DNA Methylation Changes Regulated by Melatonin was Involved in Postharvest Ripening of Tomato Fruit

Shuangshuang Shan
Hainan University
Zhiqiang Wang
Hainan University
Huili Pu
Hainan University
Wenhui Duan
Hainan University
Jixin Tian
Hainan University
Jiangkuo Li
Tianjin Academy of Agricultural Sciences
Xiangbin Xu (xbxuibcas@126.com)
Hainan University https://orcid.org/0000-0002-6160-9579

Original Research Paper

Keywords: Melatonin, DNA methylation, Fruit ripening, Postharvest tomato

Posted Date: February 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-180786/v1

Abstract

The regulation mechanism of the levels of DNA methylation of CpG islands of ethylene signaling genes induced by melatonin on postharvest ripening of tomato fruit were studied. The ripening of tomato fruit was significantly promoted by the melatonin treatment, as revealed by the appearance color, the lycopene and total soluble solids content of the fruit. In the melatonin treated fruit, the DNA methylation levels of CpG island of *SlACS10* and *SlERF-A1* were decreased, and the DNA methylation level of CpG island of *LeCTR1* was increased. In addition, melatonin treatment increased the expression level of *SlACS10, LeEIN3, SlERF-A1* and *LeERT10*, inhibited the expression level of *LeCTR1*, and by which the ethylene signaling was activated and the ripening was promoted. The present study provided valuable information for understanding the essential role of DNA methylation in the postharvest ripening of tomato fruit.

Introduction

DNA methylation is an epigenetic modification that adds a methyl group to the cytosine base, and is related to gene silencing in eukaryotes (Law and Jacobsen 2010). It is catalyzed by a series of DNA methyltransferases, namely, the Domain Rearranged Methyltransferases, Chromomethylases and the DNA Methyltransferase 1. In plants, it usually occurs in all sequences of cytosine bases: symmetrical (CG or CHG) and nonsymmetrical (CHH) contexts (with H: A, T or C) (Lang et al. 2016). DNA methylation involved in multiple functions, such as act as an imprinting signal, arrest transcript elongation, suppress homologous recombination and inhibit transcription initiation. The dynamic changes of DNA methylation play a vital role in fleshy fruit ripening. In grape berries, azacytidine (a methyltransferase inhibitor) treatment reduced the DNA methylation level and promoted berry ripening 20 d earlier than the control (Guo et al. 2019). During the development and ripening of sweet orange fruit, DNA methylation increased globally, and the application of DNA methylation inhibitor delayed the ripening (Huang et al. 2019). In addition, the treatment of immature tomato fruit with 5-azacytidine led to DNA hypomethylation and promoted the premature ripening of fruit before seed maturity (Zhong et al. 2013). These researches indicated the important function of DNA methylation in fruit ripening.

Melatonin or N-acetyl-5-methoxytryptamine, is a derivative of tryptophan, and its chemical structure is classified as biogenic indoleamine (Lerner et al. 1958). It is named melatonin due to its ability to lighten the skin in certain reptiles, amphibians and fish. The biosynthesis of melatonin begins with tryptophan, at least six different enzymes participated in this process through four different pathways, and N-acetyltryptamine is an essential intermediate in the synthesis. Melatonin is an effective scavenger of hydroxyl radicals, which can be considered as a natural preparation to protect organism from oxygen radicals. Melatonin also exerted the neuroprotective effect on spinal cord injury. In plants, melatonin resists different biotic or abiotic stresses as a signal molecule, and also stimulates growth and development, involves in a variety of physiological activities, including photosynthesis, seed germination, fruit ripening and senescence. The accumulation patterns of melatonin during fruit ripening are different in varies fruit species, the mechanism by which melatonin regulates fruit ripening is complex. In banana, melatonin is an indicator of the fruit ripening. Exogenous application of melatonin caused a delay in

banana ripening after harvest (Hu et al. 2017). In addition, melatonin promoted postharvest ripening and improved fruit quality of tomato fruit (Sun et al. 2015). The physiological and molecular mechanisms of tomato fruit ripening mediated by melatonin are revealed by proteomic analysis (Sun et al. 2016). However, to date, the epigenetic mechanism by which melatonin promotes tomato fruit ripening remains unknown.

Tomato is a typical respiratory climacteric fruit. The ripening of climacteric fruit is initiated and regulated by plant hormone ethylene. Due to the vital role of ethylene for fruit ripening, ethylene biosynthetic and signal pathway have become the attractive target for research aimed at manipulating and understanding the control of fruit ripening. The direct precursors of ethylene synthesis are *ACS* and *ACO* genes (Trivellini et al. 2011). In the ethylene signal transduction pathway, receptor and *CTR1* play a negative regulatory role (Wang et al. 2003). Transcription factors (EIN3/EIL and ERF) are activated by activated EIN2, the transcription reaction of ethylene is initiated, and the expression of target genes is activated (Chen et al. 2005). In the present study, the postharvest ripening of tomato fruit treated by melatonin was studied, and the dynamic changes of DNA methylation in CpG islands of the genes involved in ethylene biosynthesis and signal pathway, including *SlACS10, LeCTR1, LeEIN3, SlERF-A1* and *LeERT10*, were detected, respectively.

Materials And Methods

Fruit and treatment

Fresh green ripe tomato fruit (*Solanum lycopersicum* cv. Fen Gui Fei 455) were harvested from modern agricultural science and technology demonstration park (Weifang Academy of Agricultural Sciences, Shandong Province, China). The fruit having uniform weight, size, shape without any defects or injuries were selected and washed with tap water. All fruit were randomly divided into two batches. The first batch of fruit was immersed in a 0.5 mM melatonin solution at 25 ^{o}C for 10 minutes. Under the same conditions, another batch of fruits were soaked in distilled water as the control group. Every six fruits placed in a clean polypropylene plastic box, which sealed with a 0.02 mm thick low-density polyethylene bag. Finally, all fruit stored at 10 ^{o}C. Fruit samples were taken for measurement before treatment and every 5 d during storage. In addition, the fruit sampled was stored at -80 ^{o}C for enzyme and gene assays. To confirm reproducibility, each experiment was repeated three times, and the entire experiment was repeated twice.

Lycopene And Total Soluble Solids Content

According to the manufacturer's instructions, the lycopene content was detected by Plant lycopene ELISA kit (Jingkang Biological Engineering Co. Ltd. Shanghai, China). The result of lycopene content was expressed as mg per 1 kg FW. The content of TSS of tomato fruit was measured by 0.2 mL juice of each sample fruit by saccharimeter (WY032R) and expressed as %.

Methylase And Demethylase Activity

The activities of DNA methylase and demethylase were detected by using the plant methylase kit and the plant demethylase kit (Gelatins Biotechnology Co. Ltd. Shanghai, China), respectively, and were measured at 450 nm wavelength with a microplate reader. The concentration of DNA methylase and demethylase activity were calculated by standard curve, which was expressed by U kg^{-1}.

