Critical function of DNA methyltransferase 1 in tomato development and regulation of the DNA methylome and transcriptome

Running title: SlMET1 regulates tomato development and methylome

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Abstract

DNA methylation confers epigenetic regulation on gene expression and thereby on various biological processes. Tomato has emerged as an excellent system to study the function of DNA methylation in plant development. To date, regulation and function of DNA methylation maintenance remains unclear in tomato plants. Here we report the critical function of tomato (Solanum lycopersicum) Methyltransferase 1 (SIMET1) in plant development and DNA methylome and transcriptome regulation. Using CRISPR-Cas9 gene editing, we generated slmet1 mutants and observed severe developmental defects with a frame-shift mutation, including small and curly leaves, defective inflorescence, and parthenocarpy. In leaf tissues, mutations in SIMET1 caused CG hypomethylation and CHH hypermethylation on a whole-genome scale, leading to a disturbed transcriptome including ectopic expression of many RIN target genes such as ACC2 in leaf tissues, which are normally expressed in fruits. Neither the CG hypomethylation nor CHH hypermethylation in the slmet1 mutants is related to tissue culture. Meanwhile, tissue culture induces non-CG hypomethylation, which occurs more frequently at gene regions than at TE regions. Our results depict SIMET1- and tissue culture-dependent tomato DNA methylomes, and that SIMET1 is required for maintaining a normal transcriptome and normal development of tomato.

INTRODUCTION

DNA methylation at the fifth position of cytosine is an epigenetic mark present in animals and plants. Through regulation of nuclear transcriptional activities, DNA methylation is critical for a wide range of cellular functions such as genome stability and defense, imprinting, X chromosome inactivation, paramutation, tissue-specific gene regulation, carcinogenesis, and aging (Bird 2002; Bender 2004; Zhu 2009; Zhao et al. 2018). In plants, DNA methylation exist in all cytosine contexts, i.e., CG, CHG (H is A, T, or C), and CHH (Law and Jacobsen 2010). In mammal, de novo DNA methylation is catalyzed by DNMT3 and is maintained by DNMT1 (Bestor 2000; Goll and Bestor 2005). In Arabidopsis, de novo methylation can be established by DRM2 (a homologue of DNMT3) through the RNA-directed DNA methylation pathway.
(RdDM) (Law and Jacobsen 2010). Once established, CG methylation in Arabidopsis is maintained by MET1, which is homologous to mammalian DNMT1 and recognizes hemi-methylated \textit{m}^4CG/GC generated by DNA replication and methylates the unmodified cytosine; meanwhile, CHG methylation is maintained by the plant specific methyltransferase CMT3 or CMT2. Maintenance of the asymmetric CHH methylation in plants is achieved by constant de novo methylation, either through the RdDM pathway or through an alternative maintenance pathway that depends on the methyltransferase CMT2 and the chromatin remodeling factor DDM1 (Zemach et al. 2013; Fu et al. 2018; Long et al. 2018; Tan et al. 2018).

Tomato (\textit{Solanum lycopersicum}) is an important vegetable crop with a genome of \textasciitilde900 Mb on 12 chromosomes (Consortium 2012; Zhong et al. 2013). DNA methylation levels decreases during tomato fruit ripening, as revealed by the first genome-wide mapping of tomato DNA methylome (Zhong et al. 2013). Consistent with DNA hypomethylation during tomato fruit ripening, further studies demonstrated that active DNA demethylation is responsible for the epigenetic control of ripening in tomato (Liu et al. 2015; Giovannoni et al. 2017). RNAi knockdown of \textit{SlDMLs}, which are DNA demethylases in tomato, inhibited fruit ripening as a result of DNA hypermethylation and repression of ripening-related genes including transcription factors and rate-limiting enzymes of key biochemical processes (Liu et al. 2015). In \textit{sldml2} loss-of-function mutants generated by CRISPR-Cas9 gene editing, increased DNA methylation was found not only in hundreds of ripening-induced genes but also in many ripening-repressed genes (Lang et al. 2017), demonstrating the importance of removing pre-existing DNA methylation during tomato fruit ripening. These recent discoveries collectively highlighted the function of DNA methylation removal; in contrast, regulation and function of DNA methylation maintenance in tomato has been unclear.

MET1 is a conserved key DNA methylase responsible for maintaining CG methylation in plants. Mutations in \textit{MET1} caused DNA hypomethylation in the CG context in both \textit{Arabidopsis thaliana} and rice (\textit{Oryza sativa L}), whereas the concomitant DNA non-CG hypermethylation in gene body regions was observed only in Arabidopsis but not in rice (Finnegan et al. 1996; Mathieu et al. 2007b; Lister et al. 2008; Hu et al. 2014), demonstrating the important yet
variable effects of *met1* mutations in different plant species. Tomato *SIMET1* (Solyc11g030600) is a single-copy gene homologous to Arabidopsis *MET1* (Cao et al. 2014). In this study, we generated *slmet1* mutants by using CRISPR-Cas9 gene editing, and observed pleiotropic developmental phenotypes in *slmet1* mutants. Genome-wide investigations revealed significant impacts of *slmet1* mutation on methylome and transcriptome of tomato. In addition to the genome-wide characteristics of SIMET1-dependent DNA methylation, our analyses also revealed global patterns of tissue culture-induced alterations on tomato methylome.

**RESULTS**

**SIMET1 is essential for tomato plant development**

Using CRISPR-Cas9 gene editing, we generated transgenic tomato plants that harbored mutations in the *SIMET1* gene. Among the T0 mutants, *slmet1-T0-A* exhibited combinational *met1* mutations, almost all of which are 1bp or 4bp nucleotide insertion at the second exon of *SIMET1*, leading to frame shift of the encoded protein (Figure 1A, B). The *slmet1-T0-A* mutant was characterized by small and curly leaves (Figure 1C), indicating an important role of SIMET1 for normal leaf development in tomato. More importantly, the *slmet1-T0-A* mutant plant displayed severely defective inflorescence and produced only a few flowers, most of which failed to develop into fruits (Figure 1D). Moreover, although a couple of flowers eventually yielded fruits, the fruits were all parthenocarpic (Figure 1E). Together these features demonstrate an essential role of SIMET1 in tomato plant development and the yield of normal fruits.

Frame-shift mutation was also detected in *slmet1-T0-B*, another T0 transgenic plant that is a bi-allelic mutant of *SIMET1*. In *slmet1-T0-B*, one of the two *SIMET1* alleles had a 1bp insertion in the second exon and was consequently frame-shifted, while the other allele had a 3bp deletion, resulting in deletion of one amino acid (Figure 1B). Unlike *slmet1-T0-A*, *slmet1-T0-B* did not show dwarfism or failure in productivity. However, by using more than 90 T1 progenies of the *T0-B* bi-allele mutant, we were not able to isolate any T1 plant that was
homozygous with the 1bp insertion that causes SlMET1 frame shift; in fact, all these T1 plants harbored either the homozygous 3bp deletion or bi-allelic mutation, with a ratio of approximately 1:2, indicating that tomato with the homozygous frame shift mutation in SlMET1 is not viable.

