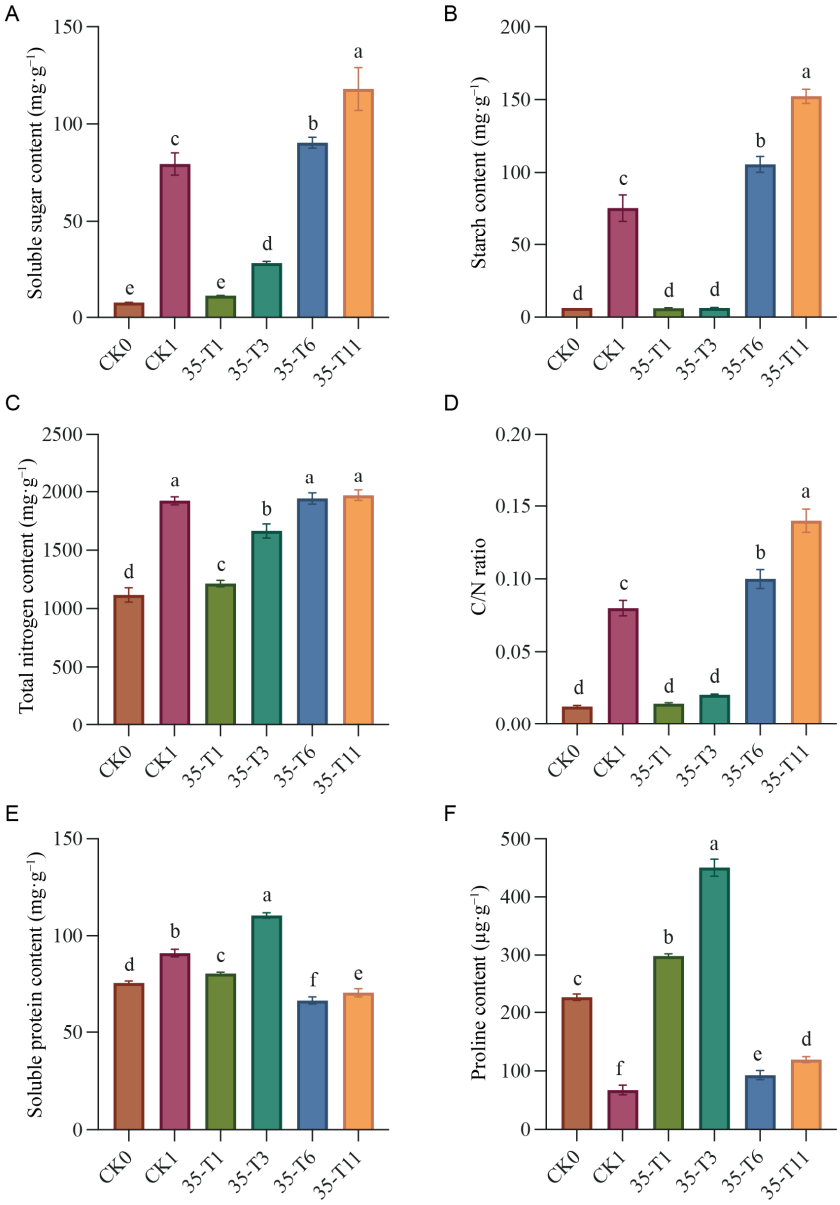


Figure 3. Dynamics changes in key metabolites in passionfruit apical meristems under 35 °C treatments. (**A**–**D**) Changes in soluble sugar content (**A**), starch content (**B**), total nitrogen content and C/N ratio (**D**) in passionfruit apical meristems under 35 °C treatments. (**E**,**F**) Changes in soluble protein content (**A**) and proline content (**F**) in passionfruit apical meristems under 35 °C treatments. Letters above bars indicate significant differences among treatments ($p < 0.05$, one-way ANOVA, Tukey's HSD post hoc test). CK0: Control group (25 °C), apical meristem without floral bud collected after 8 days. CK1: Control group (25 °C), apical meristem with floral bud collected after 8 days. 35-T1: 35 °C treatment for 1 h/day; meristem with floral bud collected on day 8. 35-T3: 35 °C treatment for 3 h/day; meristem with floral bud collected on day 8. 35-T6: 35 °C treatment for 6 h/day; meristem with floral bud collected on day 8. 35-T11: 35 °C treatment for 11 h/day; meristem without floral bud collected on day 8.

