Comparative WGBS identifies genes that influence non-ripe phenotype in tomato epimutant *Colourless non-ripening*

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Short title: Epi-genotype to epi-phenotype in *Cnr* fruits

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Whole-genome bisulfite sequencing (WGBS) allows single-base resolution and genome-wide profiling of DNA methylation in plants and animals. This technology provides a powerful tool to identify genes that are potentially controlled by dynamic changes of DNA methylation and demethylation. However naturally occurring epimutants are rare and genes under epigenetic regulation as well as their biological relevances are often difficult to define. In tomato, fruit development and ripening are a complex process that involves epigenetic control. We have taken the advantage of the tomato epimutant *Colourless non-ripening* (*Cnr*) and performed comparative mining of the WGBS datasets for the *Cnr* and *SlCMT3*-silenced *Cnr* fruits. We compared DNA methylation profiles for the promoter sequences of approximately 5,000-bp immediately upstream of the coding region of a list of 20 genes. Differentially methylated regions were found for some of these genes. Virus-induced gene silencing (VIGS) of differentially methylated gene *SlDET1* or *SlPDS* resulted in unusual brown pigmentation in *Cnr* fruits. These results suggest that comparative WGBS coupled with VIGS can be used to identify genes that may contribute to the colourless unripe phenotype of fruit in the *Cnr* epimutant.

Tomato *Cnr, SlCMT3, SlDET1, SlPDS*, DNA Methylation, WGBS, VIGS

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a nutritional fruit and vegetable crop that is consumed worldwide. It also serves as a model species to investigate mechanisms involved in the modulation of fleshy fruit ripening (Klee and Giovannoni, 2011). From characterisation of various ripening mutants, it has become evident that fruit ripening is a complex process at physiological, genetic and molecular levels (Seymour et al., 2013). For instance, mapping the *ripening inhibitor* (*rin*) locus reveals a gene that encodes a MADS-box protein designated SlMADS-RIN (Vrebalov et al., 2002), and that SlMADS-RIN acts as a master transcription factor (TF) in regulation of many ripening-related genes in tomato (Martel et al., 2011; Zhou et al, 2012; Fujisawa et al., 2013). The class-I homeodomain leucine zipper protein SlHB-1 is another TF that regulates fruit ripening through its direct binding to the 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene promoter, resulting in a tight transcriptional control of *ACO1* expression. The latter is an essential component in the

genetic pathway for biosynthesis of the ripening hormone ethylene (Lin et al., 2008). Several other TFs including SlAP2a and SlTAGL1 have also been showed to play important roles in fruit ripening, indicating existence of a genetic regulatory network associated with ripening in tomato. However, how these ripening TFs are regulated and which genes are targeted and regulated by these TFs remain largely to be elucidated in tomato (Karlova et al., 2014).

Another key tomato ripening gene, namely the SQUAMOSA Promoter Binding Protein (SBP)-box gene *SlSPL-CNR* resides at the *Colourless non-ripening* (*Cnr*) locus (Manning et al., 2006; Kong et al., 2013). *Cnr* is a spontaneous pleiotropic epimutant in which *SlSPL-CNR* expression is affected by DNA methylation in its promoter region and is also finely tuned by miR157 (Manning et al., 2006; Chen et al., 2015a). *Cnr* fruit cannot ripen and remains colourless. Its texture alters due to loss of cell-to-cell adhesion in fruit tissues (Eriksson et al., 2004). Intriguingly, enzymes that are required for RNA-directed DNA methylation (RdDM) and for methylation maintenance including DOMAINS REARRANGED METHYLTRANSFERASE 7 (SlDRM7), METHYLTRANSFERASE 1 (SlMET1) and CHROMOMETHYLASEs (SlCMT2 and SlCMT3), in particular SlCMT3, are essential to maintain the *Cnr* epiallele and the colourless non-ripening epi-phenotype (Chen et al., 2015b). Consequently, repression of these methylation genes by virus-induced gene silencing (VIGS) can lead *Cnr* fruits to ripen (Chen et al., 2015b). These findings clearly demonstrate that an epigenetic mechanism is involved in *Cnr* fruit development and ripening. This view is further supported by the finding that dynamic changes in the tomato epigenomes occur at different stages of fruit development and ripening in wild-type tomato as well as in *rin* and *Cnr* mutants (Zhong et al., 2013; Chen et al., 2015b; Zhang et al., 2016). Moreover active DNA demethylation has also been found to play a vital role in modulation of tomato fruit ripening (Liu et al., 2015; Lang et al., 2017). These recent whole-genome studies have revealed that tomato development and fruit ripening are not only genetically but also epigenetically programmed, and can be influenced by many potential genes that may be affected by DNA methylation and demethylation (Gullasci et al., 2016; Giovannoni et al., 2017). However specific fruit ripening genes under epigenetic regulation remain to be identified and functionally characterized in tomato.

