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# Ethylene promotes pectin degradation and fruit softening by negatively regulating *PavSPL7* in sweet cherry (*Prunus avium*)

Yueting Sun<sup>1,2</sup>, Yuqin Xiao<sup>1</sup>, Xiang Zhang<sup>1</sup>, Bingyang Du<sup>1</sup>, Maihemuti Turupu<sup>1</sup>, Xinyu Wang<sup>1</sup>, Qiaoqiao Zhang<sup>1</sup>, Qisheng Yao<sup>1</sup> and Tianhong Li<sup>1\*</sup>

## Abstract

Fruit softening significantly impacts the shelf-life and economic value of fruits. Sweet cherries (*Prunus avium* L.) are particularly prone to damage during transportation due to softening. While ethylene regulates cell wall degradation and fruit softening in various crops, its role and underlying mechanism in sweet cherry softening remain largely unclear. This study demonstrates that 1-aminocyclopropane-1-carboxylic acid (ACC), a key precursor in the synthesis of ethylene, steadily increases throughout sweet cherry fruit development and ripening, while ethylene treatment reduces fruit firmness. Ethylene treatment negatively regulates the transcriptional level of *PavSPL7*, a gene encoding a plant-specific SQUAMOSA Promoter Binding Protein-Like 7 (SPL) family protein. Overexpression of *PavSPL7* suppresses the transcriptional levels of genes involved in cell wall loosening (the expansin A6 gene *PavEXPA6*), pectin degradation (the pectin methyl esterase gene *PavPME12* and the pectate lyase gene *PavPL8*), and ethylene biosynthesis (the ACC synthase gene *PavACS7*), thereby inhibiting fruit softening. These results suggest that ethylene and *PavSPL7* antagonistically regulate fruit softening in sweet cherry through negative feedback loop. It is thus proposed that ethylene promotes pectin degradation by downregulating *PavSPL7* expression, thereby facilitating fruit softening. Our work gains insight into molecular mechanism underlying ethylene-mediated regulation of fruit softening in sweet cherry and provides potential targets for manipulation of fruit development and ripening.

**Keywords** Sweet cherry, Fruit softening, Ethylene, Cell wall, Pectin degradation, *PavSPL7*

## Introduction

Fruit softening is a critical constraint to extending the shelf-life of fruits during storage and transport (Gapper et al. 2013). Rapid softening often results in significant economic losses (Wang et al. 2024a). Sweet cherry (*Prunus avium* L.) is a nonclimacteric fruit that lacks strong storability, as it softens easily during storage and

transport post-harvest (Zhai et al. 2022). Therefore, understanding the regulatory mechanisms behind fruit softening is imperative to extend the shelf-life of sweet cherries.

Fruit softening occurs through the degradation of the cell wall, which involves the depolymerization and breakdown of polymers in the primary cell wall and middle lamella. These polymers mainly consist of cellulose microfibrils, hemicellulose, and a structurally diverse mixture of pectin (Toivonen and Brummell 2008; Gapper et al. 2013; Posé et al. 2019). Pectin, a polysaccharide, plays a pivotal role in fruit softening (Rose et al. 1998). Pectin in plant cells includes protopectin, water-soluble pectin (WSP), ion-bound pectin (ISP), and covalently bound pectin (CSP). During softening, insoluble pectin

\*Correspondence:

Tianhong Li  
lith@cau.edu.cn

<sup>1</sup> Frontiers Science Center for Molecular Design Breeding, College of Horticulture, China Agricultural University, Beijing 100193, People's Republic of China

<sup>2</sup> Institute of Citrus Research, Zhejiang Academy of Agricultural Sciences, Taizhou 318026, People's Republic of China

is converted to WSP and pectic acid, which is further degraded to galacturonic acid (GalA) (Rose et al. 1998). Enzymes such as polygalacturonase (PG), pectin methylesterase (PME or PE), and pectate lyase (PL) mediate these transitions, promoting fruit softening (Li et al. 2010; Wang et al. 2024a).

Wild strawberry (*Fragaria vesca*) accelerates fruit softening under PL gene *FvPLA* overexpression, while *FvPLA* silencing enhances fruit firmness (Zhang et al. 2022). Down-regulation of the *SIPL* gene in tomato (*Solanum lycopersicum*) increases fruit firmness, thereby effectively extending the fruit's shelf-life (Yang et al. 2017). Similarly, silencing *FaPG1* in strawberry (*Fragaria × ananassa*) reduces pectin dissolution in cell wall extracts, resulting in firmer fruit (Quesada et al. 2009). Expansins (EXPs), which are involved in cell growth and cell wall loosening, regulate fruit softening in tomato and apple (*Malus domestica* Borkh.) (Perini et al. 2017; Chen et al. 2022). In sweet cherry, the transcriptional levels of genes linked to pectin degradation, such as *QUARTET 3* (*PavQRT3*), *PavPME44*, *PavPL18*, *PavEXPA6*, *PavPMEI2*, and *PavPL8*, increase during fruit development and maturation (Zhai et al. 2022), indicating their potential role in fruit softening. These studies identify enzymes associated with the cell wall and pectin degradation are crucial in initiating fruit softening.

The expression of genes involved in cell wall degradation is regulated by various transcription factors. In tomato, the ripening-inhibitor (RIN), nonripening (NOR)-like1, SINAC1, and LATERAL ORGAN BOUNDARY 1 (SILOB1) positively regulate fruit softening by upregulating genes such as *SIEXP1*, *SIPL*, and *XYLOSIDASE 1* (*SIXYL1*) (Vrebalov et al., 2002; Ma et al. 2014; Gao et al. 2018; Shi et al. 2021). In banana (*Musa acuminata*), the inhibition of fruit softening by BRASSINAZOLE-RESISTANT 1 (MaBZR1) and MaBZR2 is achieved by repressing the expression of *MaEXP2*, *MaPL2*, and *XYLOGLUCAN ENDOTRANSGLYCOSYLASE 5* (*MaXET5*) (Shan et al. 2020). FvWRKY48, a WRKY-family TF in wild strawberry, promotes fruit softening by directly activating the expression of *FvPLA*, which is involved in pectin degradation (Zhang et al. 2022). In sweet cherry, PavDof2, PavDof6, and PavDof15 regulate the expression of genes like *PavQRT3*, *PavPME44*, and *PavPL18*, influencing fruit softening (Zhai et al. 2022).

Plant-specific SQUAMOSA promoter binding protein (SBP)-like (SPL) transcription factors have been proposed to regulate various aspects of plant growth and development (Schwarz et al. 2008). SPL-type transcription factors also participate in the processes of fruit ripening and softening. For instance, in tomato, the processes of fruit development and ripening are under the positive

regulatory control of the SPL COLORLESS NON-RIPENING (CNR) (Manning et al. 2006). In papaya (*Carica papaya* L.), CpSBP1 represses the expression of *CpPME1* and *CpPME2*, thereby delaying the process of fruit softening (Han et al. 2019).

The process of fruit softening is modulated by essential plant hormones such as abscisic acid (ABA) and ethylene (Giovannoni 2004; Gu et al. 2019). In persimmon (*Diospyros kaki* L.) and blueberry (*Vaccinium corymbosum*), ABA treatment significantly reduces fruit firmness by promoting cell wall metabolism (Han et al. 2016; Zhou et al. 2021). Despite the well-established role of ABA in regulating fruit maturation and softening in nonclimacteric species (Ren et al. 2010; Shen et al. 2014), recent researches have emphasized the critical functions of ethylene in various stages of nonclimacteric fruits, including development, maturation, and softening (Farneti et al. 2022; Wang et al. 2022). For instance, ethephon (ETH) treatment accelerates anthocyanin accumulation and ethylene biosynthesis in grape (*Vitis vinifera*) berries (Wang et al. 2022). In sweet cherry, ethylene treatment lowers fruit titratable acidity, while treatment with ethylene inhibitor 1-methylcyclopropene (1-MCP) increases fruit firmness and an extended shelf-life (Serradilla et al. 2019). Additionally, VcSPL12 suppresses the expression of *VcACS1* and *1-aminocyclopropane-1-carboxylic acid* (ACC) OXIDASE 6 (*VcACO6*), thereby inhibiting ethylene biosynthesis and fruit coloration in blueberry (Li et al. 2024).

Our previous research has identified 12 *PavSPLs* in sweet cherry, some of which are associated with the processes of fruit development and ripening (Sun et al. 2023). However, the role of these *PavSPLs* in ethylene-mediated fruit softening remains unclear. This study indicates that ethylene promotes fruit softening in sweet cherry by downregulating *PavSPL7*. In turn, *PavSPL7* acts as a repressor for key genes of cell wall loosening and degradation, such as *PavEXPA6*, *PavPMEI2*, and *PavPL8*, thereby suppressing fruit softening. Moreover, *PavSPL7* inhibits ethylene biosynthesis by suppressing *PavACS7* expression. This negative feedback regulation suggests an antagonistic relationship between ethylene and *PavSPL7* in controlling fruit softening in sweet cherry. These findings uncover provide novel insights into the role of ethylene in the regulation of fruit softening in sweet cherry.

## Materials and methods

### Plant materials and treatments

Sweet cherry fruits were harvested at the large green (BG), yellow (YW), full red (FR), and dark red (DR) stages (Zhai et al. 2022) from 18-year-old 'Zaodaguo' trees cultivated at the Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Sciences (Beijing, China).

Fruits of uniform size were harvested at the BG stage and incubated in the dark for 1 d to eliminate any potential environmental variation. Ethylene treatment was applied by immersing the fruit stems in ETH solution (10 mg/L, Aladdin, Shanghai, China) for 4 days, with water-treated fruits serving as controls. We maintained all the fruits under controlled conditions (23°C, 90% RH). After treatment, the samples were flash-frozen in liquid nitrogen and kept at −80°C. Thirty fruits were used per treatment, with 10 fruits per treatment considered one biological replicate, and three biological replicates were performed.

