



Phytosulfokine α treatment induces osmotic regulation to alleviate postharvest chilling injury of peach fruit by PpbHLH62

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ABSTRACT

Peach fruit is highly susceptible to chilling injury during postharvest cold storage, which affects its shelf life and market value. This study examined the impact of Phytosulfokine α (PSK- α) on chilling injury in peach fruit. RNA-seq analysis revealed significant enrichment in the 'Arginine and proline metabolism' pathway, which is crucial for osmotic regulation and plant response to abiotic stress, suggesting its role in PSK- α -mediated cold resistance. Further analysis showed that PSK- α treatment increased γ -aminobutyric acid (GABA), polyamine, and proline metabolism. GABA content rose due to reduced glutamic acid and GABA transaminase (GABA-T) activity, alongside enhanced glutamate decarboxylase (GAD) activity. PSK- α also elevated putrescine and spermidine levels, likely by activating arginine decarboxylase (ADC), ornithine decarboxylase (ODC), polyamine oxidase (PAO), and diamine oxidase (DAO) activities. Moreover, proline levels increased, possibly due to enhanced pyrroline-5-carboxylate synthase (P5CS) and ornithine aminotransferase (OAT) activities and suppressed proline dehydrogenase (PDH) activity. Additionally, PpbHLH62 regulates *PpGAD4*, *PpP5CS*, and *PpOAT*, further contributing to cold resistance. This study provides a new perspective on how PSK- α improves cold resistance in peach fruit by regulating the content of osmotic regulators.

1. Introduction

The flesh of peach fruit is juicy and rich in nutrients, making it highly favored by consumers. However, when harvested peach stored improperly, especially at temperatures between 2 °C and 8 °C, chilling injury (CI) easily occurs. The occurrence of CI is often accompanied by oxidative stress. To avoid chilling damage, commercial storage is usually kept close to 0 °C. Oxidation damages the cell membrane, resulting in the loss of cell structure integrity (Zhao et al., 2025b). When the cell membrane is damaged, the outflow of intracellular substances causes a sudden drop in intracellular solute concentration, leading to osmotic imbalance (Zhang et al., 2024). Cells undergo rapid changes in osmotic pressure, which further exacerbate cell damage. Therefore, maintaining osmotic balance and reducing oxidative stress are crucial to delaying CI and preventing further cell damage (Dong et al., 2020; Ghosh et al., 2024).

The primary substances involved in osmotic regulation include γ -aminobutyric acid (GABA), proline, and polyamines (PAs, including putrescine, spermidine, and spermine). GABA serves an essential role in

plants, acting both as a signaling molecule for information transmission and as an osmotic regulator that enhances resistance to environmental stresses (Ahmad and Fariduddin, 2024; Li et al., 2019). GABA is synthesized and degraded through two pathways. The first involves glutamic acid as the substrate, catalyzed by glutamic acid decarboxylase (GAD) to form GABA. Simultaneously, GABA is degraded into succinic acid semialdehyde through the action of GABA transaminase (GABA-T) (Zhao et al., 2025a). The second pathway for GABA synthesis occurs through PA degradation. Putrescine (Put), a key substrate in the PAs degradation pathway, is mainly synthesized from ornithine through the action of ornithine decarboxylase (ODC) or from arginine via arginine decarboxylase (ADC). Then Put is oxidized by diamine oxidase (DAO), while other PAs, such as spermine (Spm), are processed by polyamine oxidase (PAO) to form 4-aminobutyraldehyde, which is subsequently dehydrogenated by 4-aminobutyraldehyde dehydrogenase (AMADH) to produce GABA (Min et al., 2021). Additionally, the synthesis and degradation of proline play a crucial role in cellular osmotic pressure regulation. There are two main pathways involved in proline synthesis. In the cytoplasm, glutamic acid serves as the substrate, which is

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converted into glutamate- γ -semialdehyde (GSA) through the catalytic activity of pyrroline-5-carboxylate synthase (P5CS). The other pathway occurs in the mitochondria, where ornithine acts as the substrate and is converted into GSA via ornithine aminotransferase (OAT). Both pathways ultimately lead to proline (Huang et al., 2025). On the other hand, proline degradation is primarily catalyzed by proline dehydrogenase (PDH). Several studies have shown that some preservation treatments could reduce CI in peach fruit by increasing osmoregulatory substances. For example, exogenous indole-3-acetic acid (IAA) could significantly enhance GABA accumulation in peach and reduce the occurrence of CI (Zhou et al., 2023). 1-Methylcyclopropene (1-MCP) treatment of peach enhanced the enzyme activity of the PA biosynthetic enzymes and inhibited PDH activity, promoting the PAs accumulation and delaying the onset of CI (Qian et al., 2021). Modified atmosphere packaging (MAP) with 3 % O₂ delayed CI in peach, with significantly higher GABA and proline contents compared to the control (Ai et al., 2024). When measuring the proline content of Chinese olive under different storage temperatures, it was found that storage at 2 °C reduced proline levels by inhibiting P5CS and OAT activities while enhancing PDH activity. This process disrupted cell membrane integrity, reduced cold resistance, and accelerated the onset of postharvest CI (Chen et al., 2024).

Cold stress influences gene expression and protein synthesis at the molecular level, with transcription factors (TFs) playing a regulatory role in this process. Studies have shown that various TFs, such as the C-repeat (CRT)-binding factors/dehydration-responsive element (DRE) binding factors (CBF/DREBs), inducer of CBF expression (ICE), NAM, ATAF1/2, CUC1/2 (NACs), WRKYs, MYBs, and basic Helix-Loop-Helix (bHLHs), are involved in responses to cold stress (Manna et al., 2021). The bHLH proteins constitute the second most abundant TFs in plants. To date, more than 120 members have been identified in peach, playing roles in fruit development, postharvest stress resistance, and physiological metabolism regulation. MabHLH060 and MabHLH183 play a key role in ethylene-mediated alleviation of CI in banana by regulating the expression of phosphatidic acid biosynthesis genes (*MaDGK2*, *MaPLD δ 1*, and *MaPLD δ 5*) (Zhou et al., 2022). In loquat fruit, methyl jasmonate pretreatment alleviates CI-induced lignification, potentially through a regulatory pathway involving the bHLH transcription factor EjbHLH14, which represses EjbHLH1-mediated activation of the lignin-related gene *EjPRX12* (Zhang et al., 2022). While bHLHs have been shown to play crucial roles in the regulation of CI, attention has also turned to the development of novel preservation agents as complementary strategies to enhance cold tolerance in postharvested fruit.

Recently, many physical and chemical preservation methods aimed at delaying deterioration caused by CI in peach such as hydrogen sulfide (H₂S) (Wang et al., 2023c), melatonin (Li et al., 2024), microwave irradiation (Wang et al., 2023b), heat shock (Zhao et al., 2025) and cold shock (Jia et al., 2023). Phytosulfokine- α (PSK- α , [Y(SO₃H)IY(SO₃H)TQ]) is a sulfated pentapeptide known to stimulate cell proliferation (Liu et al., 2024b). Exogenous PSK- α consistently enhances postharvest quality and chilling tolerance in diverse fruits and vegetables. In loquat, it boosts ATP utilization and the activities of succinate dehydrogenase (SDH), cytochrome c oxidase (CCO), Ca²⁺-ATPase and H⁺-ATPase, thereby alleviating CI (Song et al., 2017). PSK- α delays strawberry senescence and decay at 4 °C by down-regulating *PARP1*, improving NAD⁺ recycling, elevating key antioxidant enzymes, and increasing phenolics, flavonoids, anthocyanins and melatonin (Aghdam and Alikhani-Koupaei, 2021; Aghdam et al., 2021). The same concentration induces PAL and CHS activity to raise polyphenol and flavonoid levels in broccoli under cold stress (Aghdam et al., 2020), and enhances peach cold tolerance via strengthened antioxidant defense and cell-wall stability (Zeng et al., 2024). Previous work also linked PSK- α -mediated CI mitigation in loquat to higher osmotic regulator concentrations, such as sugars and proline (Liu et al., 2024b). However, studies investigating the mechanisms by which PSK- α mitigates quality deterioration caused by CI in peach through osmotic regulation remain limited and underexplored, although such pathways have been more extensively explored in

other fruit species such as loquat and strawberry.

Therefore, this study investigated the effects of different concentrations (100, 200, 300, 400 nmol L⁻¹) of PSK- α treatment on delaying CI in peach. The CI index, firmness, relative electric conductivity and extractable juice were compared to determine the optimal concentration. Changes in osmotic regulatory substance metabolism were examined for their role in delaying CI. This study found that PSK alleviated CI in peach fruit by increasing the contents of GABA, proline and PAs. The results provide a new perspective on PSK- α treatment's role in alleviating CI in peach.

2. Materials and methods

2.1. Materials and treatment

The peach (*Prunus persica* (L.) Batsch. cv. 'Hujingmilu') used in this study had a soluble solids content of 12 % (°Brix) and a firmness of 32.78 $\pm$ 0.78 N, grown by the Jiangsu Academy of Agricultural Sciences, was delivered to the laboratory within 3 h of harvesting. Fruits from 50 trees were taken for this experiment. Peach with uniform size and maturity, no physical damage and no pest or disease, were selected as experimental materials. PSK- α was bought from Suzhou Pepmic Co., Ltd.

We selected 100, 200, 300, 400 nmol L⁻¹ PSK- α for 10 min immersion treatments on peaches, and the control peach was treated with distilled water. The control group included 100 peaches, while each of the treatment groups had 80 peaches. The treated samples were stored at 5 °C for 28 d with a relative humidity of 85 %–90 %. Parameters were measured on 0, 7, 14, 21, and 28 d. Every 7 d, 10 peaches were transferred from 5 °C to 25 °C for 3 d and then assessed for CI index and firmness. Based on the results of the preliminary experiment, we selected 200 nmol L⁻¹ PSK- α treatment for peach fruit. 60 peaches were randomly selected and reserved as the untreated control. The remaining fruits (n = 560) were randomly allocated into two treatment groups (n = 280 per group): one immersed in distilled water and the other in a 200 nmol L⁻¹ PSK- α for 10 min. Following treatment, all samples were stored at 5 °C (relative humidity 85 %–90 %) for 28 d. At 7 d intervals, 60 fruits were removed from cold storage, flash-frozen in liquid nitrogen, and stored at -80 °C for subsequent biochemical analyses. In parallel, ten fruits from each treatment were transferred every 7 d to 25 °C for 3 d to assess browning index according to established protocols. The treatment method followed Zeng et al. (2024).

2.2. Browning index, firmness, relative electric conductivity, and extractable juice

The 10 peaches were sliced on both lateral sides at one-quarter of the distance from the edge to the center, and browning on the cut surfaces was visually evaluated. Internal browning was then scored according to the following criteria: level 0 for no browning, level 1 for 0–5 % browning area, level 2 for 5 %–25 % browning area, level 3 for 25 %–50 % browning area, and level 4 for 50 %–100 % browning area (Ling et al., 2018). The browning index was calculated as follows: browning index = $\sum$ [(browning area level) $\times$ (number of fruit at the browning area level)] / (4 $\times$ number of fruit per treatment) $\times$ 100 %.

Firmness was measured using a puncture test with probe P/0.6. The initial force was set at 0.1 N, the puncture speed at 120 mm min⁻¹, and the puncture depth at 5 mm. 6 peaches from each group were selected, and 4 points around the equator of each fruit were randomly tested.

Relative electric conductivity was measured using a conductivity meter. From each fruit, six discs (5 mm in diameter) were prepared and placed into a test tube containing 20 mL of distilled water. Three test tubes were prepared per treatment group. The initial conductivity was measured and recorded as P₀. After standing for 30 min, the conductivity was measured again as P₁. Finally, the test tube was boiled for 15 min. The samples were allowed to cool to room temperature, and the

conductivity was measured as P_2 . Relative electric conductivity was calculated as follows: relative electric conductivity = $(P_1 - P_0) / (P_2 - P_0) \times 100$.

Extractable juice was determined following the method of Zhao et al. (2023). The absorbent paper was placed in a tube, and the weight was recorded as M_0 . 6 flesh discs of uniform size were placed into the tube, and the weight was recorded as M_1 . After centrifuging at 4000 g for 10 min, the discs were removed and the weight was recorded as M_2 . The Extractable juice was calculated as follows: extractable juice = $(M_2 - M_0) / (M_1 - M_0) \times 100$.

2.3. PSK- α content

1 g of frozen mesocarp tissue was homogenized in 9 mL of 0.01 M potassium phosphate buffer (pH 7.3). The homogenate was centrifuged at 12,000 g for 20 min, and the supernatant was collected for analysis. PSK- α concentration was measured using an ELISA kit (Jiangsu Meimian Industrial Co., Ltd., China) with 10 μ L of the supernatant. Results were expressed on a fresh weight (FW) basis as ng kg⁻¹.

2.4. RNA isolation and sequencing

RNA was extracted from 2 g of mesocarp tissue per sample. Each biological replicate was obtained from a single, independently sampled peach, with three biological replicates per treatment group (control and treated).

RNA samples were collected at 6 h and 7 d after the onset of cold storage. A total of 1 μ g of RNA was extracted and processed to prepare the mRNA sequencing library using the MGI Easy RNA Directional Library Prep Kit (MGI, Shenzhen) following the manufacturer's protocol. The sequencing was performed on the MGI-2000 platform (MGI, Shenzhen), utilizing 150 bp paired-end reads to obtain high-quality data for further analysis.

2.5. Identification of differentially expressed genes (DEGs)

Clean reads were aligned to the reference genome using Bowtie for further analysis (Langmead and Salzberg, 2012), and gene expression for each sample were then quantified using RSEM (Li and Dewey, 2011). The DESeq2 approach, which utilizes the negative binomial distribution, was applied to detect differentially expressed genes, adhering to the methodology outlined by Love et al. (2014). Fold change (FC) of FPKM (PSK- α) and FPKM (Control) ≥ 1.2 or ≤ 0.8 and Q-value ≤ 0.05 were considered significantly upregulated or downregulated.