In this article, we report characterization of genes that are associated with tomato ripening through comparative mining of the whole-genome bisulfite sequencing (WGBS) datasets that we previously generated for the epiallele *Cnr* non-ripe fruits and the *SlCMT3*-silenced *Cnr* ripening fruits (Chen et al., 2015b). In particular we compared DNA methylation profiles for the promoter sequences of approximate 5,000-bp immediately

upstream of the coding region of each gene. Differentially methylated regions (DMRs) were found for some of these genes and silencing of two of the differentially methylated genes by VIGS affected pigmentation in *Cnr* fruits. These results suggest that comparative WGBS coupled with VIGS can be used to identify genes that may contribute to colourless non-ripe epi-phenotype in *Cnr* fruits.

RESULTS

Cnr with an epigenome of hypermethylation is caused by a spontaneous epimutation that blocks the expression of *SlSPL-CNR* at different stages of fruit development and ripening (Manning et al., 2006; Zhong et al., 2013; Figure 1A). We previously demonstrated that suppression of *SlCMT3* by VIGS caused *Cnr* fruits to ripen, likely due to a specific reduction of the DNA methylation level in the *SlSPL-CNR* promoter coupled with an overall decrease of methylation in the *Cnr* epigenomes (Chen et al., 2015b). Repeating our VIGS experiments, we further established that *SlCMT3* VIGS is responsible for the ripening reversion in the treated *Cnr* fruits (Figure 1B). Using the latest tomato genome and epigenome databases, we also confirmed that silencing of *SlCMT3* reduced the DNA methylation level in the two differentially methylated regions (DRM1 and DRM2) within the *SlSPL-CNR* promoter in the VIGS fruits compared to non-VIGS *Cnr* controls (Figure 1C). Although *Cnr* is a dominant epiallele and *SlSPL-CNR* is primarily responsible for the phenotypic colourless non-ripening in *Cnr* fruits, we reason that other genes might also contribute to the development of such epi-phenotypes and those genes can be identified via comparative mining of the WGBS datasets generated from the *Cnr* non-ripe fruits and the *SlCMT3*-silenced *Cnr* ripening fruits.

To test this, we compiled a list of 20 genes (Table 1) and these genes were chosen because they have been implicated to be directly or indirectly associated with tomato development and fruit ripening (Karlova et al., 2014). We examined changes of the DNA methylation patterns for each of these selected genes, particularly in the 5,000-bp promoter sequences prior to the gene coding region. One or more obvious DMRs were readily identified in 12 genes including *SlSPL-CNR*, *SlNOR*, *SlMADS-RIN*, *SlTAGL1* and *SlPDS* known to be linked with tomato fruit ripening (Table 1). These DMRs were hypermethylated in normal *Cnr* fruits compared to that in wild-type tomato Ailsa Craig (AC) and mutant *rin* fruits. However in the *SlCMT3*-silenced *Cnr* fruits with ripening phenotypes (Figure 1B), DNA methylation levels in each of the corresponding DMRs were clearly reduced. For instance, one such DMR was found in the promoter region of the MADS-box gene

SlFUL1/SlTDR4 and three (DMR1, DMR2 and DMR3) in the promoter region of the GRAS family *SlGRAS* gene (Figure 2A and B). Expression levels of both *SlFUL1/SlTDR4* (Figure 2C) and *SlGRAS* (Figure 2D) revealed by RNAseq were constantly very low in *Cnr* fruits at different days post anthesis (DPA). However, in AC fruits only hypomethylation was observed in these DMRs (Figure 2A and B) and expression levels of the two genes ware relatively high at later stages of fruit development and ripening (Figure 2C and D). We interpret these results to mean that such ripening-associated genes are epigenetically regulated and that they may contribute to the colourless non-ripening epi-phenotypes in *Cnr* fruits.