Fruits from 18-year-old sweet cherry cv. ‘Rainier’ trees were used for transient transformation. The ‘Rainier’ trees were cultivated at the Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Sciences (Beijing, China). Wild-type tomato (*Solanum lycopersicum* cv. ‘Micro-Tom’) and transgenic *PavSPL7*-OE tomato lines (#1, #13, and #23) were grown in growth chambers under a 16/8-h light/dark photoperiod at 23°C.

*N. benthamiana* plants used for transient expression were cultivated in growth chambers set to 23°C with a 16/8-h light/dark photoperiod.

#### Measuring fruit ripening parameters

Fruit firmness was measured *via* a texture analyzer (TA.XT, Godalming, UK). The fruit was indented 2 mm with a 2 mm diameter probe. Dual measurements were made on opposite sides of each fruit equator line. Prior to testing, the texture analyzer was locked at 1.0 mm s<sup>−1</sup>.

The cell wall materials were extracted and analyzed following a protocol previously described (Zhai et al. 2022). Each experiment was repeated with three biologically independent sets, and 10 fruits per treatment were considered one biological replicate.

#### Measurement of ACC and ethylene contents

The ACC content was quantified according to a published method (Yue et al. 2023). Fruit samples (0.5 g) were extracted using a solution of isopropanol, distilled water, and concentrated HCl (2:1:0.002 v/v/v). The mixtures were incubated at 180 rpm for 1 h at 4°C on an orbital shaker. Following the addition of 4 mL of dichloromethane, the mixtures were shaken for an additional hour (180 rpm) followed by centrifugation (12,000 rpm, 10 min, 4°C). Following collection, the lower phase was concentrated and dried under a nitrogen stream. The final extract was taken up in 200 µL of methanol. ACC levels were determined *via* a UHPLC/MS system (Thermo, USA). A standard curve was constructed using ACC standard samples (Sigma, USA) at various concentrations, and the ACC levels in the samples were

determined on the basis of the integrated peak area. Three biological replicates were performed.

Ethylene production was measured by weighing the fruits, sealing them in a vacuum desiccator for 2 h, and then collecting a 1 mL sample for injection into an SP-3420A gas chromatograph (Beijing Beifen-Ruili, China). The column oven was held at 100°C, the injector was held at 100°C, and the detector was held at 380°C, with N<sub>2</sub> flowing at 40 mL min<sup>−1</sup>. Each measurement included three biological replicates (n = 10 fruits per replicate).

#### RNA extraction and RT-qPCR

Total RNA from sweet cherry and tomato fruits was obtained with an OminiPlant RNA Kit (CW BIO, Taizhou, China). HiScript II Q RT SuperMix plus gDNA Wiper (Vazyme, Nanjing, China) was used to convert 1 µg of RNA to first-strand cDNA. cDNA synthesis reactions were prepared in 10 µL volumes *via* 2×HQ SYBR qPCR Mix (Vazyme, Nanjing, China) and subjected to quantitative PCR on a Rotor Gene Q cycler (QIAGEN, Germany). Protocol details for the reaction were taken directly from Yue et al. (2020). *PavActin1* was used as the internal control gene for sweet cherry (Wang et al. 2023), and *SICAC* (SGN-U314153) was used as the internal control for tomato (Expósito-Rodríguez et al. 2008). Each experiment included three biological replicates.

#### Transcriptional activation activity assay in yeast cells

Transactivation assays were performed according to the Yeast System Handbook (Clontech Laboratories 2009). The *PavSPL7* coding sequence (CDS) was cloned and inserted into the pGBKT7 yeast expression vector, with the empty vector serving as a background control. MsDREB6.2, previously identified in apple as a positive control (Liao et al. 2017), served as the positive control. AH109 was transformed with the resulting plasmid. The transformants were subsequently recovered on SD/− Trp media and further tested on SD/− Trp/− His/− Ade media to identify confirmed positives.

#### Transcriptional activation activity assay in *N. benthamiana*

For the transcriptional activation activity assay in *N. benthamiana*, vectors were constructed according to a published method (Xu et al. 2022). The pBD-SK vector was generated by inserting the yeast GAL4 DNA-binding domain (BD) CDS into pGreenII 0029 62-SK. The full-length CDS of *PavSPL7* was inserted into the pBD-SK vector to produce the *35S:BD-PavSPL7* effector construct. Additionally, five copies of the GAL4 upstream activating sequence (5×GAL4 UAS) were cloned upstream of the *firefly luciferase* (*LUC*) gene in the pGreenII0800-LUC vector to create the *5×UAS:LUC*

reporter construct. These constructs were individually transferred into *A. tumefaciens* strain GV3101 (pSoup). Dual-LUC assays were performed in *N. benthamiana* plants transiently expressing these constructs according to a published method (Wang et al. 2020b). LUC signals in *N. benthamiana* leaves were imaged *via* a CCD camera (5200 Multi, Tanon, Shanghai, China), and LUC and *Renilla* luciferase (REN) activities were measured *via* the Promega Dual-LUC Reporter Assay System (Madison, WI, USA). The pBD-SK empty vector functioned as a negative reference.

#### Subcellular localization analysis

We amplified the full-length CDS (excluding the stop codon) and inserted it into the pCambia1302-green fluorescent protein (GFP) vector to investigate its subcellular localization. The resulting 35S:*PavSPL7*-GFP construct, along with the empty pCambia1302-GFP vector, was subsequently transformed into *Agrobacterium* strain GV3101. Positive colonies were re-suspended in infiltration buffer and introduced into four-week-old *N. benthamiana* leaves. Nuclear visualization was achieved with an mCherry-tagged fluorescent marker (Zhang et al. 2019). After infiltration, the leaves were incubated overnight at room temperature, transferred to light conditions, and incubated for a total of 48 h. Concurrent detection of GFP and mCherry fluorescence signals was performed under an FluoView 3000 confocal laser-scanning microscope (Olympus, Japan) with the following settings: detector, photomultiplier tube (PMT); intensity, 5%; gain, 570–770. GFP was imaged with 488 nm excitation and 500–540 nm emission, whereas mCherry was imaged with 561 nm excitation and 570–670 nm emission.

#### Transient transformation of sweet cherry fruits with *PavSPL7*

Separate transformations were performed to introduce either the 35S:*PavSPL7*-GFP fusion construct or the pCambia1302 empty vector into *Agrobacterium* strain EHA105. Bacterial cultures (100 µL) containing each plasmid were separately injected into sweet cherry fruits at the BG stage, following a previously described method (Shen et al. 2014; Qi et al. 2020; Zhai et al. 2022; Wang et al. 2023). At least two hundred fruits were used for each injection combination, with samples collected during the color transition phase (approximately 30 d post-injection). After malformed fruits were removed, a subset was selected for DNA extraction to amplify the hygromycin (Hyg) fragment for identification. The remaining fruits were divided into four aliquots for measurement of firmness, paraffin sectioning, cell wall composition analysis, and gene expression analysis. Each assay included

three biological replicates; each replicate contained at least five individual fruits.

#### Stable genetic transformation of *PavSPL7* in tomato

To generate *PavSPL7*-overexpressing (*PavSPL7*-OE) transgenic tomato plants, the full-length *PavSPL7* CDS (excluding the stop codon) was constructed and inserted into the pBI121 vector. The resulting 35S:*PavSPL7*:GUS construct was introduced into *Agrobacterium* strain EHA105 and used to transform wild-type tomato plants following standard protocols (Fillatti et al. 1987). Transgenic seedlings from the T0 and T1 generations were verified *via* PCR and GUS staining. All the tomato materials used in the experiments were from the T2 generation.

#### Yeast one-hybrid (Y1H) assays

DNA–protein interaction analysis was performed *via* Y1H assays *via* the EGY48 Chemically Competent Cell Yeast Transformation System (Weidi, Shanghai, China). The full-length *PavSPL7* CDS was inserted into the pB42AD vector, and the promoters of *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7* were inserted into the pLacZi2µ vector (primers listed in Table S2). The pB42AD empty plasmid served as a negative control. The resulting constructs were cotransformed into the yeast strain EGY48 and plated onto SD/–Trp/–Ura media. After 3–4 days of growth, appropriately sized colonies were selected and streaked onto yeast chromogenic media supplemented with X-gal, after which color development was recorded.

#### Electrophoretic mobility shift assay (EMSA)

The full-length *PavSPL7* CDS was constructed and inserted into pGEX-4 T-1. Recombinant *PavSPL7* with a glutathione S-transferase (GST) tag was expressed in *E. coli* BL21 (DE3), followed by purification with a GST 4FF Sefinose Resin Kit (Songon, Shanghai, China). The DNA-binding ability was assessed *via* EMSAs *via* optimized protocols (Feng et al. 2023). Probes encompassing the GTAC motifs within the promoters of *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7* were synthesized and biotin labeled at the 5' end (DIA-UP Biotech, Beijing, China). Unlabeled probes were used as competitors, and mutant probes containing a CCCC sequence instead of GTAC were synthesized, with the remainder of the sequence identical to that of the general probes. Binding and competition reactions were carried out *via* a Chemiluminescent EMSA Kit (Beyotime, Shanghai, China) following established electrophoretic mobility optimization procedures.

### Dual-LUC reporter assay

To examine the regulation of downstream genes by PavSPL7, its full-length CDS was constructed and inserted into the pGreenII 0029 62-SK vector, while 2-kb fragments of the *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7* promoters were individually cloned and inserted into the pGreenII 0800-LUC vector. The resulting constructs were subsequently transformed into *Agrobacterium* strain GV3101 (with the pSoup plasmid). The dual-LUC assay was conducted *via* the *N. benthamiana* transient expression system. LUC and REN activities were quantified *via* the Dual-LUC Reporter Assay System (Promega, USA). The relative LUC/REN ratios of the 62-SK and LUC empty vectors were used as reference controls.