2.6. Enrichment analyses of KEGG

DEGs were functionally classified based on Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway annotations. And their enrichment was performed using the phyper function in R, calculating P-values, which were then adjusted for FDR to obtain Q-values. Generally, Q-value ≤ 0.05 was considered significant.

2.7. Quantitative real-time PCR validation of RNA-seq data and metabolic enzyme-related genes

Using qRT-PCR, 8 DEGs associated with the synthesis of secondary metabolites were further analyzed. The corresponding GenBank accession numbers and DEG IDs are listed as follows: 18768825 (XM_007200391.2), 18788738 (XM_007223068.2), 18790877 (XM_020555722.1), 18770262 (XM_007204587.2), 18782650 (XM_007215909.2), 18791668 (XM_007225935.2), 18781341 (XM_007212112.2), 8788985 (XM_007223539.2). Meanwhile, the relative expression levels of genes encoding synthetic enzymes involved in metabolism were also assessed. Relative gene expressions were normalized by comparison with the expression of Actin

(XM_007204300.2) and analyzed using the $2^{-\Delta\Delta CT}$ method. The sequences of oligonucleotide primers are listed in Table S1.

2.8. GABA-T and GAD activity, glutamic acid, and GABA content

The glutamic acid content was determined following (Al-Quraan et al., 2011) method with modifications. 1 g sample was homogenized in 5 mL of 75 % methanol, and the absorbance was measured at 340 nm. A standard curve was created using NADH as the standard to calculate glutamic acid content, and results were expressed as mg kg⁻¹.

GABA content and GAD activity were measured following the method described by Shan et al. (2016). GABA content was determined from 5 g of fruit tissue using a GABA standard curve and expressed in mg kg⁻¹. GAD activity was measured from 2 g of tissue based on the amount of GABA production, with 1 U GAD activity defined as the amount required to produce 1 g GABA per min.

The activity of GABA-T was measured using an enzyme-linked immunosorbent assay (ELISA) kit. 1 g of tissue was ground thoroughly in 7 mL of phosphate buffer (pH 7.3). After centrifuging at 12,000 g for 20 min at 4 °C, the supernatant was collected as the extract for GABA-T activity measurement. Following the kit instructions, each reagent was added to the reaction mixture along with 10 μ L of the extract, and absorbance was measured at 450 nm.

2.9. DAO, PAO, ADC, and ODC activity

DAO and PAO activities were measured following the method of Wang et al., (2017). 2 g sample was ground in 5 mL of 100 mmol L⁻¹ potassium phosphate buffer (pH 6.5). For the reaction system, 0.1 mL of 20 mmol L⁻¹ putrescine, 0.1 mL of 20 mmol L⁻¹ Spd and Spm were added to supernatant from grinding. Absorbance change of 0.001 per min at 550 nm was defined as 1 U of DAO or PAO activity.

ADC and ODC activities were determined with modifications to the method (Zuo et al., 2021). 1 g sample was ground in 5 mL of 100 mmol L⁻¹ potassium dihydrogen phosphate buffer. After centrifugation at 12,000 g for 20 min, the supernatant was collected. Then, 0.2 mL of 50 μ mol L⁻¹ L-arginine and L-ornithine were added as substrates. 1 U of ADC or ODC activity was defined as a 0.001 change per min in absorbance at 254 nm per gram.

2.10. Putrescine (Put) and spermine (Spm) content

The method for PA content determination is based on Bokhary et al., (2020), with modifications. 2 g of sample were homogenized with 5 mL 5 % perchloric acid, incubated on ice for 1 h, and centrifuged with 12,000 g for 20 min at 4 °C to collect the supernatant. Two milliliters of the extract were combined with 1 mL of 2 mol L⁻¹ NaOH and 10 μ L benzoyl chloride. The mixture was thoroughly mixed and incubated at 30 °C for 30 min. Afterward, 2 mL of saturated NaCl solution was added. It was then extracted with 2 mL of ether, vortexed for 10 s, and centrifuged at 4000 g for 5 min. The ether layer was separated and dried under a stream of N₂ gas. The residue was dissolved in 1 mL methanol and filtered using a 0.45 μ m organic microporous filter. PA content analysis was performed using high performance liquid chromatography (HPLC, Agilent 1290) with a C18 reverse-phase column (Rapid Resolution HD 2.1 $\times$ 55 mm, 1.8 μ m) and UV detection at 254 nm. The mobile phase consisted of 65 % methanol (A) and 35 % water (B), with a flow rate of 0.8 mL/min. The injection volume was 20 μ L. Results were calculated based on standard curves for Put and Spm.

2.11. Proline content and P5CS, OAT and PDH activity

The proline content and the activities of P5CS, OAT, and PDH were determined following the method described by Sánchez et al. (2001).

For proline content, 2 g tissue was shaken and extracted in sulfosalicylic acid solution for 10 min. After centrifugation at 3500 g for

10 min, the supernatant was stored at 4 °C for measurement. Absorbance was measured at 520 nm, and results were calculated based on a standard curve constructed with proline, expressed as mg kg⁻¹ FW.

For OAT activity, 1 g peach tissue was ground in 5 mL 50 mM phosphate buffer (pH 8.0) and centrifuged at 12,000 g for 25 min at 4 °C. 0.1 mL aliquot of the supernatant was added to 0.9 mL phosphate buffer containing 35 mM L-ornithine for the reaction. 1 U OAT activity was defined as the production of 1 μM Δ-1-pyrroline-5-carboxylate (P5C) per min.

For P5CS and PDH activities, 5 g peach tissue was ground in 10 mL 50 mM Tris-HCl buffer (pH 7.2). After centrifugation, the supernatant was collected as the crude extract for P5CS and PDH activity assays. The activity of P5CS is calculated based on NADP⁺ production, with 1 U of enzyme activity defined as the amount that forms 1 μmol NADP⁺ per second. PDH activity is calculated by measuring NADPH production at 340 nm, with one unit of enzyme activity defined as the amount that generates 1 μmol NADPH per second.

2.12. Phylogenetic tree and amino acid sequence analysis of PpbHLH62

The phylogenetic tree of PpbHLH62 was constructed using the Neighbor-Joining method in MEGA 11. Amino acid sequence alignment of PpbHLH62 was performed with DNAMAN 8 software.

2.13. Subcellular localization

The full-length CDS of PpbHLH62 (excluding the stop codon) was cloned into the pCambia1305 vector to generate the fusion construct PpbHLH62-GFP. This construct was introduced into *Agrobacterium tumefaciens* strain GV3101 and subsequently infiltrated into *Nicotiana benthamiana* leaves using a 1-mL needleless syringe. After 48 h, GFP fluorescence signals were detected using a fluorescence microscope (DM4000 B, Leica, Germany) following DAPI staining (10 μg mL⁻¹, C0065, Solarbio, China).

2.14. Dual-luciferase reporter (DLR) assay

The full-length CDS of PpbHLH62, lacking the stop codon, was inserted into the pGreenII 62-SK vector to serve as the effector construct. Promoter regions upstream of *PpGAD1*, *PpGAD4*, *PpP5CS*, *PpPAO2*, and *PpOAT* were individually cloned into the pGreenII 0800-LUC vector as reporter constructs. All recombinant plasmids were introduced into *Agrobacterium tumefaciens* strain GV3101. The effector and reporter strains were mixed at a volume ratio of 8:1 (v/v) and co-infiltrated into *Nicotiana benthamiana* leaves for transient expression. At 60 h post-infiltration, the ratio of firefly luciferase (LUC) and renilla luciferase (REN) was quantified using a Dual Luciferase Reporter Assay Kit (DL101-01, Vazyme, China) by the luminescence detector (GloMax 20/20, Promega, USA). Fluorescence signals were further observed using a luminescence imaging system (LB 985, Berthold, Germany).

2.15. Statistical analysis

Each sample in the experiment was analyzed with 3 biological replicates, and all data were expressed as mean ± standard deviation. Data analysis and plotting were performed using Origin 2024 (OriginLab, Massachusetts, USA), while significant differences were determined by independent sample T-tests using IBM SPSS Statistics 24 (SPSS Inc., Chicago, USA).

3. Results

3.1. Effects of different concentrations of PSK-α on CI of peach fruit during low temperature storage

As shown in Fig. 1, different concentrations of PSK-α treatment

exhibited varied effects on the quality of peach fruit. In Fig. 1A, it is evident that all treatment groups exhibited different levels of browning by the 21 + 3 d. At the end of storage, the control group showed the most severe internal browning, followed by the 400 nmol L⁻¹ group, and the 200 nmol L⁻¹ group displayed the least browning. Internal browning is the most evident manifestation of CI in peach fruit, with the browning index quantifying the extent of browning. As shown in Fig. 1B, the browning index increases with prolonged storage. At 28 + 3 d, the lowest browning index is observed in the 200 nmol L⁻¹ group, followed by the 100 nmol L⁻¹ and 300 nmol L⁻¹ groups, while the control and 400 nmol L⁻¹ groups exhibit the most severe CI. This result is consistent with the apparent

The decrease in firmness is a characteristic feature of the fruit ripening stage. As shown in Fig. 1C, at 28 d, the firmness of the control group decreased by 64.1 % compared to 0 d, while the firmness of the other treatment groups decreased by 82.4 %, 54.7 %, 68.1 %, and 86.4 %, respectively, from low to high concentrations. This suggests that the 200 nmol L⁻¹ group maintained significantly greater firmness compared to both the control and other treatment groups. Relative electric conductivity, an indicator of membrane integrity, increases with storage time across all groups, reflecting increasing cell membrane damage. The 200 nM PSK-α consistently exhibits significantly lower relative electric conductivity values compared to the control and other treatments, suggesting better maintenance of cell membrane stability under chilling conditions. Extractable juice first increased and then declined during storage. By the end of storage, the 200 nM treatment group showed significantly lower value compared to the other treatment and the control. The 200 nM treatment group retains the highest juicy content at later stages, particularly at 21 d and 28 d, significantly outperforming the control group and most other treatments.

In summary, different concentrations of PSK-α treatment exhibited significant differences in preserving peach fruit quality. Based on internal browning, browning index, and firmness, 200 nM of PSK-α was determined to be the optimal treatment concentration.

3.2. Effects of PSK-α treatment on PSK-α content and relative gene expression of PpPSK2, PpPSK3, and PpTPST in peach fruit stored at low temperature

During storage, the PSK-α content in peach fruit increased significantly at 6 h and then returned to the original level. Notably, PSK-α levels in the treated group were significantly higher than those in the control at 6, 12, and 24 h (Fig. 2A).

PpPSK2 and *PpPSK3* were significantly upregulated by PSK-α treatment, with peak expression at 6 h and 12 h, respectively (Fig. 2B and C). *PpTPST* expression also increased transiently, showing 3.5, 1.9, and 2.3 fold elevations over the control at 6, 12, and 24 h, respectively (Fig. 2D).

3.3. DEGs of RNA-seq

RNA-seq analysis was performed on peach fruit samples treated with 200 nmol L⁻¹ of PSK-α after 6 h and 7 d of low-temperature storage. To assess the reliability of the RNA-seq, eight highly expressed DEGs in peach were selected for qRT-PCR. The relative expression levels of each gene were plotted on the X against FPKM values on the Y, as shown in Fig. S1. The RNA-seq and qRT-PCR results were positively correlated, with an R² = 0.8361, indicating that the RNA-seq results are accurate and reliable.

Up-regulated genes are defined as those with FPKM(PSK-α)/FPKM(Control) ≥ 1.2, while down-regulated genes are those with FPKM(PSK-α)/FPKM(Control) ≤ 0.8. For the 6 h and 7 d samples analyzed (Fig. 3A), a total of 3262 DEGs were identified at 6 h after treatment, comprising 1687 upregulated genes and 1575 downregulated genes. At 7 d, there were 1645 DEGs, including 607 up-regulated genes and 1038 down-regulated genes. Finally, the union of DEGs identified at 6 h and 7 d comprised a total of 4169 genes, while the intersection between the

