DNA Methylation Changes Were Involved in Inhibiting Ethylene Signaling and Delaying Senescence of Tomato Fruit Under Low Temperature

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Research Article

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Abstract

Objectives To comprehend the epigenetic mechanism of low temperature in delaying senescence of fruit, the changes of DNA methylation patterns of genes related to ethylene biosynthesis and signaling were analyzed in tomato fruit.

Results In the present results, the expression level of *LeEIN3*, *SIERF-A1* and *LeERT10* decreased, and the expression level of *LeCTR1* increased in tomato fruit stored at the low temperature of 11 °C. Meanwhile, the DNA methylation level of CpG island of *LeEIN3*, *SIERF-A1* and *LeERT10* increased, and the DNA methylation level of CpG island of *LeCTR1* decreased in tomato fruit, respectively. The low temperature suppressed ethylene signaling via changing DNA methylation and gene expression, and delayed senescence of tomato fruit.

Conclusions The present study offered valuable information for understanding the role of DNA methylation in senescence of fruit, and provided a foundation for genetic modifying the epigenetic target sites and controlling fruit senescence.

Introduction

The cold chain is a supply chain of perishable products, which are stored and transported in a low temperature environment and a specific range of humidity conditions (Bogataj et al. 2005). In the food supply system, the cold chain has the capacity to maintain the quality and safety of food and reduce food loss, which was diffusely applied to delay fruit senescence and rot (Hu et al. 2019). Fruit senescence was aggravated through all kinds of internal and external factors, such as the insufficient energy supply, damage of membrane system, mechanical injury (Chen et al. 2020). In general, a decrease in product temperature by 10 °C from environmental conditions doubles the shelf life, because the metabolism and the related physiological reactions of the product are slowed down (Wu et al. 2019). On the premise of maintaining normal life activities of agricultural products, the low temperature weakens postharvest physiological processes such as respiratory intensity and energy dissipation, delays fruit senescence, reduces postharvest losses; besides, the low temperature decreases the rate of biochemical reactions, such as browning reactions, lipid oxidation and pigment degradation; and inhibits growth and propagation of microorganisms (James and James 2010; Macheka et al. 2017). Fresh agricultural products are necessities for the daily lives of people and have a steady large-scale demand. In practice, postharvest losses are a primary obstacle in achieving sustainable fresh produce chains (Hodges et al. 2011).

DNA methylation is an epigenetic modification which adds a methyl group to cytosine bases (Schuebeler 2015). The DNA methylation participated in the regulation of gene expression during plant development and stress, and increased the adaptation of plants to various abiotic and biotic factors (Marfil et al. 2019; Zhang et al. 2010). The effect of external factors, such as low temperature, exogenous hormone and environmental stress, induced variation of DNA methylation and influenced gene expression (Klebaner et

al. 2016). DNA methylation of nuclear genomes involved a more widespread range of methylation sites in plants than that in animals (Vanyushin and Ashapkin 2011). The CG, CHG, and CHH (H is A, T or C) are common target sites of cytosine DNA methylation in plant genomes (Dubrovina and Kiselev 2016). In plants, DNA methylation of promoter regions ordinarily restrains transcription, but methylation in coding regions frequently either no affect the gene expression, or only has a medium influence (Zilberman et al. 2007). It had revealed that fruit senescence was under epigenetic control mediated by changes of DNA methylation and distribution, besides genetic and hormonal controls (Gapper et al. 2013). The changes of DNA methylation played an important role in the regulation of the tomato fruit softening and ripening (Zhong et al. 2013).

Tomato is among the most popular vegetables in the worldwide range. As the typical respiratory climacteric fruit, tomato can release ethylene to accelerate the senescence during storage and transportation stages. It is commonly harvested at the early mature stage, and supplies various regions by cold chain logistics (Tigist et al. 2013). Understanding the epigenetic mechanism of low temperature in delaying senescence of tomato fruit may provide valuable information for reducing postharvest fruit losses. In the present study, the changes of DNA methylation patterns of CpG islands of genes related to ethylene biosynthesis and signaling responded to low temperature in tomato fruit were analyzed.