In addition to genetic dysfunction of DNA methyltransferase, DNA hypomethylation can also be induced by chemical treatments with DNA methylation inhibitors such as 5-aza-2-deoxycytidine. To examine whether chemical inhibition of DNA methylation can mimic SlMET1 dysfunction, we applied 5-aza-2-deoxycytidine to the apical meristem of wild type tomato plants. As expected, 5-aza-2-deoxycytidine inhibited the development of inflorescence and resulted in growth retardation in tomato plants (Figure S1), supporting a fundamental role of DNA methylation in determining tomato development and productivity.

**SIMET1 dysfunction reduces genome-wide CG methylation but increases CHH methylation**

To explore the SIMET1-dependent DNA methylome, we performed whole genome bisulfite sequencing of the slmet1 T0 mutants. Tissue culture and regeneration can alter genome-wide DNA methylation patterns in plants such as rice (Stroud et al. 2013). Since transgenic tomato plants were obtained from callus with regeneration, we used regenerated wild type tomato as the reference. The bisulfite conversion efficiency is similar among all the samples (Table S1). Global DNA methylation levels in the CG context were detected as 77.08% in the regenerated wt T0 (wtR0) plant; in comparison, genome-wide CG methylation level was significantly reduced to 57.81% in slmet1-T0-A(Figure 2A). CG hypomethylation was observed across each of the 12 chromosomes in slmet1-T0-A compared to wtR0 (Figures 2B, S2). The strong reduction in global CG methylation level is consistent with the function of MET1 being responsible for CG methylation in plants (Lister et al. 2008; Hu et al. 2014).

In addition to genome-wide CG hypomethylation, slmet1-T0-A mutant also displayed substantially increased DNA methylation levels in the CHH context (Figures 2A, B, S2). The overall CHH methylation level was 9.98% in slmet1-T0-A and 4.95% in wtR0 (Figure 2A),
showing a hypermethylation pattern in the mutant that was ubiquitous across each chromosome in the tomato genome (Figures 2B, S2). On the whole, slmet1-T0-A showed stronger impacts on genome-wide DNA methylation compared to slmet1-T0-B (Figures 2A, B, S3); this seems to reflect the higher proportion of frame-shift mutations in slmet1-T0-A than in slmet1-T0-B (Figure 1B). On a whole-genome scale, gene regions and transposable element (TE) regions both exhibited CHH hypermethylation in the slmet1-T0-A and slmet1-T0-B mutants (Figure 2C), with CHH hypermethylation observed in all the nine CHH subcontexts (Figures S4-S6). Thus, disruption of SIMET1 function extensively alters tomato DNA methylome as a result of integrated regulation of DNA methylation in different cytosine contexts.

Since SIMET1 dysfunction differentially affects DNA methylation in CG, CHG and CHH cytosine contexts, we searched differentially methylated bins (DMB) of CG, CHG, and CHH separately throughout the genome (Tables S2-S13) using DMB caller (Zabet et al. 2017). In the CG context, both slmet1-T0-A and slmet1-T0-B show much more hypo DMBs than hyper DMBs (Figure 2D); whereas in the CHH context, both slmet1-T0-A and slmet1-T0-B show much more hyper DMBs than hypo DMBs (Figure 2D). These patterns are consistent with the assessment of global DNA methylation levels shown in Figure 2A to 2C. That CG DMBs in both slmet1-T0-A and slmet1-T0-B are overwhelmingly dominated by hypomethylation supports the role of SIMET1 as a CG methylase. We further categorized DMBs into three types, i.e., genes, TEs, and others (Tables S2-S19). Among the slmet1-T0-A CG hypo DMBs, gene and TE accounted for 13.49% and 68.11%, respectively (Figure 2D). Meanwhile, in the slmet1-T0-A CHH hyper DMBs, gene and TE accounted for 6.20% and 68.75%, respectively. Similarly in slmet1-T0-B DMBs, TE also accounted for a higher proportion than gene among either CG hypo DMBs or CHH hyper DMBs (Figure 2D). This pattern is consistent with previous reports that the majority of tomato genome contains heavily methylated TEs particularly in pericentromeric regions, whereas gene-rich euchromatic regions are less methylated (The Tomato Genome Consortium. 2012; Zhong et al. 2013).
Compared to slmet1-T0-A, slmet1-T0-B showed less CG hypo DMBs and CHH hyper DMBs (Figure 2D), indicating that the latter is a weaker allele. At CG, CHG, and CHH DMBs identified in slmet1-T0-A, DNA methylation levels are similarly altered in slmet1-T0-B, albeit the degrees of methylation changes are less obvious in slmet1-T0-B than slmet1-T0-A (Figure 2E). In slmet1-T0-A, CG hypo DMBs also showed CHH hypermethylation; the same genetic loci similarly showed coexistence of CG hypomethylation and CHH hypermethylation in slmet1-T0-B (Figure S7). It is possible that CG hypomethylation results in de-silencing of transposons, which can lead to elevated siRNA levels and thereby enhanced CHH methylation through RdDM (Nuthikattu et al. 2013; McCue et al. 2015).

Pleiotropic growth phenotype and DNA methylome in slmet1-T0-BT1 progeny

Individual plants of slmet1-T0-BT1 progeny displayed pleiotropic growth features, most notably as growth retardation, curly and thick leaves, reduced floral buds, and parthenocarpic fruits (Figures 3A, S8A; Table S20). One of the slmet1-T0-BT1 plants with homozygous AAG deletion (assigned as slmet1-1) and with growth abnormality (Figure S8A), was named slmet1-1-T1-B (hereafter named T1-B briefly), were subsequently compared to its control counterpart, which was T1 of the regenerated wild type wtR0 (named hereafter wtR1).

Compared to wtR0, T1-B displayed over 20,000 CG hypo DMBs, the majority of which are associated with TEs (Figure S8B), although genome-wide CG methylation levels are similar between T1-B and wtR0 (Figure S8C, D). Like slmet1-T0-A and slmet1-T0-B, the T1-B also showed CHH hyper DMBs in addition to CG hypo DMBs (Figure S8D) and the CHH hyper DMBs are dominated by TE regions (Figure S8C). At the CG hypo DMBs that are shared by slmet1-T0-A and slmet1-T0-B (Tables S14-S19), decreased CG methylation levels were observed in T1-B (Figure 3B), whereas at the CHH hyper DMBs shared by the two T0 lines (Tables S14-S19), increased CHH methylation levels were observed in T1-B (Figure 3B). Meanwhile, at the CG hypo DMBs identified in T1-B, CG methylation levels in slmet1-T0-A and slmet1-T0-B are decreased compared to wtR0; whereas at the CHH hyper DMBs identified in
T1-B, the two T0 lines showed increased CHH methylation levels (Figure S8E). In the CG, CHG, CHH contexts, 3677, 2094, and 4637 DMBs were identified, respectively, as commonly present in the slmet1-T0-A, slmet1-T0-B and T1-B mutants (Tables S27-S32). These common DMBs also showed obvious CG hypo-methylation and CHH hyper methylation pattern. In the CG context, there are 3583 CG hypo DMBs and only 94 CG hyper DMBs. In contrast, in the CHH context, there are 4606 hyper DMBs and only 31 hypo DMBs (Figure 3C). Two representative loci showing consistent CG DNA hypo methylation in the slmet1T0 and T1 mutants are shown in Figure 3D, and the reduction in DNA methylation levels was confirmed by Chop-qPCR (Figure 3E). Therefore, DNA methylation patterns in the slmet1 T0 and T1 mutants collectively support a crucial role of SlMET1 in ensuring a proper DNA methylome in tomato.