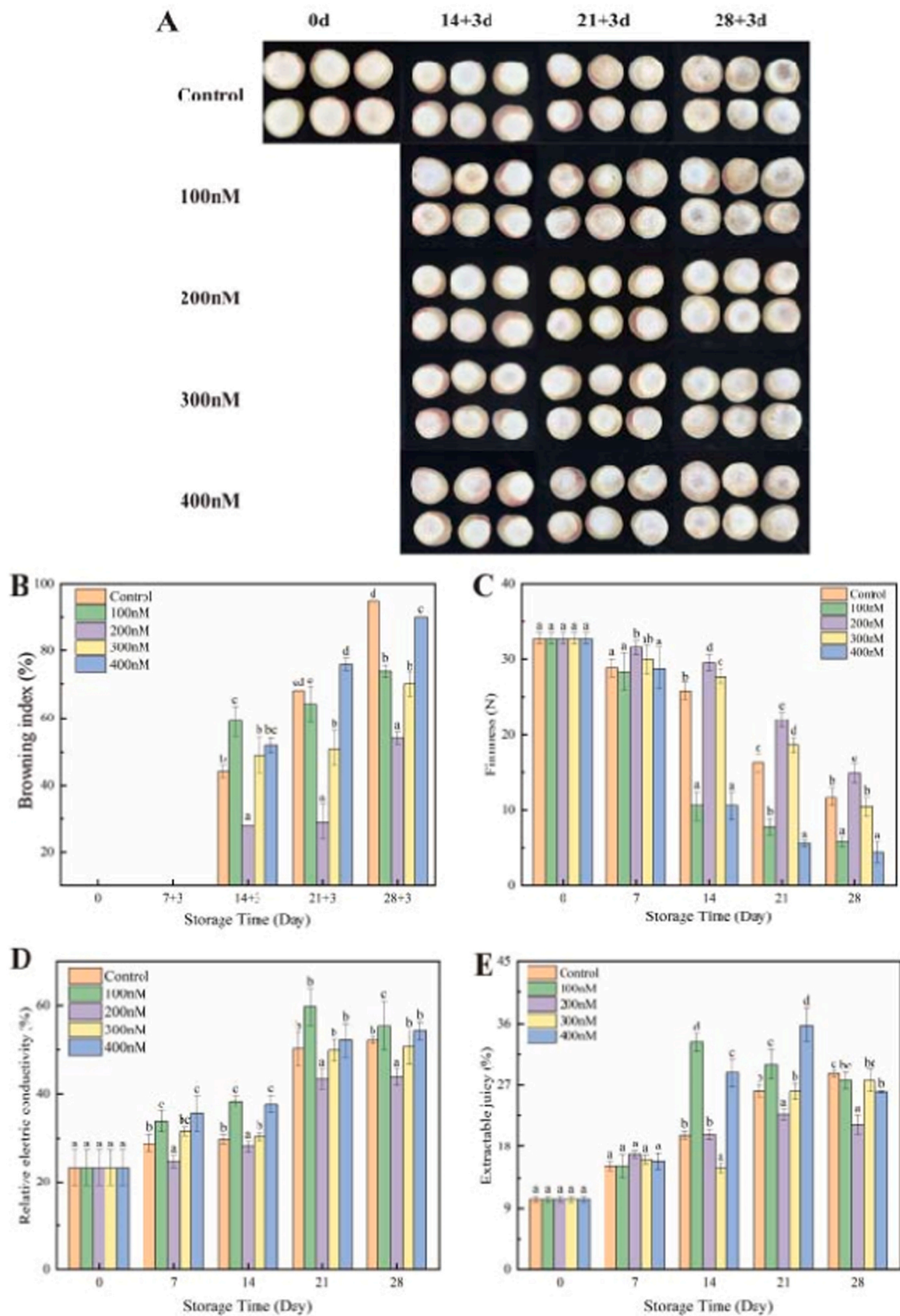


Fig. 1. Effects of different concentrations of PSK- α treatment on CI quality in peach. (A) Internal browning. (B) Browning index. (C) Firmness. (D) Relative electric conductivity (E) Extractable juice. Different letters indicate significant differences at the same storage time with $P < 0.05$.

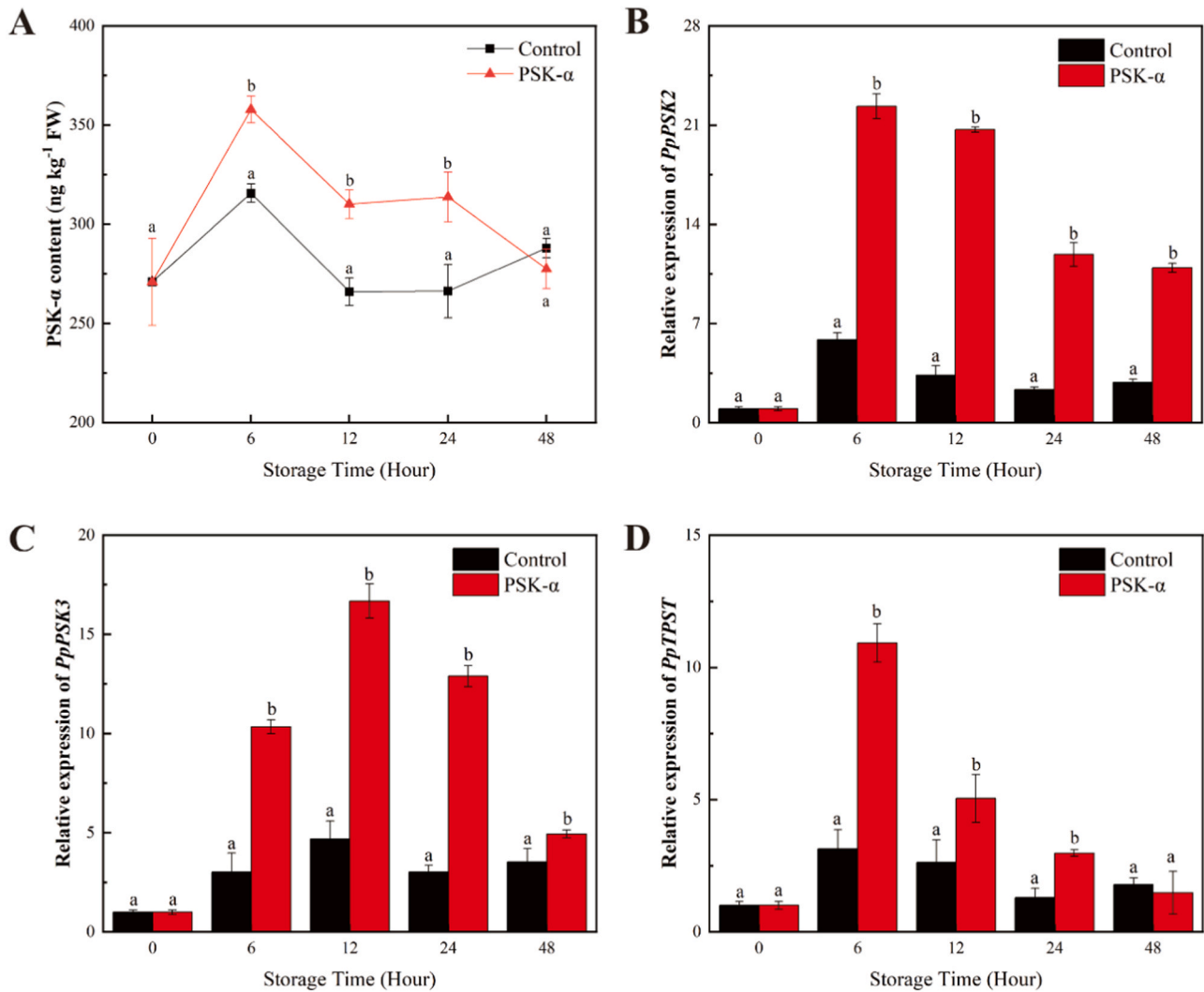


Fig. 2. Effects of different concentrations of PSK-α treatment on PSK-α content. (A) PSK-α content. (B) Relative gene expression of *PpPSK2*. (C) Relative gene expression of *PpPSK3*. (D) Relative gene expression of *PpTPST*.

two time points included 738 shared DEGs (Fig. 3B).

3.4. Enrichment analysis of KEGG and GO pathway

4169 DEGs were selected as candidates for KEGG and GO pathway enrichment analysis. Among them, 1168 genes were annotated to KEGG pathways. The five pathways with the highest significance (Q-value) were carbon metabolism, biosynthesis of amino acid, plant hormone signal transduction, propanoate metabolism, and ribosome (Fig. 3C).

Fig. 3D presents the significant enrichment of DEGs across three dimensions: biological processes, molecular functions, and cellular components, based on GO enrichment analysis results. Firstly, in biological processes, the pathways with the highest number of enriched genes are fatty acid metabolic process and proteolysis. Secondly, in molecular functions, the pathways with the most enriched genes are protein binding and nucleotide binding. Finally, for cellular components, the pathways with the highest number of enriched genes are the cytosolic small ribosomal subunit and the plasma membrane.

In addition, Arginine and proline metabolism also showed a high enrichment level (Q-value = 0.03) and contained a significant number of DEGs (number of gene = 21) (Fig. 3E). This pathway is involved in plant responses to abiotic stress, particularly playing a critical role in osmoregulation, nitrogen metabolism, and antioxidant responses. Therefore, selecting the Arginine and proline metabolism pathway for further in-

depth analysis will help reveal the effects of PSK-α treatment on the plant stress resistance mechanism. This aims to further elucidate the role of this metabolic pathway in the mechanism by which PSK-α mediates the alleviation of CI in peach fruit.

3.5. Effects of PSK-α treatment on GABA metabolism and relative gene expression of *PpGAD1*, *PpGAD4*, and *PpGABA-T3* in peach fruit stored at low temperature

The glutamic acid-dependent GABA metabolic pathway plays a dominant role in GABA biosynthesis (Bai et al., 2009). Glutamic acid is the precursor for GABA synthesis. Glutamic acid is converted into γ -aminobutyric acid (GABA) through the catalytic action of glutamic acid decarboxylase (GAD). As shown in Fig. 4A and B, the glutamic acid content decreased with the extension of storage time. The PSK-α treatment group maintained a lower glutamic acid content, possibly due to higher GAD activity compared to the control group, leading to greater conversion of glutamic acid.

GABA helps in scavenging reactive oxygen species (ROS) and maintaining intracellular osmotic balance to withstand stress, which is beneficial for peach fruit in resisting CI. GABA-T plays a critical role in GABA catabolism by catalyzing the conversion of GABA into succinic semialdehyde, which then enters the tricarboxylic acid (TCA) cycle. As shown in Fig. 4C and D, after 14 d, the PSK-α treatment group exhibited

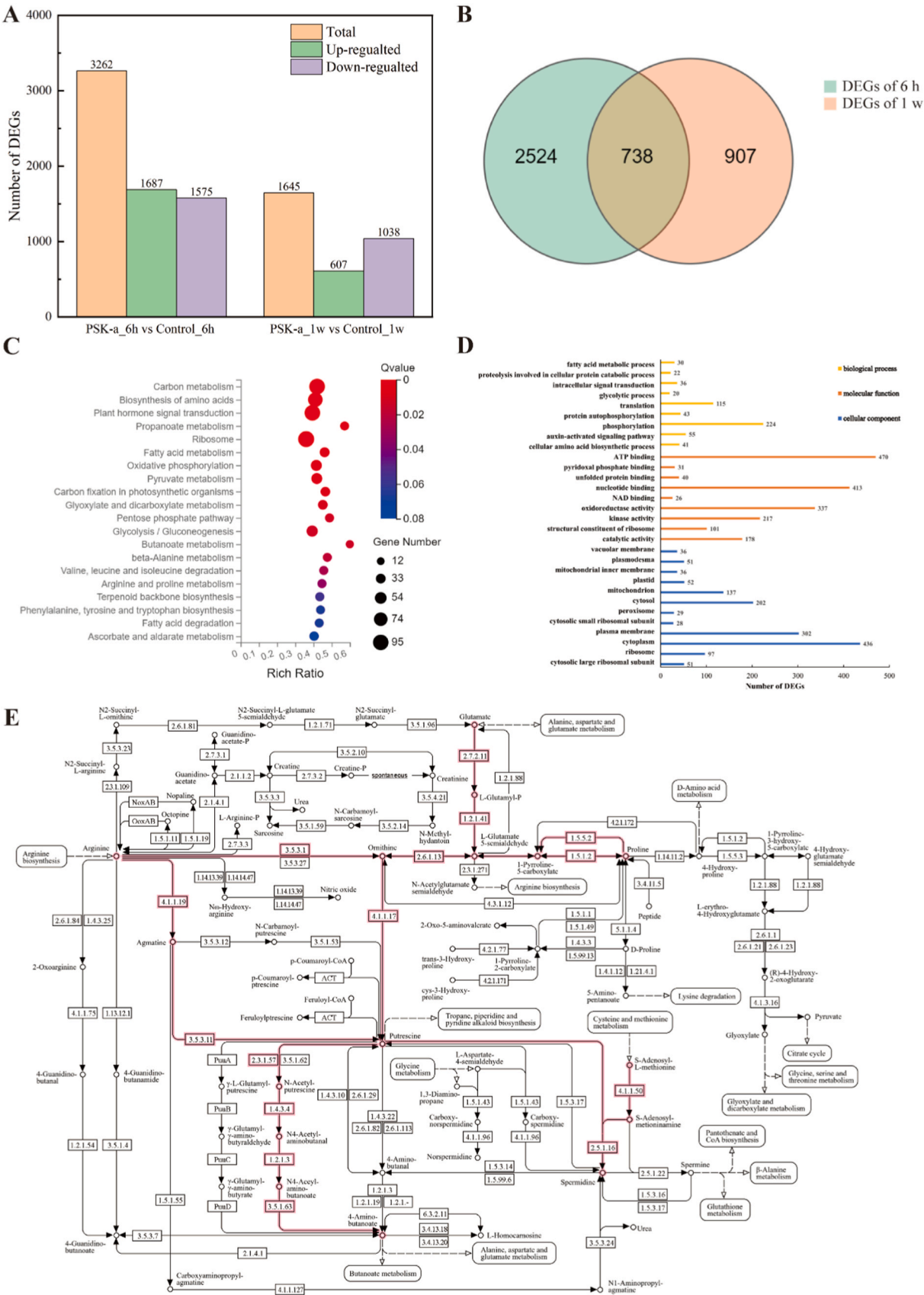


Fig. 3. Different expression genes (DEGs) analysis of RNA-seq. (A) Number of DEGs. (B) Venn of DEGs. (C) KEGG Pathway enrichment analysis (the bubble size indicates the number of DEGs annotated to the KEGG categories, and the bubble color represents the enrichment Q-value). (D) GO Pathway enrichment analysis. (E) MAP00330 Arginine and proline metabolism. The key sub-pathways are indicated by red lines.