Materials And Methods

Plant Material

Tomato (*Solanum lycopersicum* cv. Fen Gui Fei 455) fruit were harvested from modern agricultural science and technology demonstration park (Weifang, Shandong province, China) and transported to the laboratory. All fruit were sorted by means of size without physical injuries or infections, and were put in a plastic box to retain relative humidity (85–90%), then stored at 11 °C and 25 °C for 0, 3, 6, 9 and 12 d, respectively. The pericarp tissues were collected with well mixed to freeze in liquid nitrogen, and then were stored at -80 °C.

Measurement of the Content of Total Chlorophyll and Lycopene

Frozen pericarp tissues were ground powder in the liquid nitrogen, then weigh 0.5 g powder and dissolve in 1.5 mL phosphate-buffered saline. Following the manufacturer's instructions, the content of total chlorophyll and lycopene was measured via Plant Total Chlorophyll ELISA Kit (Jining Biological Technology Co. Ltd., Shanghai, China) and Plant Lycopene ELISA Kit (Jining Biological Technology), respectively. Each sample was performed three replications, and the OD value was measured by spectrophotometer at a wavelength of 450 nm.

Measurement of the Content of Vitamin C and Soluble Solid, the activities of Polygalacturonase (PG) and Cellulase

The content of Vitamin C was measured by Plant Vitamin C ELISA Kit (Jining Biological Technology) as per the manufacturer's recommendations. The content of the soluble solid of tomato fruit was measured with a saccharimeter (WY032R). The activities of PG and cellulase were discovered by Plant Polygalacturonase ELISA Kit and Plant Cellulase ELISA Kit (Jingkang Biotechnology Co. Ltd., Shanghai, China), respectively. Each sample was performed three replications, and the OD value was measured by spectrophotometer at a wavelength of 450 nm.

Measurement of energy status

The extract of tomato fruit was measured by HPLC (Waters e2695, America) equipped with a C18 inverting column (5 μm $\times$ 250 mm $\times$ 4.6 mm) and UV (254 nm) detector via the method of Liu et al. (2006). The contents of ATP, ADP, and AMP were calculated through the external standard method and were denoted mg kg^{-1} basis on fresh weight. EC was calculated as: $\text{EC} = (\text{ATP} + 0.5 \times \text{ADP}) / (\text{ATP} + \text{ADP} + \text{AMP})$.

Measurement of the Activities of Methylase and Demethylase

The activities of DNA methylase and demethylase was measured through the method of Pu et al. (2020).

Gene Expression Assay by RT-qPCR

The sequence of *SIACS10*, *LeCTR1*, *LeEIN3*, *LeERT10* and *SIERF-A1* genes (Supplementary) were inquired by NCBI web (<https://www.ncbi.nlm.nih.gov/>). The forward and reverse primers were devised according to the sequence of genes (Table 1). The reaction system was measured applying the iCycler iQ real-time PCR detection system (Bio-Rad, Berkeley, CA, USA) in three replicates and GAPDH was used as an internal reference. Following to the way of Livak and Schmittgen (2001), the relative expression level of the gene was calculated.

Table 1
primers used for expression analyses of genes by qRT-PCR

Gene	primer	Sequence(5'-3')
<i>SIACS10</i>	Forward Primer	GGTCGCAGAGGCAATCAAGC
	Reverse Primer	GCCACAGCCCTCATTCTTATGC
<i>LeCTR1</i>	Forward Primer	AAGAGAACCTGGCATCCG
	Reverse Primer	TGAGGCAGACAGCGTTAC
<i>LeEIN3</i>	Forward Primer	CATTGAAGCCGCCGTACAGA
	Reverse Primer	TCAACCAATCTCACCTCGAAAGC
<i>LeERT10</i>	Forward Primer	CGTGCCGTAGCGATTGAGC
	Forward Primer	CCAAACCGTCGATACGTCCAAA
<i>SIERF-A1</i>	Forward Primer	CACAGCCACTCAGAAGACCGTT
	Reverse Primer	AGCACTTTCCCTACAGCCTTGG
Universal	Forward Primer	CCAAGGCTGTAGGGAAAGTGCTA
<i>GAPDH</i>	Reverse Primer	TCAACCACGGACACATCAACAGT

DNA Methylation Level Analysis by Bisulfite Sequencing PCR (BSP)

DNA was purified by SDS solubilization and phenol-chloroform extraction. The primers were designed according to the sequence of the CpG island of genes (Table 2). Bisulfite mix reagent was added to high-quality DNA, transformed on a circulating heater and completed DNA modification. After PCR reaction, product cloning was connected and converted in Trans-T1 Phage Resistant competent cell, and selectively grew on LB agar plate contain *Ampicillin*. Plasmids sequenced by Instrument (3730XL DNA analyzer, ABI Company).