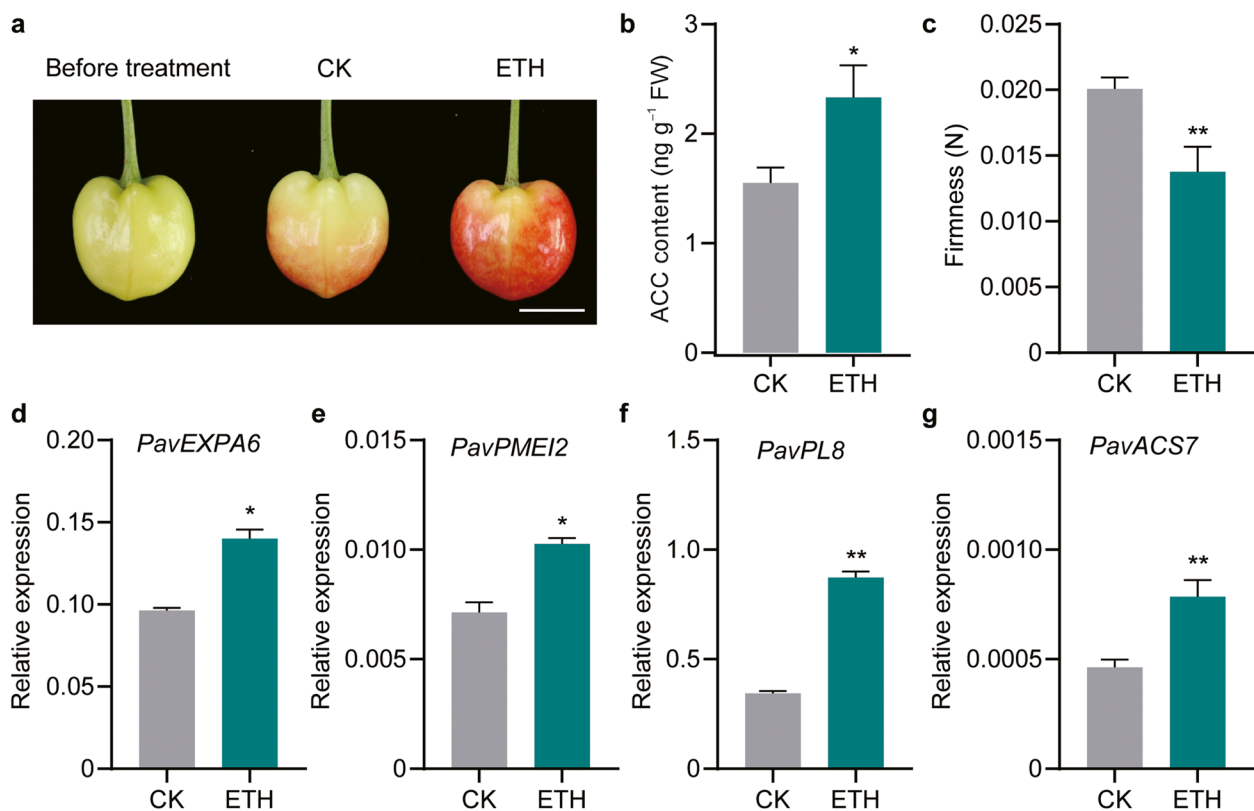
### Statistical analysis

GraphPad v8.4.3 was used for data visualization and statistical analysis. Student's *t* test was used for two-group comparisons, whereas one-way ANOVA followed by Tukey's range test was used for multiple comparisons.

## Results

### Ethylene promotes fruit softening in sweet cherry

To investigate the relationship between fruit softening and ethylene accumulation in sweet cherry (*Prunus avium* cv. 'Zaodaguo'), changes in fruit firmness and ACC levels, a key ethylene precursor, were measured during fruit development and maturation. Fruit firmness decreased as ripening progressed (Fig. S1a). ACC content increased from the BG stage to the early ripening stage (FR), followed by a reduction at the full ripening stage (DR) (Fig. S1b). Reverse-transcription quantitative PCR (RT-qPCR) was assessed to evaluate the expression of genes associated with cell wall loosening, degradation, and ethylene biosynthesis. Generally, the expression levels of *PavEXPA6*, *PavPMEI2*, and *PavPL8*, three structural genes related to fruit softening that have been identified in a previous study, increased during ripening (Fig. S1c–e). Transcriptome data for the 'Zaodaguo' cultivar at various developmental stages showed that *PavACS7*, a key ethylene biosynthesis gene, exhibited the most significant differential expression (Fig.



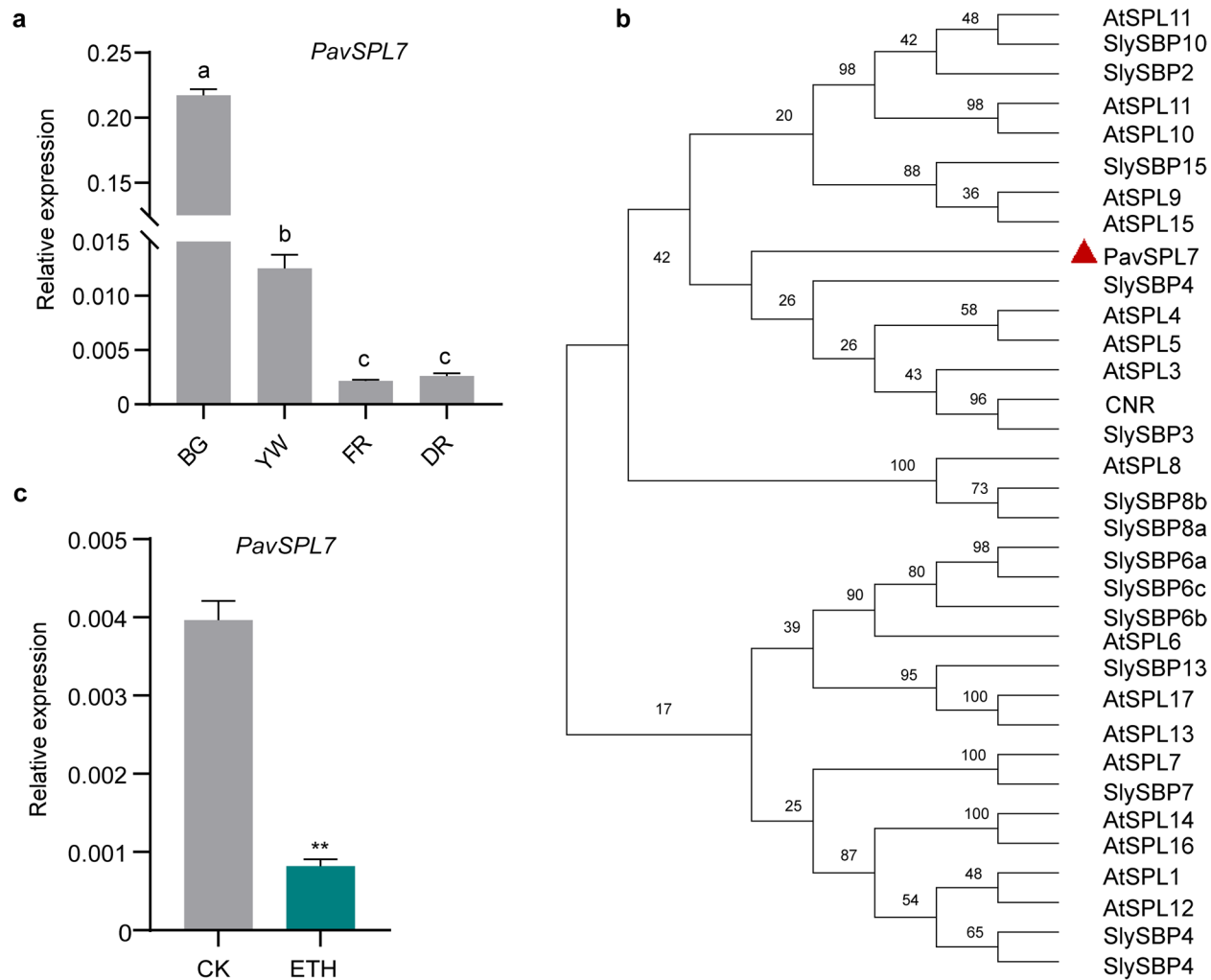
**Fig. 1** Ethylene promotes fruit softening in sweet cherry. **a–g** Response of 'Zaodaguo' sweet cherry fruits to ethephon (ETH) treatment. Fruits were harvested at the large green (BG) stage and treated with ETH for four days before sampling, compared to water treatment (CK). Representative photograph (**a**) (Bar = 1 cm), ACC content (**b**), fruit firmness (**c**), and transcript levels of *PavEXPA6* (**d**), *PavPMEI2* (**e**), *PavPL8* (**f**), and *PavACS7* (**g**) examined by RT-qPCR. Data are presented as mean  $\pm$  SE from three biological replicates. Statistically significant differences are indicated (\*  $P < 0.05$ , \*\*  $P < 0.01$ ; Student's *t*-test). ACC, 1-aminocyclopropane-1-carboxylic acid; FW, fresh weight; *PavEXPA6*, *Prunus avium* Expansins A6; *PavPMEI2*, Pectin Methylesterase Inhibitor 2; *PavPL8*, Pectate Lyase 8; *PavACS7*, ACC Synthase 7

S2), mirroring the expression patterns of *PavEXPA6*, *PavPMEI2*, and *PavPL8* (Fig. S1f). These results suggest a positive correlation between ethylene and fruit softening.