**SIMET1 is required for proper transcriptional regulation of key genes for tomato productivity**

To examine how SIMET1 mutation affects gene regulation in tomato plants, we compared the leaf transcriptome of the homozygous T1-B, with its wild type control wtR1. The T1-B showed 2526 upregulated DEGs and 1972 downregulated DEGs (fold change>2, FDR<0.01) (Tables S33, S34). At these 2526 up DEGs, T1-B displayed a minor reduction in the average DNA methylation levels, especially in the CG context (Figure 4A), indicating a potential correlation between DNA hypomethylation and elevated gene expression in the slmet1 mutants. For instance, elevated transcript levels of Solyc01g109720 and Solyc02g068680 genes were observed in T1-B, accompanied by reduced DNA methylation in the gene region (Figure 4B). More example genes were listed in TableS35.

During tomato fruit ripening, active DNA demethylation regulates many ripening-related genes such as RIN (RINPENING INHIBITOR), which is a key transcriptional regulator of fruit ripening with 292 known target genes (Martel et al. 2011; Zhong et al. 2013). Gene expression of RIN was not altered in T1-B; however, 82 RIN target genes were identified as DEGs (fold change>2, FDR<0.01) in T1-B, among which 65 genes showed increased expression levels;
when relaxing the threshold of DEG to FDR<0.01, 154 RIN target genes were identified as DEGs, among which 111 genes were upregulated in T1-B (Figure 4C; TableS36). These observations suggested that the ripening related RIN target genes are repressed by SIMET1 in the leaf tissues.

Some of the up-regulated ripening-related genes showed DNA hypomethylation in the T1-B mutant. For instance, T1-B displayed decreased DNA methylation as well as increased gene expression of the RIN target gene ACC2 (Figure 4D, E), which functions in the biosynthesis of ethylene that promotes fruit ripening (Barry and Giovannoni 2007). Meanwhile, ACC2 gene expression level was elevated in 5-aza-2-deoxycytidine-treated plants compared to the control plants (Figure 4F), supporting a potential correlation between DNA methylation and gene expression of ACC2.

**Tissue culture alters tomato DNA methylome independently of SIMET1 function or environmental stimuli**

Plants regenerated from calli may show a different DNA methylome from that of inbred lines (Stroud et al. 2013). To examine whether SIMET1-dependent DNA methylation interacts with the potential impact of tissue culture on tomato plant DNA methylome, we first compared the methylomes of wtR0 and wtR1 with the wild type Ohio8245 inbred line (wtO8). Global DNA methylation levels in CG context are similar between regenerated plants and wtO8, while the CHG and CHH methylation levels showed decreases in wtR0 and wtR1 compared to the wtO8 (Figure 5A). We identified the CG, CHG, and CHH DMBs in wtR0 and wtR1 independently compared to wtO8 (TablesS37-S54). Both wtR0 and wtR1 showed similar numbers of hypo and hyper DMBs in the CG context (Figure 5B). In contrast to the balanced CG methylation, CHG and CHH methylation both showed approximately 6 folds higher numbers of hypo DMBs than hyper DMBs (Figure 5B), indicating that CHG and CHH methylation may be repressed during regeneration. Several representative loci showing the CHG and CHH hypo-methylation are shown in Figure 5C.
Neither wtR0 nor wtR1 showed alteration in genome-wide CG methylation levels compared to wtO8 (Figure 5D). In contrast, global reduction in CHH methylation level was observed across each chromosome in wtR0 compared to wtO8, but to a greater extent at the ends of chromosomes (Figures 5D, S10), where gene-rich euchromatic regions are located (Zhong et al. 2013). In contrast to wtR0, wtR1 displayed CHH hypomethylation only at the ends of chromosome arms, instead of across the whole chromosome (Figure 5D). Such a difference indicates that inbreeding of the regenerated wtR0 can, at least partially, restore CHH methylation that is lost during tissue culture. Meanwhile, both wtR0 and wtR1 displayed CHG hypomethylation at the ends of chromosomes, which are the same regions where significant CHH hypomethylation was observed (Figures 5D, S10, S11). In the tomato epigenome, the ends of chromosome arms contain less methylation especially in the CHG and CHH contexts, compared to the rest of the chromosome (Figure S12; (Zhong et al. 2013)). Therefore, it appears that the less methylated gene-rich regions are more susceptible to tissue culture-induced DNA hypomethylation, and that DNA hypomethylation in these regions, compared to that in other chromosomal regions, is more resistant to restoration through subsequent inbreeding.

In wtR0 and wtR1, regions categorized as gene or TE together account for the majority of the regeneration-induced hypo CHG and CHH DMBs (Figure 5B). At gene regions, average methylation levels in the CHG and the CHH contexts were decreased in both wtR0 and wtR1 compared to wtO8 (Figure 5E). At TE regions, the average CHH methylation level was decreased in wtR0 compared to wtO8 or wtR1, whereas the average CHG methylation levels were similar between the regenerated and inbred plants (Figure 5E). Together these patterns further support the notion that tissue culture-induced DNA hypomethylation not only prefers genes over TEs, but also is more stable in the former than the latter.

Next we checked the methylation levels of CHG and CHH subcontexts in wtR0 and wtR1 compared to wtO8. All three CHG subcontexts (CAG, CCG and CTG) showed decreased methylation levels at gene-rich euchromatic regions (Figures S13-S15). At gene regions, the 9 CHH subcontexts showed decreases similarly in both wtR0 and wtR1 (Figure S15), whereas at
TE regions, the CHH subcontexts showed similar change in wtR0 but not in wtR1, particularly, the methylation levels of six CHH subcontexts (CAA, CAC, CAT, CTA, CTC, CTT) in wtR1 are similar to wtO8, while the CCA, CCC and CCT methylation levels remain lower than wtO8 (FigureS15).