Table 2
primers used for DNA methylation analyses of genes by BSP

Gene	primer	Sequence(5'-3')
<i>SlACS10</i>	Forward Primer	GGTTAGGTAGTTGATTGA(C/T)GTTATATT
	Reverse Primer	CAAATACCTAAAATTACCCAATAATT
<i>LeCTR1</i>	Forward Primer	GTATTTGATTTGGATTTGATGGATT
	Reverse Primer	TACCAATACATCAATCACAAAATCC
<i>LeEIN3</i>	Forward Primer	GTGGAGTTTAAGAAGTTGAGTATAAGT
	Reverse Primer	CATCATTTTCAACATATACTTCAATAT
<i>LeERT10</i>	Forward Primer	TGCAATCTCTATGTGATGAAATCAACT
	Forward Primer	TCATAACTAGTCATTTCAAGTTCAAC
<i>SlERF-A1</i>	Forward Primer	T(C/T)GGAATTTGTGGTTTTATTAGAGA
	Reverse Primer	ATTACTTTATTCCAC(G/A)ACAACCTTC

Statistical Analysis

The data were statistically analyzed using SPSS Statistics 17.0 software (SPSS Inc., Chicago, IL) and the graphs draw by GraphPad Prism 9 (GraphPad, San Diego, CA, USA). The value of mean and standard deviation (SD) was calculated. Two-way ANOVA and statistically significant differences (Tukey's HSD) for all data were executed. Differences at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) or $P < 0.0001$ (****) were deemed as significant, and ns expressed no significant difference.

Results

Changes of Fruit Phenotype

Compared with the control fruit, low temperature treatment significantly delayed senescence of tomato fruit. The color of the control fruit became red at 3 d. However, the color in low temperature treated fruit became red only until the 9 d (Fig. 1A-C).

The Content of Total Chlorophyll and Lycopene

The content of chlorophyll decreased with fruit senescence, and it was higher in low temperature treated fruit than those in control. The content of chlorophyll in low temperature treated fruit reduced from 33.13 to 27.56 mg kg⁻¹ during storage from 3 to 12 d, while the chlorophyll content in the control fruit reduced from 27.83 to 7.9 mg kg⁻¹ (Fig. 1D).

Simultaneously, the low temperature treated fruit had lower lycopene content compared with that in the control fruit. The content of lycopene in the control fruit was 2.11 times that of the low temperature treated fruit after stored for 3 d. The lycopene content in low temperature treated fruit raised to 8.40 mg kg⁻¹ after stored for 9 d, while the control fruit only increased to 4.52 mg kg⁻¹ (Fig. 1E).

The Contents of Vitamin C and Soluble Solid

The content of vitamin C decreased with fruit senescence from 3 to 12 d. The content of Vitamin C in the low temperature treated fruit reduced from 112.22 to 83.68 mg kg⁻¹, while the control fruit reduced from 104.90 to 73.38 mg kg⁻¹ (Fig. 2A). The content of soluble solid in control fruit decreased from 3.70–3.00% with fruit senescence during storage from 3 to 12 d, but increased from 3.47–3.88% in low temperature treated fruit (Fig. 2B).

The Activities of PG and Cellulase

The activities of PG and cellulase increased significantly with fruit senescence. In low temperature treated fruit, the activities of PG and cellulase were both lower than those in control fruit. The PG activity in low temperature treated fruit raised from 313.03 to 479.43 U kg⁻¹ during storage from 3 to 12 d, and raised from 376.93 to 628.61 U kg⁻¹ in control fruit (Fig. 2C). The activity of cellulase in low temperature treated fruit enhanced from 56.52 to 114.30 mU kg⁻¹ during storage from 3 to 12 d, and enhanced from 72.57 to 143.56 mU kg⁻¹ in control fruit (Fig. 2D).