Sweet cherry fruits were harvested at the BG stage and treated with ETH for four days before sampling (Fig. 1a). ACC levels were higher in ETH-treated fruits compared to controls (CK), confirming the success of the treatment (Fig. 1b). ETH treatment accelerated fruit coloration and significantly reduced firmness (Fig. 1a, c). Expression levels of *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7* were upregulated by ETH treatment, aligning with changes in fruit firmness and ACC content (Fig. 1d–g). Our results demonstrate that ethylene promotes fruit softening in sweet cherry by upregulating a regulatory network of genes involved in cell wall loosening, degradation, and ethylene biosynthesis.

***PavSPL7* expression is negatively regulated by ethylene**

Previous research demonstrated the involvement of PavSPLs in sweet cherry fruit development and maturation (Sun et al. 2023). This prompted an investigation into whether these genes regulate fruit softening. Promoter sequence analysis of *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7* revealed several GTAC elements, known binding sites for SPLs (Fig. S3). To further elucidate their roles, the expression patterns of *PavSPLs* across various stages of fruit development and maturation were examined (Fig. S4). *PavSPL1* expression increased during



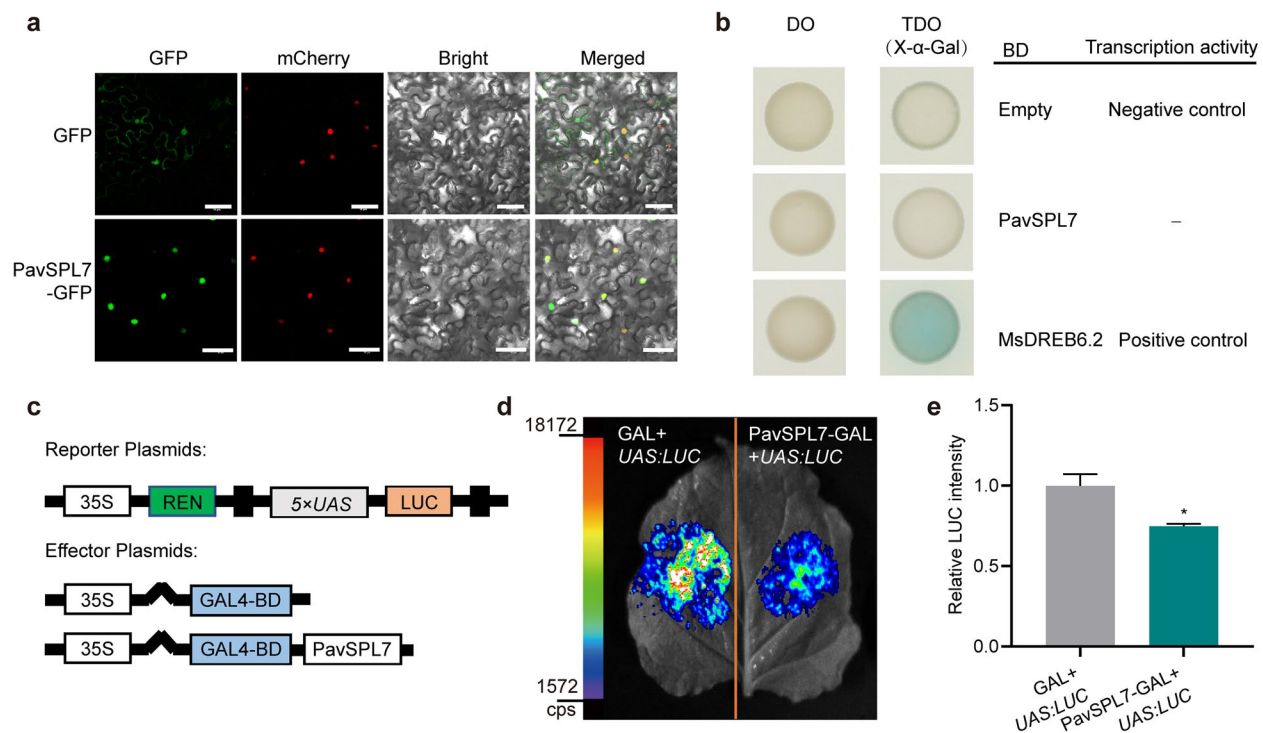
**Fig. 2** Expression analysis of *PavSPL7*. **a** Relative transcript levels of *PavSPL7* during fruit ripening. **b** Phylogenetic tree of SPL proteins, including *PavSPL7*, AtSPLs from *Arabidopsis*, SQUAMOSA promoter binding protein (SISBPs), and COLORLESS NON-RIPENING (CNR) from tomato (*Solanum lycopersicum*), reconstructed using the neighbor-joining method. The sequences and origins of the *Arabidopsis* and tomato proteins are listed in Table S1. **c** Repression of *PavSPL7* transcript levels following ethephon (ETH) treatment. *PavActin1* was used as an internal control. Data are presented as mean ± SE from three biological replicates. Statistically significant differences are indicated: \*\**P* < 0.01; Student's *t*-test. *PavSPL7*, *Prunus avium* SQUAMOSA Promoter Binding Protein-Like 7

fruit ripening, while *PavSPL2* and *PavSPL8* exhibited stable expression across all stages. In contrast, the transcript levels of *PavSPL3–7* and *PavSPL9–12* consistently decreased throughout development. Notably, *PavSPL7* showed the highest expression levels and the most significant changes during fruit ripening, making it a prime candidate for further analysis (Fig. S4, Fig. 2a). To investigate the evolutionary relationship between *PavSPL7* and *SPL* genes from other species, a phylogenetic tree was generated in MEGA 7.0 using the protein sequences of AtSPLs from *Arabidopsis* and SISBPs from tomato. *PavSPL7* is closely related to SISBP4, AtSPL3–5, SISBP3, and CNR (Fig. 2b). In tomato, CNR is a known regulator of fruit ripening (Manning et al. 2006), while in papaya, its AtSPL3–5 homolog CpSBP1 negatively regulates fruit softening (Han et al. 2019). The present study further examined *PavSPL7* expression in response to ETH treatment, observing significant repression of its expression (Fig. 2c).

To characterize the functional properties of the transcription factor *PavSPL7*, subcellular localization and

transcriptional activity assays were conducted. For subcellular localization, a *35S:PavSPL7-GFP* fusion protein was transiently introduced into *Nicotiana benthamiana* leaves via *Agrobacterium tumefaciens*-mediated infiltration. Observation under confocal laser scanning microscopy revealed stable green fluorescence from *PavSPL7-GFP*, localized specifically to the nucleus of infiltrated leaves, confirming that *PavSPL7* is predominantly nuclear (Fig. 3a).

To evaluate the transcriptional activity of *PavSPL7*, we constructed a pGADT7-*PavSPL7* fusion plasmid and expressed it in the *Saccharomyces cerevisiae* strain AH109. Yeast cells expressing *PavSPL7* did not grow on triple dropout (TDO) medium, whereas the positive control thrived, demonstrating that *PavSPL7* does not function as a transcriptional activator in the yeast system (Fig. 3b). To further validate its transcriptional regulatory function, a dual-LUC reporter assay was conducted. The LUC reporter gene was cloned downstream of five copies of the yeast *GAL4* upstream activating sequence (*5xUAS*), and the effector construct consisted



**Fig. 3** Subcellular localization and transactivation assay of *PavSPL7*. **a** Subcellular localization of *PavSPL7* in *N. benthamiana* leaves analyzed via transient expression. A *35S:PavSPL7-green fluorescent protein (GFP)* construct was infiltrated into *N. benthamiana* leaves, and GFP fluorescence was observed. mCherry was used as a nuclear marker. Scale bar = 50 μm. **b** Transcriptional activity of *PavSPL7* in yeast. Yeast colonies were selected from synthetic defined media lacking tryptophan (SD/–Trp), and *PavSPL7* transcriptional activity was tested on SD/–Trp/–Leu/–Ade media (triple dropout [TDO]). The empty pGBKT7 vector served as a negative control, and MsDREB6.2 was the positive control. β-glucuronidase (GUS) activity in yeast cells was visualized using X-α-gal as a substrate. **c–e** Constructs used for the transactivation assay in *N. benthamiana* leaves (**c**), firefly luciferase (LUC) signals in *N. benthamiana* leaves (**d**), and LUC/*Renilla* luciferase (REN) ratios (**e**). Data are presented as means ± SEs from three biological replicates. Statistically significant differences are indicated (\* *P* < 0.05; Student's *t*-test). *PavSPL7*, *Prunus avium* SQUAMOSA Promoter Binding Protein-Like 7; DO, dropout; BD, DNA-binding domain; UAS:LUC, Upstream Activating Sequence:LUC

