

Tissue dependent variations of DNA methylation and endoreduplication levels during tomato fruit development and ripening

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Abstract Tomato fruit cells are characterized by a strong increase in nuclear ploidy during fruit development. Average ploidy levels increased to similar levels (above 50C) in two distinct fruit tissues, pericarp and locular tissue. However, ploidy profiles differed significantly between these two tissues suggesting a tissue-specific control of endoreduplication in tomato fruit. To determine possible relationships between endoreduplication and epigenetic mechanisms, the methylation status of genomic DNA from pericarp and locular tissue of tomato fruit was analysed. Pericarp genomic DNA was characterized by an increase of CG and/or CNG methylation at the 5S and 18S rDNA loci and at gypsy-like retrotransposon sequences during fruit

growth. A sharp decrease of the global DNA methylation level together with a reduction of methylation at the rDNA loci was also observed in pericarp during fruit ripening. Inversely, no major variation of DNA methylation either global or locus-specific, was observed in locular tissue. Thus, tissue-specific variations of DNA methylation are unlikely to be triggered by the induction of endoreduplication in fruit tissues, but may reflect tissue-specific ploidy profiles. Expression analysis of eight putative tomato DNA methyltransferases encoding genes showed that one chromomethylase (CMT) and two rearranged methyltransferases (DRMs) are preferentially expressed in the pericarp during fruit growth and could be involved in the locus-specific increase of methylation observed at this developmental phase in the pericarp.

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Abbreviations

B	Breaker
dpa	Days post anthesis
DMT	DNA methyltransferase
HPLC	High-performance liquid chromatography
MG	Mature green
5mC	5-Methylcytosine
RR	Red ripe
RT-PCR	Reverse transcription-polymerase chain reaction
SE	Standard error
T	Turning

Introduction

In eukaryotic nuclei, DNA is tightly wrapped around octamers of histones to form the chromatin, a highly organised structure. Chromatin organisation is essential for the control of gene expression and other nuclear processes such as DNA replication or transposon stability. Changes in chromatin structure are determined by protein complexes involved in the ATP-dependent repositioning of nucleosomes, in histone posttranslational modifications, establishing the so-called “histone code” and in the methylation of cytosine residues (Richards and Elgin 2002). Together, the histone code and the DNA methylation status constitute the epigenetic marks which may vary with development both in animals and plants (Li 2002; Finnegan et al. 1998). Functional studies of epigenetic regulators have highlighted the essential role of epigenetic modifications in plant development (Hsieh and Fischer 2005). Yet, there was no evidence that epigenetic mechanisms play any function during fruit development until the recent characterization of a tomato epimutant, which is impaired in fruit ripening (Manning et al. 2006).

Tomato is one of the major model systems to study fleshy fruit development. Tomato fruit development can be divided into three main phases that precede fruit ripening (Gillpasy et al. 1993). Fruit set which corresponds to phase I, follows carpel development and leads to the decision to set fruit. During phase II, ovary tissues undergo an intense mitotic activity. Phase III corresponds to fruit growth essentially due to cell expansion concomitant to a dramatic increase in nuclear ploidy level (Joubes et al. 1999). The effect of this huge increase in DNA amount on genome functioning and chromatin organisation is largely unknown. Yet, it is now well established that epigenetic marks play a major role in both processes during the development of eukaryotic organisms. However, the function of epigenetic modifications in the context of endoreduplicated nuclei has not been investigated.

To investigate the possible relationships between endoreduplication and epigenetic mechanisms, we have analysed DNA methylation of genomic DNA in two fruit

tissues, the pericarp and the locular tissue, in relation to their ploidy level. The ploidy profiles were analysed separately in pericarp and locular tissue using flow cytometry. Southern experiments with methyl-sensitive restriction enzymes and HPLC analysis of total DNA were used to demonstrate tissue-specific variations in DNA methylation level. Finally, tomato DNA methyltransferases gene expression studies revealed complex expression profiles in the different fruit tissues. The results obtained are consistent with tissue-specific epigenetic modifications during fruit development and ripening.

Materials and methods

Plant material and growth conditions

Tomato plants (*Solanum lycopersicum* cv. Ailsa Craig, provided by UC Davis, CA, USA) were grown in a greenhouse during the spring season with a 15-h light (approximately 270 W/m²)/9-h dark photoperiod. In average, the temperature was 25°C during the day and 16°C during the night. Young non-dissected leaves (2–4 cm), mature leaves (limb of fully expanded leaves), stem apex, stems, closed flowers (0.5 cm), open flowers (1 cm) and fruits at 3 days post anthesis (dpa), 5, 10, 20 and 30 dpa, mature green (40 dpa, MG), breaker (B), turning (T) and red ripe (RR) were collected. Fruits of 20 dpa and more were dissected immediately after harvest to separate pericarp and locular tissue. Plant material was immediately frozen in liquid nitrogen and stored at –80°C.