Energy Status

The contents of ATP in the low temperature treated fruit changed slightly over the entire storage, while decreased from 33.39 to 25.71 µg g⁻¹ in the control fruit (Fig. 3A). The ADP content of both groups displayed an enhanced trend. ADP content was 18.83 µg g⁻¹ in control fruits after stored for 12 d, but low temperature treatment was 15.45 µg g⁻¹ (Fig. 3B). The low temperature treatment effectively prevented the increase of AMP content. The AMP content of the control fruit raised from 7.28 to 43.36 µg g⁻¹ during the whole storage, while the low temperature treatment only was 9.35 µg g⁻¹ at the end of storage (Fig. 3C). According to the variations of ATP, ADP and AMP content, EC in tomato fruit reduced with prolonging storage time. The EC value of the low temperature treatment was 1.75 times that of the control after stored for 12 d (Fig. 3D).

The Activities of DNA Methylase and Demethylase

The activity of DNA methylase in low temperature treated fruit reached a maximum after stored for 6 d, which was 551.33 U kg⁻¹, and then decreased to 341.33 U kg⁻¹ after stored for 12 d. The activity of DNA methylase in the control fruit increased from 291.33 to 784.67 U kg⁻¹ during storage from 6 to 12 d (Fig. 4A). The activity of DNA demethylase in low temperature treated fruit was lower than those in

control fruit. The activity of demethylase in low temperature treated and control fruit reached the maximum of 459.78 and 644.57 U kg⁻¹ after stored for 12 d, respectively (Fig. 4B).

Genes Expression and DNA Methylation Levels

The expression level of the *SIACS10* gene decreased with fruit senescence. During storage from 6 to 12 d, the expression level of *SIACS10* in low temperature treated fruit reduced from 0.32 to 0.13, while the control fruit reduced from 0.45 to 0.23 (Fig. 5A). Moreover, the methylation level of *SIACS10* only appeared at 12 d, and the methylation level of the control fruit was 4.29 times that of those in low temperature treatment (Fig. 5B and C).

The expression level of *LeCTR1* in the control fruit was 0.55 after stored for 6 d. While the expression level of *LeCTR1* in the low temperature treated fruit enhanced and then reduced at the whole storage period, reached a maximum value of 1.29 at 6 d, and reduced to 1.05 at 12 d (Fig. 6A). The DNA methylation rate of CpG island of *LeCTR1* in the low temperature treated fruit both were 0.6% at 6 and 12 d, while DNA methylation rate of CpG island of *LeCTR1* in control fruit merely arose at 6 d (Fig. 6B and C).

The expression of *LeEIN3* in low temperature treated fruit was down-regulated. The expression level of *LeEIN3* of control fruit was 9.55 times than those in low temperature treatment after stored for 6 d. The expression level of *LeEIN3* of control fruit was 22.21 times than those in low temperature treatment after stored for 12 d (Fig. 6D). The DNA methylation rate of *LeEIN3* in the low temperature treated fruit was higher than those in the control (Fig. 6E and F). The DNA methylation rate of *LeEIN3* in low temperature treatment was 1.61 times than those in control after stored for 12 d (Fig. 6E).

The expression level of *SIERF-A1* in both groups was up-regulated, but the low temperature treated fruit was slightly lower than those in control. The expression of *SIERF-A1* in low temperature treated and control fruit was 1.49 and 1.91 at 12 d, respectively (Fig. 7A). After stored for 6 d, the methylation rate of CpG island of *SIERF-A1* in low temperature treated and control fruit reached a peak value of 1.7% and 0.6%, respectively. After stored for 12 d, DNA methylation was not located in low temperature treated fruit, while the methylation rate of CpG island of *SIERF-A1* in the control fruit reduced to 0.3% (Fig. 7B and C).