In contrast to the CHG and CHH contexts which showed DNA hypomethylation, global CG methylation is not affected by tissue culture (Figure 5D, E), indicating that tissue culture does not contribute to the genome-wide CG hypomethylation in the slmet1 mutants. Compared to the inbred line wtO8, the slmet1-T0-A still showed a robust CG hypomethylation pattern on a genome-wide scale (FigureS16A-D). At slmet1-T0-A VS wtO8 DMBs, the slmet1-T0-B also showed similar change pattern (FigureS16E). Although tissue culture causes a genome-wide reduction in CHH methylation, global DNA methylation level in the CHH context was still clearly elevated as observed in slmet1 mutants compared to the inbred control wtO8 (FigureS16B). Therefore, the patterns of CG hypomethylation and CHH hypermethylation in the slmet1 mutants are sufficiently robust to overcome the tissue culture-triggered methylation change.

Since DNA methylation at tissue culture-induced DMRs was not stable during the tissue culture processes, we wondered whether DNA methylation at these loci may be affected by environmental stress conditions. Tomato plants were exposed to various environmental stress or phytohormone stimuli, including cold, heat, drought, salinity, abscisic acid (ABA), and indole-3-acetic acid (IAA). As revealed by methylation-sensitive enzyme digestion, DNA methylation levels at the examined tissue culture-induced DMRs were mostly insensitive to the environmental stimuli within the experimental time frame (Figure 5F). Thus, tomato DNA methylation that can be altered by tissue culture appears to be stable under environmental stress conditions.

DISCUSSION

Tomato is one of the most important vegetable crops in the world. DNA methylation confers important epigenetic regulation in tomato, as have been demonstrated by several recent
studies that collectively highlighted the necessity of an active DNA demethylation process for tomato fruit ripening (Zhong et al. 2013; Liu et al. 2015; Lang et al. 2017). In this study, the correlation between the frame shift mutation and the severely defective production of flowers and fruits in slmet1-T0-A, together with the absence of homozygous frame-shift mutation in the T1 progeny of slmet1-T0-B, indicates that DNA methylation not only is important for fruit ripening but also can be crucial for normal development of the productive organs in tomato. Thus, our findings and previous reports collectively suggest that DNA methylation and demethylation may be two processes sequentially required for normal production of tomato fruits, i.e., SIMET1-dependent DNA methylation ensures normal production of flowers and fruits and then SIDML2-dependent DNA demethylation mediates fruit ripening.

Although MET1 is a conserved key DNA methylase in plants, the impacts of MET1 mutations on DNA methylomes in different plant species can have varying patterns. In Arabidopsis met1 mutants, decreased CG methylation is accompanied by increased CHG methylation in gene body regions in the first generation, as well as by increased CHH methylation in the successive generations, indicating the possibility that non-CG methylation may be increased as a compensatory response to the loss of CG methylation (Lister et al. 2008). Indeed, because the loss of CG methylation in met1 mutants triggers genome-wide alternative epigenetic mechanisms, it has been proposed that CG methylation evolved not only to provide a level of epigenetic regulation, but also to coordinate and stabilize epigenetic memory required for its transcriptional inheritance (Mathieu et al. 2007b). Similar to Arabidopsis, tomato met1 mutants displayed CHH hypermethylation across chromosomes in both the T0 and T1 generations. In contrast to Arabidopsis and tomato, the rice met1 mutant showed hypo-methylation in all the CG, CHG and CHH contexts (Hu et al. 2014). Therefore, it seems interesting to examine in the future whether MET1 mutations lead to non-CG hypermethylation only in dicot species but not in monocot plants.

In Arabidopsis, maintenance of CHH methylation on the euchromatic chromosome arms is RdDM-dependent, while CHH methylation in the heterochromatic pericentromeric regions is maintained by CMT2 without RdDM (Zemach et al. 2013). On the other hand, the DNA
demethylase ROS1 can antagonize methylation at both RdDM-dependent and RdDM-independent loci (Tang et al. 2016). In tomato plants, it has been suggested that CMT2-dependent CHH methylation exists independently of the RdDM pathway (Gouil and Baulcombe 2016), and that at least one functional ROS1 homolog is present (Liu et al. 2015; Lang et al. 2017). In this study, we observed that in the slmet1 mutants, CHH methylation was increased in both pericentromeric regions and chromosome arms (Figure 2B), and that all the CHH subcontexts exhibited similar hypermethylation patterns (Figures S4-S6), suggesting that both RdDM-dependent and CMT2-dependent CHH methylation was affected in the slmet1 mutants. Therefore, there are two possible reasons why slmet1 plants are hypermethylated in the CHH context. First, CG hypomethylation in slmet1 mutants may desilence transposable elements and trigger siRNA production, resulting in an increase of RdDM mediated CHH DNA methylation. This possibility is supported by the observation that CHH methylation levels were increased in slmet1-$T0-A$ CG hypothesis DMBs (Figure S7). In fact, CHH hypermethylation is also observed in the successive generations of the Arabidopsis met1-3 mutant (Mathieu et al. 2007a), which is at least partially due to siRNA accumulation in CG hypomethylated loci and dependent on DRM2 (Mathieu et al. 2007b). However, this possibility cannot explain CHH hypermethylation in RdDM-independent loci. A second possible reason for CHH hypermethylation is the repression of the DNA demethylase DMLs. In both Arabidopsis and rice met1 mutants, gene expression of DMLs are repressed. We checked the FPKM values of the tomato DML genes from our RNA seq data. Among the four tomato DML genes, DML2 and DML3 showed reduction in $T1-B$ mutant (Figure S17). Therefore, it seems that siRNA accumulation and demethylase repression may both contribute to the aberrant CHH hypermethylation.

Consistent with a previous report (Gouil and Baulcombe 2016), we observed that tomato methylation level is lower in the CCG subcontext than CAG and CTG at heterochromatin (Figure S18). It was also reported that Arabidopsis met1 mutation specifically caused the loss of CCG methylation across the chromosomes, and that this effect is more pronounced in heterochromatic regions, which may be caused by MET1-mediated mCGG and CmCG that
recruit SUVH5/6 that would catalyze H3K9me2, resulting in subsequent recruitment of CMT3 to methylate the first cytosine of CCG (Gouil and Baulcombe 2016). Yet it is still unclear whether tomato slmet1 mutation can cause such an effect. The slmet1-T0-A, which is the strongest slmet1 mutant obtained in this study, showed very slight decrease of CCG methylation levels in heterochromatic regions (Figures S4, S19) compared to wtR0, while the milder mutant slmet1-T0-B and its T1 progeny T1-B did not show any decrease of CCG methylation in heterochromatic regions when compared to their control counterparts wtR0 and wtR1, respectively (Figures S21, S22). Interestingly, both slmet1-T0-A and slmet1-T0-B showed CCG hyper-methylation in the chromosome arms including gene flanking regions compared to wtR0 (Figures S4, S19, S21). However, this pattern is weak in the T1 progeny T1-B plant (Figure S22). Because none of our slmet1 mutants is a loss-of-function allele, and because the control wtR0 already has robust CCG hypomethylation in euchromatic regions when compared to the inbred line wtO8 (Figures S13-S15), it is still unclear whether a complete loss of SlMET1 function can cause CCG hypomethylation as was observed in the Arabidopsis met1 mutant.