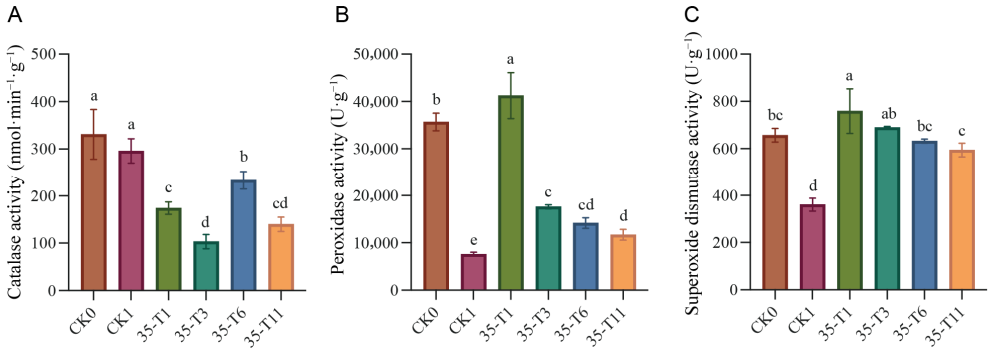


Figure 4. Dynamics changes in antioxidant enzyme activity of passionfruit apical meristems under 35 °C treatments. (**A**–**C**) Changes in CAT activity (**A**), POD activity (**B**) and SOD activity (**C**) in passionfruit apical meristems under 35 °C treatments. Letters above bars indicate significant differences among treatments ($p < 0.05$, one-way ANOVA, Tukey's HSD post hoc test).

3.4. Comparative Transcriptome Analysis of Passionfruit Meristems Under High Temperature

To elucidate the molecular response of passionfruit floral initiation to prolonged high-temperature stress, a comparative transcriptome analysis was performed on apical meristems subjected to 35 °C for varying durations. Key genes and transcription factors associated with heat-inhibited floral bud formation were identified. Significant differential gene expression was observed across all comparisons. The T0 vs. T6 comparison exhibited the highest number of differentially expressed genes (DEGs) with 7616 (3611 up-regulated and 4005 down-regulated). The T6 vs. T11 comparison showed 7399 DEGs (3666 up-regulated and 3733 down-regulated), while T0 vs. T11 had the fewest, with 3892 DEGs (1909 up-regulated and 1983 down-regulated) (Figure 5A,B; Tables S2–S4). This indicates that both 6 h and 11 h of heat stress substantially affect the transcriptome of apical meristems and impair floral development.

Gene Ontology (GO) enrichment analysis revealed distinct functional categories among DEGs from different comparisons. In T0 vs. T6, cell component terms were most represented (4256 genes), with significant enrichment in cell periphery (927), plastid (692), chloroplast (675), and plastid stroma (231). Biological processes included response to stimulus (1576), response to stress (899), and response to abiotic stimulus (639). Only one molecular function term, structural molecule activity (100 genes), was enriched (Figures 5C and S1A; Table S5). In contrast, the T0 vs. T11 comparison, biological processes constituted the most represented category, with 28 terms encompassing 9386 genes. Notable enriched terms included response to stimulus (833 genes), developmental process (478 genes), and response to stress (470 genes). Only one cellular component term was significantly enriched, corresponding to the cell periphery (472 genes). Similarly, a single molecular function term was identified: transcription regulator activity (221 genes) (Figures 5D–F and S1B; Table S6). In the T6 vs. T11 comparison showed a strong enrichment in biological processes, with 20 terms and 9380 genes. Highly represented terms included response to stimulus (1548 genes), response to chemical (918 genes), and response to abiotic stimulus (644 genes). Cellular component analysis revealed 7 enriched terms, including cell periphery (945 genes), plasma membrane (819 genes), and cell junction (267 genes). Molecular function enrichment comprised 3 terms, with transcription regulator activity (400 genes), DNA-binding transcription factor activity (374 genes), and transmembrane transporter activity (285 genes) being most prominent (Figure S1C,D; Table S7).