The expression level of *LeERT10* in the low temperature treated fruit was significantly lower than those in the control. The expression level of *LeERT10* in low temperature treated fruit reduced from 0.24 to 0.10 during storage from 6 to 12 d, while the control fruit reduced from 0.48 to 0.15 (Fig. 7D). The DNA methylation rate of CpG island of *LeERT10* in the low temperature treated fruit was higher than those in control. The methylation rate of CpG island of *LeERT10* in low temperature treated fruit increased to 1.7% after stored for 12 d, while DNA methylation was not located in the control (Fig. 7E and F).

Discussion

In cold chain logistics, low temperature plays an important role in transporting perishable foods from production to consumption while ensuring the quality and safety of foods. Low temperature storage

could significantly reduce the expression level of numerous genes related to the aroma volatiles synthesis during the storage of tomato fruit (Zou et al. 2018). The postharvest fruit, as independent existence, infinitely depleted nutrient to result in irreversible senescence until death (Wang et al. 2021a). In the present study, the low temperature (11 °C) treatment effectively delayed the senescence of tomato fruit (Fig. 1A-C). The content of total chlorophyll in the low temperature treated fruit was higher than that in the control fruit, and the lycopene content in the low temperature treated fruit was lower than those in control fruit, which cause a noticeable color difference on tomato fruit peel (Fig. 1D and E).

The fruit ripening and senescence connected with degradation of the cell wall, which the enhancement of water-soluble pectin and the reduction cellulose gradually caused fruit to soften (Wang et al. 2021b). Cellulose, as the main component of plant cell walls, can maintain the strength of the cell wall (Wang et al. 2021b). PG was the major hydrolysis enzyme of modifying pectin, which can further decompose pectin acid-generating from that pectin methylesterase (PME) catalyzed pectin demethylation, finally resulting in the structure of cell wall loosening and fruit softening (Rugkong et al. 2010). The better fruit quality was maintained by lower fruit softening enzyme activity (Adhikary et al. 2021). In this study, the PG and cellulase activities in the low temperature treated fruit were also lower than those in the control fruit (Fig. 2C and D), which indicated that low temperature effectively restrained pectin degradation and cell wall injury to delay senescence and soften of tomato fruit.

Similarly, the higher energy consumption can cause the increase of respiratory rate in postharvest fruit to accelerate the development of senescence and disease (Li et al. 2020). ATP was the center of energy conversion within cells, which produce chemical energy through catabolic and anabolic that was used immediately in plant (Vichaiya et al. 2020). The membrane system integrity was connected with cellular energy status. The continuous consumption of cellular energy may lead to membrane damage, while higher-level ATP content can preserve the membrane potential and maintain the membrane system integrity, to delay fruit senescence and to retain qualities (Aghdam et al. 2018). The higher accumulation of energy-matter in kiwifruit could keep an integral cell membrane (Wang et al. 2020). In this study, low temperature treated fruit retained higher ATP content and EC level compared with the control fruit (Fig. 3), which may be contributing to restraining the activities of PG and CE, to delay the fruit senescence. Previous reports showed that MeJA treatment delays the postharvest softening of blueberry and pineapple fruit by altering cell wall modification and energy metabolism (Boonyariththongchai and Supapvanich 2017). The senescence process of the litchis (Tang et al. 2020), longans (Li et al. 2020) and broccoli (Huang et al. 2021) accompany the reduction of ATP, ADP content and EC level, and the enhancement of AMP.

The process of fruit ripening and senescence is closely related to the biosynthetic and signaling pathway of ethylene. Among the biosynthetic pathways upstream of ethylene, ACS and ACO enzymes are encoded by multigene families. They were differentially expressed in various tissues at different developmental phases (Nakatsuka et al. 1998). The *ETR* and *CTR1* both are a negative regulatory element in the ethylene signaling pathway, which can activate the positive regulatory factor *EIN2*, pass the downstream *EIN3/EILs*, promote the expression of the transcription factor *ERF* and transcript *ERT*, and ultimately

express ethylene-related response genes (Guo et al. 2018; Hu et al. 2012; Trentmann 2000). DNA methylation and demethylation were requested for both the activation of ripening induced genes and the suppression of ripening repressed genes (Lang et al. 2017).