Expression Analyses Of Ethylene Signaling Genes

Total RNA extraction and quantitative real-time PCR (qRT-PCR) were performed as previously described Xu et al. (2013), and the expression of five genes, including *SlACS10, LeCTR1, LeEIN3, SlERF-A1* and *LeERT10*, was examined using qRT-PCR. The comparative Ct method ($2^{-\Delta\Delta Ct}$) was adopted to calculate the expression data with the gene *GAPDH* as an endogenous control. The primers used for qRT-PCR analysis were designed with Primer 5.0 and listed in Table 1.

Table 1
Primers used in the qRT-PCR.

Gene	Primer	Sequence (5'-3')
GAPDH	Forward Primer	CCAAGGCTGTAGGGAAAGTGCTA
	Reverse Primer	TCAACCACGGACACATCAACAGT
SlACS10	Forward Primer	GGTCGCAGAGGCAATCAAGC
	Reverse Primer	GCCACAGCCCTCATTCTTATGC
LeCTR1	Forward Primer	AAGAGAACCTGGCATCCG
	Reverse Primer	TGAGGCAGACAGCGTTAC
LeEIN3	Forward Primer	CATTGAAGCCGCCGTACAGA
	Reverse Primer	TCAACCAATCTCACCTCGAAAGC
SlERF-A1	Forward Primer	CACAGCCACTCAGAAGACCGTT
	Reverse Primer	AGCACTTTCCCTACAGCCTTGG
LeERT10	Forward Primer	CGTGCCGTAGCGATTGAGC
	Reverse Primer	CCAAACCGTCGATACGTCCAAA

Bisulfite Sequencing Pcr And Dna Methylation Analysis

According to the method of Murray and Thompson (1980), total DNA was extracted from tomato tissues. The CpG islands of five genes (*SlACS10, LeCTR1, LeEIN3, SlERF-A1* and *LeERT10*) were predicted by

online software (http://www.bioinformatics.org/ sms2/cpg_islands.html). The details of the size and location of CpG island of genes are described in the Supplementary 1. The primers for amplifying the target DNA regions in the control and treated tomato fruit are attached in Table 2. The PCR products were cloned into the vector, and more than ten clones of each derivative were sequenced. Clones with the minimum 95% bisulfite conversion rate and complete sequencing data were aligned and analyzed through BiQ analyzer.

Table 2
Primers used in the bisulfite sequencing PCR.

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

Statistical analysis

All data were analysed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncans multiple range test.

Results

Effects of melatonin on fruit ripening, lycopene and TSS content

To monitor the effect of melatonin on the ripening process of tomato fruit, mature green tomato fruit was treated with 0.5 mM melatonin. The most obvious change of tomato fruit during ripening is the alteration of color. After stored for 10 d, the control fruit began to turn red (Fig. 1A). Compared with that of the control fruit, the melatonin treated fruit maturity advanced by 5 d, and reached full maturity early at 20 d. Massive amounts of lycopene were accumulated in mature tomato fruit and mainly responsible for the red color. In the present results, melatonin treatment increased the lycopene and TSS content compared

with the control tomato fruit (Fig. 1B and C). During stored from 5 to 20 d, the lycopene content in melatonin treated fruit increased from 8.2 to 11.3 mg kg^{-1}, while the lycopene content in the control fruit increased from 7.6 to 11.8 mg kg^{-1}. The content of TSS in melatonin treated tomato fruit was higher than that in the control fruit. During the whole storage, the TSS content in the control fruit was increased from 4.01 to 4.56%. Compared with that in the control fruit, the TSS content in the melatonin treated fruit was increased from 4.01 to 4.97%.

Effects Of Melatonin On Methylase And Demethylase Activities

As shown in the Fig. 2A, melatonin treatment increased the activity of methylase in tomato fruit compared with that in the control fruit. Significant differences between the melatonin treated and control fruit were observed. The activity of methylase in control fruit was 19.2, 65.3 and 84.8 U kg^{-1} at 5, 15 and 20 d, respectively. Compared with that in the control fruit, the activity of methylase in melatonin treated fruit was 32.0, 71.1 and 93.3 U kg^{-1} at 5, 15 and 20 d, respectively. The demethylase activity in the melatonin treated fruit was significantly increased and reached a peak value of 76.5 U kg^{-1} at 15 d. While, the demethylase activity in the control fruit was 56.2 U kg^{-1} at 15 d.

Effects of melatonin on the levels of genes expression and DNA methylation

In order to understand the regulation mechanism of DNA methylation mediated by melatonin in tomato fruit ripening, the levels of genes expression and DNA methylation of ethylene signal related genes, which including *SlACS10, LeCTR1, LeEIN3, SlERF-A1* and *LeERT10*, were examined.

As shown in the Fig. 3A, the expression levels of *SlACS10* were 0.20 and 0.55 at 15 and 20 d, respectively. While the expression level of the *SlACS10* showed a down-regulation in the control fruit at the whole storage period, which was 0.17 and 0.16 at 15 and 20 d, respectively. Compared with that in the control fruit, melatonin treatment decreased the DNA methylation level of CpG island of *SlACS10* to 0.4% in tomato fruit at 15 d (Fig. 3B). The DNA methylation level of CpG island of *SlACS10* in control fruit was 1.1% at 15 d. As compared to the control fruit, the changes of DNA methylation sites in CpG island of SlACS10 of melatonin treated fruit was observed (Fig. 3C).

The expression levels of the *LeCTR1* were 0.11 and 0.33 at 15 and 20 d, respectively (Fig. 4A). Compared with that in the control fruit, the expression level of *LeCTR1* in the melatonin treated fruit was increased to 0.86 at 15 d and decreased to 0.19 at 20 d (Fig. 4A). The DNA methylation of CpG island of *LeCTR1* was not occurred in the control fruit at 0 and 15 d (Fig. 4B and C). While, in the melatonin treated fruit, the DNA methylation level of CpG island of *LeCTR1* was increased to 1.3% at 20 d (Fig. 4B).

During the whole storage time, the expression levels of the LeEIN3 showed a down-regulation in the control fruit, which were 0.18 and 0.04 at 15 and 20 d, respectively (Fig. 5A). Compared with that in the

control fruit, melatonin treatment increased the DNA methylation level of CpG island of *LeEIN3* in tomato fruit to 25.6% at 15 d (Fig. 5B). In the control fruit, the DNA methylation level of CpG island of *LeEIN3* was 12.2% at 15 d. In melatonin treated fruit, the changes of DNA metylation sites in CpG island of *LeEIN3* was observed at 15 and 20 d (Fig. 5C).