Analysis of cytosine methylation by HPLC and Southern-blot hybridization

Genomic DNA was prepared according to Doyle and Doyle (1990). Absolute methylation levels were measured by HPLC after enzymatic hydrolysis as described in Causevic et al. (2005). For each sample, four replicates were performed with two independent genomic DNA preparations. Statistical data were calculated from these eight measurements ($n = 8$).

Methylation state of specific genomic sequences was investigated by Southern-blot hybridizations using *Hpa*II and *Msp*I (Promega) and the following PCR-DIG labelled probes (Roche): rDNA 5S and 18S from *A. thaliana* (Vongs et al. 1993), TY3/gypsy-like retrotransposon from tomato (Su and Brown 1997).

RNA extraction and RT-PCR analysis

Based on homology with the *Arabidopsis thaliana* DMT cDNAs using TBLASTN, 8 putative tomato DMTs were

identified in the tomato EST databases (TIGR data base [http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=tomato], Solanaceae Genome Network [http://www.sgn.cornell.edu/]).

Total RNA was isolated from dehydrated powder using the SV Total RNA isolation system (Promega). Semi-quantitative RT-PCR were performed as described in (Télef et al. 2006). The number of PCR cycles was adjusted for each gene to avoid reaching saturation. PCR reactions were loaded on 1.5% (w/v) ethidium bromide-stained agarose gel. Gene-specific primers used for PCR amplification are listed in Table 1.

DNA ploidy level analysis

Nuclei were prepared and analysed as described by Cheniclet et al. (2005). For each developmental stage, a minimum of 3 fruits was analysed with two independent quantifications. Calibration of C value was made with nuclei from young leaves. Ploidy histograms were quantitatively analysed with

DPAC software (Partec). The mean C value (MCV) of each histogram was calculated as the sum of each C value class weighted by its frequency. Statistical data were obtained from three independent measures (n = 3).

Results

Analysis of the ploidy level of pericarp and locular tissue cells

We have separately analysed the nuclear ploidy levels in pericarp and locular tissue harvested at various stages of tomato fruit development (Fig. 1). As shown in Fig. 2, the average ploidy level (mean C value, MCV) increased during fruit development and ripening and reached similar values in pericarp and locular tissue. However, in locular tissue the MCV increased rapidly from 25.9 ± 4.0 (20 dpa) to reach a maximum value of 54.6 ± 2.9 at 30 dpa. No significant variations were observed at later developmental

Table 1 Tomato DNA methyltransferase genes

		Accession numbers	Forward primer	Reverse primer	Homologues from <i>A. thaliana</i>
Class I	SIMET1	GenBank : AJ002140	ATTGGCGTGACCTTGAAG	CTGTGCTTGTGCAAGATG	DMT1
	SICMT2	TIGR : TC184438; TC170932 SGN-U320922	GAATTGTAATGAAGTGTCAC	AATGTTACATGGAATTGAATG	CMT1 CMT2 CMT3
Class II	SICMT3	TIGR : TC157511 SGN-U314390	GACGAATCTCTAGATAAGG	GAAATAAGCTTTGCATATAAG	
	SICMT4	SGN-U345626	GATTCTCAGTCTGGTTCC	CAGACAATGGGTCATCAG	
	SIDRM5	GenBank : EU344815	AAGACATGTTCCAAATGGG	CGCCACATGAATCTATCAG	DMT7 DMT9 DMT10
Class III	SIDRM6	TIGR : TC167190 SGN-U321564	ATTGTTGCATTGATCACCG	AGAAGACCAACTATCATCC	
	SIDRM7	TIGR : TC161581	TGGAAGTAACAGGGTGAC	ACAATGAGAAGTTCAGCAG	
	SIDRM8	SGN-U325992	ATTCATGCAGGAGCCTTG	GTCTCAGAATTGATGCCG	
EF1α		GGTTAAGATGATTCCCACC	CCAGTTGGGTCCTTCTTG		

Based on homology with the *Arabidopsis thaliana* DMT cDNAs, 8 tomato putative DMTs were identified in the tomato EST databases (TIGR data base, Solanaceae Genome Network). Complete open reading frames were obtained for *SIMET1* (GenBank), *SIDRM6* and *8* (SGN), and *SIDRM5* (this work). Partial sequences were obtained for the three chromomethylases and for *SIDRM7*