As one of the key enzymes, ACS tightly regulated ethylene biosynthesis (Barry and Grierson, 2000). Among the *ACS* genes that have been cloned from tomato, *LeACS2* and *LeACS4* are mainly expressed during the climacteric phase of tomato fruit, resulting in a large amount of ethylene and a characteristic of auto-catalytic ethylene of system II (Anugerah et al. 2015). During the natural ripening of watermelon, the expression of two *ACS* isoforms, *Cla022653* and *Cla011522*, were significantly up-regulated in 97103 flesh, which play an important role in ethylene biosynthesis and ripening control in watermelon flesh (Zhou et al. 2016). Here, low temperature treatment reduced the expression of the *SlACS10* gene (Fig. 5), which might involve inhibiting the ethylene biosynthesis and delaying the senescence of tomato fruit. The DNA methylation level of the CpG island of *SlACS10* in low temperature treated fruit was reduced compared with control, the changes of seven DNA methylation sites in the CpG island of *SlACS10* might have no critical importance on its expression.

CTR1 is a negative regulator of ethylene signaling, and its interaction with ETR1 is required for the negative regulation of ethylene signaling (Kieber et al. 1993). *LeCTR1* silenced tomato plants induce the expression of ethylene-responsive genes, such as *ERF5*, *EIN2*, and *EIN3* (Chandan et al. 2019). The amino acid sequence of *MhCTR1* showed 55% homology to *LeCTR1*, as well as the changes of *MhCTR1* expression in the pulp were closely related to the regulation of the banana senescence process (Hu et al. 2012). Here, the DNA methylation level of CpG island of *LeCTR1* in the low temperature treated fruit decreased than those in control fruit at 6 d, and by which raised the expression of the *LeCTR1* gene (Fig. 6A-C), and might suppress ethylene signaling. These may be one of the mechanisms of low temperature delayed senescence of tomato fruit.

EIN3 is a key to activate the ethylene transcription factors *ERF*. EIN3 proteins bind directly to primary ethylene response element (PERE) motifs to regulate gene expression (Solano et al. 1998). In *Arabidopsis*, *EIN3*, *ORE1* and *CCG* work together to regulate ethylene-mediated chlorophyll degradation during leaf senescence (Qiu et al. 2015). *CpEIN3a* was found to increase and participate in carotenoid accumulation during fruit ripening and senescence in papaya (Fu et al. 2017). Compared with the control, low temperature treatment raised the DNA methylation level of *LeEIN3* in tomato fruit, reduced the expression of *LeEIN3* (Fig. 6D-E), and suppressed ethylene signaling. The increased DNA methylation of *LeEIN3* may be involved in low temperature delaying senescence of tomato fruit.

ERFs are DNA-binding proteins belonging to the AP2/ERF family, and coordinate transcription of diverse ethylene-responsive genes (Gu et al. 2002). The expression levels of the four *ERF* genes in pear increased significantly during fruit senescence. *Pbr012024.1* responded to the ethylene signal, and the *Pbr022708.1* can be induced by ethylene and is the only ethylene response factor that regulates fruit senescence (Hao et al. 2017; Xu et al. 2018). In tomato, overexpression of *LeERF1* accelerated fruit senescence (Li et al. 2007). *LeERF2* is induced by ethylene and suppressed in ripening inhibited mutants (Wu et al. 2002).

Here, compared with that in control fruit, the DNA methylation levels of *SIERF-A1* in low temperature treated fruit reached maximum at 6 d, the expression of *SIERF-A1* gene was significantly decreased at 6 and 12 d (Fig. 7A-C), and by which the ethylene signaling might be suppressed. These may be one of the mechanisms of low temperature delayed tomato fruit senescence.

ERT encodes transcripts that regulate ethylene transcription regulation, and participates in the cascade of constitutive cellular factors related to ethylene signal transduction (Trentmann 2000). 49 putative ethylene-responsive transcripts (ERTs) were isolated from etiolated seedlings of *Arabidopsis*, and ethylene-regulated nuclear protein (ERN) was isolated and cloned from *ERT2*. Evidence had shown that *ERN1* encoded downstream targets of EIN3 protein as did *ERF1* (Solano et al. 1998). Hot water treatment delayed the ripening of tomato fruit, which process accompanies the reduction of the expression level of *LeERT10* and increase of methylation level (Pu et al. 2020). In the present study, the low temperature treatment enhanced the DNA methylation level of *LeERT10* and significantly reduced the expression of the *LeERT10* gene in tomato fruit (Fig. 7D-F), and suppressed ethylene signaling. The enhanced DNA methylation level of *LeERT10* may be involved in low temperature delaying senescence of tomato fruit.