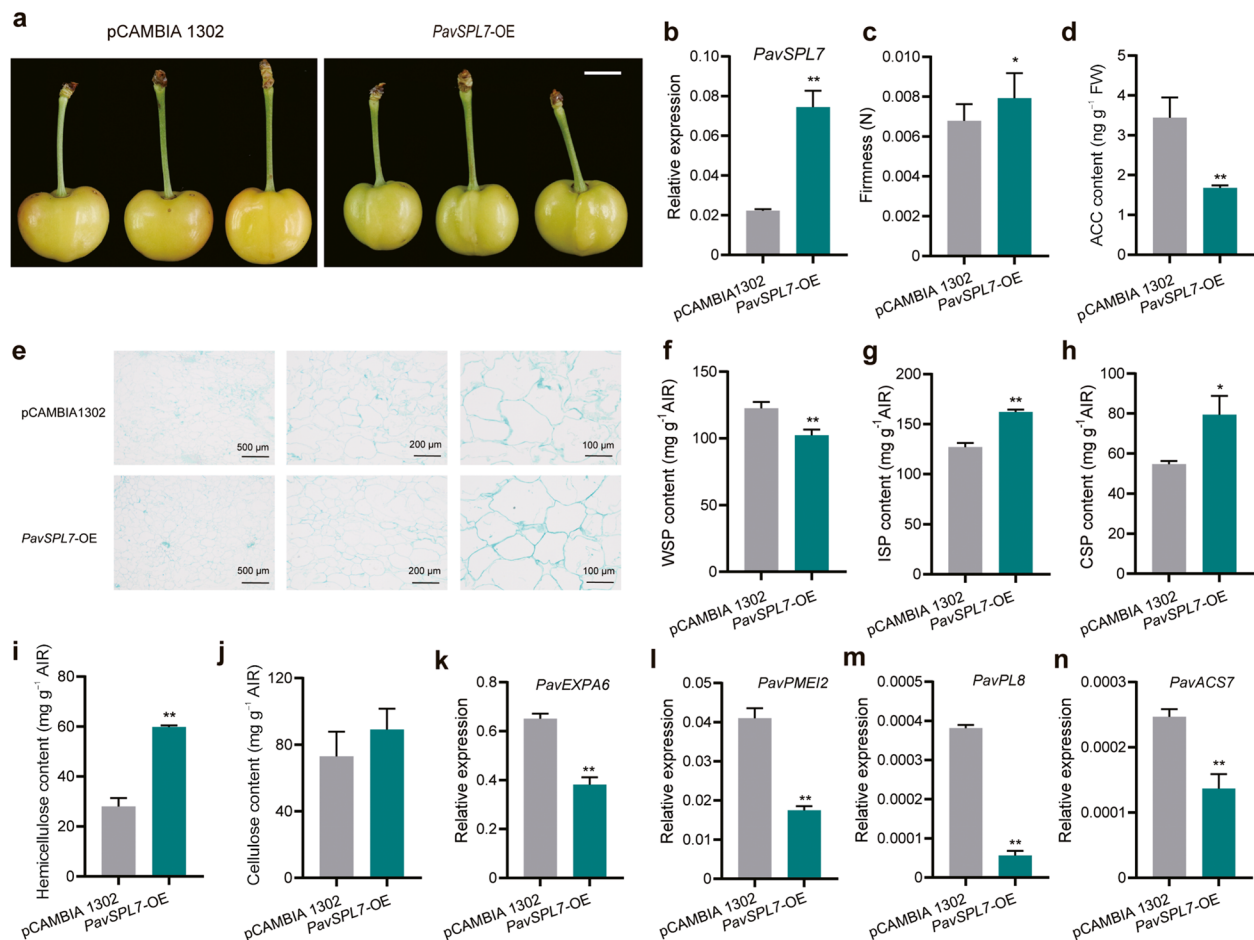
of *PavSPL7* cloned in-frame downstream of the GAL4 DNA-BD under the 35S promoter. Co-infiltration of the *LUC* reporter and *GAL4-BD-PavSPL7* effector in *N. benthamiana* leaves showed a decrease in LUC chemiluminescence and lower LUC/REN ratios in the presence of *PavSPL7*, indicating that *PavSPL7* acts as a transcriptional repressor (Fig. 3c–e).

#### **PavSPL7 represses fruit softening in sweet cherry**

To determine the role of *PavSPL7* in softening, we performed transient overexpression of *PavSPL7* in sweet cherry fruits (*PavSPL7*-OE, Fig. 4a). Successful transformation was confirmed *via* PCR amplification of the

Hyg resistance gene carried by the overexpression vector (Fig. S5). *PavSPL7* expression was significantly higher in *PavSPL7*-OE fruits compared to empty vector control fruits (Fig. 4b). Compared to control fruits, *PavSPL7*-OE fruits exhibited significantly greater firmness and lower ACC accumulation (Fig. 4c, d). Microscopic analysis revealed that cell structures were more tightly packed, while cell wall degradation was less pronounced in *PavSPL7*-OE fruits than in control fruits (Fig. 4e).

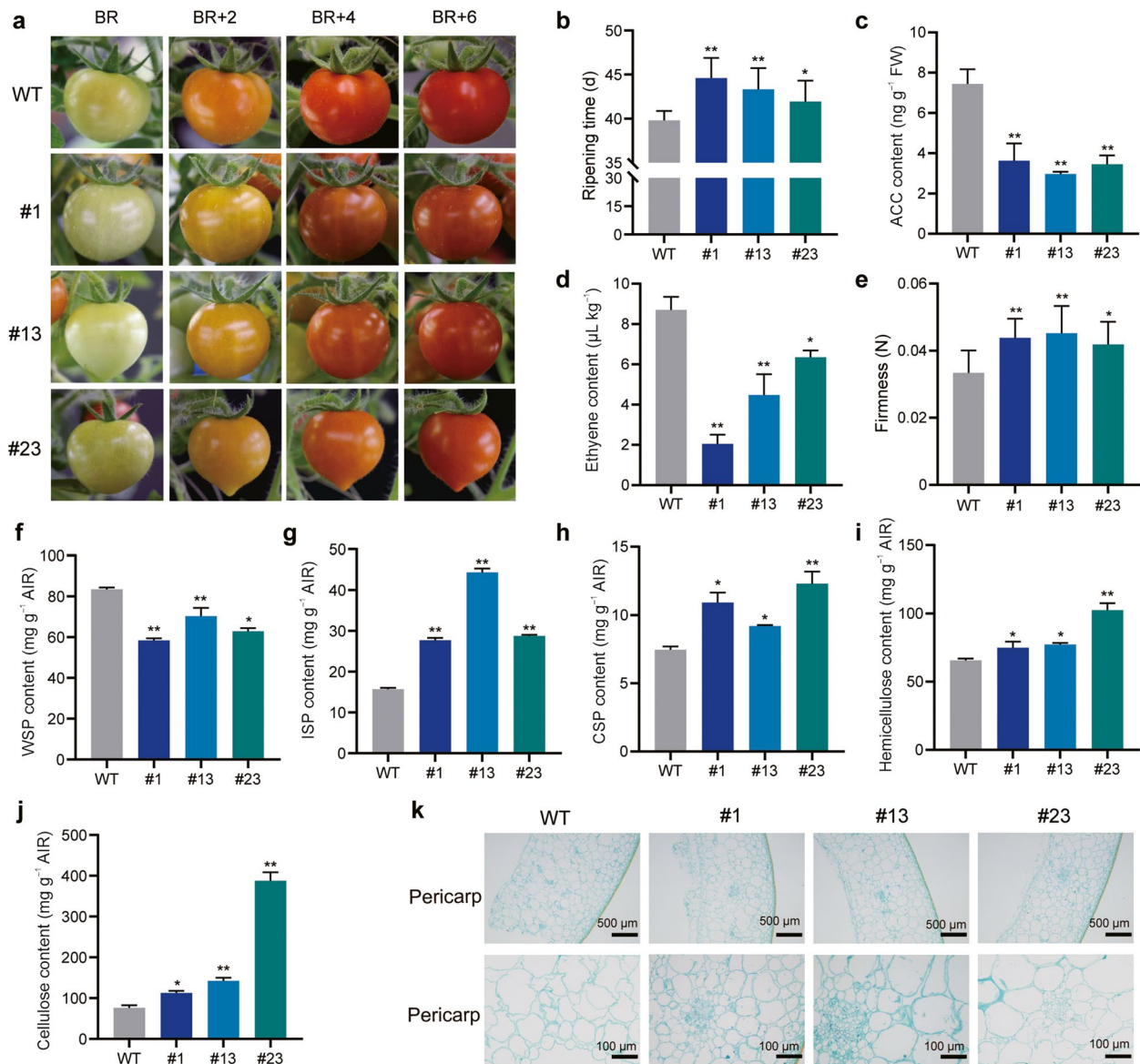
Next, the levels of key cell wall components (hemicellulose, cellulose, and pectin) were assessed in the fruits. WSP levels were reduced in *PavSPL7*-OE fruits relative to controls (Fig. 4f). Fruits overexpressing *PavSPL7*



**Fig. 4** Transient expression of *PavSPL7* in sweet cherry fruits delays ripening. **a** Phenotypes of 'Rainier' sweet cherry fruits subjected to agroinfiltration-mediated transient overexpression of *PavSPL7*. The empty vector pCambia1302 was used as a negative control (Bar = 1 cm). **b–d** Relative *PavSPL7* transcript levels (**b**), fruit firmness (**c**), and 1-aminocyclopropane-1-carboxylic acid (ACC) content (**d**) in the fruits shown in (**a**). **e** Microscopic observation of cells in *PavSPL7*-overexpressing (*PavSPL7*-OE) sweet cherry fruits. **f–j** Contents of water-soluble pectin (WSP, **f**), ion-bound pectin (ISP, **g**), covalently bound pectin (CSP, **h**), hemicellulose (**i**), and cellulose (**j**) in transgenic sweet cherry fruits. AIR, alcohol-insoluble residue. **k–n** Expression of *PavEXPA6* (**k**), *PavPMEI2* (**l**), *PavPL8* (**m**) and *PavACS7* (**n**), genes associated with cell wall degradation or ethylene biosynthesis in the transgenic fruits. *PavEXPA6*, *Prunus avium* Expansins A6; *PavPMEI2*, *Pectin Methylsterase Inhibitor 2*; *PavPL8*, *Pectate Lyase 8*; *PavACS7*, *ACC Synthase 7*. Data are presented as means  $\pm$  standard errors (SEs) from three biological replicates. Statistically significant differences are indicated (\*  $P < 0.05$ , \*\*  $P < 0.01$ ; Student's *t*-test). *PavSPL7*, *Prunus avium* SQUAMOSA Promoter Binding Protein-Like 7

contained significantly more ISP, CSP, and hemicellulose, while cellulose content showed no significant change (Fig. 4g–j). The expression of genes regulating cell wall loosening, degradation, and ethylene biosynthesis was then examined. *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7* were downregulated in *PavSPL7*-OE fruits compared to controls (Fig. 4k–n). Additionally, other

genes regulating cell wall loosening and degradation, including *PavQRT3*, *Mannanase-encoding gene 7* (*PavMAN7*), *PavPLL19*, *Expansin-like A2* (*PavEXLA2*), and *PavEXPA4*, were downregulated in *PavSPL7*-OE fruits (Fig. S6). These results indicate that *PavSPL7* inhibits fruit softening by repressing cell wall degradation and ethylene biosynthesis in sweet cherry.