However, not all of the ripening genes showed changes in DNA methylation in the *SlCMT3*-silenced *Cnr* ripening fruits. Indeed, no obvious DMR could be located for genes such as *SlAP2a*, *SlMADS1*, *SlFUL2*, *SlSEP3* and *SlFBP24-Like* through scrutinising the *Cnr* and *SlCMT3*-silenced *Cnr* WGBS datasets (Table 1). These analyses imply that although the five genes may play important roles in fruit ripening in wild-type tomato, in *Cnr* they are not necessarily under the RdDM-mediated epigenetic control and they are unlikely to contribute to the unripe epi-phenotypes. On the other hand, in the case of *SlTAG1*, we observed that decrease and increase in DNA methylation concurrently occurred within the two DMRs in the *SlCMT3*-silenced *Cnr* ripening fruits (Figure 3A). Interestingly, the level of the *SlTAG1* RNA transcripts was relatively abundant in both *Cnr* and AC, but higher in *Cnr* than AC at 7, 17 and 47-DPA; similar at 37 and 42-DPA; however at 27-DPA more expression of *SlTAG1* was observed in AC (Figure 3C). It seems that there is no clear correlation between *SlTAG1* expression, *SlTAG1* promoter methylation and fruit ripening in *Cnr*, *SlCMT3*-silenced *Cnr*, *rin* and AC. A similar pattern of DNA methylation changes in DMRs was also observed for *SlANT1* in the *SlCMT3*-silenced *Cnr* fruits (Table 1). Taken together, our analyses suggest both *SlTAG1* and *SlANT1* may not make significant contributions to the development of epi-phenotypes in *Cnr* fruits.

Through mining the WGBS datasets, we also identified an MYB TF gene *SlAN2* which is involved in regulating expression of genes required for anthocyanidin biosynthesis (Povero et al., 2010; Kiferle et al., 2015). Compared to AC and *rin*, the DMR in the *SlAN2* gene promoter was almost completely free of DNA methylation in normal *Cnr* fruit. However in the *SlCMT3*-silenced *Cnr* ripening fruits, we observed an escalated methylation level in this DMR (Figure 3B). Moreover the *SlAN2* mRNA level was extremely low in *AC*, *Cnr* and

all other tomato mutant fruits (Figure 3D). These findings may imply that a highly methylated DMR cannot be accessed by a ripening-attenuator(s) in AC, *SlCMT3*-silencing *Cnr*, or even *rin* fruit. However in normal *Cnr*, such a ripening-attenuator may bind to a methylation-free "DMR" in the *SlAN2* promoter to affect *SlAN2* expression, subsequently contributing to the colourless non-ripening epi-phenotypes. In addition, it should be noted that the level of anthocyanidin in tomato fruits is low and the role of anthocyanidin as well as relevant genes including *SlAN2* for anthocyanidin biosynthesis in ripening remains unclear in tomato (Mathews et al., 2003; Povero et al., 2010).

Our analyses reveal that the 20 targeted genes fall into four different groups with respect of their DMRs in response to suppression of *SlCMT3* expression in *Cnr*; and that in addition to *SlSPL-CNR*, other Group-I genes are also likely to be associated with the *Cnr* epi-phenotypes (Table 1). To test this prediction drawn from our comparative mining of the WGBS datasets, we used VIGS to silence *SlDET1* and *SlPDS*, two Group-I genes in *Cnr* (Table 1; Figure 4; Figure 5). As observed among other Group-I genes, two DMRs (DMR1 and DMR2) were identified in the *SlDET1* promoter and their methylation levels reduced in the *SlCMT3*-silenced ripening *Cnr* fruits (Figure 4A and B), but only one such DMR was found in the *SlPDS* promoter (Figure 5A). *SlDET1* expression level was found to be low at different ripening stages in AC, *Cnr*, *high pigment* (*hp*) or other ripening mutant tomato fruits (Figure 4C). It should be pointed out that the high pigmentation phenotype of the tomato *hp-2* mutant is caused by a mutation in the *SlDET1* gene (Mustilli et al., 1999). We then cloned two fragments, one covering the 5'-end and the other covering the 3'-end of the *SlDET1* gene into a potato virus X–based VIGS vector (van Wezel et al., 2002) to produce PVX/SlDET1n and PVX/SlDET1c, respectively (Figure 4D). We generated RNA transcripts of PVX/SlDET1n, PVX/SlDET1c or PVX by *in vitro* transcription and injected viral RNAs into the carpopodium of *Cnr* fruits attached to the plant. In repeated VIGS experiments, fruits were injected at various stages of development on different trusses on the same plant and on different plants. We found that *Cnr* fruits that were injected with free PVX virus developed non-ripening phenotype, typical of normal *Cnr* fruits (Figure 4E). However on fruits injected with either PVX/SlDET1n (Figure 4F and G) or PVX/SlDET1c (Figure 4H and I), we observed the appearance of intensified brown pigmentation. Similarly, we also cloned a fragment of the *SlPDS* gene into PVX vector to generate PVX/SlPDS (Figure 5B). Sectors on *Cnr* fruits that were injected with PVX/SlPDS recombinant viral RNA transcripts developed