KEGG pathway analysis highlighted consistent enrichment across comparisons in biosynthesis of secondary metabolites, plant hormone signal transduction, starch and sucrose metabolism, and phenylpropanoid biosynthesis (Figure S2A–C; Tables S8–S10).

These results suggest that under 6 h and 11 h of heat stress, passionfruit meristems activate hormone signaling, enhance carbohydrate metabolism, and initiate synthesis of protective secondary metabolites to cope with thermal stress.

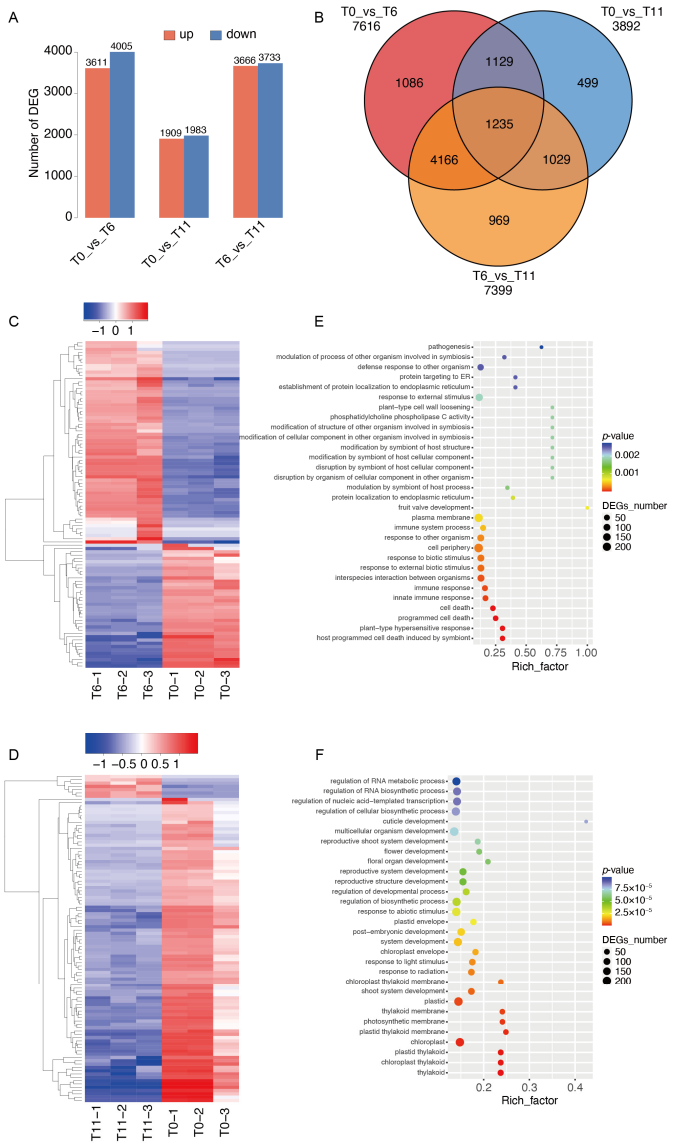


Figure 5. Transcriptomic profiling and identification of differentially expressed genes (DEGs) in passionfruit under heat stress. (**A**) Number of significantly up- and down-regulated DEGs across comparison groups. (**B**) Venn diagram illustrating overlaps among DEG sets from different comparisons. (**C**,**D**) Hierarchical clustering of DEGs in the T0_vs_T6 (**C**) and T0_vs_T11 (**D**) comparisons. (**E**,**F**) Top significantly enriched biological process (GO) terms for down-regulated (**E**) and up-regulated (**F**) genes in the T0_vs_T11 comparison.

3.5. Regulation of Floral Bud Formation by Salicylic Acid Signaling Under Heat Stress

Based on transcriptomic findings indicating pronounced hormone and stress responses, we quantified endogenous phytohormones. The auxin (IAA) content was highest in the CK1 group (6.37 ng·g^{-1}), significantly greater than in bud-lacking CK0 and all heat-treated groups (Figure 6A). Gibberellic acid (GA_3) was significantly higher in CK1 than in CK0 but comparable to 35-T6 (Figure 6B). Cytokinin (CTK) was lowest in CK1 (0.04 ng·g^{-1}), significantly lower than in CK0 and heat-treated groups, and varied in a "decrease–increase–decrease" pattern among treated groups (Figure 6C). Abscisic Acid (ABA) was higher in CK1 than CK0 and fluctuated with extended heat exposure (Figure 6D). Jasmonic acid