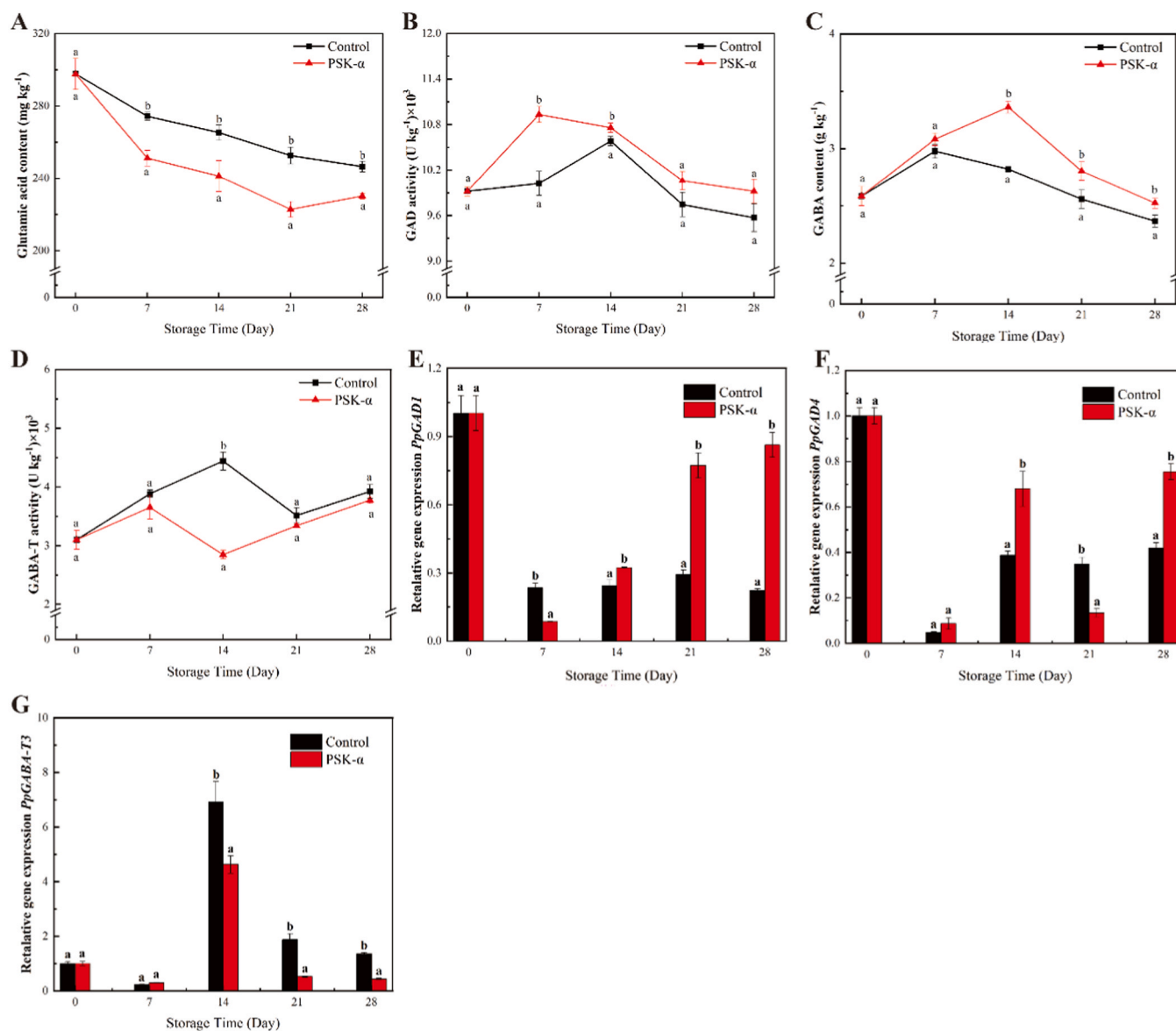


Fig. 4. Effect of PSK- α treatment on GABA metabolism. (A) Glutamic acid content. (B) GAD activity. (C) GABA content. (D) GABA-T activity. (E) Relative gene expression of *PpGAD1*. (F) Relative gene expression of *PpGAD4*. (G) Relative gene expression of *PpGABA-T3*. Different letters indicate significant differences at the same storage time with $P < 0.05$.

significantly greater GABA content compared to the control group. On one hand, GAD activity was greater in the treatment group compared to the control, leading to increased GABA production. On the other hand, the activity of GABA-T in the treatment group was lower than that in the control group, consuming less GABA.

PSK- α treatment significantly activated the expression of *PpGAD1* and *PpGAD4* (Fig. 4E and F), with *PpGAD1* showing significantly higher expression than the control at 7 d and 14 d, and *PpGAD4* at 14 d and 28 d. This suggests that PSK- α enhances GABA synthesis, which plays a vital role in stress signaling and alleviating storage-related stress. Obviously, the expression level of *PpGABA-T3* was lower in the treatment group from 14 d to 28 d, indicating that PSK- α inhibited GABA catabolism (Fig. 4G).

In conclusion, PSK- α treatment maintained higher GABA content in the GABA shunt, which was beneficial for enhancing fruit stress resistance.

3.6. Effects of PSK- α treatment on PA degradation pathway and relative gene expression of *PpADC* and *PpPAO2* in peach fruit stored at low temperature

In peach fruit, the Put content in the PSK- α treatment group continuously increased during storage and showed significant differences from the control group, except on 0 d and 21 d (Fig. 5A). By contrast, Spm levels in the treatment group remained consistently and significantly higher compared to those in the control group (Fig. 5B).

ODC and ADC catalyze the formation of putrescine from ornithine and arginine, respectively. Figs. 5C and 5D show that the activities of ODC and ADC in the treatment group remained significantly higher than those in the control group. PAO and DAO oxidize Spm and Put, respectively, leading to the production of GABA. Fig. 5E illustrates that PAO activity in the treatment group exhibited an increasing trend and was notably higher than in the control group, except on 14 d. DAO activity increased and then declined, reaching its peak on 14 d. From 14 d onwards, activity in the PSK- α treatment group was significantly greater than that in the control group (Fig. 5F).

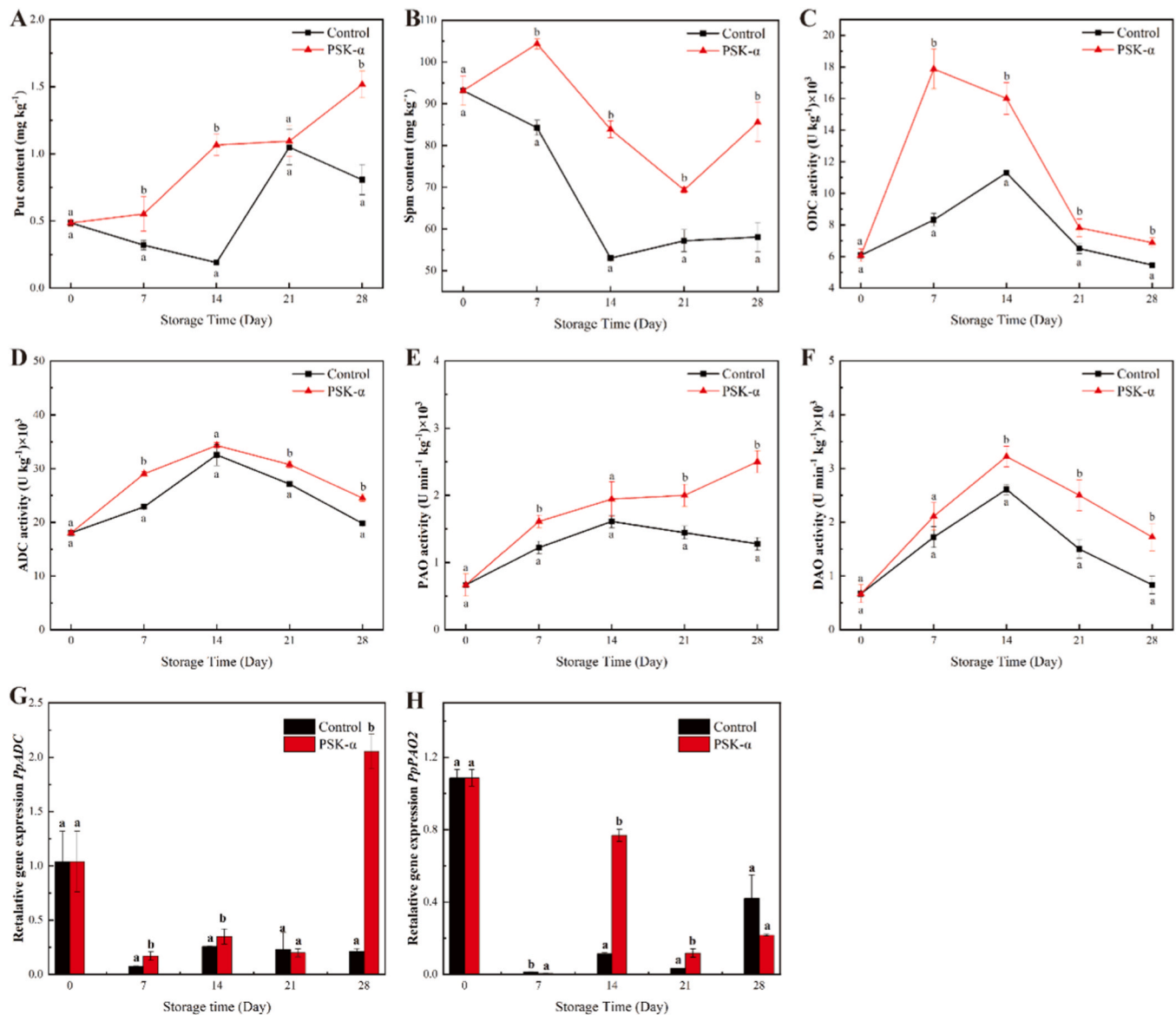


Fig. 5. Effect of PSK-α treatment on polyamines metabolism. (A) Put content. (B) Spm content. (C) ODC activity. (D) ADC activity. (E) PAO activity. (F) DAO activity. (G) Relative gene expression of *PpADC*. (H) Relative gene expression of *PpPAO2*. Different letters indicate significant differences at the same storage time with $P < 0.05$.

PpADC expression was significantly upregulated in the PSK-α-treated group at 7 d, 14 d, and 28 d (Fig. 5G). The expression of *PpPAO2* was significantly upregulated in the PSK-α treated group compared to the control, especially on 14 d and 21 d (Fig. 5H).

PSK-α treatment enhances Put and Spm levels, increases ODC and ADC activities, and promotes GABA production through elevated PAO and DAO activities in peach fruit during cold storage.

3.7. Effects of PSK-α treatment on proline metabolism and relative gene expression of *PpOAT*, *PpP5CS*, and *PpPDH2* in peach fruit stored at low temperature

OAT and P5CS respectively make ornithine and glutamic acid as substrates to produce P5C, which P5C is then converted into proline through further enzymatic reactions. As shown in Fig. 6A, OAT activity exhibited an upward trend in the early storage period, followed by a gradual decline. OAT activity in the PSK-α treatment group was significantly elevated compared to the control group. Additionally, P5CS showed a similar trend to OAT (Fig. 6B).

Evidently, due to the upregulation of OAT and P5CS activities, the treatment group maintained a significantly elevated proline content compared to the control group throughout the storage period (Fig. 6C). As shown in Fig. 6D, proline degrading enzyme PDH showed high activity in the control group, which limited proline accumulation within the fruit.

In the Fig. 6E, the expression of *PpOAT* was significantly elevated in the PSK-α-treated group on 21 d and 28 d, showing a 3.5- and 1.8-fold increase compared to the control. *PpP5CS* expression was markedly higher at 21 d compared to the control, with an increase of approximately 3.3-fold (Fig. 6F). For the expression of *PpPDH2* (Fig. 6G), a key enzyme in proline catabolism, the PSK-α-treated group showed significantly higher expression on 21 d and 28 d.

Compared to the control, the PSK-α treatment had higher proline-producing enzymes OAT and P5CS activities and lower activity of the degrading enzyme PDH, resulting in higher proline content.

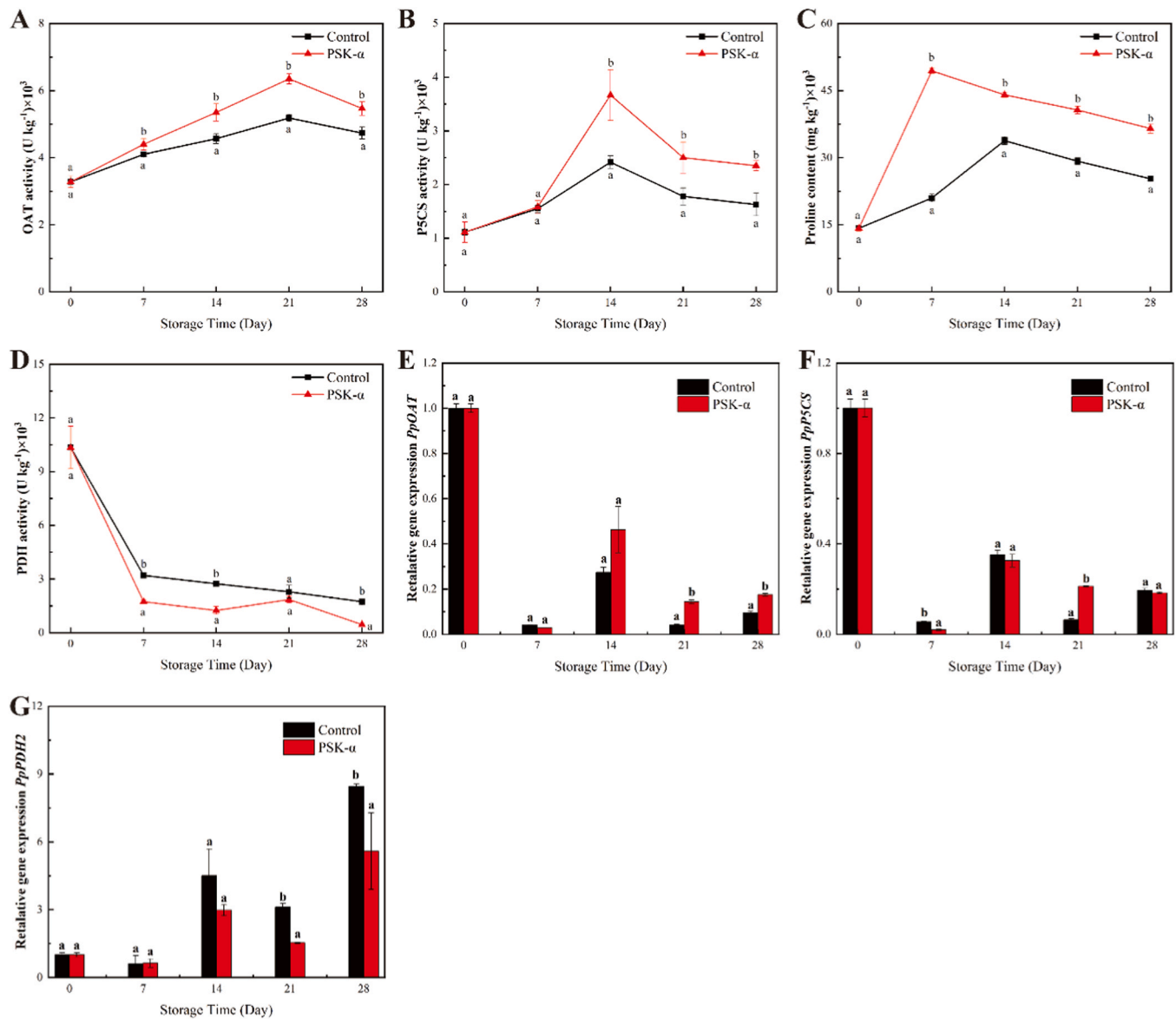


Fig. 6. Effect of PSK- α treatment on proline metabolism. (A) OAT activity. (B) P5CS activity. (C) Proline content. (D) PDH activity. (E) Relative gene expression of *PpOAT*. (F) Relative gene expression of *PpP5CS*. (G) Relative gene expression of *PpPDH2*. Different letters indicate significant differences at the same storage time with $P < 0.05$.