brown pigmentation (Figure 5C-E). These data clearly demonstrate that silencing of either *SlDET1* or *SlPDS* can disrupt development of the epi-phenotypes in *Cnr* fruits.

DISCUSSION

In plants, epigenetic RdDM takes place at cytosines in CG, CNG and CNN contexts (where N is A, T or C) and such methylation can be maintained through the combined enzymatic activity of DRMs, MET1 and CMTs. In *Arabidopsis* DRM2 catalyses *de novo* CG, CNG and CNN methylation; whilst CMT2 is also involved in establishing non-symmetrical CNN methylation. Maintenance of cytosine methylation at the CG, CNG or CNN site is mediated by MET1, CMT3 or DRM2, respectively. Such epigenetic modification can have a profound influence on plant growth and development as well as plant responses to stress and adaptation to changing environments (Gallusci et al., 2016; Giovannoni et al., 2017). However, naturally occurring epimutation is sporadic and genes under epigenetic regulation as well as their biological significances are often difficult to delineate (Manning et al., 2006). WGBS permits genome-wide DNA methylation profiling at single-base resolution and is a powerful technology to identify genes that may be controlled by dynamic changes of DNA methylation and demethylation (Zhong et al., 2003; Lang et al., 2017). We used a rare tomato epimutant *Cnr* and performed comparative mining of the WGBS datasets for the *Cnr* and *SlCMT3*-silenced *Cnr* fruits (Chen et al., 2015b), in order to identify genes, in addition to the dominant *SlSPL-CNR*, that may be associated with the development of the *Cnr* epi-phenotypes.

Our analyses revealed that the 20 genes included in current study fall into four different groups with respect of their DMRs in response to suppression of *SlCMT3* expression in *Cnr* (Table 1). Group-I includes *SlSPL-CNR* and 11 other ripening-associated genes, which showed a reduced methylation level in the DMRs in the *SlCMT3*-silenced *Cnr* ripening fruits. These genes are likely to be epigenetically regulated and DNA methylation-mediated down-regulation of expression of these genes may play a role in development of the non-ripening epi-phenotypes in *Cnr*. Using VIGS, we were able to functionally confirm that Group-I genes such as *SlDET1* and *SlPDS* can affect the development of the *Cnr* phenotypes (Figure 4; Figure 5). However the impact of Group-II genes on *Cnr* fruit development and (non-)ripening is difficult to predict due to fluctuating up- and down-changes in DNA methylation within their DMRs. On the other hand, the unexpected increase in methylation level for the Group-III gene DMR in the *SlCMT3*-silenced *Cnr* is intriguing, and may suggest a different mechanistic mode for this group of genes or genes with similar DMR methylation patterns, to

epigenetically regulate epi-phenotypic development compared to Group-I genes in *Cnr*. Finally Group-IV genes, even they are known to be involved in ripening in wild-type or other ripening-mutant tomatoes, would be unlikely to play a role in the development of the *Cnr* epi-phenotypes (Table 1).

In summary, through mining WGBS datasets for the epiallele *Cnr* non-ripe fruits and the *SlCMT3*-silenced *Cnr* ripening fruits as well as gene functional analysis via the efficient virus-induced gene silencing system, we were able to characterize genes that are under RdDM-mediated epigenetic modulation and associated with development of the *Cnr* epi-phenotypes.

MATERIALS AND METHODS

Plant materials and growth

Tomato (*Solanum lycopersicum* cultivar Ailsa Craig, AC) and the *Cnr* epimutant plants were grown in insect-free glasshouses at 25^0C during daytime with supplementary lighting to give a 16-h photoperiod, and at 20^0C overnight (8-h).