(JA) did not differ significantly between CK1 and CK0 but was significantly lower in all treated groups, with the minimum in 35-T11 (0.39 ng·g^{-1}) (Figure 6E). Salicylic Acid (SA) was significantly higher in CK1 than CK0, but both were lower than most treated groups. Among heat-treated groups, SA increased initially and then decreased with prolonged exposure (Figure 6F). Under optimal conditions, successful bud formation in CK1 was associated with significantly higher levels of IAA, GA_3 and SA compared to the bud-lacking control CK0. By contrast, CTK content was significantly lower in CK1 than in CK0. Abscisic acid showed a distinct pattern, with the lowest concentrations observed in CK0 among all treatment groups. Notably, GA_3 levels in CK1 were statistically similar to those in the 35-T6 treatment group. Under heat stress, elevated SA levels in short-term treatment groups (35-T1, 35-T3, and 35-T6) were correlated with active bud initiation, suggesting a promotive role of SA in this process. In contrast, prolonged heat exposure (35-T11) resulted in reduced SA accumulation and concomitant inhibition of bud formation.

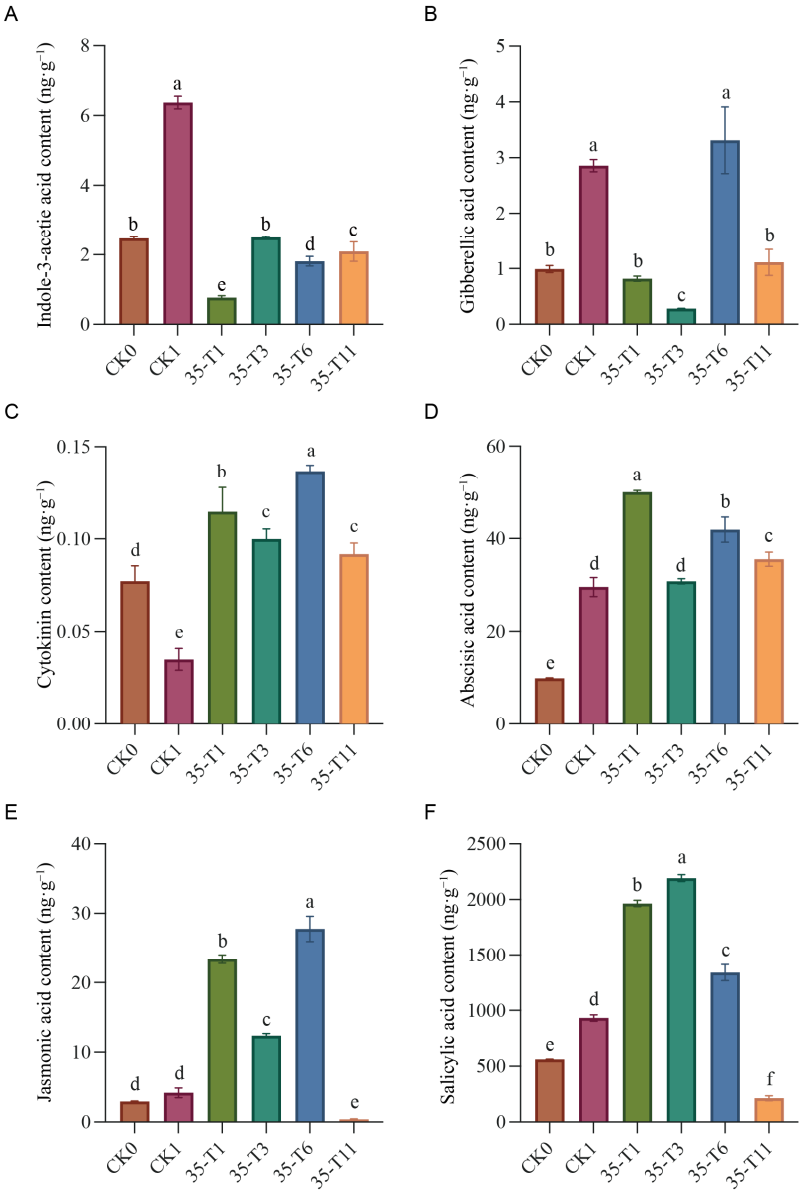


Figure 6. Dynamic changes in endogenous hormone levels in passionfruit apical meristems under 35 °C treatments. (**A**–**F**) Contents of auxin (**A**), gibberellic acid (**B**), cytokinin (**C**), abscisic acid (**D**), jasmonic acid (**E**) and salicylic acid (**F**). Different lowercase letters indicate significant differences among treatments ($p < 0.05$, one-way ANOVA, Tukey's HSD post hoc test).