3.8. Effect of PSK- α treatment on gene expression of *PpbHLH62*, subcellular localization and bioinformatics analysis of *PpbHLH62*

qRT-PCR was used to determine the relative expression of *PpbHLH62* in treated and control group during storage. As shown in Fig. 7A, *PpbHLH62* expression initially increased and then plateaued. Notably, on 7 d, the treatment group exhibited significantly higher expression than the control.

The *PpbHLH62*-GFP and Empty-GFP plasmids were transiently expressed in tobacco leaves by *Agrobacterium* infiltration. Fluorescence microscopy (Fig. 7B) revealed that *PpbHLH62* localizes to the nucleus, which may facilitate its function as a transcriptional activator.

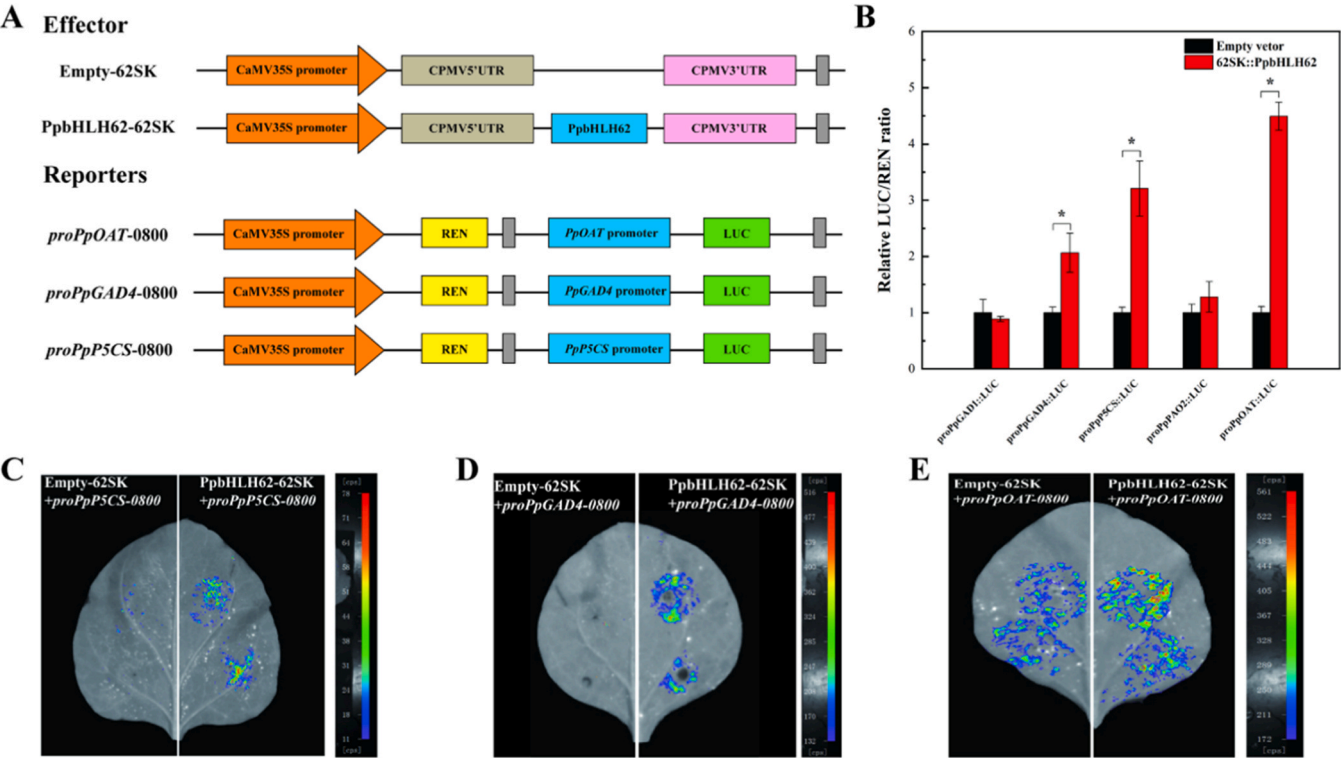
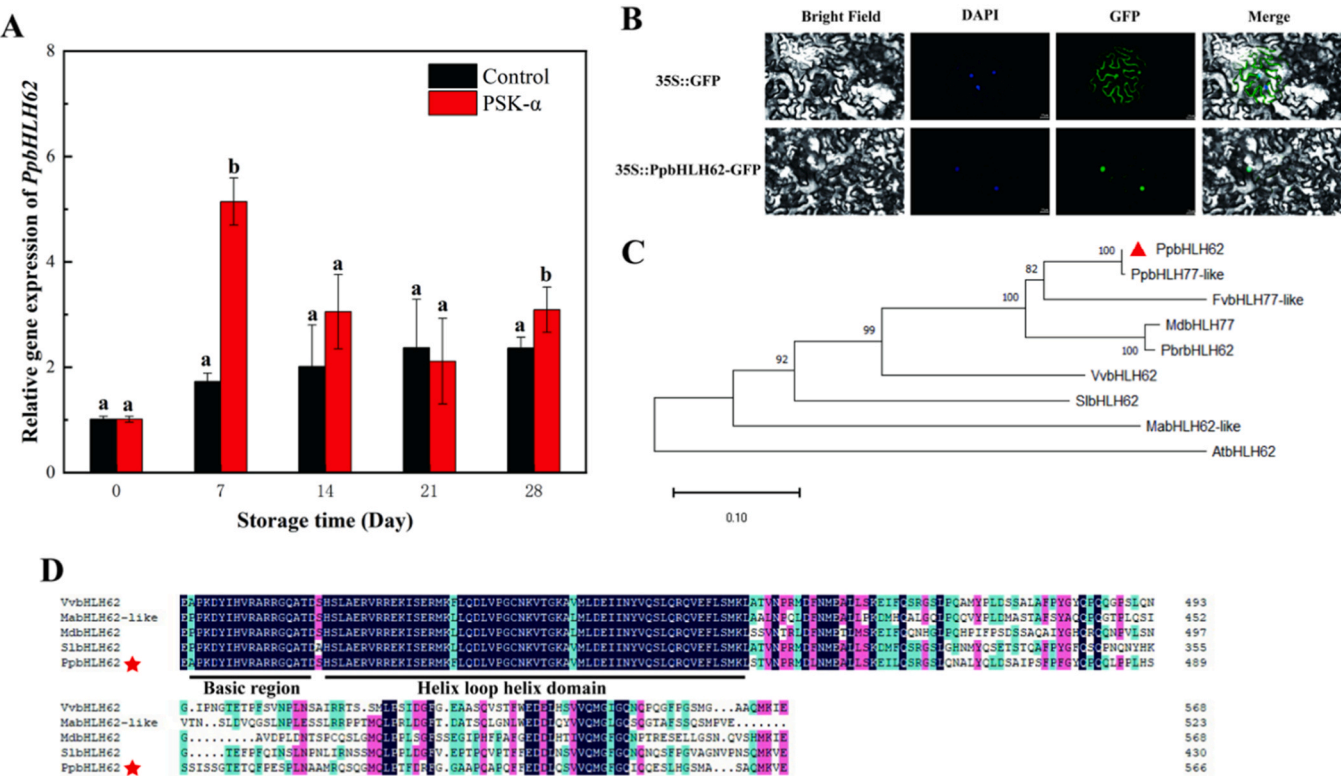
In MEGA-X, a Neighbor-Joining tree (with homology values indicated at each node) was constructed to compare the *bHLH62* sequences from *Malus domestica*, *Fragaria vesca*, *Vitis vinifera*, *Solanum lycopersicum*, *Pyrus bretschneideri*, *Arabidopsis thaliana*, and *Musa acuminata* with that of *Prunus persica* *PpbHLH62* in Fig. 7C. The results indicate that, aside from its high similarity with the *PpbHLH72*-like sequence in the same species, *PpbHLH62* exhibits the highest homology with *FvbHLH62*.

Additionally, *PpbHLH62* shows considerable similarity to *bHLH72* in *Malus domestica* and *bHLH62* in *Pyrus pyrifolia*, suggesting that these proteins may share similar functions.

Amino acid sequences of *PpbHLH62* and *bHLH62* or *bHLH62*-like proteins from other species were aligned using DNAMAN 8 (Fig. 7D). The Basic region and Helix–Loop–Helix (HLH) domain were annotated below the alignment. Conserved residues within these regions indicate the presence of characteristic motifs of the *bHLHs* across homologous proteins.

3.9. Transcriptional activation of *PpbHLH62* on *PpGAD4*, *PpP5CS*, and *PpOAT*

Vectors for DLR assays were constructed as illustrated in Fig. 8A. As shown in Fig. 8B, *PpbHLH62* was able to bind to the promoters of *PpGAD4*, *PpP5CS*, and *PpOAT*, thereby activating the transcription of the downstream LUC reporter gene. Compared with the Empty-62SK control, *PpbHLH62* significantly enhanced LUC expression by 2.06, 3.61, and 4.50 fold, respectively. These findings were further supported by in



vivo bioluminescence imaging assays (Fig. 8C–E).

4. Discussion

Low-temperature storage is an important method for extending fruit quality. However, peach fruit stored at low temperatures for extended periods is prone to CI. Typical CI symptoms in peach fruit include mealiness, impaired ripening, surface pitting, and internal browning. Among these, internal browning is one of the most visually alarming and commercially significant manifestations, as it directly affects consumer acceptance and marketability. In this study, browning symptoms were used as the primary indicator to evaluate the severity of CI and the efficacy of PSK- α treatment. Compared to other CI symptoms, browning is closely associated with osmotic imbalance and cellular metabolic disruption during cold stress. Therefore, our focus on browning provides valuable insights into how the regulation of osmolyte metabolism contributes to enhancing cold tolerance in postharvest peach fruit. (Cantín et al., 2010; Wang et al., 2024a). In this study, 200 nM PSK- α treatment significantly inhibits internal browning and reduced softening in peach during CI (Fig. 1A, B and C). Notably, a significant decline in fruit firmness was observed in the 100 nM and 400 nM PSK- α treatment groups at 14 d. This may be attributed to the concentration-dependent effect of PSK- α . At suboptimal concentrations, the treatment may fail to activate the fruit's defense system against CI. At higher concentrations, PSK- α may induce physiological stress or disrupt the balance of endogenous hormones, thereby promoting the expression of cell wall-degrading enzymes. Both scenarios could contribute to the acceleration of fruit softening. At the same time, the relative electrical conductivity and extractable juice also showed varying degrees of reduction in the 200 nM PSK- α treatment group, indicating that the 200 nM treatment maintained cellular integrity (Fig. 1D and E). As extractable juice is closely linked to mealiness, a key CI-related texture disorder, it was used to assess internal quality changes. In our study, extractable juice increased during the development of CI, particularly in the early to mid storage period. This may be attributed to CI-induced membrane damage and tissue loosening, which promote intracellular fluid leakage and facilitate juice release. Therefore, the extractable juice reflects structural disruption rather than improved fruit quality, and may serve as an early indicator of chilling-induced physiological damage. The same results were confirmed in experiments where PSK- α treatment delayed CI in bananas and loquats (Liu et al., 2024b; Wang et al., 2022).