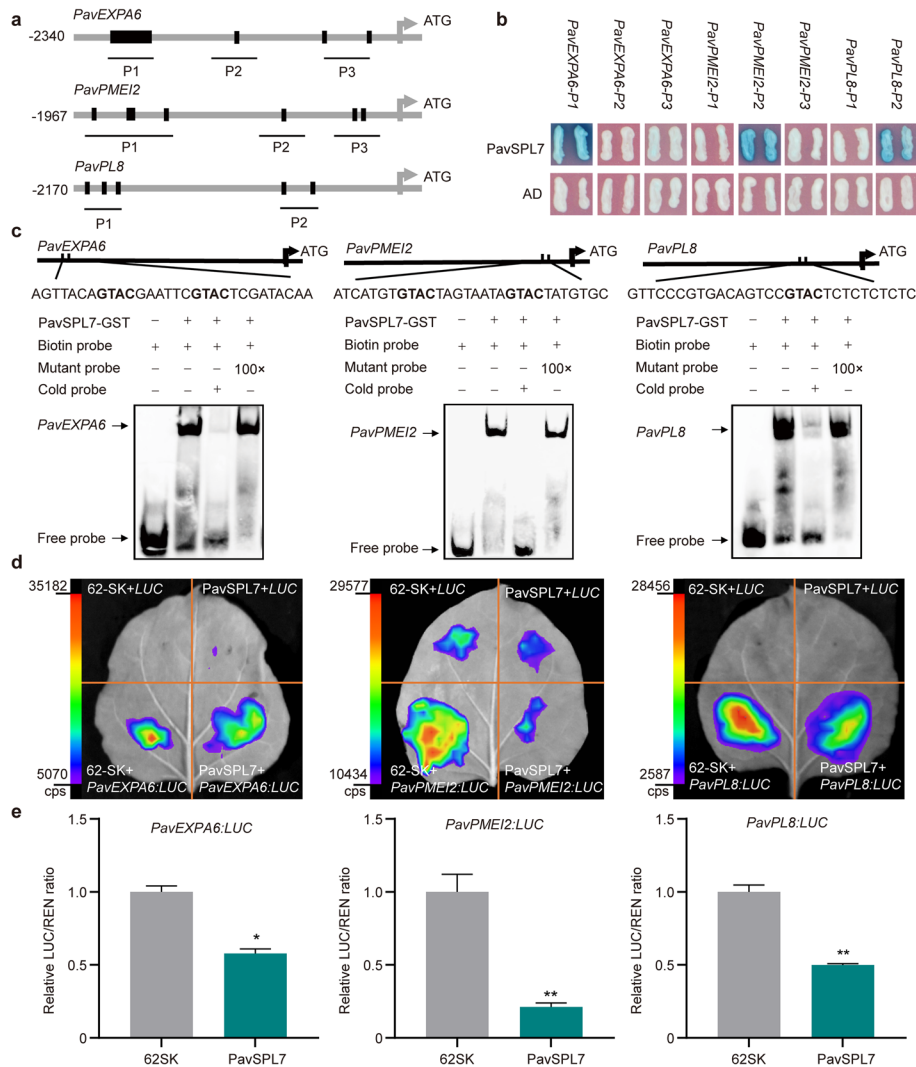


**Fig. 5** Effects of heterologous expression of *PavSPL7* on fruit development and quality in tomato. **a** Ripening-related phenotypes of fruits from WT, *PavSPL7*-overexpressing (*PavSPL7*-OE) #1, *PavSPL7*-OE #13, and *PavSPL7*-OE #23 tomato lines at the BR to BR+6 stages. BR, breaker stage. **b** Ripening time of fruits. **c** 1-aminocyclopropane-1-carboxylic acid (ACC) levels. **d** Ethylene levels. **e** Fruit firmness at BR+4. **f–j** Contents of water-soluble pectin (WSP, **f**), ion-bound pectin (ISP, **g**), covalently bound pectin (CSP, **h**), hemicellulose (**i**), and cellulose (**j**) in *PavSPL7*-OE tomato fruits at BR+4. AIR, alcohol-insoluble residue. **k** Paraffin sections of pericarps from WT and *PavSPL7*-OE fruits at the BR+4 stage. Data are presented as means  $\pm$  SEs from three biological replicates. Statistically significant differences are marked (\*  $P < 0.05$ , \*\*  $P < 0.01$ ; Student's *t*-test). *PavSPL7*, *Prunus avium* SQUAMOSA Promoter Binding Protein-Like 7; WT, wild type

PavSPL7 delays fruit ripening and softening in tomato fruits

To further investigate the mechanistic role of PavSPL7 in fruit softening, *PavSPL7* was heterologously expressed in tomato. Six *PavSPL7*-OE lines were obtained, and lines #1, #13, and #23 were selected for analysis based on their relatively high *PavSPL7* transcript levels (Fig. S7). In *PavSPL7*-OE fruits, the ripening stage (breaker stage; BR) occurred 43 days after anthesis, a 3-day delay compared to wild-type (WT)

fruits, indicating that PavSPL7 retards fruit ripening (Fig. 5a, b). Consistent with this observation, heterologous expression of *PavSPL7* in tomato resulted in reduced accumulation of ACC and ethylene, alongside increased fruit firmness (Fig. 5c–e). Compared to WT fruits, *PavSPL7*-OE fruits exhibited lower WSP content, while ISP, CSP, hemicellulose, and cellulose levels remained unchanged. Additionally, the cell arrangement was denser, and cell wall degradation was less pronounced in *PavSPL7*-OE fruits (Fig. 5f–k).



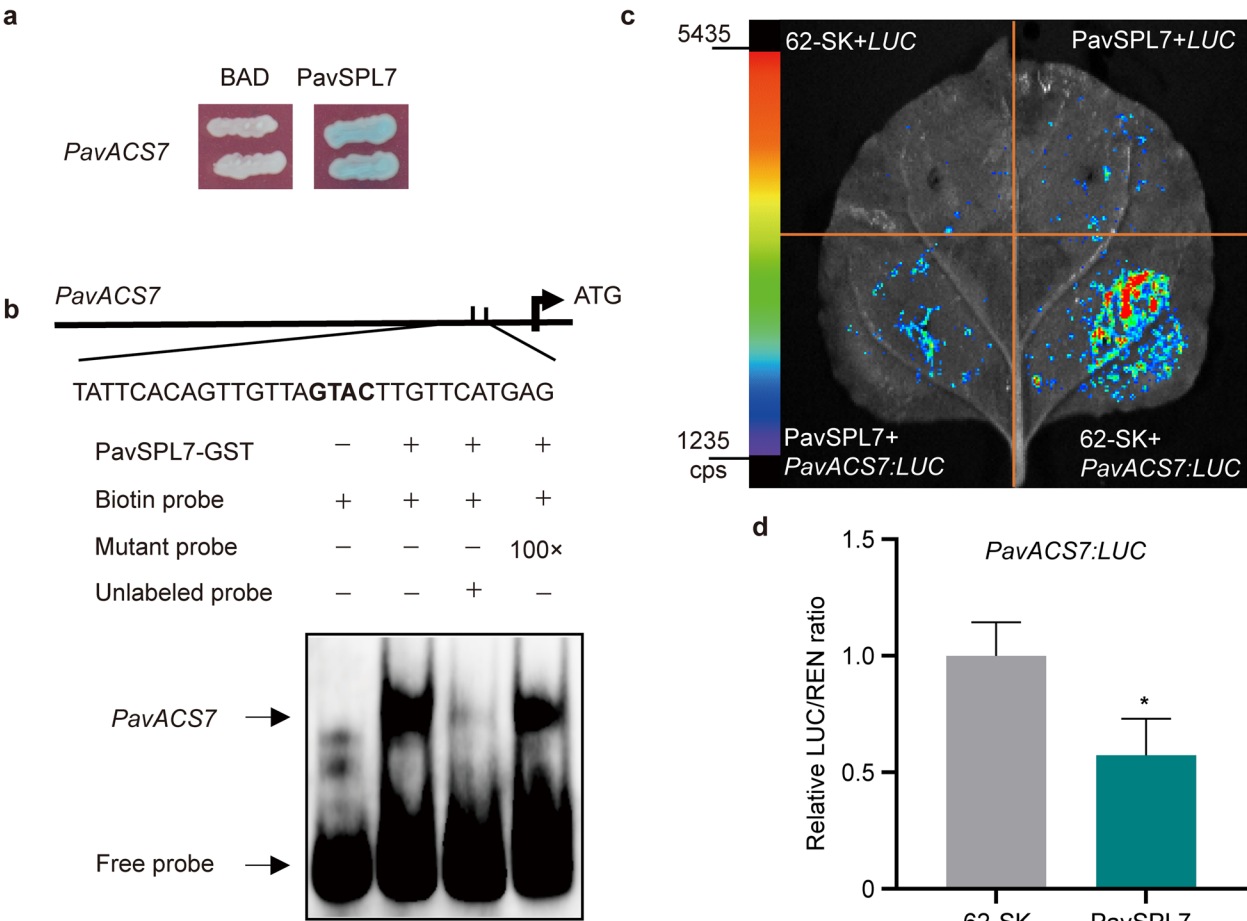
**Fig. 6** PavSPL7 represses transcription by binding to the promoters of softening-related genes. **a** Diagram of the *PavEXPA6*, *PavPMEI2*, and *PavPL8* promoters used in the yeast one-hybrid (Y1H) assays. **b** Y1H assay shows that PavSPL7 binds to the *PavEXPA6*, *PavPMEI2*, and *PavPL8* promoters. AD, pB42AD empty vector (negative control). **c** Electrophoretic mobility shift assay (EMSA) showing the direct binding of recombinant purified PavSPL7-glutathione S-transferase (GST) to probes derived from the *PavEXPA6*, *PavPMEI2*, and *PavPL8* promoters. The absence and presence of binding are represented by the symbols – and +, respectively. **d** *N. benthamiana* leaves were coinfiltrated with effector and reporter constructs, and LUC signals were detected 48 h postinfiltration. **e** Relative LUC/REN ratios in *N. benthamiana* leaves detected via a dual-luciferase reporter assay. The value of 62SK + *PavACS7pro*:LUC was set to 1. Data are presented as means ± SEs from three biological replicates. Statistically significant differences are indicated (\*  $P < 0.05$ , \*\*  $P < 0.01$ ; Student's *t*-test). PavSPL7, *Prunus avium* SQUAMOSA Promoter Binding Protein-Like 7; *PavEXPA6*, *Prunus avium* Expansins A6; *PavPMEI2*, Pectin Methylesterase Inhibitor 2; *PavPL8*, Pectate Lyase 8; *PavACS7*, 1-aminocyclopropane-1-carboxylic acid Synthase 7; LUC/REN, firefly luciferase/*Renilla* luciferase