Given that both CK1 (T0) and 35-T6 (T6) had higher SA levels than bud-deficient 35-T11 (T11), we analyzed key SA biosynthesis and signaling genes (Figure 7A). SA biosynthesis genes *PePAD4*, *PeEDS1.2*, and *PeICS1* were highly expressed in T6, whereas *PeEDS1.2* and *PeICS1* were nearly suppressed in T11, correlating with SA abundance (Figure 7B,D). This implies that *PeEDS1.2* and *PeICS1* are potential regulators promoting SA accumulation and bud formation under short-term stress, but are inhibited under prolonged heat. In the signaling pathway, key components (*PeNPR1*, *PeTGA*, and *PePR-1*) were significantly down-regulated in T11 (Figure 7B,D), indicating disrupted SA signaling under low SA conditions. Co-expression analysis suggested potential interactions between three WRKY transcription factors PeWRKY11, PeWRKY15, and PeWRKY16 and *PePAD4* (Figure 7C). The high expression levels of these WRKY genes under short-term heat stress (T6) imply a possible role in regulating PePAD4-mediated SA biosynthesis (Figure 7B,D), which may contribute to floral initiation under moderately high-temperature conditions.

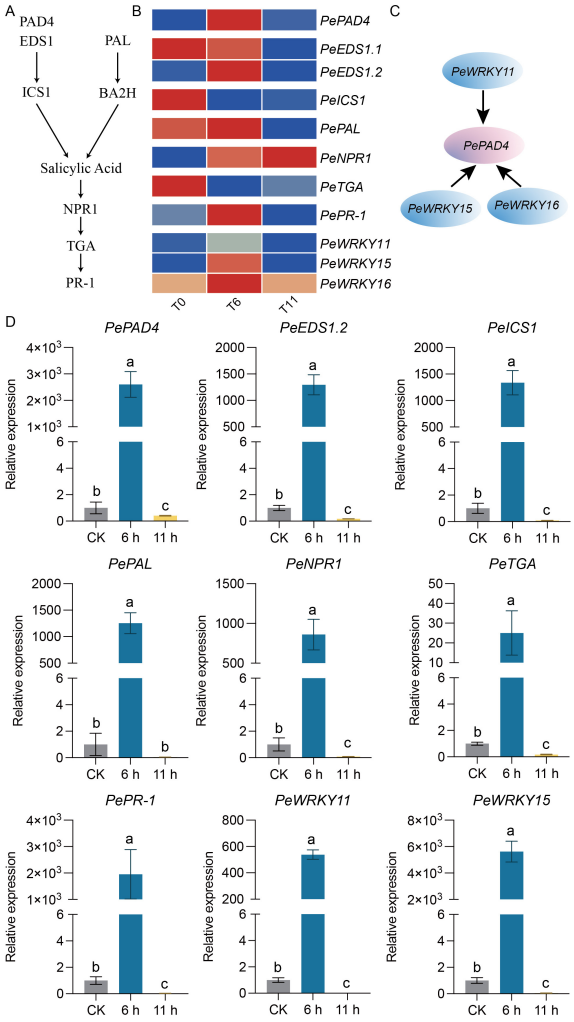


Figure 7. Dynamic Molecular mechanisms of salicylic acid biosynthesis and signaling in passionfruit under heat stress. (**A**) Schematic representation of the SA biosynthesis and signaling pathway. (**B**) Expression heatmap of key genes involved in SA biosynthesis and signaling across different treatment groups. Red indicates up-regulated gene expression, blue indicates down-regulated gene expression, and color intensity reflects the magnitude of expression change. (**C**) Predicted protein interaction network between the SA biosynthesis gene *PePAD4* and transcription factors PeWRKY11, PeWRKY15, and PeWRKY16. (**D**) Expression profiles of significantly expressed genes in apical meristems under 35 °C treatments. Statistically significant differences are indicated with different lowercase letters ($p < 0.05$, one-way ANOVA, Tukey's HSD post hoc test).