Our RNA seq data using leaf tissues showed that a considerable proportion of fruit ripening related RIN target genes were upregulated in the T1-B mutant. This indicates that the SIMET1 is required to repress these RIN target genes in the vegetative tissues, so that these genes are only activated at certain stage such as during fruit ripening. Besides, plant NAM (NO APICAL MERISTEM) proteins are conserved regulators of various developmental processes including formation of the shoot apical meristem and floral organs (Ernst et al. 2004). The T1-B DEGs contain 29 NAM genes, most of which displayed higher gene expression levels compared to wtR1 (Table S55). Although functions of these tomato NAM genes remain to be demonstrated, alterations in the NAM gene expression seem consistent with the interrupted leaf and flower development (Figures 3A, S8A) in slmet1 mutant. Therefore, SIMET1 seems to affect tomato development by controlling proper expression of many development-related genes. However, only some of the RIN target genes and NAM genes show DNA hypomethylation in T1-B
mutant compared to wtR1, indicating that the SlMET1 may also repress their gene expression indirectly.

The regenerated tomato plants are characterized by DNA hypomethylation compared to the inbred plants. This pattern is consistent with observations in other plant species that also went through tissue culture (Guo et al. 2007; Stroud et al. 2013; Stelpflug et al. 2014). In rice, plants regenerated from tissue culture showed significant reduction in DNA methylation levels compared to the inbred plants, with hypomethylation mostly occurring near genes (Stroud et al. 2013). This feature is consistent with our observation that, in regenerated tomato compared to its inbred counterpart, DNA hypomethylation is more obvious in gene-rich euchromatic regions. Meanwhile, tissue culture-induced DNA hypomethylation in tomato also exhibited distinctive patterns from those in rice. Regenerated rice plants were significantly enriched with CG hypomethylation DMRs, although loss of DNA methylation also occurs in non-CG contexts (Stroud et al. 2013); in contrast, regenerated tomato plants clearly showed DNA hypomethylation in non-CG contexts but not in the CG context, indicating that tissue culture-induced DNA hypomethylation in tomato and rice is mediated through different mechanisms. Interestingly, the wtR0 showed CHH methylation decrease across both the RdDM-dependent euchromatins and RdDM-independent heterochromatin regions. However, in the next generation wtR1, the CHH methylation in heterochromatin regions is recovered to the wtO8 level while the CHH methylation in euchromatin regions is not recovered. The CHG methylation at euchromatin was also decreased in regenerated plant wtR0, and was not recovered in the next generation wtR1. Currently it is unclear why the CHG and CHH methylation in euchromatin regions was not recovered in wtR1.

MATERIALS AND METHODS

Plant materials and CRISPR-CAS9 gene editing

The tomato Solanum lycopersicum cv. Ohio 8245 was used in this study. A 19bp sgRNA oligo (GCATCTGCAACTGGTAAGA) targeting the 2nd exon of the SlMET1 gene (Solyc11g030600) was cloned into the psgR-Cas9-At vector as described previously (Feng et al. 2013; Yanfei
The sgRNA–Cas9 cassette fragment was then sub-cloned into plant expression vector pCAMBIA1300 vector which was digested by HindIII and EcoRI. The construct was transformed into tomato cotyledons by Agrobacterium-mediated transformation as previously described (Eck et al. 2006). The slmet1-T0-A and slmet1-T0-B were the regenerated T0 plants, which were genotyped by primers of Tmet1-SQ-F: gatgttcacttccacttcatgc and Tmet1-SQ-R: catatgacaggagttccatcttc. The control wild type plants wtO8 and wtR0 were an inbred line and a T0 plant regenerated without Cas9, respectively. The wtR1 is the T1 progeny of wtR0. The slmet1-1-T1-B is the T1 progeny of slmet1-T0-B mutant which contain 3 bp deletion. Tomato plants were grown initially in growth medium and subsequently transferred to soil in a growth room with the condition of 14/10 h and 25/20 °C for day/night.

**Whole genome bisulfite sequencing and data analysis**

For whole genome bisulfite sequencing, the mature leaves from tomato plants of different genotypes were taken, and the genomic DNA was extracted by DNeasy Plant Mini Kit (Qiagen). The bisulfite conversion, library construction, and deep sequencing were performed by the Core facility of the genomics in Shanghai Center for Plant Stress Biology.

The data analysis pipeline was performed according to the methods described previously (Lang et al. 2017). In brief, for data analysis, low-quality sequences (q < 20) were trimmed using trim in BRAT-BW (Harris et al. 2012), and clean reads were mapped to the reference genome using BRAT-BW, allowing two mismatches. The reference genome version is SL2.50 (ftp://ftp.solgenomics.net/tomato_genome/assembly/build_2.50/) (Consortium 2012). To remove potential PCR duplicates, the remove-dupl command of BRAT-BW was used. The methylation level is defined as “weighted methylation level” according to Schultz et al (Schultz et al. 2012). For example, if a region has 3 CHH sites: first site has 10 reads covered and 3 out of the 10 reads are methylated; second site has 5 reads covered and 1 out of the 5 reads is methylated; the third site has 5 reads covered and none of them are methylated. Then the methylation level for CHH is calculated as \((3+1+0)/(10+5+5) = 20\%\).
Identification of DMBs was conducted using Bioconductor package “Differentially Methylated Regions caller” (Zabet et al. 2017). For each context, “computeDMRs” function in DMRcaller library was used with parameters “method = "bins", binSize = 100, test = "score", pValueThreshold = 0.01, minCytosinesCount = 4, minGap = 200, minSize = 50, minReadsPerCytosine = 4". The min Proportion Difference cutoff for CG, CHG, and CHH is 0.4, 0.4, and 0.2. For genome-wide methylation ratio figures, the tomato genome was divided into 100 kb bins and the methylation levels were calculated for each bin.

mRNA seq and data analysis

For mRNA seq, total RNA was extracted from wtR1 and slmet1-1-T1-B by Trizol Reagent (Ambion) using the same leaf material as for the DNA extraction. The library construct and deep sequencing were then performed at the Core Facility of Genomics in Shanghai Center for Plant Stress Biology.

For RNA-seq data processing, quality control was checked using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc). The reads were aligned to the tomato genome using TopHat2 with "--b2-very-sensitive" option(Kim et al. 2013). The ITAG2.4 annotation gff3 file was supplied as the "-G" option to TopHat2. The program featureCounts(Liao et al. 2014) was used to count the mapped fragments for each gene. The "-p" parameter is set to to count fragments instead of reads. The output count table was used as the input for edgeR(Robinson et al. 2010). According to the user guide, "estimateCommonDisp", "estimateTagwiseDisp" and "exactTest" in edgeR were used with default parameters. The differentially expressed genes (DEGs) were genes with fold change > 2 and FDR < 0.01.