In this study, we observed a significant increase in PSK- α content at 6 h during the early stage of cold storage, followed by a gradual decline toward baseline levels (Fig. 2A). This transient accumulation reflects an early physiological response of the fruit to CI and PSK- α treatment. The expression patterns of *PpPSK2*, *PpPSK3*, and *PpTPST* further support this observation. *PpPSKs* encode precursor peptides that require post-translational modifications to become biologically active. Specifically, tyrosine residues must be sulfated by tyrosylprotein sulfotransferase (TPST), followed by proteolytic cleavage by subtilisin-like serine proteases to release the mature PSK- α peptide (Yang et al., 1999). In our study, *PpPSK2* and *PpPSK3* were rapidly induced during early storage, suggesting enhanced precursor synthesis (Fig. 2B and C). Meanwhile, *PpTPST* was significantly upregulated between 6 and 24 h, indicating activation of the sulfation process (Fig. 2D). These findings suggest that exogenous PSK- α treatment not only stimulates transcription of its precursor genes but also promotes the enzymatic machinery required for PSK- α maturation, potentially forming a positive feedback loop that contributes to the fruit's early defense response against CI. Further studies in peach have shown that PSK- α treatment induced an increase in endogenous PSK- α content, enhancing signal transduction (Jiao, 2022). Research suggests that the increase in endogenous PSK- α levels may be achieved by upregulating the expression of *FaPSK3*, *FaPSK6*, and *FaTPST* in strawberries (Aghdam et al., 2021). Similar findings have also been observed in loquats (Liu et al., 2024b). For signal transduction, PSK- α mainly interacts with the PSKR (PSK- α receptor) on the cell

membrane. Earlier research indicates that the intracellular region of PSKR has a kinase domain responsible for conveying phosphorylation signals (Kaufmann et al., 2017; Wang et al., 2023a). An alternative post-translational modification of PSKR has also been confirmed to mediate defense responses to necrotrophic fungus *Botrytis cinerea* via PUB-mediated ubiquitination (Hu et al., 2023). In addition to its role in signal transduction, PSK- α is also associated with active substances such as auxin, melatonin, and abscisic acid (ABA). Studies have reported that PSK- α enhances tomato leaf resistance to gray mold by promoting auxin synthesis (Zhang et al., 2018). Meanwhile, PSK- α also increases endogenous melatonin levels by upregulating the expression of melatonin-synthesizing enzyme-related genes, thereby improving cold tolerance in strawberry fruit (Aghdam et al., 2021). The above results indicate that PSK- α treatment delays the process of CI by significantly inhibiting internal browning, reducing the browning index, and delaying fruit softening and keeping cell integrity.

Although studies have identified that PSK- α can delay CI in peach fruit, its underlying molecular mechanism remains unclear. Transcriptomics, as a high-throughput sequencing technology, can be used to analyze changes in gene expression in peach fruit during the CI process as storage progresses. Sample of RNA-seq were collected at 6 h and 7 d. These time points were selected to represent the early signal response phase (6 h) and an early molecular response phase prior to the onset of visible chilling injury symptoms (7 d), based on preliminary observations that CI symptoms typically appear after 14 d at 5 °C. This study investigated the changes in gene expression between the treatment and control groups at 6 h and 7 d post-treatment, and conducted a comprehensive analysis. The results indicated that carbon metabolism, biosynthesis of amino acid, and plant hormone signal transduction were key pathways through which PSK- α delays CI in peach (Fig. 3C). Arginine and proline metabolism is involved in plant responses to abiotic stress, particularly playing a critical role in osmoregulation, nitrogen metabolism, and antioxidant responses. Exposure to abiotic stress, the pathways involved show varying responses, indicating their roles in stress responses (Xu et al., 2022). Along with cold stress, the leakage of intracellular substances following cell membrane damage aggravates osmotic imbalance, intensifying the adverse effects. During osmotic stress, increased concentrations of amino acids such as arginine and proline correlate with their enhanced utilization as substrates in the TCA cycle, thereby contributing to stress tolerance in plants (Batista-Silva et al., 2019). However, in pathway MAP00330, we observed that the sub-pathways M00015 Proline biosynthesis, M00972 Proline metabolism, M00133 Polyamine biosynthesis, M00134 Polyamine biosynthesis, and M00135 GABA biosynthesis contained a higher number of enriched genes. Notably, PAs, GABA, and proline in these pathways are key substances involved in regulating osmotic balance and redox homeostasis. For example, glutamic acid treatment in plum fruit has been shown to delay CI symptoms by influencing GABA metabolism (Hou et al., 2024). Previous studies have shown that ABA treatment regulates the transcription of genes involved in proline metabolism, thereby delaying browning in peach fruit (Yang et al., 2024). NaHS treatment can enhance the antioxidant capacity and cold tolerance of zucchini by inducing the accumulation of proline and PAs, thereby delaying CI in zucchini fruit (Yi et al., 2024). Therefore, further investigation into the synthesis and degradation pathways of PAs, GABA, and proline will help explain the effects of PSK- α on the CI process.

GABA plays a key role in helping plants cope with low-temperature stress. Under such conditions, GABA primarily enhances plant cold tolerance through several mechanisms. The accumulation of GABA within cells helps maintain osmotic balance, prevents water loss, and alleviates osmotic imbalance caused by low temperatures. The accumulation of GABA within cells helps to reduce water loss and osmotic imbalance caused by low temperatures (Liu et al., 2024a). Secondly, low temperatures lead to increased ROS activity, which easily cause cellular damage. Under cold conditions, GABA helps mitigate oxidative damage by clearing ROS through antioxidant mechanisms (Fan et al., 2024). In

our study, the significantly higher GABA content in the PSK- α treatment group compared to the control group could be explained by the treatment group's increased GAD enzyme activity and reduced GABA-T activity relative to the control (Fig. 4). These findings are consistent with the results observed in loquat and banana, where PSK- α treatment effectively delayed CI (Liu et al., 2024b; Wang et al., 2022). A study on wheat revealed that GABA began to accumulate 28 h after osmotic stress, suggesting that GABA may be the only amino acid intermediate responding to osmotic stress (Bartyzel et al., 2003). In a study on MeJA treated prune fruit, Zhao et al. (2025a) found that GABA content positively correlated with the activities of APX, SOD, and CAT, suggesting that the enhanced ROS scavenging observed in postharvest treatments under cold stress may be attributed to increased GABA content. It has been reported that increased endogenous GABA content can also enhance the activities of enzymes in the non-enzymatic antioxidant system, including glutathione peroxidase, glutathione reductase, monodehydroascorbate reductase, and glutathione S-transferase (Shelp et al., 2021). Postharvest melatonin treatment promoted GABA accumulation in yellow-flesh peach fruit by upregulating GABA biosynthesis genes, suppressing GABA degradation, and supporting related metabolic pathways during cold storage (Wu et al., 2023). Therefore, the delay in CI in peach fruit treated with PSK- α may be attributed to increased GAD activity, suppressed GABA-T activity, and the resulting accumulation of GABA.

In addition to the pathway that generates GABA from glutamic acid, the PA degradation pathway is considered an alternative route for GABA production (Bhardwaj et al., 2024). In this pathway, apart from producing GABA, PAs themselves exhibit various biological functions. Studies have shown that exogenous application of Put, Spm, and Spd could significantly enhance the tolerance of various plants to different stresses, such as drought, salinity, and low temperatures. External Put effectively enhances drought tolerance in sugar beet by boosting antioxidant enzyme activity, regulating ROS homeostasis, and promoting physiological and biochemical adaptations (Islam et al., 2022). Exogenous Spd enhanced salt-alkali stress tolerance in tomatoes by regulating PA metabolism and related enzyme activities in roots, exhibiting differential effects on tomato varieties with varying salt tolerance (Hu et al., 2012). Exogenous Spm alleviated CI in cold-stored immature soybeans by regulating the antioxidant defense system, maintaining cellular integrity under low-temperature conditions (Song et al., 2018). In this study, Put and Spm contents in the PSK- α treatment remained significantly higher than those in the control throughout the storage period, with both peaking around 14 d before declining (Fig. 5A and B). This indicates that PSK- α treatment effectively promotes PAs accumulation, potentially playing an important role in regulating physiological metabolism during cold storage. These results are in agreement with previous reports (Jia et al., 2022). In the early storage period, ODC and ADC activities were significantly higher in the PSK- α treatment compared to the control. This suggests that PSK- α treatment effectively enhances Put synthesis by upregulating ODC and ADC activities, with a more pronounced impact on PA metabolism during the initial storage phase (Fig. 5C and D). These findings are consistent with the results of Song et al. (2022), who reported that melatonin maintained the post-harvest quality of eggplants during cold storage. PAO and DAO activities were consistently higher in the PSK- α treatment group than in the control, peaking at 14 d and declining more gradually during later storage (Fig. 5E and F). This indicates that PSK- α treatment promotes GABA synthesis by enhancing PA catabolism. PAs accumulation, induced by higher ADC and ODC activity, enhances ROS scavenging and reduces electrolyte leakage and MDA levels. These effects help protect cells from chilling or stress-induced damage (Dai et al., 2024). At 28 d, *PpADC* expression was strongly induced in the PSK- α -treated group, coinciding with the highest Put content observed during storage (Fig. 5g). As *PpADC* encodes a key enzyme in Put biosynthesis, this suggests that PSK- α may promote late-stage Put accumulation, potentially enhancing membrane stability and chilling tolerance. Thus, it can be inferred that

exogenous PSK- α enhances cold tolerance in peach fruits by increasing ADC and ODC activity to elevate Put levels, while also promoting GABA production through elevated PAO and DAO activity.

Peach fruits treated with exogenous 200 nM PSK- α exhibited higher proline accumulation, which could be attributed to the increased activities of OAT and P5CS and the decreased activity of PDH in the treatment (Fig. 6). These results are consistent with findings that PSK- α treatment delays CI in bananas and loquats (Liu et al., 2024b; Wang et al., 2022). To cope with postharvest stress, triggering endogenous proline accumulation is crucial for enhancing cold tolerance in fruits and vegetables. Proline degradation via PDH transfers electrons to coenzyme FAD through the electron transport chain, resulting in ATP generation (Chen et al., 2024). Furthermore, its precursor, P5C, is oxidized by P5C dehydrogenase (P5CDH) to produce glutamic acid, which enters the TCA cycle to generate additional energy (Kavi Kishor and Sreenivasulu, 2013). Proline accumulation under stress conditions mitigates oxidative stress and osmotic imbalance, while its post-stress degradation provides cellular energy through these two pathways. Studies have shown that transcription factors regulate proline content through transcriptional control, thereby enhancing fruit storage tolerance. For example, glycine betaine-mediated *PpHsfA2a* improve cold tolerance in peach fruit by transcriptionally regulating proline metabolism-related enzymes (Song et al., 2025). Additionally, tomato plants with the *SlWRKY51* knocked out exhibit lower proline content and reduced cold tolerance (Wang et al., 2024b). In summary, PSK- α enhances proline content by increasing the activity of proline biosynthetic enzymes and reducing the activity of proline-degrading enzymes, thereby improving the cold tolerance of peach fruit.

As the second largest transcription factor family in plants, bHLHs regulate a wide range of physiological processes. In this study, expression analysis revealed that *PpbHLH62* was significantly induced by PSK- α treatment, with its transcript level on 7 d markedly higher than that of the control (Fig. 7A). Moreover, during cold storage at 5 °C, *PpbHLH62* expression on 7 d was significantly elevated compared to 0 d. These results suggest that *PpbHLH62* may play a crucial role in the PSK- α -mediated alleviation of CI in peach fruit. The bHLHs is categorized into typical bHLH proteins, which contain a basic region, and atypical bHLH proteins, which lack this region. The primary distinction between the two lies in the presence or absence of a DNA-binding domain. bHLHs containing basic region are capable of directly binding to promoter sequences to participate in transcriptional regulation, whereas those lacking this region cannot bind DNA on their own and instead influence downstream transcriptional processes by interacting with other proteins. Conserved domain analysis of *PpbHLH62* (Fig. 7D) revealed the presence of DNA-binding domain, indicating its potential to function as a transcription factor. Given the presence of a conserved DNA-binding domain, bHLHs have been shown to participate directly in transcriptional regulation.

To further elucidate their functional roles, especially under abiotic stress conditions, several studies have investigated how specific bHLH members regulate gene expression in response to CI. *MaMYC2* not only responds strongly to MeJA under cold stress but also interacts with *MaICE1*, suggesting that the interaction between these two transcription factors containing bHLH domains enhance the expression of downstream cold-responsive genes, thereby improving the fruit's resistance to CI (Zhao et al., 2013). The *MabHLH355* modulates CI in banana peel by regulating ROS metabolism; its downregulation under cold stress reduces *MaGSTF12* and *MaGSTT1* expression, impairing ROS scavenging and aggravating cold damage (Lin et al., 2024). bHLHs enhance cold tolerance in sweetpotato by regulating stress-responsive genes, particularly through E-box binding activity. Some, like *IbHLH8* and *IbHLH92*, are involved in stress-related gene networks, while *IbbHLH79*, an ICE1-like bHLH, activates the CBF pathway and significantly improves cold resistance when overexpressed (Jin et al., 2021). Promoter sequence analysis of key enzyme-encoding genes involved in GABA metabolism, polyamine catabolism, and proline metabolism revealed

that the promoters of *PpGAD1*, *PpGAD4*, *PpP5CS*, *PpPAO2*, and *PpOAT* contain one or more bHLH-binding motifs (CANNTG), suggesting potential direct regulation by bHLH transcription factors. Therefore, DLR assays were performed to evaluate the potential transcriptional targets of PpbHLH62, revealing that it significantly regulates *PpGAD4*, *PpP5CS*, and *PpOAT* (Fig. 8B). This regulatory effect was further confirmed by in vivo bioluminescence imaging experiments (Fig. 8B, C, and D). The CabHLH035 directly binds to the promoter of the *CaP5CS* and activates its expression, thereby promoting proline biosynthesis. Overexpression of *CabHLH035* leads to increased proline accumulation under salt stress, while silencing *CabHLH035* reduces proline content. This indicates that bHLHs enhance stress tolerance by regulating proline metabolism (Zhang et al., 2022a). Similar to the findings of this study, related results also support the key role of bHLHs in regulating amino acid-related secondary metabolism and enhancing stress tolerance in fruits and vegetables.

5. Conclusion

In this study, we demonstrated that PSK- α treatment enhanced the cold tolerance of peach fruit and delays CI symptoms by altering GABA metabolism, polyamine degradation pathways, and proline metabolism. By regulating the activities of related enzymes and maintaining higher levels of GABA, Put, Spm, and proline, the osmotic and redox balance in cells is preserved, alleviating CI in peach fruit under low-temperature conditions. The basic helix-loop-helix (bHLH) transcription factors play a crucial role in the regulation of nitrogen metabolism in plants. Studies have shown that both PSK- α treatment and low-temperature conditions induce the expression of *PpbHLH62*, which may regulate the biosynthesis of proline and GABA through its transcriptional activation function, thereby promoting their accumulation under abiotic stress and enhancing plant stress tolerance. In summary, this study provides new insights into how PSK- α and PpbHLH62 preserve the quality of peach fruit during CI.