4. Discussion

The duration of high-temperature exposure is a critical factor influencing floral initiation in plants. Beyond visible morphological responses, prolonged heat stress triggers a series of internal physiological adaptations, including alterations in nutrient allocation, osmolyte accumulation, and hormonal balance, all of which contribute to thermotolerance. Previous studies on 'Passion Dream' passionfruit have demonstrated impaired flowering under temperatures exceeding 28 °C [16]. In the present study, plant mortality under 40 °C/35 °C treatment likely resulted from cumulative heat injury beyond the threshold of metabolic recovery. Our experiments involving 30 °C and 35 °C treatments at varying durations revealed that extended heat exposure significantly delayed floral initiation, as indicated by the increased time to first bud formation. Notably, bud formation still occurred within 16 h of heat exposure, suggesting that short-term stress may be partially mitigated by innate heat defense mechanisms.

Our physiological measurements revealed dynamic changes in key metabolites and enzyme activities under heat stress. Carbohydrate and total nitrogen content generally increased across all heat-treated groups. Soluble protein and proline levels exhibited an initial rise followed by a decline and subsequent minor recovery, whereas CAT activity showed a fluctuating trend and POD and SOD activities declined progressively with prolonged stress. These responses indicate that soluble sugars and starch accumulate over time under heat stress, while osmoprotectants and antioxidant enzyme activities decrease after an initial increase, reflecting possible resource exhaustion or metabolic disruption.

These observed shifts in metabolic profiles align with established heat-response mechanisms. The initial increase in soluble proteins and proline is consistent with their known roles in osmotic adjustment and protein protection under heat stress [20,21]. However, their subsequent decline, coupled with the progressive decrease in POD and SOD activities, suggests a potential breakdown of defense mechanisms under prolonged stress [22,23], leading to the metabolic disruption and oxidative damage we documented. Furthermore, heat stress disrupts ROS homeostasis, which can interfere with normal bud differentiation [24]. Phytohormone also mediate thermotolerance and reproductive development. Increased ABA and decreased CTK levels under heat stress have been reported in maize, wheat, and tomato [25–27], consistent with our observations of hormonal changes in passionfruit. Phytohormones also mediate the critical trade-off between survival and reproductive success under heat stress [28]. The observed increase in ABA is indicative of a conservative stress-avoidance strategy; it likely promotes stomatal closure to reduce water loss and redirects resources away from growth [29,30], thereby helping to maintain cellular viability at the expense of developmental processes like flowering. Concurrently, the decline in CTK, a promoter of cell division and nutrient mobilization, may reflect a deliberate suppression of meristematic activity and sink strength in the apical bud. While this hormonal shift enhances short-term survival chances under severe stress, it directly compromises reproductive investment by inhibiting cell proliferation and metabolic activities essential for floral bud initiation and development, ultimately leading to the observed bud abortion.

Based on these findings, a comprehensive cascade of heat response in passionfruit can be proposed, initiating from transcriptomic reprogramming, which alters hormonal signaling and carbon/nitrogen metabolic fluxes. These changes subsequently induce physiological adjustments in osmolyte accumulation and antioxidant capacity, ultimately manifesting as the morphological outcome of bud abortion or survival. This integrated view connects molecular initiation to phenotypic consequence, providing a systematic understanding of heat inhibition. Furthermore, the pivotal role of SA uncovered in this cascade suggests a promising practical application: the exploration of exogenous SA

application as a potential strategy to mitigate heat stress and secure stable flower production in passionfruit cultivation [31,32].