Melatonin treatment increased the expression level of *SlERF-A1* in tomato fruit (Fig. 6A). In the control fruit, the expression level of *SlERF-A1* was first decreased and then remained flat at 0.93 at 15 and 20 d. In the melatonin treated fruit, the expression level of *SlERF-A1* was 1.22 and 1.48 at 15 and 20 d, respectively. The DNA methylation level of CpG island of *SlERF-A1* in control fruit was first increased and then remained flat at 0.6% at 15 and 20 d (Fig. 6B). Compared with that in control fruit, the DNA methylation level of CpG island of *SlERF-A1* in melatonin treated fruit was decreased to 0 and 0.3% at 15 and 20 d, respectively. The location and distribution of the DNA methylation sites of the CpG island of *SlERF-A1* were altered by melatonin treatment at 20 d (Fig. 6C).

During the whole storage period, the expression level of *LeERT10* in the control fruit was gradually decreased to 0.03 at 20 d (Fig. 7A). The expression level of *LeERT10* in the melatonin treated fruit was first decreased slightly to 0.03 at 15 d and then increased to 0.09 at 20 d. The DNA methylation of CpG island of *LeERT10* in control fruit was not occurred at 15 d (Fig. 7B). In the melatonin treated fruit, the DNA melthylation of CpG island of *LeERT10* was increased to 0.8%. Compared with that in the control fruit, the DNA methylated sites of CpG island of *LeERT10* were changed in the melatonin treated fruit (Fig. 7C).

Discussion

Melatonin affects fruit ripening and senescence by regulating signaling molecules. Such as melatonin treatment improved the ripening and quality of tomato fruit by enhanced the expression of genes related ethylene signal transduction (Sun et al. 2015). In berry ripening, the high concentration of ABA induced by melatonin promoted the ethylene production (Xu et al. 2018). Melatonin repressed ethylene biosynthesis and delayed the natural variation of postharvest ripening and quality of banana fruit via repressed the expression of *MaACS1* and *MaACO1* (Hu et al. 2017). In the present results, the color changes and the content accumulations of lycopene and TSS in tomato fruit indicated the role of melatonin in promoting fruit ripening (Fig. 1). Melatonin treatment also altered the activities of methylase and demethylase, demonstrated that the co-regulation of methylase and demethylase is the key element affecting the DNA methylation level in tomato fruit (Lang et al. 2017). In addition, the expression level and the DNA methylation profile of CpG island of genes related to ethylene biosynthesis and signal significantly changed in melatonin treated tomato fruit (Figs. 3, 4, 5, 6 and 7).

Numerous biological functions of ethylene have been found, the most studied function of ethylene is the promotion in fruit ripening (Gontia-Mishra et al. 2014). In the ethylene biosynthetic pathway, ACS catalyze the reaction from S-adenosylmethionine to ACC. From the molecular level, the role of positive feedback regulation of ACS in ethylene biosynthesis has been elucidated in fruit, such as apple, plum and

watermelon fruit (Yang et al. 2013; El-Sharkawy et al. 2008; Zhou et al. 2016). In the ripening of tomato fruit, the abundance of *LeACS2* and *LeACS4* increased with the soaring in ethylene production (Nakatsuka 1998). In the present study, the decrease of DNA methylation level of CpG island may lead to the increase of *SlACS10* expression, which might be one of the reasons for melatonin treatment promoted the ripening of tomato fruit.

CTR1 is a putative MAP-kinase kinase kinase that acts downstream of the receptor in the ethylene signal transduction pathway and suppress the ethylene signal cascade. In Arabidopsis, *AtCTR1* is a negative regulatory gene of ethylene signal transduction and is involved in the regulation of senescence (Kou et al. 2012). Similarly, *LeCTR1* plays a negative role in the ethylene signaling pathway and participates in the ripening process of tomato fruit. In the present results, the expression level of *LeCTR1* in the melatonin treated fruit was increased at 15 d (Fig. 4A). The most reasonable explanation is that, in the ripening fruit, the induction of negative expression regulator may be a damping mechanism to response the sharp increase in ethylene production to slow down the ripening process (Klee 2002). A probably correlation between the methylation level of CpG island and the gene expression level was observed in *LeCTR1* at 20 d. The inhibition of gene expression might be associated with the increase of the DNA methylation of CpG island of *LeCTR1.* This is probably also one of the reasons for melatonin treatment positively regulates tomato fruit ripening.

EIN3 is an important downstream component of ethylene signal pathway and the transcription factor which regulates the transcription of ethylene responsive genes (Guo and Ecker 2004). The EIN3/EIN3-like (EIL) transcription factors converges on ethylene signaling to regulate a series of plant developmental processes. *PpEIN3* involved in the development and ripening of peach fruit (Zhang et al. 2019). *LeEILs* are positive regulators of ethylene responses of the tomato fruit ripening. In the present study, melatonin treatment increased the expression level of *LeEIN3* in tomato fruit at 20 d as that compared with the control fruit. The increased gene expression of *LeEIN3* may related to changes of the DNA methylated sites, which may be one of the reasons for melatonin positively regulates tomato fruit ripening.

The ethylene response factor genes (ERF) represents one of the largest gene families of plant transcription factors, which is downstream of ethylene signal pathway and activated by a linear transduction pathway of ethylene signaling (Pirrello et al. 2012). Many reports indicate that, *ERF* genes have been shown associated with fruit ripening. *MaERFs* were involved in banana fruit ripening through interaction with ethylene biosynthesis genes or transcriptional regulation (Xiao et al. 2013). *ERF4* controls maturity date of various climacteric fruit such as peach and apricot. In the ripening of apple fruit, the increased expression of *MdERF1* and *MdERF2* was inhibited via 1-methylcyclopropene treatment, indicating that transcription is positively regulated by the ethylene signaling system (Wang et al. 2007). *SlERF-A1* is the member of the B3 subgroup of *ERF* genes in tomato. In the present study, melatonin treatment increased the expression of *SlERF-A1* at 15 and 20 d. The increased expression level of *SlERF-A1* may be due to the decreased level of DNA methylation of its CpG island. This is probably also one of the reasons for melatonin positively regulates tomato fruit ripening.

ERT genes were the clone series of Early Ripening Tomato (ERT) in tomato fruit, and the mRNAs of *ERT* in wild-type immature green fruit were present at a substantial level (Picton et al. 1993). The mRNAs homologous to *LeERT10* in tomato fruit showed mature specific accumulation, and reached the peak value at 3 to 5 d postbreaker (Picton et al. 1993). In the melatonin treated fruit, the expression level of *LeERT10* was increased at 20 d. The variations of the expression level of *LeERT10* in melatonin treated fruit might relate to the changes in methylated CpG sites, which may be related to melatonin promoting fruit ripening.

In summary, the DNA methylation changes of CpG islands of genes in ethylene biosynthesis and signal pathway were involved in regulating ripening of melatonin treated fruit. The present results provide foundation for understanding the epigenetic target sites of fruit ripening and controlling fruit ripening and senescence.