Constructs of VIGS vectors

Non-translatable fragments corresponding to the 5'-end (463bp) or 3'-end (390bp) of *SlDET1* were PCR-amplified using two sets of primers PP376/*Cla*I (5'-atttcgATCGATgtggaatgaagctgaccaaac-3') and PP377/*Eag*I (5'-gaaggaCGGCCGgactgacaactacaaggcaaggaa-3') or PP374/*Cla*I (5'-attcatATCGATgagacaacccaatatcctgaaat 3') and PP375/*Eag*I (5'-taatccCGGCCGccatactaaccgtcttggcactct-3') and cloned into the *Cla*I/*Eag*I sites of the Potato virus X (PVX) vector (van Wezel et al., 2002) to generate PVX/SlDET1n and PVX/SlDET1c, respectively. Similarly, a fragment of 483bp of the *SlPDS* gene was PCR-amplified using a pair of primers PP383/*Cla*I (5'-gccaggATCGATgagccgctttgatttct-3') and PP383/*Eag*I (5'-tcgtaaCGGCCGtctgacttggccacctttgactc-3') and cloned into the *Cla*I/*Eag*I sites of the PVX vector to produce PVX/SlPDS. All constructs were verified by sequencing. PVX/SlCMT3 was generated in our previous study (Chen et al., 2015b).

Whole-genome bisulfite sequencing (WGBS) datasets and bioinformatics analysis

WGBS datasets for *Cnr*, *SlCMT3*-silenced *Cnr* as well as the wild-type tomato Ailsa Craig (AC) and the *rin* mutant were available from our previous studies (Zhong et al., 2013; Chen et al., 2015b; http://www.epigenome.cuhk.edu.hk/encode.html). Bioinformatics analysis was performed as previously described (Zhong et al., 2013). Specific gene identification numbers were obtained from the tomato genome database (https://solgenomics.net/search/locus). Transcript expression levels of each gene at different days post-anthesis were calculated from the RNA transcriptome databases (http://www.epigenome.cuhk.edu.hk/ZhongWeb/tomato/tomato_index.jsp).

Virus-induced gene silencing (VIGS)

PVX-based VIGS in *Cnr* fruits was performed as described (Chen et al., 2015b). Viral RNA transcripts were generated by *in vitro* transcription as described (Zhou et al., 2012). The carpopodium of *Cnr* fruits at 5–15 days post anthesis was needle-injected with recombinant viral RNAs for each of the PVX-based VIGS constructs. After injection *Cnr* fruits were routinely examined and photographed with a Nikon Coolpix995 digital camera (Chen et al., 2015b).

Compliance and ethics *The authors declare that they have no conflict of interest.*

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Figure legends

Figure 1 Differential DNA methylation in the *SlSPL-CNR* promoter affects fruit ripening in the tomato epimutant *Cnr*. A, Expression level of *SlSPL-CNR* during fruit ripening in wild-type tomato Ailsa Craig (AC), epimutant *Cnr* and mutants *rin*, *hp* and *Nor*. RPKM (Reads Per Kilobase of transcript per Million mapped reads) represents the relative expression level of *SlSPL-CNR* transcript in RNAseq that is proportional to the number of cDNA fragments that originate from it. B, Ripening reversion in *SlCMT3*-silenced *Cnr* fruits. Virus-induced *SlCMT3* gene silencing occurred in *Cnr* fruits that were injected with PVX/SlCMT3 and ripened. Fruits were photographed at 54-days post anthesis (DPA). C, Reduction of DNA methylation in the specific promoter regions DMR1 and DMR2 of the *SlSPL-CNR* gene in *SlCMT3*-silenced *Cnr*. DMR refers to differentially methylated region. WGBS datasets for AC, *rin*, *Cnr* and *SlCMT3*-silenced *Cnr* fruits at 42-DPA were used for comparative bioinformatics analysis. Gene ID for *SlSPL-CNR* and its coordinate on tomato chromosome 2 are indicated.