Chop-qPCR

Genomic DNA was extracted by Qiagen Dneasy Plant mini Kit from true leaves of the tomato plants. 300ng DNA were subjected to HpyCH4IV or Alu1 digestion in 30µl volume at 37°C overnight, followed by 65°C (HpyCH4IV) or 80°C (Alu1) inactivation for 20min. The non-digestion control was performed by adding no enzyme. 1.5µl digested product or
non-digestion control was used for qPCR in 20µl volume. Real-time PCR was carried out using iQ SYBR Green Supermix (Biorad) on a CFX96 real-time PCR detection system (Bio-RAD). The non-digestion control was used as the loading control. All primers were listed in TableS56. Error bars indicate SD from 3 biological replicates.

**Quantitative real time PCR**

Total RNA was extracted using the Trizol reagent (Ambion) from true leaves of the tomato plants, followed by DNase digestion using TURBO DNA-free™ Kit (Ambion). 2µg of RNA was then subjected to reverse transcription using the SuperScript™ III Reverse Transcriptase (Invitrogen) following the manufacturer's instructions in 20ul volume. Real-time PCR was carried out using iQ SYBR Green Supermix (Biorad) on a CFX96 real-time PCR detection system (Bio-RAD). A housekeeping gene, EF1a, was used as the internal control for all reactions. The fold change of gene expression value was calculated as described previously (Li et al. 2016). All primers were listed in TableS56.

**5-aza-2-deoxycytidine treatment and multiple stress treatment of tomato plants**

For the 5-aza-2-deoxycytidine treatment experiment, 100µl of 2mM 5-aza-2-deoxycytidine was added on the shoot apical meristem of the 3-week-old tomato plants (Ohio8245), and the treatment was repeated once every week. Photos were taken two weeks after the treatment.

For the multiple stress treatments, 12-day-old tomato plants growing in soil was subjected to multiple stress treatments. For cold and heat stresses, the plants was transferred to a cold room (4°C) or a 38°C chamber, respectively, and the plants grown under normal condition (25°C) were used as control; For ABA and IAA treatments, the plant leaves were sprayed everyday with 100µM ABA or 100µM IAA, respectively. The plants sprayed with water were used as control; For salt stress, the plants were watered with 150mM NaCl every day or distilled water as control; For drought stress, stop watering the plants for 7 days, and plants well-watered were used as control. 7 days after stress treatments, the leaves of the
stress-treated and control plants were harvested and frozen in liquid nitrogen immediately for further study.

**ACCESSION NUMBER**

The datasets generated in this study are available at the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE102273.

**ACKNOWLEDGEMENTS**

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**AUTHOR CONTRIBUTIONS**

H.Z., J.K.Z. and Y.Y. conceptualized the research; Y.Y., T.U.D., W.L., S.L., Z.L., X.W., J.G., W.W., W.N., and H.Z. performed the experiments; K.T., Y.Y., T.U.D., Z.C., A.K.H., and H. Z. analyzed the data; Y.Y., K.T., J.K.Z., and H.Z. wrote the manuscript. All authors read and approved the paper.

**REFERENCES**


SUPPORTING INFORMATION

Figure S1. DNA methylation inhibitor 5-aza-2-deoxycytidine treatment inhibited the occurrence of inflorescence and caused growth retardation of tomato

100µl of 2mM 5-aza-2-deoxycytidine was added on the shoot apical meristem of 3-week-old tomato plants (Ohio8245), and the treatment was repeated after one week. Photos were taken two weeks after the initial treatment.

Figure S2. Comparison of methyl cytosine levels in slmet1-T0-A versus wtR0

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in slmet1-T0-A mutant versus that in wtR0. Cytosine levels in the CG, CHG, and CHH contexts are indicated by red, blue, and greencolors, respectively.

Figure S3. Comparison of methyl cytosine levels in slmet1-T0-B versus wtR0

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in slmet1-T0-B mutant versus that in wtR0. Cytosine levels in the CG, CHG, and CHH contexts are indicated by red, blue, and greencolors, respectively.
Figure S4. Genome-wide average methylation levels of subcontexts of CG, CHG, CHH and total C in gene regions and TE regions, each with their contiguous 2kb upstream and downstream flank regions

Data of wtR0, slmet1-T0-A, and slmet1-T0-B are presented in gray, purple, and green colors, respectively.

Figure S5. Comparison of methyl cytosine levels of all CHH subcontexts in slmet1-T0-A mutant versus wtR0

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in slmet1-T0-A mutant versus that in wtR0. The color 1 indicates mCAA, color 2 indicates mCAC, color 3 indicates mCAT, color 4 indicates mCCA, color 5 indicates mCCC, color 6 indicates mCCT, color 7 indicates mCTA, color 8 indicates mCTC, color 9 indicates mCTT.

Figure S6. Comparison of methyl cytosine levels of all CHH subcontexts in slmet1-T0-B mutant versus wtR0

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in slmet1-T0-B mutant versus that in wtR0. The color 1 indicates mCAA, color 2 indicates mCAC, color 3 indicates mCAT, color 4 indicates mCCA, color 5 indicates mCCC, color 6 indicates mCCT, color 7 indicates mCTA, color 8 indicates mCTC, color 9 indicates mCTT.

Figure S7. Overall DNA methylation levels in total C, CG, CHG and CHH contexts at CG, CHG and CHH hyper and hypo DMBs that were identified in slmet1-T0-A compared to wtR0

Figure S8. Morphological phenotypes and methylome of the slmet1-1-T1-B mutant
(A) The *T1-B* mutant showed growth retardation, curly and thick leaves, reduced floral buds, and parthenocarpic fruits. (B) Numbers and composition of *T1-B* DMBs compared with wtR1 in the CG, CHG and CHH contexts. (C) Cytosine (C) methylation levels of CG, CHG, CHH, and total C in wtR1 and *T1-B*. (D) Genome-wide average methylation levels of CG, CHG, CHH and total C in gene regions and TE regions, each with their contiguous 2kb upstream and downstream flank regions. (E) Overall DNA methylation levels in the CG, CHG and CHH contexts at hyper and hypo DMBs identified in *T1-B* compared to wtR1.

**Figure S9. Comparison of methyl cytosine levels in *T1-B* versus wtR1**

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in *T1-B* versus that in wtR1.

**Figure S10. Comparison of methyl cytosine levels in wtR0 versus that in wtO8**

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in wtR0 versus that in wtO8.

**Figure S11. Comparison of methyl cytosine levels in wtR1 versus that in wtO8**

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in wtR1 versus that in wtO8.

**Figure S12. Distribution of methylcytosines in wtO8 on all the 12 chromosomes of tomato.** Red indicates CG; blue indicates CHG; and green indicates CHH.