The expression of genes involved in cell wall degradation and fruit ripening was examined in tomato fruits overexpressing *PavSPL7*. The expression of genes such as *SlEXP1*, *SlXYL1*, *SlPL*, *xyloglucan endotransglucosylase/hydrolase 5 (SlXTH5)*,  $\beta$ -galactosidase 4 (*SlTBG4*), and *cellulase-like 2 (SlCEL2)* (Fig. S8), as well as ethylene biosynthesis and ripening-related genes like *SlACO1*, *SlACS2*, *SlACS4*, *ethylene insensitive 4 (SlETR4)*, *RIN*, and *NOR* (Fig. S8), was down-regulated in these fruits compared to the WT. *PavSPL7*-OE tomato fruits were stored for 30 d, after which similar rates of fruit collapse were observed between *PavSPL7*-OE and WT fruits, but water loss was significantly lower in the *PavSPL7*-OE fruits (Fig. S9). These results suggest that *PavSPL7* functions as a

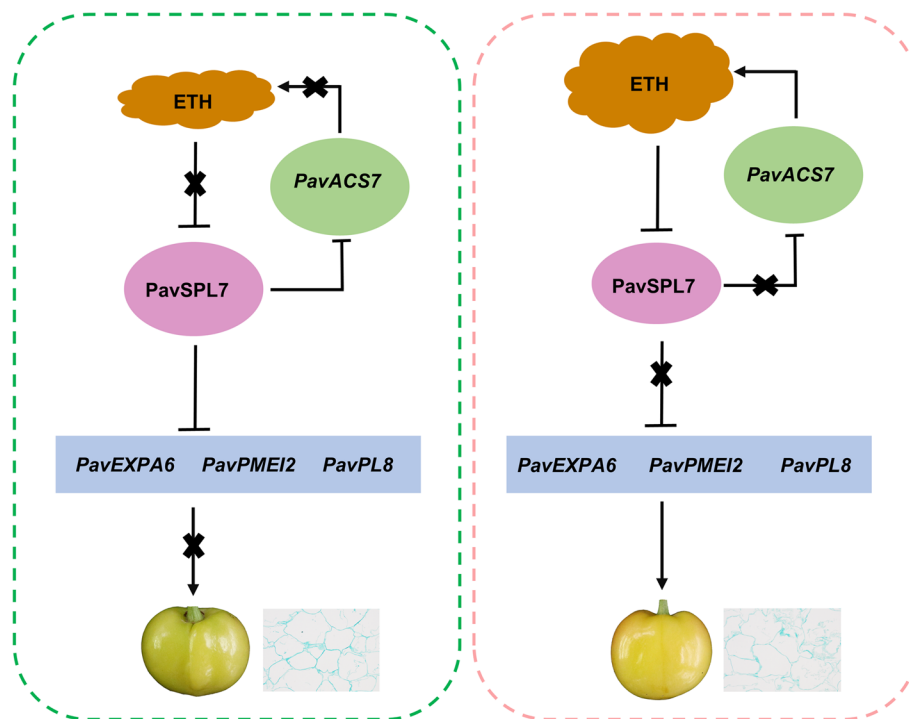
repressor of tomato fruit ripening and softening by its inhibitory effect on cell wall degradation and ethylene biosynthesis.

**PavSPL7 represses genes associated with cell wall degradation and ethylene biosynthesis**

*PavEXPA6*, *PavPMEI2*, and *PavPL8* expression levels were suppressed in *PavSPL7*-OE fruits versus wild-type controls fruits. To determine whether these genes are directly regulated by *PavSPL7*, the promoters of *PavEXPA6*, *PavPMEI2*, and *PavPL8* were cloned and divided into two or three fragments based on the distribution of GTAC elements, followed by yeast one-hybrid assays (Fig. 6a; Fig. S2). The results showed that *PavSPL7* bound to the P1 fragment of the *PavEXPA6* promoter, the P2



**Fig. 7** PavSPL7 represses the transcription of ethylene biosynthesis genes by binding to their promoters. **a** yeast one-hybrid (Y1H) assays showing that PavSPL7 binds to the 1-aminocyclopropane-1-carboxylic acid Synthase 7 (*PavACS7*) promoter. **b** Electrophoretic mobility shift assay (EMSA) demonstrating the direct binding of recombinant purified PavSPL7-glutathione S-transferase (GST) to a probe derived from the *PavACS7* promoter. The symbols – and + denote absence and presence, respectively. **c** *N. benthamiana* leaves were coinfiltrated with effector and reporter constructs, and LUC signals were detected at 48 h postinfiltration. **d** Relative LUC/REN ratios in *N. benthamiana* leaves detected by dual-luciferase reporter assay. The value of 62SK + *PavACS7pro:LUC* was set to 1. Data are presented as means ± SEs from three biological replicates. Statistically significant differences are indicated (\*  $P < 0.05$ , \*\*  $P < 0.01$ ; Student's *t*-test). PavSPL7, *Prunus avium* SQUAMOSA Promoter Binding Protein-Like 7; BAD, pB42AD; LUC/REN, firefly luciferase/*Renilla* luciferase



**Fig. 8** A proposed model on the role of PavSPL7 in regulating ethylene-mediated fruit softening. In the early stage of sweet cherry fruit development when ethylene level is low, PavSPL7 is abundantly expressed and represses the expression of *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7*, thereby preventing fruit softening. During fruit development and maturation, ethylene is increased to downregulate *PavSPL7*, leading to activation of softening-related genes and promotion of fruit softening. Positive and negative effects are denoted by arrows and blunt ends, respectively; crosses symbol represent inhibited pathways. PavSPL7, *Prunus avium* SQUAMOSA Promoter Binding Protein-Like 7; *PavEXPA6*, *Prunus avium* Expansins A6; *PavPMEI2*, Pectin Methylesterase Inhibitor 2; *PavPL8*, Pectate Lyase 8; *PavACS7*, 1-aminocyclopropane-1-carboxylic acid Synthase 7

fragment of the *PavPMEI2* promoter, and the P2 fragment of the *PavPL8* promoter (Fig. 6b). We further confirmed by EMSAs with recombinant purified GST-tagged PavSPL7 protein (Fig. 6c). To explore how PavSPL7 regulates these genes, dual-LUC reporter assays were conducted. The results showed that PavSPL7 significantly diminished the transcriptional activation of the *PavEXPA6*, *PavPMEI2*, and *PavPL8* promoters (Fig. 6d, e), indicating that PavSPL7 negatively regulates the transcription of these genes.

These results demonstrate that ethylene promotes fruit softening in sweet cherry, and that *PavACS7* expression is repressed by *PavSPL7* overexpression (Figs. 1 and 4; Fig. S1). To determine whether PavSPL7 directly regulates *PavACS7* expression, Y1H assays and EMSAs were performed. PavSPL7 was shown to bind to the GTAC element within the *PavACS7* promoter in both assays (Fig. 7a, b). Dual-LUC reporter assays further confirmed that PavSPL7 represses *PavACS7* promoter activity (Fig. 7c). These results demonstrate that the direct repression of cell wall degradation and ethylene biosynthesis genes is mediated by PavSPL7.

## Discussion

### Ethylene plays important roles in fruit ripening and softening in sweet cherry

Fruit softening is a highly complex process that involves the coordination of various factors, primarily driven by critical changes in cell wall structure and composition. In climacteric fruits such as tomatoes (Liu et al. 2015), apples (Wang et al. 2024b), and bananas (Song et al. 2022; Liu et al. 2023), ethylene serves as a primary trigger that promotes ripening and softening. Recent studies have also highlighted ethylene's role in regulating fruit maturation and softening in nonclimacteric species (Upadhyay et al. 2023; Liu et al. 2024; Wang et al. 2024c). In blueberry, for example, ethylene induces the expression of *PE* and *PG* genes, which accelerates pectin degradation and fruit softening (Wang et al. 2020a). Furthermore, treatment with the competitive ethylene inhibitor 1-MCP increases fruit firmness in sweet cherry (Seradilla et al. 2019). Ethylene production in grape berries steadily increases during fruit development and ripening (Chervin et al. 2008; Wang et al. 2022).