Declarations

Acknowledgments

This study was supported by the National Natural Science Foundation of China (31872160), the open Foundation of Tianjin Key Laboratory of Postharvest Physiology and Storage of Agricultural Products (kf2019002), and the Hainan University Science Foundation (ZY2019HN09, KYQD(2k)1959).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

References

Chen YF, Etheridge N, Schaller GE (2005) Ethylene signal transduction. Ann of Bot 95: 901-915.

El-Sharkawy I, Kim WS, Jayasankar S, Svircev AM, Brown DCW (2008) Differential regulation of four members of the ACC synthase gene family in plum. J Exp Bot 59:2009-2027.

Gontia-Mishra I, Sasidharan S, Tiwari S (2014) Recent developments in use of 1-aminocyclopropane-1-carboxylate (ACC) deaminase for conferring tolerance to biotic and abiotic stress. Biotechnol Lett 36:889-898.

Guo D, Li Q, Zhao H, Wang Z, Zhang G, Yu Y (2019) The variation of berry development and DNA methylation after treatment with 5-azaC on ‘Kyoho’ grape. Sci Hortic 246:265-271.

Guo H, Ecker JR (2004) The ethylene signaling pathway: New insights. Curr Opin Plant Biol 7:40-49.

Hu W, Yang H, Tie W et al (2017). Natural variation in banana varieties highlights the role of melatonin in postharvest ripening and quality. J Agric Food Chem 65:9987-9994.

Huang H, Liu R, Niu Q et al (2019) Global increase in DNA methylation during orange fruit development and ripening. Proc Natl Acad Sci U S A116:1430-1436.

Klee HJ (2002) Control of ethylene-mediated processes in tomato at the level of receptors. J Exp Bot 53:2057-2063.

Kou X, Watkins CB, Gan S (2012) *Arabidopsis AtNAP* regulates fruit senescence. J Exp Bot 63:6139-6147.

Lang Z, Wang Y, Tang K et al (2017) Critical roles of DNA demethylation in the activation of ripening-induced genes and inhibition of ripening-repressed genes in tomato fruit. Proc Natl Acad Sci U S A 114:E4511-E4519.

Lang Z, Xie S, Zhu J (2016) The 1001 *Arabidopsis* DNA Methylomes: An Important Resource for Studying Natural Genetic, Epigenetic, and Phenotypic Variation. Trends Plant Sci 21:906-908.

Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 11:204-220.

Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W (1958) Isolation of melatonin, the pineal gland factor that lightens melanocytes. J Am Chem Soc 80:2587-2587.

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321-4325.

Nakatsuka A (1998) Differential Expression and Internal Feedback Regulation of 1-Aminocyclopropane-1-Carboxylate Synthase, 1-Aminocyclopropane-1-Carboxylate Oxidase, and Ethylene Receptor Genes in Tomato Fruit during Development and Ripening. Plant Physiol 118:1295-1305.

Picton S, Gray J, Barton S, Abubakar U, Lowe A, Grierson D (1993) cDNA cloning and characterisation of novel ripening-related mRNAs with altered patterns of accumulation in the ripening inhibitor (rin) tomato ripening mutant. Plant Mol Biol 23:193-207.

Pirrello J, Prasad BN, Zhang W et al (2012) Functional analysis and binding affinity of tomato ethylene response factors provide insight on the molecular bases of plant differential responses to ethylene. BMC Plant Biol 12:1-15.

Silvia F, Angela R, Serena V, Bonghi C (2017) Rosaceae Fruit Development, Ripening and Post-harvest: An Epigenetic Perspective. Front Plant Sci 8:1247.

Sun Q, Zhang N, Wang J et al (2015) Melatonin promotes ripening and improves quality of tomato fruit during postharvest life. J Exp Bot 66:657-668.

Sun Q, Zhang N, Wang J et al (2016) A label-free differential proteomics analysis reveals the effect of melatonin on promoting fruit ripening and anthocyanin accumulation upon postharvest in tomato. J

Pineal Res 61:138-153.

Trivellini A, Ferrante A, Vernieri P, Serra G (2011) Effects of abscisic acid on ethylene biosynthesis and perception in *hibiscus rosa-sinensis* L. flower development. J Exp Bot, **62**, 5437-5452.

Wang A, Tan D, Takahashi A, Li TZ, Harada T (2007) MdERFs, two ethylene-response factors involved in apple fruit ripening. J Exp Bot 58:3743-3748.

Wang W, Hall AE, O'Malley R, Bleecker AB (2003) Canonical histidine kinase activity of the transmitter domain of the ETR1 ethylene receptor from *Arabidopsis* is not required for signal transmission. Proc Natl Acad Sci U S A 100:352-357.

Xiao Y, Chen J, Kuang J et al (2013) Banana ethylene response factors are involved in fruit ripening through their interactions with ethylene biosynthesis genes. J Exp Bot 64:2499-2510.

Xu L, Yue Q, Xiang G, Bian F, Yao Y (2018) Melatonin promotes ripening of grape berry via increasing the levels of ABA, H2O2, and particularly ethylene. Hortic Res 5:41.

Xu X, Yin L, Ying Q et al (2013) High-throughput sequencing and degradome analysis identify miRNAs and their targets involved in fruit senescence of *Fragaria ananassa*. PlOS One 8:1.

Yang X, Song J, Campbell-Palmer L, Fillmore S, Zhang Z (2013) Effect of ethylene and 1-MCP on expression of genes involved in ethylene biosynthesis and perception during ripening of apple fruit. Postharvest Biol Technol 78:55-66.

Zhang Y, Huang Y, Wang C et al (2019) Characterization and identification of *PpEIN3* during the modulation of fruit ripening process by ectopic expressions in tomato. Plant Genome 12:0.

Zhong S, Fei Z, Chen Y (2013) Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat Biotechnol 31:154-159.

Zhou M, Guo S, Zhang J (2016) Comparative dynamics of ethylene production and expression of the *ACS* and *ACO* genes in normal-ripening and non-ripening watermelon fruits. Acta Physiol Plant 38:228.