CRediT authorship contribution statement

Peng Jin: Supervision, Methodology, Conceptualization. **Yinqiu Bao:** Writing – original draft, Investigation, Conceptualization. **Zheng-guo Wu:** Writing – review & editing. **Yonghua Zheng:** Writing – review & editing. **Yaqin Zhao:** Investigation. **Liuhua Xiao:** Data curation. **Liangyi Zhao:** Investigation. **Yu Liu:** Data curation. **Yuan Zeng:** Writing – original draft, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Peng Jin reports financial support was provided by National Natural Science Foundation of China. Peng Jin reports a relationship with the National Natural Science Foundation of China that includes: funding grants.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.postharvbio.2025.113746](https://doi.org/10.1016/j.postharvbio.2025.113746).

Data availability

No data was used for the research described in the article.

References

- Aghdam, M.S., Alikhani-Koupaei, M., 2021. Exogenous phyto-sulfokine α (PSK- α) applying delays senescence and relief decay in strawberry fruits during cold storage by sufficient intracellular ATP and NADPH availability. *Food Chem.* 336, 127685. <https://doi.org/10.1016/j.foodchem.2020.127685>.
- Aghdam, M.S., Sayyari, M., Luo, Z.S., 2020. Exogenous application of phyto-sulfokine α (PSK α) delays yellowing and preserves nutritional quality of broccoli florets during cold storage. *Food Chem.* 333, 127481. <https://doi.org/10.1016/j.foodchem.2020.127481>.
- Aghdam, M.S., Sayyari, M., Luo, Zs, 2021. Exogenous phyto-sulfokine α application delays senescence and promotes antioxidant nutrient accumulation in strawberry fruit during cold storage by triggering endogenous phyto-sulfokine α signaling. *Postharvest Biol. Technol.* 175, 111473. <https://doi.org/10.1016/j.postharvbio.2021.111473>.
- Ahmad, S., Fariduddin, Q., 2024. Deciphering the enigmatic role of gamma-aminobutyric acid (GABA) in plants: Synthesis, transport, regulation, signaling, and biological roles in interaction with growth regulators and abiotic stresses. *Plant Physiol. Biochem.* 208, 108502. <https://doi.org/10.1016/j.plaphy.2024.108502>.
- Ai, S.J., Xu, S.M., Wu, C.X., Grierson, D., Chen, K.S., Xu, C.J., 2024. Novel insights into modified atmosphere mediated cold tolerance in peach fruit during postharvest storage. *Postharvest Biol. Technol.* 218, 113187. <https://doi.org/10.1016/j.postharvbio.2024.113187>.
- Al-Quraan, N.A., Locy, R.D., Singh, N.K., 2011. Implications of paraquat and hydrogen peroxide-induced oxidative stress treatments on the GABA shunt pathway in *Arabidopsis thaliana* calmodulin mutants. *Plant Biotechnol. Rep.* 5, 225–234. <https://doi.org/10.1007/s11816-011-0174-3>.
- Bai, Q.Y., Chai, M.Q., Gu, Z.X., Cao, X.H., Li, Y., Liu, K.L., 2009. Effects of components in culture medium on glutamate decarboxylase activity and γ -aminobutyric acid accumulation in foxtail millet (*Setaria italica* L.) during germination. *Food Chem.* 116, 152–157. <https://doi.org/10.1016/j.foodchem.2009.02.022>.
- Bartzel, I., Pelczar, K., Paszkowski, A., 2003. Functioning of the γ -aminobutyrate pathway in wheat seedlings affected by osmotic stress. *Biol. Plant* 47, 221–225. <https://doi.org/10.1023/B:BIOP.0000022255.01125.99>.
- Batista-Silva, W., Heinemann, B., Rugen, N., Nunes-Nesi, A., Araújo, W.L., Braun, H.P., Hildebrandt, T.M., 2019. The role of amino acid metabolism during abiotic stress release. *Plant Cell Environ.* 42, 1630–1644. <https://doi.org/10.1111/pce.13518>.
- Bhardwaj, R., Sati, H., Oberoi, H.S., Pareek, S., 2024. The mechanisms of melatonin in low-temperature stress tolerance in postharvest fruits and vegetables. *Future Postharvest Food* 1, 180–197. <https://doi.org/10.1002/fpf2.12013>.
- Bokhary, S.U.F., Wang, L., Zheng, Y.H., Jin, P., 2020. Pre-storage hot water treatment enhances chilling tolerance of zucchini (*Cucurbita pepo* L.) squash by regulating arginine metabolism. *Postharvest Biol. Technol.* 166, 111229. <https://doi.org/10.1016/j.postharvbio.2020.111229>.
- Cantín, C.M., Crisosto, C.H., Ogundiwon, E.A., Gradziel, T., Torrents, J., Moreno, M.A., Gogorcena, Y., 2010. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. *Postharvest Biol. Technol.* 58, 79–87. <https://doi.org/10.1016/j.postharvbio.2010.06.002>.
- Chen, G., Kuang, X.Y., Fan, Z.Q., Chen, Y.Z., Lin, Y.F., Wang, H., Chen, Y.H., Lin, H.T., 2024. Energy and proline metabolisms participate in the occurrence of chilling injury in cold-stored Chinese olive fruit. *Postharvest Biol. Technol.* 214, 112955. <https://doi.org/10.1016/j.postharvbio.2024.112955>.
- Dai, Hy, Li, J., Tao, Jq, Song, Z.C., Ji, S.J., Zhou, X., Li, M.L., Zhou, Q., 2024. Exogenous spermidine treatment delays the softening of postharvest blueberry fruit by inhibiting the accumulation of abscisic acid. *Future Postharvest Food* 1, 252–262. <https://doi.org/10.1002/fpf2.12024>.
- Dong, H.B., Yang, J.L., Wang, Y., Jiang, Y., Chen, J., Zhang, W.N., Lu, Y.M., Chen, L., Chen, Y., 2020. Polysaccharide SAF from *Sarcodon aspratus* attenuates oxidative stress-induced cell damage and bleomycin-induced pulmonary fibrosis. *Int. J. Biol. Macromol.* 164, 1215–1236. <https://doi.org/10.1016/j.ijbiomac.2020.07.120>.
- Fan, Z.Q., Lin, B., Lin, Y.F., Chen, Y.Z., Chen, G., Chen, J.Y., Wang, H., Chen, Y.H., Lin, H.T., 2024. Alleviation of chilling injury in cold-stored Chinese olive (*Canarium album* Lour.) fruit by γ -aminobutyric acid treatment in relation to ROS metabolism. *Sci. Hortic.* 327, 112851. <https://doi.org/10.1016/j.scienta.2024.112851>.
- Ghosh, D., Das, T., Paul, P., Dua, T.K., Roy, S., 2024. Zinc-loaded mesoporous silica nanoparticles mitigate salinity stress in wheat seedlings through silica-zinc uptake, osmotic balance, and ROS detoxification. *Plant Physiol. Biochem.* 211, 108693. <https://doi.org/10.1016/j.plaphy.2024.108693>.
- Hou, Y.Y., Deng, R., Shataer, D., Hong, J.Y., Wang, L., Jin, P., Zhao, Y.T., 2024. L-Glutamate treatment alleviates chilling injury of prune (*Prunus domestica* L.) fruit by regulating ROS homeostasis, GABA shunt, and energy metabolism. *Food Chem.* 461, 140899. <https://doi.org/10.1016/j.foodchem.2024.140899>.
- Hu, Z.J., Fang, H.M., Zhu, C.G., Gu, S.H., Ding, S.T., Yu, J.Q., Shi, K., 2023. Ubiquitylation of PHYTOSULFOKINE RECEPTOR 1 modulates the defense response in tomato. *Plant Physiol.* 192, 2507–2522. <https://doi.org/10.1093/plphys/kiad188>.
- Hu, X.H., Zhang, Y., Shi, Y., Zhang, Z., Zou, Z.R., Zhang, H., Zhao, J.Z., 2012. Effect of exogenous spermidine on polyamine content and metabolism in tomato exposed to salinity-alkalinity mixed stress. *Plant Physiol. Biochem.* 57, 200–209. <https://doi.org/10.1016/j.plaphy.2012.05.015>.
- Huang, S., Bi, Y., Cao, S.F., Li, X.W., Zhang, Q., Lei, Y.X., Jia, M.H., Zhang, M.M., Liu, Q., Wang, X.B., Gao, Z.C., Chen, H.X., Wang, J., 2025. Melatonin regulates proline metabolism by modulating CmDREB1A/E to enhance cold tolerance in cantaloupe fruit. *Plant Physiol. Biochem.*, 110027 <https://doi.org/10.1016/j.plaphy.2025.110027>.