Figure 2 Differential DNA methylation profiles of Group-I genes. A, Reduction of DNA methylation in the specific promoter region DMR of the *SlFUL1*/*SlTDR4* gene in *SlCMT3*-silenced *Cnr*. B, Decrease of DNA methylation in the specific promoter regions DMR1, DMR2 and DMR3 of the *SlGRAS* gene in *SlCMT3*-silenced *Cnr*. DMR refers to differentially methylated region. WGBS datasets for AC, *rin*, *Cnr* and *SlCMT3*-silenced *Cnr* fruits at 42 days post anthesis (DPA) were used for comparative bioinformatics analysis. Gene IDs and their coordinates on tomato chromosomes are indicated. C and D, Expression level of *SlFUL1/SlTDR4* (C) and *SlGRAS* (D) during fruit ripening in wild-type tomato Ailsa Craig (AC), epimutant *Cnr* and mutants *rin*, *hp* and *Nor*. RPKM (Reads Per Kilobase of transcript per Million mapped reads) represents the relative expression level of mRNA transcript in RNAseq that is proportional to the number of cDNA fragments that originate from it.

Figure 3 Differential DNA methylation profiles of Group-II and Group III genes. A, DNA methylation dynamics in the specific promoter regions DMR1 and DMR2 of the *SlTAG1* gene in *SlCMT3*-silenced *Cnr*. B, Increase of DNA methylation in the specific promoter region DMR of *SlAN2* gene in *SlCMT3*-silenced *Cnr*. DMR refers to differentially methylated region. WGBS datasets for AC, *rin*, *Cnr* and *SlCMT3*-silenced *Cnr* fruits at 42 days post anthesis (DPA) were used for comparative bioinformatics analysis. Gene IDs and

their coordinates on tomato chromosomes are indicated. C and D, Expression level of *SlTAG1* (C) and *SlAN2* (D) during fruit ripening in wild-type tomato Ailsa Craig (AC), epimutant *Cnr* and mutants *rin*, *hp* and *Nor*. RPKM (Reads Per Kilobase of transcript per Million mapped reads) represents the relative expression level of mRNA transcript in RNAseq that is proportional to the number of cDNA fragments that originate from it.

Figure 4 VIGS of Group-I gene *SlDET1* affects pigmentation in *Cnr* fruits. A and B, Reduction of DNA methylation in the specific promoter regions DMR1 and DMR2 of the *SlDET1* gene in *SlCMT3*-silenced *Cnr*. DNA methylation profiles are shown for the entire gene (A) and for an enlarged section to show the reduced methylation in DMR1 and DMR2 (B). DMR refers to differentially methylated region. WGBS datasets for AC, *rin*, *Cnr* and *SlCMT3*-silenced *Cnr* fruits at 42 days post anthesis (DPA) were used for comparative bioinformatics analysis. Gene IDs and their coordinates on tomato chromosomes are indicated. **C,** *SlDET1* expression level at different stages of fruit ripening in AC, epimutant *Cnr* and mutants *rin*, *hp* and *Nor*. RPKM (Reads Per Kilobase of transcript per Million mapped reads) represents the relative expression level of mRNA transcript in RNAseq that is proportional to the number of cDNA fragments that originate from it. D, VIGS constructs PVX/SlDET1n and PVX/SlDET1c. The PVX genome organization (RDRP: RNA-dependent RNA polymerase; the triple-gene block encodes 25K, 12K and 8K movement proteins; CP: coat protein) and the two restriction enzymes *Cla*I and *Eag*I are indicated. E-I, Phenotypes in control and *SlDET1*-silenced *Cnr* fruits. *Cnr* fruits were injected with the control PVX (E), PVX/SlDET1n (F and G), of PVX/SlDET1c (H and I), and photographed at 50-DPA.

Figure 5 VIGS of *SlPDS* affects pigmentation in *Cnr* fruits. A, Reduction of DNA methylation in differentially methylated region (DRM) of the *SlPDS* gene promoter in *SlCMT3*-silenced *Cnr*. WGBS datasets for AC, *rin*, *Cnr* and *SlCMT3*-silenced *Cnr* fruits at 42 days post anthesis (DPA) were used for comparative bioinformatics analysis. Gene IDs and their coordinates on tomato chromosomes are indicated. B, VIGS construct PVX/SlPDS. The PVX genome organization (RDRP: RNA-dependent RNA polymerase; the triple-gene block encodes 25K, 12K and 8K movement proteins; CP: coat protein) and the two restriction enzymes *Cla*I and *Eag*I are indicated. C-D, Phenotypes in *SlPDS*-silenced *Cnr* fruits. Fruit photograph was taken at 50-DPA.