Figures

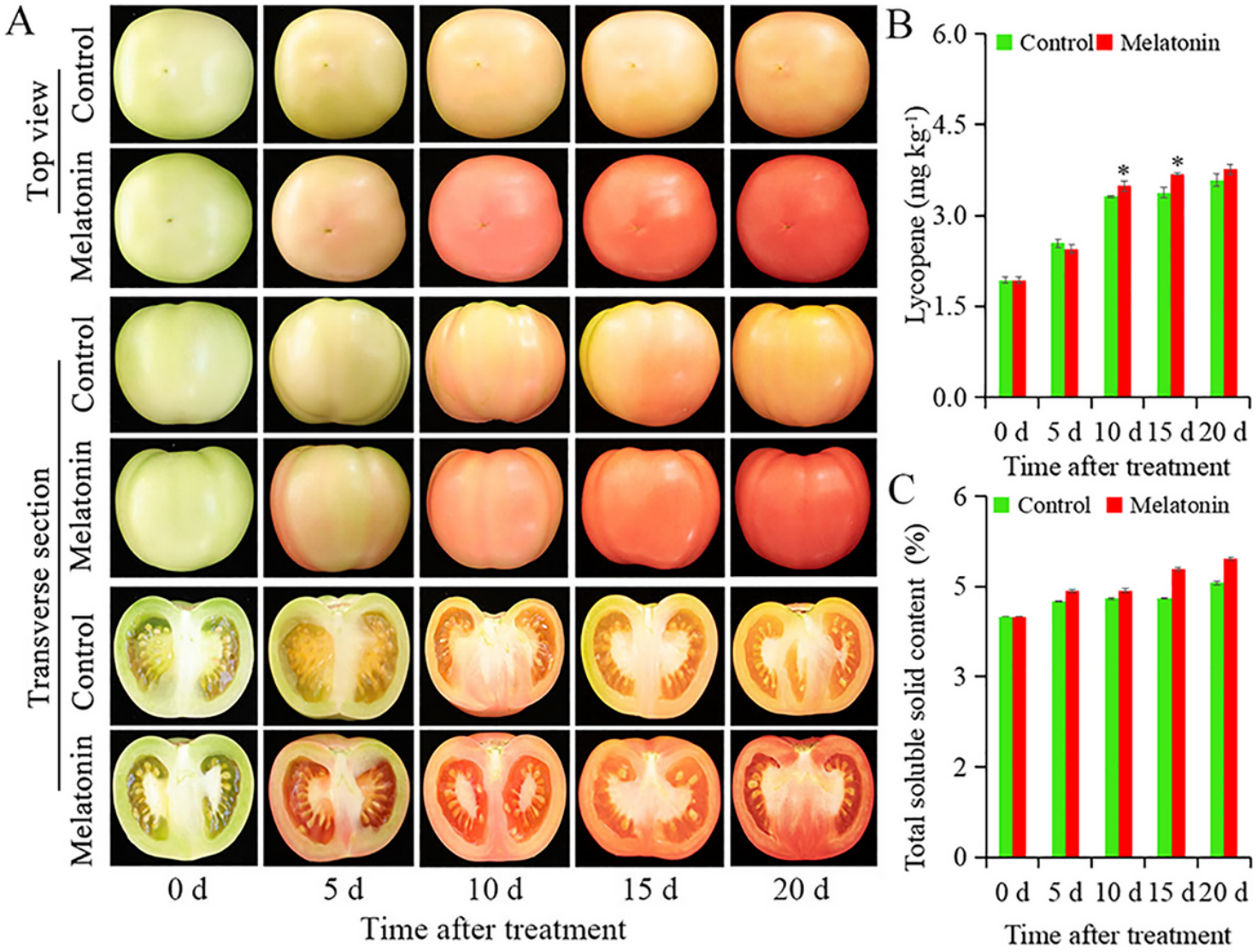

Figure 1

Effects of melatonin on (A) ripening phenotype changes, (B) lycopene content and (C) TSS content of tomato fruit. Vertical bars represent standard deviations of the mean. Asterisks indicate statistical difference of the values at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

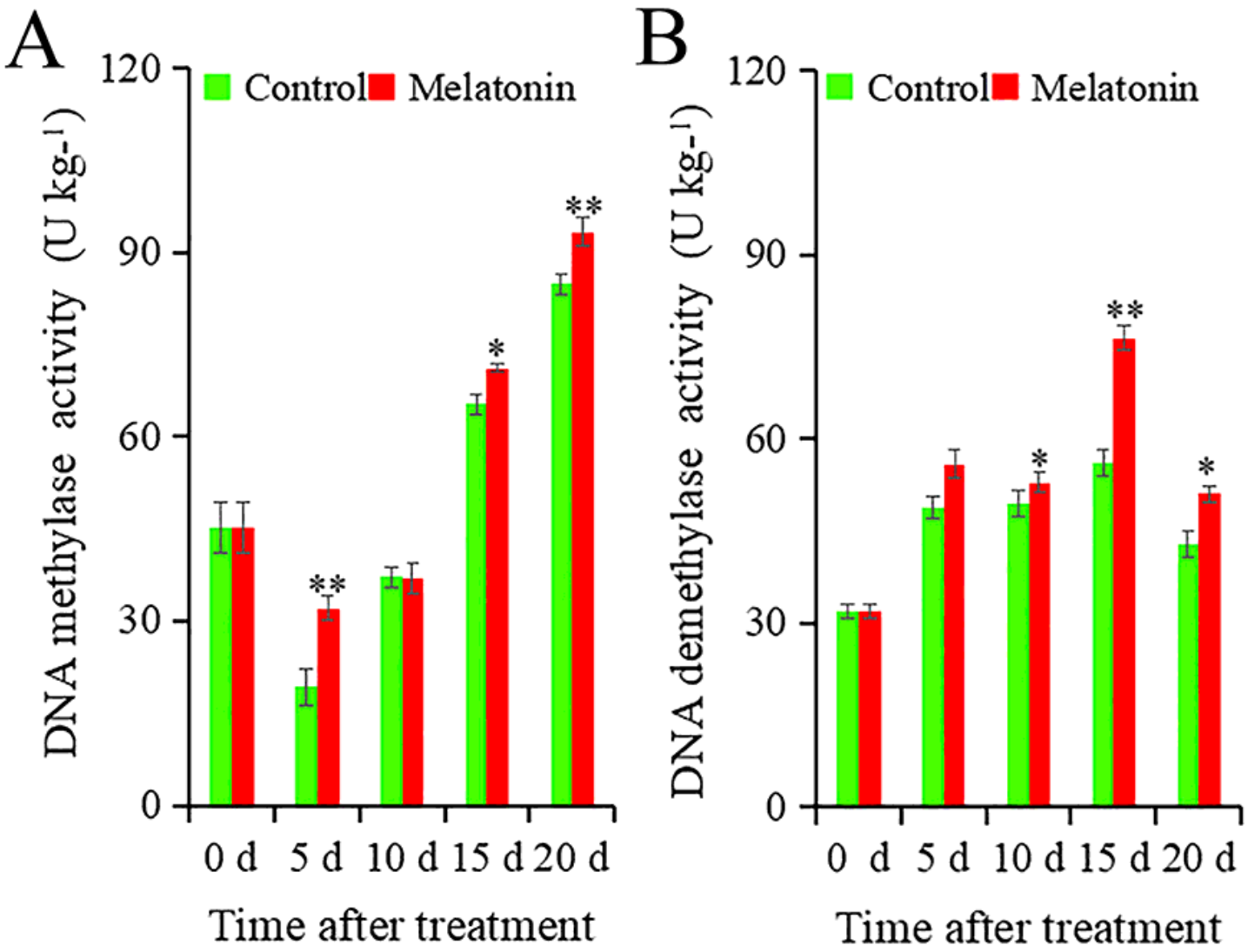

Figure 2

Effects of melatonin on methylase (A) and demethylase (B) activity of tomato fruit. Vertical bars represent standard deviations of the mean. Asterisks indicate statistical difference of the values at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