**Figure S13. Comparison of methyl cytosine levels of CHG subcontexts in wtR0 versus wtO8**

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine
numbers in \(wtr0\) versus that in \(wtO8\). Red indicates CAG, blue indicates CCG, and green indicates CTG.

**Figure S14. Comparison of methyl cytosine levels of CHG subcontexts in \(wtr1\) versus \(wtO8\)**

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in \(wtr1\) versus that in \(wtO8\).

**Figure S15. Genome-wide average methylation levels of subcontexts of CG, CHG, CHH and total C in gene regions and TE regions, each with their contiguous 2kb upstream and downstream flank regions in \(wtO8\), \(wtr0\) and \(wtr1\)**

Data of \(wtO8\), \(wtr0\), and \(wtr1\) are presented in green, purple, and gray colors, respectively.

**Figure S16. Genome-wide DNA methylation changes in \(slmet1\) mutants compared to \(wtO8\)**

(A) Cytosine (C) methylation levels of CG, CHG, CHH, and total C in \(wtO8\) and \(slmet1\) T0 mutants. (B) Ratios of the methylated cytosines in \(slmet1\) T0 mutants versus \(wtO8\). Methyl cytosines were counted per 100kb of chromosome 1. (C) Genome-wide average methylation levels of CG, CHG, CHH and total C in gene regions and TE regions, each with their contiguous 2kb upstream and downstream flank regions. (D) Numbers and composition of \(slmet1-T0-A\) and \(slmet1-T0-B\) DMBs, as well as their overlapped DMBs compared with \(wtO8\) in CG, CHG and CHH contexts. (E) Overall DNA methylation levels in CG, CHG and CHH contexts at hyper and hypo DMBs identified in \(slmet1-T0-A\) compared to \(wtO8\).

**Figure S17. DML2 and DML3 showed significant decrease in T1-B compared to wtr1**

FPKM values of \(wtr1\) and T1-B from RNA seq data are shown. Values are mean \(\pm\) SD, n=2. Asterisks indicate significant difference between \(wtr1\) and T1-B: *\(P < 0.05\); **\(P < 0.01\).

**Figure S18. Chromosome distribution of methylcytosines in CHG subcontexts in \(wtO8\)**
Red indicates CAG; blue indicates CCG; and green indicates CTG.

**Figure S19. Comparison of methyl cytosine levels of CHG subcontexts in slmet1-T0-A versus wtR0**

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in slmet1-T0-A versus wtR0.

**Figure S20. Genome-wide average methylation levels of subcontexts of CG, CHG, CHH and total C in gene regions and TE regions, each with their contiguous 2kb upstream and downstream flank regions in wtO8, slmet1-T0-A and slmet1-T0-B**

Data of wtO8, slmet1-T0-A, and slmet1-T0-B are presented in gray, purple, and green colors, respectively.

**Figure S21. Comparison of methyl cytosine levels of CHG subcontexts in slmet1-T0-B versus wtR0**

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in slmet1-T0-B versus wtR0.

**Figure S22. Comparison of methyl cytosine levels of CHG subcontexts in T1-B versus wtR1**

Methyl cytosines were counted per 100kb in all the 12 chromosomes. The horizontal axis indicates chromosomal positions, and the plotted line represents the ratios of methylcytosine numbers in T1-B versus wtR1.

**Table S1. Bisulfite conversion rates of all samples**

**Table S2. Slmet1-T0-A VS wtR0 CG hyper DMBs**

**Table S3. Slmet1-T0-A VS wtR0 CG hypo DMBs**
Table S4. *Slmet1-T0-A* VS wtR0 CHG hyper DMBs

Table S5. *Slmet1-T0-A* VS wtR0 CHG hypo DMBs

Table S6. *Slmet1-T0-A* VS wtR0 CHH hyper DMBs

Table S7. *Slmet1-T0-A* VS wtR0 CHH hypo DMBs

Table S8. *Slmet1-T0-B* VS wtR0 CG hyper DMBs

Table S9. *Slmet1-T0-B* VS wtR0 CG hypo DMBs

Table S10. *Slmet1-T0-B* VS wtR0 CHG hyper DMBs

Table S11. *Slmet1-T0-B* VS wtR0 CHG hypo DMBs

Table S12. *Slmet1-T0-B* VS wtR0 CHH hyper DMBs

Table S13. *Slmet1-T0-B* VS wtR0 CHH hypo DMBs

Table S14. *Slmet1-T0-A* and *slmet1-T0-B* CG hyper DMBs overlap (VS wtR0)

Table S15. *Slmet1-T0-A* and *slmet1-T0-B* CG hypo DMBs overlap (VS wtR0)

Table S16. *Slmet1-T0-A* and *slmet1-T0-B* CHG hyper DMBs overlap (VS wtR0)

Table S17. *Slmet1-T0-A* and *slmet1-T0-B* CHG hypo DMBs overlap (VS wtR0)

Table S18. *Slmet1-T0-A* and *slmet1-T0-B* CHH hyper DMBs overlap (VS wtR0)

Table S19. *Slmet1-T0-A* and *slmet1-T0-B* CHH hypo DMBs overlap (VS wtR0)

Table S21. *Slmet1-1-T1-B* VS wtR1 CG hyper DMBs

Table S22. *Slmet1-1-T1-B* VS wtR1 CG hypo DMBs

Table S23. *Slmet1-1-T1-B* VS wtR1 CHG hyper DMBs

Table S24. *Slmet1-1-T1-B* VS wtR1 CHG hypo DMBs
Table S25. Slmet1-1-T1-B VS wtR1 CHH hyper DMBs

Table S26. Slmet1-1-T1-B VS wtR1 CHH hypo DMBs

Table S27. Slmet1-T0-A (VS wtR0), slmet1-T0-B (VS wtR0) and slmet1-1-T1-B (VS wtR1) CG hyper DMBs overlap

Table S28. Slmet1-T0-A (VS wtR0), slmet1-T0-B (VS wtR0) and slmet1-1-T1-B (VS wtR1) CG hypo DMBs overlap

Table S29. Slmet1-T0-A (VS wtR0), slmet1-T0-B (VS wtR0) and slmet1-1-T1-B (VS wtR1) CHG hyper DMBs overlap

Table S30. Slmet1-T0-A (VS wtR0), slmet1-T0-B (VS wtR0) and slmet1-1-T1-B (VS wtR1) CHG hypo DMBs overlap

Table S31. Slmet1-T0-A (VS wtR0), slmet1-T0-B (VS wtR0) and slmet1-1-T1-B (VS wtR1) CHH hyper DMBs overlap

Table S32. Slmet1-T0-A (VS wtR0), slmet1-T0-B (VS wtR0) and slmet1-1-T1-B (VS wtR1) CHH hypo DMBs overlap

Table S33. Slmet1-1-T1-B VS wtR1 upregulated genes (fold change>2, FDR<0.01)