- Islam, M.J., Uddin, M.J., Hossain, M.A., Henry, R., Begum, M.K., Soheli, M.A.T., Mou, M. A., Ahn, J., Cheong, E.J., Lim, Y.S., 2022. Exogenous putrescine attenuates the negative impact of drought stress by modulating physio-biochemical traits and gene expression in sugar beet (*Beta vulgaris* L.). *PLoS One* 17, e0262099. <https://doi.org/10.1371/journal.pone.0262099>.
- Jia, Z.Y., Bao, Y.Q., Zhao, Y.Q., Liu, Y., Zheng, Y.H., Feng, Z.Y., Jin, P., 2023. Cold shock treatment enhances cold tolerance in peach fruit through modulating PpZIP9 and PpVIP1-mediated respiratory metabolism. *Postharvest Biol. Technol.* 204, 112421. <https://doi.org/10.1016/j.postharvbio.2023.112421>.
- Jia, Z.Y., Wang, Y., Wang, L., Zheng, Y.H., Jin, P., 2022. Amino acid metabolomic analysis involved in flavor quality and cold tolerance in peach fruit treated with exogenous glycine betaine. *Food Res. Int.* 157, 111204. <https://doi.org/10.1016/j.foodres.2022.111204>.
- Jiao, C.F., 2022. PpCBF6 is involved in phytosulfokine α -retarded chilling injury by suppressing the expression of PpLOX5 in peach fruit. *Front. Plant Sci.* 13, 874338. <https://doi.org/10.3389/fpls.2022.874338>.
- Jin, R., Kim, H.S., Yu, T., Zhang, A.J., Yang, Y.F., Liu, M., Yu, W.H., Zhao, P., Zhang, Q. Q., Cao, Q.H., Kwak, S.S., Tang, Z.H., 2021. Identification and function analysis of bHLH genes in response to cold stress in sweetpotato. *Plant Physiol. Biochem.* 169, 224–235. <https://doi.org/10.1016/j.plaphy.2021.11.027>.
- Kaufmann, C., Motzkus, M., Sauter, M., 2017. Phosphorylation of the phytosulfokine peptide receptor PSKR1 controls receptor activity. *J. Exp. Bot.* 68, 1411–1423. <https://doi.org/10.1093/jxb/erx030>.
- Kavi Kishor, P.B., Sreenivasulu, N., 2013. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant Cell Environ.* 37, 300–311. <https://doi.org/10.1111/pce.12157>.
- Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359. <https://doi.org/10.1038/nmeth.1923>.
- Li, B., Dewey, C.N., 2011. RSEM accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinf.* 12, 323. <https://doi.org/10.1186/1471-2105-12-323>.
- Li, Z., Huang, T., Tang, M., Cheng, B., Peng, Y., Zhang, X., 2019. iTRAQ-based proteomics reveals key role of gamma-aminobutyric acid (GABA) in regulating drought tolerance in perennial creeping bentgrass (*Agrostis stolonifera*). *Plant Physiol. Biochem.* 145, 216–226. <https://doi.org/10.1016/j.plaphy.2019.10.018>.
- Li, S.E., Ma, R.J., Xu, J.L., Shen, Z.J., Yu, M.L., 2024. Effect of melatonin treatment on improving jasmonates content and cell wall stability involved in enhanced chilling tolerance of peach fruit during cold storage. *Postharvest Biol. Technol.* 213, 112957. <https://doi.org/10.1016/j.postharvbio.2024.112957>.
- Lin, S.Q., Song, W., Gan, L.Z., Wei, W., Shan, W., Kuang, J.F., Chen, J.Y., Lu, W., 2024. Low temperature downregulates MabHLH355 and its associated target genes responsible for scavenging ROS in banana peel under cold stress. *Postharvest Biol. Technol.* 213, 112956. <https://doi.org/10.1016/j.postharvbio.2024.112956>.
- Ling, C., Xu, J., Shao, S., Wang, L., Jin, P., Zheng, Y.H., 2018. Effect of ultrasonic treatment combined with peracetic acid treatment reduces decay and maintains quality in loquat fruit. *J. Food Qual.* 2018, 1–8. <https://doi.org/10.1155/2018/7564056>.
- Liu, M.M., Gao, J., Wang, N., Yan, Y.G., Zhang, G., Chen, Y., Zhang, M.Y., 2024a. Effects of exogenous GABA on physiological characteristics of licorice seedlings under saline-alkali stress. *Plant Stress* 11, 100364. <https://doi.org/10.1016/j.stress.2024.100364>.
- Liu, Y., Hou, Y.Y., Yi, B.H., Zhao, Y.Q., Bao, Y.Q., Wu, Z.G., Zheng, Y.H., Jin, P., 2024b. Exogenous phytosulfokine α alleviates chilling injury of loquat fruit via regulating sugar, proline, polyamine and gamma-aminobutyric acid metabolisms. *Food Chem.* 436, 137729. <https://doi.org/10.1016/j.foodchem.2023.137729>.
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
- Manna, M., Thakur, T., Chirom, O., Mandlik, R., Deshmukh, R., Salvi, P., 2021. Transcription factors as key molecular target to strengthen the drought stress tolerance in plants. *Physiol. Plant* 172, 847–868. <https://doi.org/10.1111/ppl.13268>.
- Min, D.D., Zhou, J.X., Li, J.Z., Ai, W., Li, Z.L., Zhang, X.H., Fu, X.D., Zhao, X.M., Li, F.J., Li, X.A., Guo, Y.Y., 2021. SIMYC2 targeted regulation of polyamines biosynthesis contributes to methyl jasmonate-induced chilling tolerance in tomato fruit. *Postharvest Biol. Technol.* 174, 111443. <https://doi.org/10.1016/j.postharvbio.2020.111443>.
- Qian, C.L., Ji, Z.J., Zhu, Q., Qi, X.H., Li, Q.Q., Yin, J.D., Liu, J., Kan, J., Zhang, M., Jin, C. H., Xiao, L.X., 2021. Effects of 1-MCP on proline, polyamine, and nitric oxide metabolism in postharvest peach fruit under chilling stress. *Hortic. Plant J.* 7, 188–196. <https://doi.org/10.1016/j.hpj.2020.12.007>.
- Sánchez, E., López-Lefebvre, L.R., García, P.C., Rivero, R.M., Ruiz, J.M., Romero, L., 2001. Proline metabolism in response to highest nitrogen dosages in green bean plants (*Phaseolus vulgaris* L. cv. Strike). *J. Plant Physiol.* 158, 593–598. <https://doi.org/10.1078/0176-1617-00268>.
- Shan, Tm, Jin, P., Zhang, Y., Huang, Y.P., Wang, X.L., Zheng, Y.H., 2016. Exogenous glycine betaine treatment enhances chilling tolerance of peach fruit during cold storage. *Postharvest Biol. Technol.* 114, 104–110. <https://doi.org/10.1016/j.postharvbio.2015.12.005>.
- Shelp, B.J., Aghdam, M.S., Flaherty, E.J., 2021. Gamma-aminobutyrate (GABA) regulated plant defense: mechanisms and opportunities. *Plants* 10. <https://doi.org/10.3390/plants10091939>.
- Song, H.W., Wang, X.F., Hu, W.C., Yang, X.H., Diao, E.J., Shen, T., Qiang, Q., 2017. A cold-induced phytosulfokine peptide is related to the improvement of loquat fruit chilling tolerance. *Food Chem.* 232, 434–442. <https://doi.org/10.1016/j.foodchem.2017.04.045>.
- Song, Q.Y., Wang, L., Wang, Y.Y., Shi, K.L., Wu, T.Y., Qiu, T., Jiang, J.J., Wang, X.Y., Liu, Z.K., Jin, P., Zheng, Y.H., Chen, D., 2025. PpHsfA2a-regulated the accumulation of proline and polyamines participates in glycine betaine-enhanced chilling resistance in peach fruit. *Postharvest Biol. Technol.* 220, 113320. <https://doi.org/10.1016/j.postharvbio.2024.113320>.
- Song, J.F., Wu, G., Liu, C.Q., Li, D.J., 2018. Effect of exogenous spermine on chilling injury and antioxidant defense system of immature vegetable soybean during cold storage. *J. Food Sci. Technol.* 55, 4297–4303. <https://doi.org/10.1007/s13197-018-3372-y>.
- Song, L.J., Zhang, W.W., Li, Q., Jiang, Z.X., Wang, Y.H., Xuan, S.X., Zhao, J.J., Luo, S.X., Shen, S.X., Chen, X.P., 2022. Melatonin alleviates chilling injury and maintains postharvest quality by enhancing antioxidant capacity and inhibiting cell wall degradation in cold-stored eggplant fruit. *Postharvest Biol. Technol.* 194, 112092. <https://doi.org/10.1016/j.postharvbio.2022.112092>.
- Wang, D., Huang, H., Jiang, Y.M., Duan, X.W., Lin, X.Y., Aghdam, M.S., Luo, Z.S., 2022. Exogenous phytosulfokine α (PSK α) alleviates chilling injury of banana by modulating metabolisms of nitric oxide, polyamine, proline, and γ -aminobutyric acid. *Food Chem.* 380, 132179. <https://doi.org/10.1016/j.foodchem.2022.132179>.
- Wang, L., Huang, X.L., Liu, C.C., Zhang, C., Shi, K.L., Wang, M.L., Wang, Y.Y., Song, Q.Y., Chen, X.Y., Jin, P., Zheng, Y.H., 2023c. Hydrogen sulfide alleviates chilling injury by modulating respiration and energy metabolisms in cold-stored peach fruit. *Postharvest Biol. Technol.* 199, 112291. <https://doi.org/10.1016/j.postharvbio.2023.112291>.
- Wang, D., Li, L., Xu, Yq, Limwachiranon, J., Li, D., Ban, Z.J., Luo, Z.S., 2017. Effect of exogenous nitro oxide on chilling tolerance, polyamine, proline, and γ -aminobutyric acid in bamboo shoots (*Phyllostachys praecox* f. *prevernalis*). *J. Agric. Food Chem.* 65, 5607–5613. <https://doi.org/10.1021/acs.jafc.7b02091>.
- Wang, T.T., Liu, J.C., Yuan, Y.B., Wang, Y., Cheng, C.X., Yang, S.L., 2024a. PpWRKY65 mediates peach (*Prunus persica*) fruit lignification in both ambient temperature storage and chilling injury condition. *Postharvest Biol. Technol.* 216, 113043. <https://doi.org/10.1016/j.postharvbio.2024.113043>.
- Wang, D., Ren, X., Meng, L., Zheng, R., Li, D., Kong, Q., 2023a. Exogenous phytosulfokine α (PSK α) alleviates chilling injury of kiwifruit by regulating Ca²⁺ and protein kinase-mediated reactive oxygen species metabolism. *Foods* 12, 4196. <https://doi.org/10.3390/foods12234196>.
- Wang, Y.X., Zhang, M.H., Wu, C.Z., Chen, C., Meng, L., Zhang, G.Q., Zhuang, K.Y., Shi, Q. H., 2024b. SIWRKY51 regulates proline content to enhance chilling tolerance in tomato. *Plant Cell Environ.* 47, 5104–5114. <https://doi.org/10.1111/pce.15081>.
- Wang, K., Zhu, G., Li, Y.L., Chen, S.Q., Rashid, A., Wang, X.T., Wu, X.Y., 2023b. Non-thermal effects of microwave irradiation alleviates postharvest chilling injury of peach fruit by retarding phenolic accumulation and enhancing membrane stability. *Food Chem.* 411, 135448. <https://doi.org/10.1016/j.foodchem.2023.135448>.
- Wu, C.C., Hao, W.Z., Yan, L., Zhang, H.Z., Zhang, J., Liu, C.H., Zheng, L., 2023. Postharvest melatonin treatment enhanced antioxidant activity and promoted GABA biosynthesis in yellow-flesh peach. *Food Chem.* 419, 136088. <https://doi.org/10.1016/j.foodchem.2023.136088>.
- Xu, Y., Freund, D.M., Hegeman, A.D., Cohen, J.D., 2022. Metabolic signatures of Arabidopsis thaliana abiotic stress responses elucidate patterns in stress priming, acclimation, and recovery. *Stress Biol.* 2. <https://doi.org/10.1007/s44154-022-00034-5>.
- Yang, W.T., He, Y., Li, W.H., Dai, M., Wu, B., Zhang, Z., Shi, J.Y., Song, Z.Y., 2024. PpERF-CRF3 selected by transcriptomic analysis plays key roles in the regulation of ABA alleviating chilling injury in peach fruit. *Int. J. Biol. Macromol.* 282, 136850. <https://doi.org/10.1016/j.ijbiomac.2024.136850>.
- Yang, H.P., Matsubayashi, Y., Nakamura, K., Sakagami, Y., 1999. Oryza sativa PSK gene encodes a precursor of phytosulfokine- α sulfated peptide growth factor found in plants. *PNAS* 96, 13560–13565. <https://doi.org/10.1073/pnas.96.23.13560>.
- Yi, B.H., Liu, Y., Wu, Z.G., Zheng, Y.H., Chen, H.J., Jin, P., 2024. Hydrogen sulfide alleviates chilling injury of zucchini fruit by regulating antioxidant capacity, endogenous hydrogen sulfide, proline, and polyamine metabolism. *Postharvest Biol. Technol.* 208, 112638. <https://doi.org/10.1016/j.postharvbio.2023.112638>.
- Zeng, Y., Bao, Y.Q., Shen, X.Y., Liu, Y., Yi, B.H., Ren, H.Y., Wang, K.T., Zheng, Y.H., Jin, P., 2024. Phytosulfokine- α treatment maintains fruit quality and alleviates chilling injury of peach fruit by regulating relative oxygen species and cell wall metabolisms. *Postharvest Biol. Technol.* 216, 113073. <https://doi.org/10.1016/j.postharvbio.2024.113073>.
- Zhang, H., Guo, J., Chen, X., Zhou, Y., Pei, Y., Chen, L., Ul Haq, S., Lu, M., Gong, H., Chen, R., 2022a. Pepper bHLH transcription factor CabHLH035 contributes to salt tolerance by modulating ion homeostasis and proline biosynthesis. *Hortic. Res.* 9, uhac203. <https://doi.org/10.1093/hr/uhac203>.
- Zhang, H., Hu, Z., Lei, C., Zheng, C., Wang, J., Shao, S., Li, X., Xia, X., Cai, X., Zhou, J., Zhou, Y., Yu, J., Foyer, C.H., Shi, K., 2018. A plant phytosulfokine peptide initiates auxin-dependent immunity through cytosolic Ca²⁺ signaling in tomato. *Plant Cell* 30, 652–667. <https://doi.org/10.1105/tpc.17.00537>.
- Zhang, M., Shi, Y., Liu, Z., Zhang, Y., Yin, X., Liang, Z., Huang, Y., Grierson, D., Chen, K., 2022. An EjbHLH14-EjbHB1-EjPRX12 module is involved in methyl jasmonate alleviation of chilling-induced lignin deposition in loquat fruit. *J. Exp. Bot.* 73, 1668–1682. <https://doi.org/10.1093/jxb/erab511>.
- Zhang, W.L., Yuan, S., Zhang, H.X., Liu, N., Zhang, Y.X., 2024. Exogenous paclobutrazol effectively mitigates salt stress in 'Duli' (*Pyrus betulifolia*) seedlings by reducing Na⁺/K⁺ ratio, improving their osmotic potentials, and activating the expression level of SOS and ABA pathway genes. *Sci. Hortic.* 338, 113836. <https://doi.org/10.1016/j.scienta.2024.113836>.
- Zhao, L.Y., Cassan-Wang, H., Zhao, Y.Q., Bao, Y.Q., Hou, Y.Y., Liu, Y., Wu, Z.G., Bouzayen, M., Zheng, Y.H., Jin, P., 2025. Calcium-dependent protein kinase PpCDPK29-mediated Ca²⁺-ROS signal and PpHSA2a phosphorylation regulate

- postharvest chilling tolerance of peach fruit. *Plant Biotechnol. J.* 23, 1938–1953. <https://doi.org/10.1111/pbi.70024>.
- Zhao, M.L., Wang, J.N., Shan, W., Fan, J.G., Kuang, J.F., Wu, K.Q., Li, X.P., Chen, W.X., He, F.Y., Chen, J.Y., Lu, W.J., 2013. Induction of jasmonate signalling regulators MaMYC2s and their physical interactions with MalCE1 in methyl jasmonate-induced chilling tolerance in banana fruit. *Plant Cell Environ.* 36, 30–51. <https://doi.org/10.1111/j.1365-3040.2012.02551.x>.
- Zhao, Y.T., Wu, Y.J., Zhang, X., Zhu, X., Hou, Y.Y., Chen, J.Y., Cui, K.B., Li, X.W., Wu, W. X., 2025a. Methyl jasmonate attenuates chilling injury of prune fruit by maintaining ROS homeostasis and regulating GABA metabolism and energy status. *Postharvest Biol. Technol.* 220, 113303. <https://doi.org/10.1016/j.postharvbio.2024.113303>.
- Zhao, Z.C., Yu, M.X., Wei, Y.Y., Xu, F., Jiang, S., Chen, Y., Ding, P., Shao, X.F., 2025b. Cinnamon essential oil causes cell membrane rupture and oxidative damage of *Rhizopus stolonifer* to control soft rot of peaches. *Food Control* 170, 111039. <https://doi.org/10.1016/j.foodcont.2024.111039>.
- Zhao, Y.Q., Zhao, L.Y., Hu, S.Q., Hou, Y.Y., Wang, J.D., Zheng, Y.H., Jin, P., 2023. Hydrogen sulfide-induced chilling resistance in peach fruit is performed via sustaining the homeostasis of ROS and RNS. *Food Chem* 398, 133940. <https://doi.org/10.1016/j.foodchem.2022.133940>.
- Zhou, Q.H., Bao, Z.Y., Yu, Y., Chen, W., Yang, Z.F., Cao, S.F., Shi, L.Y., 2023. IAA regulated levels of endogenous phytohormones in relation to chilling tolerance in cold-stored peaches after harvest. *Postharvest Biol. Technol.* 205, 112490. <https://doi.org/10.1016/j.postharvbio.2023.112490>.
- Zhou, Z., Yang, Y., Shan, W., Zhang, H., Wei, W., Kuang, J., Chen, J., Lu, W., 2022. Ethylene attenuates chilling injury of banana fruit via the MabHLH060/183 module in controlling phosphatidic acid formation genes. *Postharvest Biol. Technol.* 183, 111724. <https://doi.org/10.1016/j.postharvbio.2021.111724>.
- Zuo, X.X., Cao, S.F., Zhang, M., Cheng, Z.W., Cao, T.T., Jin, P., Zheng, Y.H., 2021. High relative humidity (HRH) storage alleviates chilling injury of zucchini fruit by promoting the accumulation of proline and ABA. *Postharvest Biol. Technol.* 171, 111344. <https://doi.org/10.1016/j.postharvbio.2020.111344>.