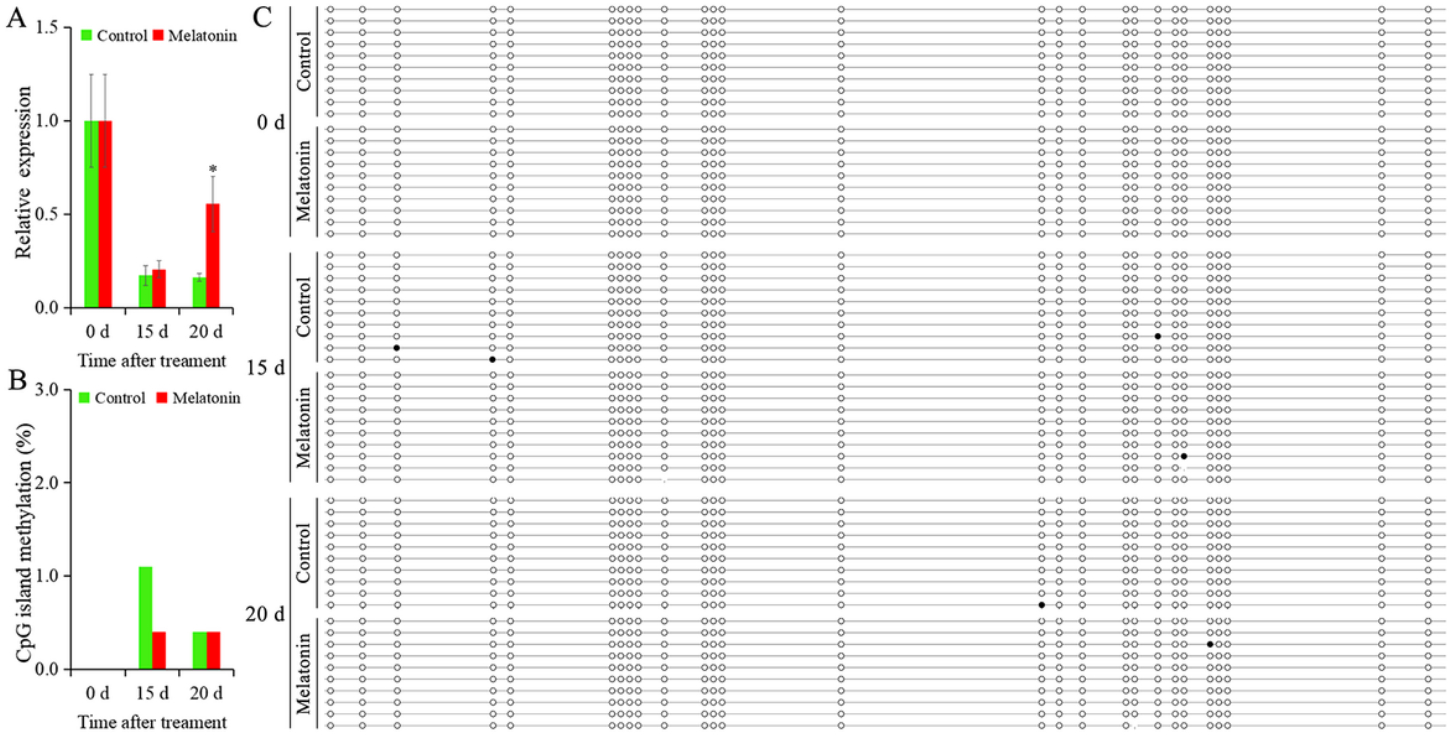

Figure 3

The expression and DNA methylation levels of SlACS10 in tomato fruit. (A) The relative expression level of SlACS10. (B) The DNA methylation level of CpG island of SlACS10. (C) Sequencing of DNA methylation sites of CpG island of SlACS10. 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 mean. Asterisks indicate statistical difference of the values at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