In this study, endogenous ACC levels increased throughout the development and maturation of sweet cherry ‘Zaodaguo’ fruit (Fig. S1), and ethylene treatment led to a significant loss of fruit firmness (Fig. 1). These findings align with results from other species, suggesting that ethylene plays a pivotal role in the ripening and softening of sweet cherry fruit. ABA is another major factor that promotes the ripening and softening of nonclimacteric fruits. ABA treatment enhances fruit softening in strawberry and blueberry (Zhou et al. 2021; Zamorano-Curaqueo et al. 2024). There is evidence of an interaction between ABA and ethylene during fruit softening (Wang et al. 2019). ABA accelerates fruit softening in sweet cherry (Zhai et al. 2022), but whether ABA and ethylene coordinate to regulate fruit softening in this species remains an intriguing topic for future research.

#### **PavSPL7 negatively regulates fruit softening and ethylene signaling**

Fruit softening during ripening is primarily driven by cell wall degradation. The breakdown of cell wall components, including pectin solubilization, reduced intercellular adhesion, and hemicellulose depolymerization, significantly impacts fruit firmness (Yan et al. 2020). SPL-type transcription factors play a pivotal role in regulating plant growth, development, and fruit ripening (Manning et al. 2006). Genes in the SPL family, such as *CpSBPI* and *CNR*, are involved in the regulation of fruit softening and cell wall degradation (Manning et al. 2006; Han et al. 2019). Our previous research identified 12 *SPL* genes in sweet cherry and investigated their roles in fruit ripening (Sun et al. 2023). In the present result, the expression of *PavSPL7* was negatively correlated with fruit ripening and softening (Fig. 2a), and its expression was repressed by ethylene treatment (Fig. 2c). Overexpression of *PavSPL7* inhibited pectin degradation and fruit softening in both sweet cherry and tomato fruits, with a denser and more compact cell arrangement observed in *PavSPL7*-OE fruits compared to controls (Figs. 4 and 5). These findings suggest that *PavSPL7* regulates fruit softening by modulating genes involved in cell wall degradation. Additionally, *PavSPL7* suppresses ethylene biosynthesis, further inhibiting fruit softening (Fig. 7). These results imply that ethylene and *PavSPL7* form a negative feedback loop that might prevent fruit from undergoing rapid softening due to excess ethylene production during sweet cherry development and maturation. In addition to *PavSPL7*, the expression of *PavSPL1* increased during fruit development, although to a lesser extent; its roles in fruit ripening and softening are worth exploring (Fig. S3).

Based on these results, we propose a model describing how ethylene promotes fruit softening by inhibiting *PavSPL7* expression in sweet cherry (Fig. 8). When

ethylene accumulates at low levels, *PavSPL7* inhibits the expression of *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7* to suppress ethylene biosynthesis and fruit softening. During fruit maturation, higher ethylene production represses the expression of *PavSPL7*, thereby alleviating the suppression of ethylene biosynthesis and cell wall degradation imposed by *PavSPL7*, thus enhancing fruit softening. We conclude that ethylene and *PavSPL7* antagonistically regulate fruit softening *via* negative feedback regulation. Our findings provide new insight into the role of ethylene in promoting fruit softening in sweet cherry.

#### **Conclusions**

In conclusion, this study proposes a model describing how ethylene regulates fruit softening by inhibiting *PavSPL7* expression in sweet cherry (Fig. 8). In the early stages of fruit development, low ethylene biosynthesis results in high levels of *PavSPL7*, which represses the expression of *PavEXPA6*, *PavPMEI2*, *PavPL8*, and *PavACS7*, thus suppressing ethylene production and fruit softening. As fruit matures, increased ethylene production downregulates *PavSPL7*, relieving its suppression of ethylene biosynthesis and cell wall degradation, thereby enhancing fruit softening. This model suggests that ethylene and *PavSPL7* regulate fruit softening through an antagonistic negative feedback mechanism, and the mechanism fine-tunes the softening process and identifies *PavSPL7* as a promising target for extending fruit shelf life. Our findings offer new insights into the role of ethylene in promoting fruit softening in sweet cherry.

#### **Supplementary Information**

The online version contains supplementary material available at <https://doi.org/10.1007/s44281-025-00089-0>.

Additional file 1: Figure S1. Changes in firmness and 1-aminocyclopropane-1-carboxylic acid (ACC) accumulation during fruit development in ‘Zaodaguo’ sweet cherry. a Changes in fruit firmness during sweet cherry ripening. b Changes in ACC content during sweet cherry fruit ripening. c–f Gene expression dynamics of cell wall degradation and hormone regulatory pathways during fruit ripening. BG, large green; YW, yellow; FR, full red; DR, dark red. Results are averaged across three biological repeats  $\pm$  SE. Significant thresholds set at \*  $P < 0.05$  by one-way ANOVA followed by Tukey’s range test

Additional file 2: Figure S2. Heatmap analysis of ethephon (ETH) biosynthetic genes at different developmental stages. Genes 524, 525, 526, 3169, 8973, and 10862 belong to the *Prunus avium* 1-aminocyclopropane-1-carboxylic acid OXIDASE 6 (*PavACO*) family

Additional file 3: Figure S3. Analysis of cis-acting elements in the promoters of genes associated with cell wall degradation and ethylene biosynthesis in sweet cherry. Vertical lines represent GTAC cis-acting elements. *PavEXPA6*, *Prunus avium* Expansins A6; *PavPMEI2*, *Pectin Methylsterase Inhibitor 2*; *PavPL8*, *Pectate Lyase 8*; *PavACS7*, 1-aminocyclopropane-1-carboxylic acid Synthase 7

Additional file 4: Figure S4. *PavSPL* expression dynamics during fruit development and maturation in sweet cherry. *PavSPLs*, *Prunus avium*

*SQUAMOSA Promoter Binding Protein-Like*. Data are presented as means  $\pm$  SE ( $n = 3$ ). Statistically significant differences are determined using one-way ANOVA followed by Tukey's range test and indicated as follows: \*  $P < 0.05$

Additional file 5: Figure S5. Confirmation of transformation in sweet cherry fruits *via* PCR. The 392 bp PCR product from genomic DNA indicates the hygromycin (Hyg) resistance gene

Additional file 6: Figure S6. Expression analysis of fruit softening-related genes in *PavSPL7*-overexpressing (*PavSPL7*-OE) sweet cherry fruits. Results are averaged across three biological repeats  $\pm$  SE. Significant thresholds set at \*\*  $P < 0.01$  by Student's *t*-test. WT, wild type

Additional file 7: Figure S7. Confirmation of transformation in tomato. a Confirmation of transformation in tomato *via* PCR; the 712-bp PCR product from genomic DNA was amplified using sequencing primers in tomato. b GUS staining of tomato flowers. c *PavSPL7* expression level in *PavSPL7*-OE lines and WT fruits. The *PavSPL7* expression value in WT fruits was set to 1. Results are averaged across three biological repeats  $\pm$  SE. Significant thresholds set at \*  $P < 0.05$ , \*\*  $P < 0.01$  by Student's *t*-test

Additional file 8: Figure S8. Expression of softening-related genes in WT and *PavSPL7*-OE tomato fruits at the BR + 4 stage, normalized to the *SICAC* reference gene. BR, breaker stage; BR + 4, 4 days after the BR stage. *SIXYL1*, *Solanum lycopersicum* XYLOSIDASE 1; *SIXTH5*, xyloglucan endotransglucosylase/hydrolase 5; *SITBG4*,  $\beta$ -galactosidase 4; *SICEL2*, cellulase-like 2; *SIETRA4*, ethylene insensitive 4; *RIN*, ripening-inhibitor; *NOR*, non-ripening. Results are averaged across three biological repeats  $\pm$  SE. Significant thresholds set at \*  $P < 0.05$ , \*\*  $P < 0.01$  by Student's *t*-test

Additional file 9: Figure S9. Postharvest shelf life and water loss of *PavSPL7*-OE tomato fruits in the T2 generation. a Fruit shelf-life of Micro Tom (wild type) and transgenic lines (*PavSPL7*#1, *PavSPL7*#13, and *PavSPL7*#23). Fruits collection was carried out at the BR+10 stage and maintained them at room temperature (RT). Phenotypes were recorded at 0 d and 30 d post-harvest. Scale bar = 1 cm. b Fruits were weighed on the indicated days post-harvest to measure water loss. Significant thresholds set at \*  $P < 0.05$ , \*\*  $P < 0.01$  by Student's *t*-test

Additional file 10: Figure S10. Raw images of yeast assays. a Raw images of Fig. 3b: Transcriptional activity assay of *PavSPL7* in yeast cells. b Raw images of Fig. 6b: *PavSPL7* binding to the *PavEXPA6*, *PavPMEI2*, and *PavPL8* promoters in the yeast one-hybrid (Y1H) system. c Raw images of Fig. 7a: *PavSPL7* binding to the *PavACS7* promoter in the Y1H system. TDO, triple dropout; BD, DNA-binding domain; AD, pB42AD empty vector

Additional file 11: Table S1. Summary of gene information for *Arabidopsis* (*AtSPL*) and tomato (*SlySBP* and *CNR*).

Additional file 12: Table S2. Primers used in this study.

## Acknowledgements

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## Authors' contributions

YTS and THL designed the research and wrote the manuscript. YQX and XYW performed sample collection and treatment. XZ and BYD contributed to the plasmid construction and protein interaction experiment. MT and QQZ performed the transient injection in sweet cherry and stable genetic transformation in tomato. QSY was responsible for the RNA extraction and gene expression analysis.

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## Data availability

All data included in this study are available within the paper and its supplementary materials.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interest

No conflict of interest declared.

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