Conclusion

The low temperature changed the DNA methylation levels of CpG island of *LeCTR1*, *LeEIN3*, *SIERF-A1* and *LeERT10* in tomato fruit, impacted their expression and suppressed ethylene signaling, by which delayed fruit senescence. The study offered useful information for comprehending the role of DNA methylation for fruit senescence, and provided a foundation for genetically modifying the epigenetic target sites and controlling fruit senescence.

Declarations

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Conflicts of Interest The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Figures

Figure 1

Senescence changes of tomato fruit. (A) Senescence phenotypic characterization of the exterior of tomato fruit. (B) Senescence phenotypic characterization of the flank of tomato fruit. (C) Senescence phenotypic characterization of the interior of tomato fruit. (D) Total chlorophyll content. (E) Lycopene content. Vertical bars represent standard deviations of the means, $n=3$. Asterisks indicate statistical

difference of the values at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) or $P < 0.0001$ (****), and ns = no significant.

Figure 2

The change of vitamin C content (A), soluble solid content (B), polygalacturonase activity (C), and cellulase activity (D) in tomato fruit. Vertical bars represent standard deviations of the means, n=3.

Asterisks indicate statistical difference of the values at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) or $P < 0.0001$ (****), and ns = no significant.

Figure 3

The change of ATP content (A), ADP content (B), AMP content (C), and Energy charge (D) in tomato fruit. Vertical bars represent standard deviations of the means, n=3. Asterisks indicate statistical difference of the values at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) or $P < 0.0001$ (****), and ns = no significant.

Figure 4

The change of the Methylase activity (A) and Demethylase activity (B) in tomato fruit. Vertical bars represent standard deviations of the means, $n=3$. Asterisks indicate statistical difference of the values at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) or $P < 0.0001$ (****), and ns = no significant.

Figure 5

Expression level and DNA methylation rate of SIACS10 in tomato fruit. (A) Relative expression level. (B) DNA methylation rate. (C) Changes of DNA methylation sites. Each line represents 1 clone, and 1 circle represents 1 CpG site. The black circle represents the methylated CG, and the white circle represents the unmethylated CG. Vertical bars represent standard deviations of the means, $n=3$. Asterisks indicate statistical difference of the values at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) or $P < 0.0001$ (****), and ns = no significant.

Figure 6

Expression level and DNA methylation rate of LeCTR1 and LeEIN3 in tomato fruit. (A) Relative expression level of LeCTR1. (B) DNA methylation rate of CpG island of LeCTR1. (C) Changes of DNA methylation sites of LeCTR1. (D) Relative expression level of LeEIN3. (E) DNA methylation rate of CpG island of LeEIN3. (F) Changes of DNA methylation sites of LeEIN3. Each line represents 1 clone, and 1 circle represents 1 CpG site. The black circle represents the methylated CG, and the white circle represents the

unmethylated CG. Vertical bars represent standard deviations of the means. Asterisks indicate statistical difference of the values at $P < 0.05$ (*) or $P < 0.01$ (**).

Figure 7

Expression level and DNA methylation rate of SIERF-A1 and LeERT10 in tomato fruit. (A) Relative expression level of SIERF-A1. (B) DNA methylation rate of CpG island of SIERF-A1. (C) Changes of DNA methylation sites of SIERF-A1. (D) Relative expression level of LeERT10. (E) DNA methylation rate of CpG

island of LeERT10. (F) Changes of DNA methylation sites of LeERT10. Each line represents 1 clone, and 1 circle represents 1 CpG site. The black circle represents the methylated CG, and the white circle represents the unmethylated CG. Vertical bars represent standard deviations of the means, $n=3$. Asterisks indicate statistical difference of the values at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) or $P < 0.0001$ (****), and ns = no significant.

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