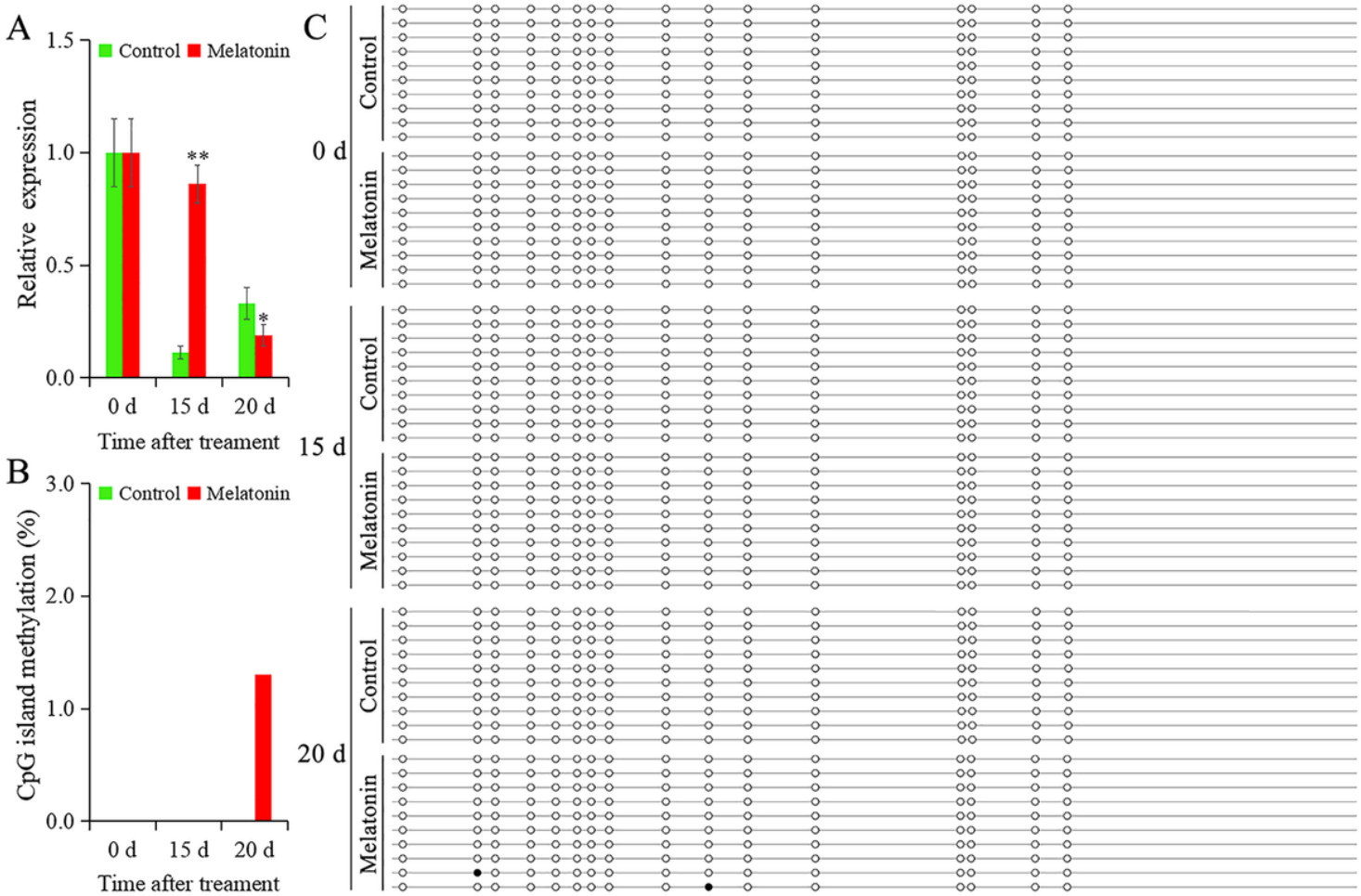

Figure 4

The expression and DNA methylation levels of LeCTR1 in tomato fruit. (A) The relative expression level of LeCTR1. (B) The DNA methylation level of CpG island of LeCTR1. (C) Sequencing of DNA methylation sites of CpG island of LeCTR1. 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 mean. Asterisks indicate statistical difference of the values at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

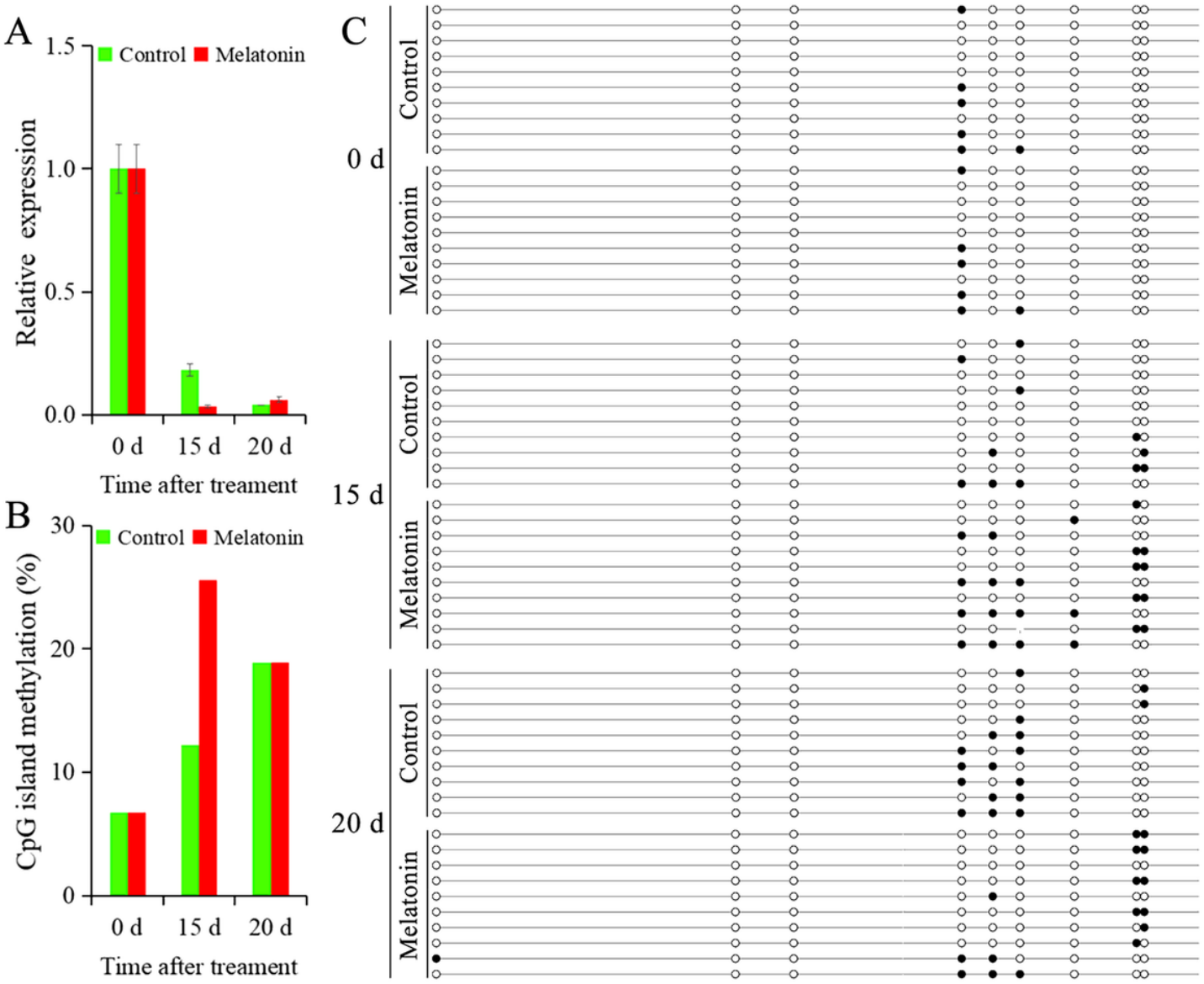

Figure 5

The expression and DNA methylation levels of LeEIN3 in tomato fruit. (A) The relative expression level of LeEIN3. (B) The DNA methylation level of CpG island of LeEIN3. (C) Sequencing of DNA methylation sites of CpG island 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 mean. Asterisks indicate statistical difference of the values at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