Table S34. Slmet1-1-T1-B VS wtR1 downregulated genes (fold change>2, FDR<0.01)

Table S35. Example genes with increased gene expression levels in slmet1-1-T1-B and decreased DNA methylation in slmet1 mutants

Table S36. RIN target genes in slmet1-1-T1-B DEG list (FDR<0.01)

Table S37. WtR0 VS wtO8 CG hyper DMBs

Table S38. WtR0 VS wtO8 CG hypo DMBs

Table S39. WtR0 VS wtO8 CHG hyper DMBs
Table S40. WtR0 VS wtO8 CHG hypo DMBs
Table S41. WtR0 VS wtO8 CHH hyper DMBs
Table S42. WtR0 VS wtO8 CHH hypo DMBs
Table S43. WtR1 VS wtO8 CG hyper DMBs
Table S44. WtR1 VS wtO8 CG hypo DMBs
Table S45. WtR1 VS wtO8 CHG hyper DMBs
Table S46. WtR1 VS wtO8 CHG hypo DMBs
Table S47. WtR1 VS wtO8 CHH hyper DMBs
Table S48. WtR1 VS wtO8 CHH hypo DMBs
Table S49. WtR0 and wtR1 CG hyper DMBs overlap (VS wtO8)
Table S50. WtR0 and wtR1 CG hypo DMBs overlap (VS wtO8)
Table S51. WtR0 and wtR1 CHG hyper DMBs overlap (VS wtO8)
Table S52. WtR0 and wtR1 CHG hypo DMBs overlap (VS wtO8)
Table S53. WtR0 and wtR1 CHH hyper DMBs overlap (VS wtO8)
Table S54. WtR0 and wtR1 CHH hypo DMBs overlap (VS wtO8)
Table S55. NAM (No apical meristem) genes in slmet1-1-T1-B DEG list (FDR<0.01)
Table S56. Primers used in this study

Figure legends

Figure 1. CRISPER-CAS9 gene editing of the tomato SIMET1
(A) Gene structure of the tomato SlMET1 and the position of the sgRNA; (B) Sequence alignments of slmet1-T0-A and slmet1-T0-B mutants with the reference; (C-E) slmet1-T0-A mutant shows severe morphological phenotypes compared to the wtO8 including curly leaf (C), abnormal flowers (D), and parthenocarpic fruit production (E).

Figure 2. SIMET1 mutation caused genome-wide DNA methylation change

(A) Cytosine (C) methylation levels of CG, CHG, CHH, and total C in wtR0 and the slmet1 T0 mutants. (B) Ratios of the methylated cytosines in the slmet1 T0 mutants versus those in wtR0. Ratios were calculated per 100kb of chromosome 1. See also Figure S2 and Figure S3 for all chromosomes. (C) Genome-wide average methylation levels of CG, CHG, CHH and total C in gene regions and TE regions, each with their contiguous 2kb upstream and downstream flanks. Data of wtR0, slmet1-T0-A, and slmet1-T0-B are presented in gray, purple, and green colors, respectively. (D) Numbers and composition of slmet1-T0-A and slmet1-T0-B DMBs (differentially methylated bins), as well as their overlapped DMBs compared with wtR0 in CG, CHG and CHH contexts. (E) Overall DNA methylation levels in CG, CHG and CHH contexts at hyper- and hypo-DMBs identified in slmet1-T0-A compared to wtR0. See also Figure S7.

Figure 3. Pleiotropic growth phenotypes and DNA methylome in slmet1-T0-B T1 progeny

(A) Pleiotropic growth phenotypes in slmet1-T0-B T1 progenies with the same SIMET1 genotype (homozygous 3bp deletion, slmet1-1); see also Figure S8A and Table S20. (B) Overall DNA methylation levels in CG, CHG and CHH contexts at slmet1-T0-A and slmet1-T0-B overlap DMBs as compared with wtR0. (C) Numbers and composition of DMBs common in slmet1-T0-A, slmet1-T0-B and the T1 progeny slmet1-1-T1-B (T1-B). (D) Snapshots of two example loci showing CG DNA hypo methylation in both the slmet1-T0 mutants (slmet1-T0-A, slmet1-T0-B) and T1-B. DNA methylation levels are indicated by vertical bars with the same scale. (E) Chop-qPCR assays of the two representative loci followed by the
methylation sensitive enzyme HpyCH4IV. SL0020 represents the locus: ch00:20,035,326-20,037,794; SL0659 represents the locus ch06:591,458-596,663.

Figure 4. SIMET1 is required for proper transcriptional regulation of key genes for tomato productivity

(A) Overall DNA methylation levels at the slmet1-1-T1-B(DEGs (differentially regulated genes) compared with wtR1. (B) Examples of genes that show increased expression levels accompanied by decreased DNA methylation. The upper panel shows their gene expression levels (FPKM) in wtR1 and slmet1-1-T1-B from mRNAseq data; the lower panel shows snapshots of whole-genome bisulfite sequencing (WGBS) of DNA methylation at these gene loci. (C) Proportions of up- and down-regulated of RIN target genes in T1-B DEGs compared to wtR1. Numbers of genes are as indicated. (D) Gene expression analysis of ACC2 by RT-qPCR in wtO8, wtR1 and T1-B. Values are normalized to wtO8 and are mean ± SD, n=3. (E) DNA hypomethylation at ACC2 gene loci in slmet1 mutants. Snapshots of WGBS are shown. (F) ACC2 gene expression levels in plants treated with and without the DNA methylation inhibitor 5-aza-2-deoxycytidine.

Figure 5. Regeneration alters tomato DNA methylome independently of SIMET1 function or environmental stimuli

(A) Cytosine (C) methylation levels of CG, CHG, CHH, and total C in wtO8, wtR0 and wtR1. (B) Numbers and composition of DMBs in regenerated plants compared with the inbred line wtO8. (C) WGBS snapshots of 3 example loci showing CHG and CHH hypomethylation in wtR0 and wtR1, compared with wtO8. (D) Ratios of the methylated cytosines in wtR0 or wtR1 versus their counterparts in wtO8. Ratios were calculated per 100kb of chromosome 1. See also Figure S10 and Figure S11 for all chromosomes. (E) Genome-wide average methylation levels of CG, CHG, CHH and total C in gene regions and TE regions, each with their contiguous 2kb upstream and downstream flanks. Data of wtO8, wtR0, and wtR1 are presented in green, purple, and gray colors, respectively. (F)
Heatmap representation of relative DNA methylation levels at a group of regeneration-induced hypo DMRs, with or without various environmental stress treatments or hormonal stimuli. DNA methylation levels were compared by means of methylation-sensitive enzyme digestion using HpyCH4IV (for the first 11 loci) or AluI (for the rest 2 loci). Values were normalized to those in the non-treated, inbred control wtO8. The color scale represents the fold change relative to the non-treated, inbred control wtO8.

Figure 1