Beyond these general metabolic responses, our findings highlight a particularly critical role for salicylic acid in coordinating passionfruit's reproductive response to heat. Notably, short-term heat treatments (T1–T6) led to significantly higher SA levels compared to both the control (CK1) and long-term treatment (T11), implying that transient SA accumulation may support floral initiation under moderate stress, whereas prolonged heat suppresses SA biosynthesis and inhibits flowering. Salicylic acid appears to function as a pivotal signaling molecule mediating the trade-off between thermotolerance and reproductive development under heat stress. This is consistent with studies in other species where SA is known to regulate stomatal closure, enhance antioxidant capacity, and promote chaperone expression, thereby mitigating heat-induced damage [33]. The transient elevation of SA may serve as an alert signal, priming meristem cells for floral transition while maintaining basal defense. However, under prolonged heat stress, the sustained suppression of SA biosynthesis and signaling, as observed in the 35-T11 group, indicates a collapse of this coordinated alert system. This failure to maintain SA-mediated defense is concomitant with the onset of systemic oxidative damage and the eventual cessation of meristem activity. The correlation between chronically low SA levels and the progression to visible tissue wilting and plant mortality suggests that the breakdown of the SA pathway may be a pivotal event in the transition from stress acclimation to irreversible cellular dysfunction and programmed cell death.

This pivotal role of SA was further substantiated at the molecular level. The suppression of SA biosynthesis genes (*PeEDS1.2* and *PeICS1*) and signaling components (*PeNPR1*, *PeTGA*, *PePR-1*) under prolonged heat aligns with the decline in SA levels and the failure of bud formation. This indicates that sustained high temperatures may disrupt SA-mediated transcriptional programming, ultimately leading to the abortion of floral initiation. The coordinated expression of WRKY transcription factors (*PeWRKY11/15/16*) and *PePAD4* under short-term stress further suggests a regulatory module fine-tuning SA accumulation in a time-dependent manner. These findings position SA not merely as a stress-responsive metabolite but as a critical regulator linking stress perception with developmental decisions. Specifically, SA exhibits a dual role in thermotolerance, promoting floral initiation under moderate stress while being suppressed under severe conditions. This context-dependent function highlights its potential as a strategic target for improving heat resilience in passionfruit and other economically important fruit crops.

5. Conclusions

This study demonstrates that prolonged high-temperature stress significantly inhibits floral initiation in passionfruit (*Passiflora edulis*) by disrupting key physiological and molecular processes, including carbohydrate and nitrogen metabolism, phytohormone homeostasis, and antioxidant enzyme activities. Most notably, we identified that short-term heat exposure induces transient accumulation of salicylic acid and upregulates its biosynthesis genes (*PeEDS1.2*, *PeICS1*) and associated transcription factors (*PeWRKY11/15/16*), which collectively promote floral bud formation. In contrast, prolonged thermal stress leads to suppression of the SA pathway and ROS homeostasis disruption, ultimately resulting in bud abortion. These results underscore the dual role of SA as a central regulator mediating the trade-off between thermotolerance and reproductive development under heat stress. The key genes and pathways identified here provide valuable targets for molecular breeding of heat-resistant passionfruit varieties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11121526/s1, Figure S1: GO functional enrichment analysis of differentially expressed genes under heat stress; Figure S2: KEGG pathway enrichment analysis of DEGs under heat stress; Table S1: Summary of transcriptome sequencing quality of passionfruit meristems samples; Table S2: Summary of Differentially Expressed Genes Identified in T0 vs. T6; Table S3: Summary of Differentially Expressed Genes Identified in T0 vs. T11; Table S4: Summary of Differentially Expressed Genes Identified in T6 vs. T11; Table S5: Gene Ontology (GO) Enrichment Analysis of Differentially Expressed Genes between T0 and T6; Table S6: Gene Ontology (GO) Enrichment Analysis of Differentially Expressed Genes between T0 and T11; Table S7: Gene Ontology (GO) Enrichment Analysis of Differentially Expressed Genes between T6 and T11; Table S8: KEGG Orthology (KO) Enrichment Analysis of Differentially Expressed Genes between T0 and T6; Table S9: KEGG Orthology (KO) Enrichment Analysis of Differentially Expressed Genes between T0 and T11; Table S10: KEGG Orthology (KO) Enrichment Analysis of Differentially Expressed Genes between T6 and T11; Table S11: List of primers.

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Data Availability Statement: The data presented in this study are openly available in the National Genomics Data Center (NGDC) and are accessible through accession number CRA030609. [National Genomics Data Center (NGDC)] [https://ngdc.cncb.ac.cn] [CRA030609].

Conflicts of Interest: The authors declare no conflicts of interest.

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