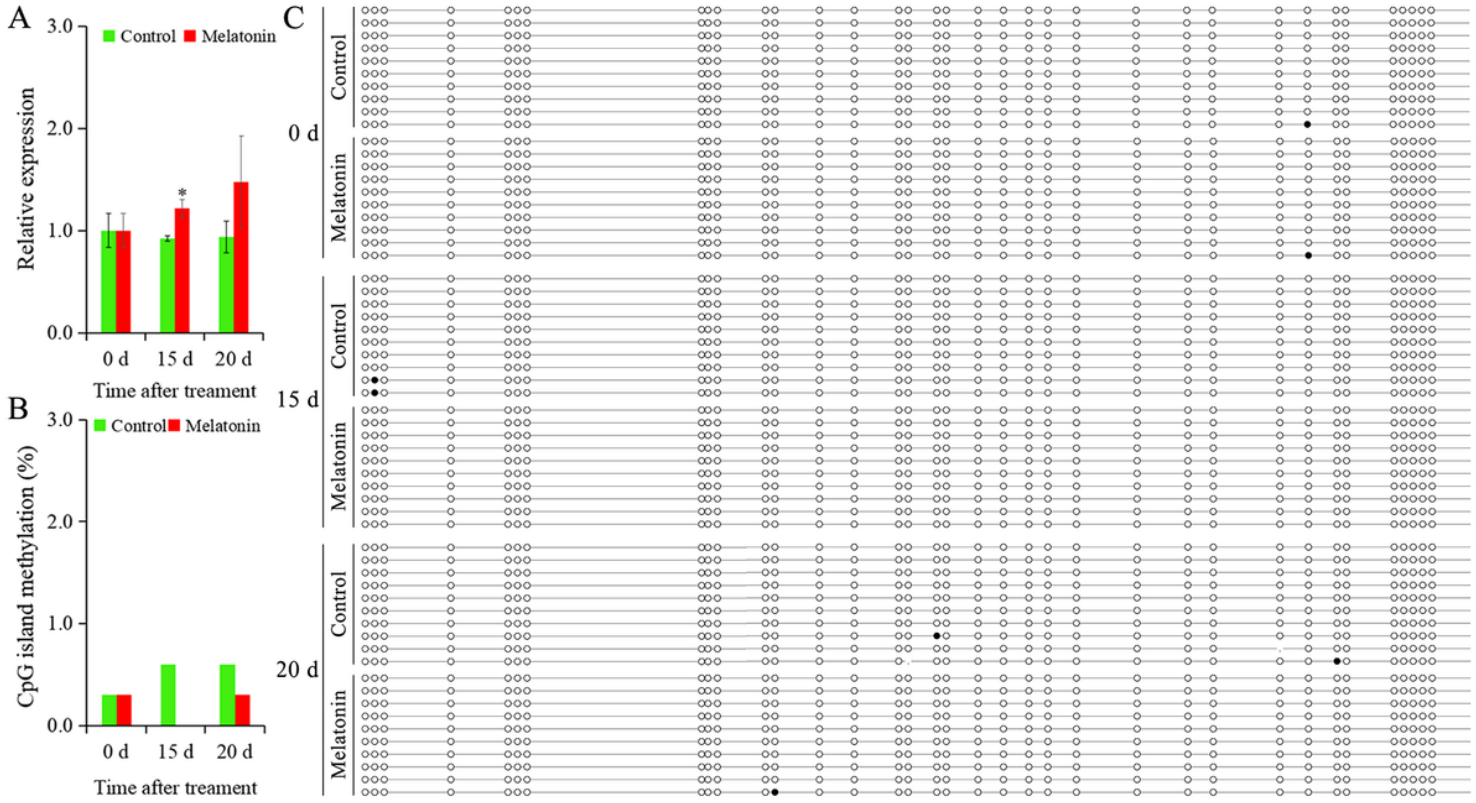

Figure 6

The expression and DNA methylation levels of SlERF-A1 in tomato fruit. (A) The relative expression level of SlERF-A1. (B) The DNA methylation level of CpG island of SlERF-A1. (C) Sequencing of DNA methylation sites of CpG island of SlERF-A1. 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 mean. Asterisks indicate statistical difference of the values at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

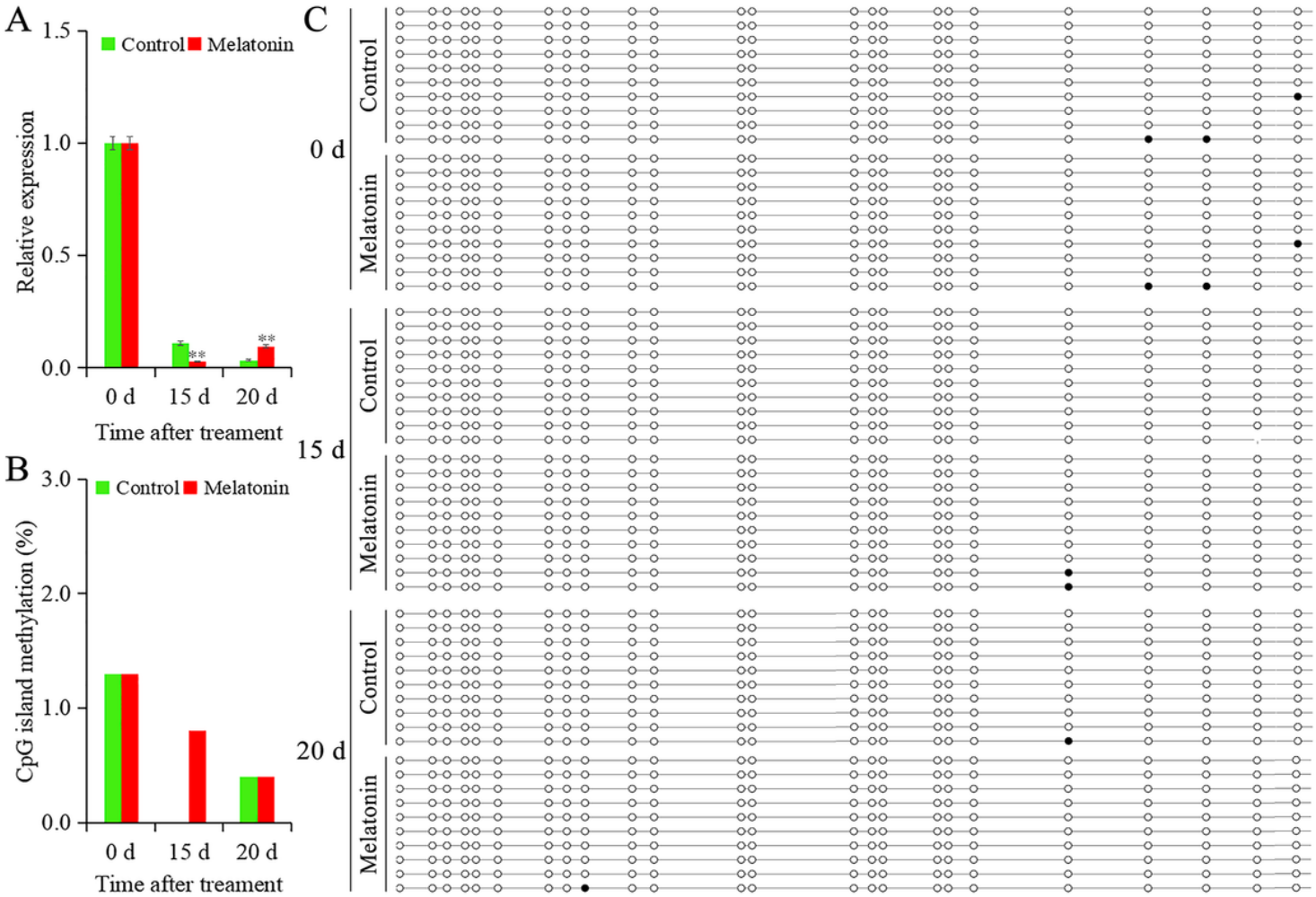

Figure 7

The expression and DNA methylation levels of LeERT10 in tomato fruit. (A) The relative expression level of LeERT10. (B) The DNA methylation level of CpG island of LeERT10. (C) Sequencing of DNA methylation sites of CpG island 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 mean. Asterisks indicate statistical difference of the values at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary1.doc