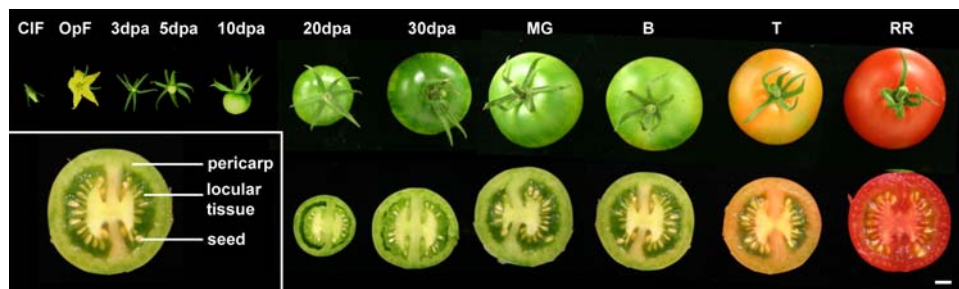
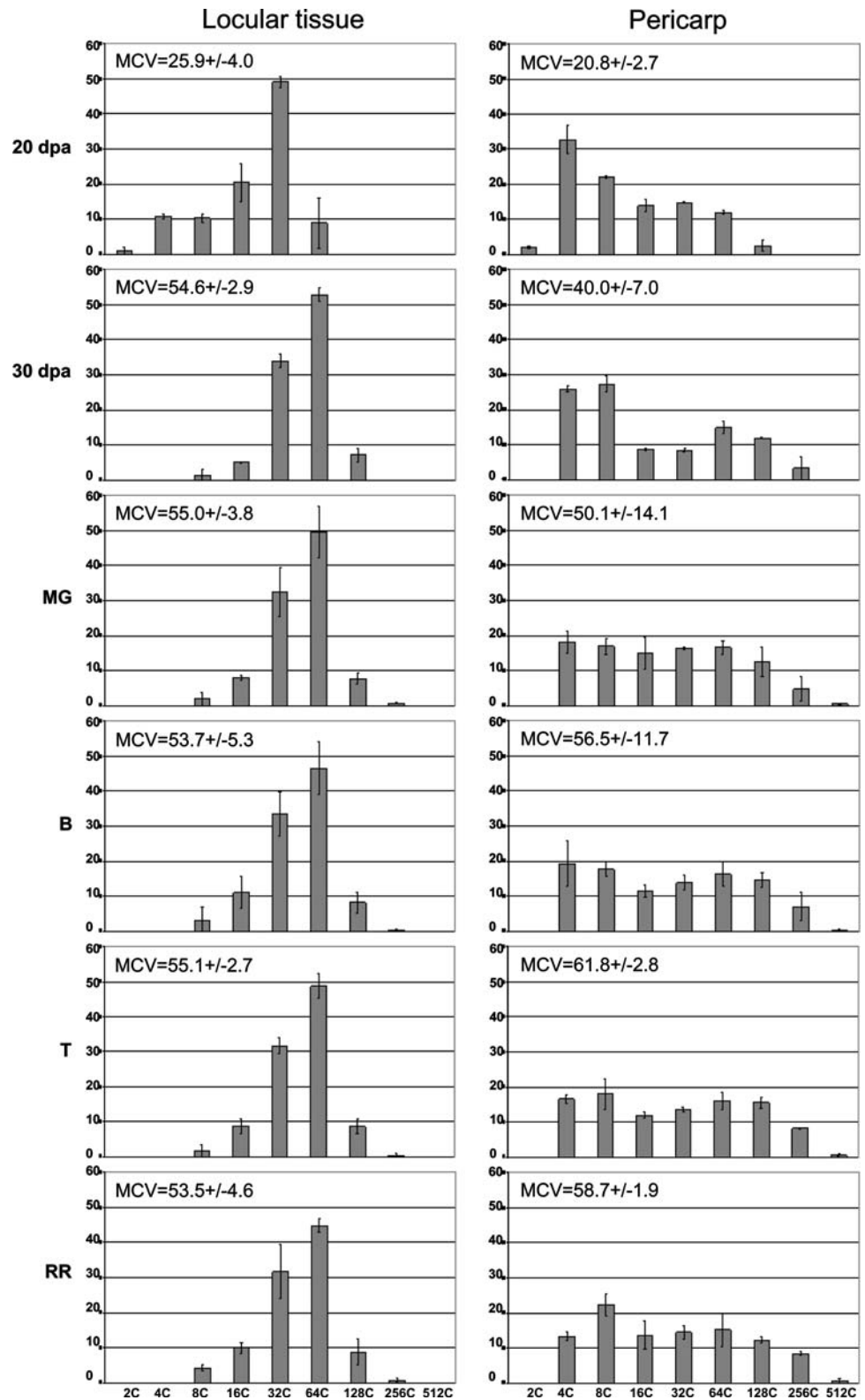


Fig. 1 Tomato flowers and fruits. The flower and fruit samples are as follows: closed flower (CIF), open flower (OpF), fruits at 3 days post anthesis (3 dpa), 5, 10, 20, 30 and 40 dpa (mature green, MG), breaker

stage (B), turning stage (T) and red ripe stage (RR). In the lower left part a transversal section in a breaker fruit is shown. Bar represents 1 cm

Fig. 2 Analysis of ploidy levels in the pericarp and locular tissue of developing tomato fruit. Ploidy was analyzed by flow cytometry. Frequency of each C value was calculated from measurements performed in duplicate on three different fruits ($n = 3$). The mean C value \pm SD (MCV) is indicated for each sample



stages. Inversely, the maximum MCV value was achieved more progressively in pericarp since MCV increased from 20.8 ± 2.7 (20 dpa) to a maximum value of 61.8 ± 2.8 at the turning stage of ripening. This suggests that the control

of endoreduplication proceeds in different ways in locular tissue and in pericarp.

Analysis of the nuclei distribution in the different ploidy classes further outlined these differences. In locular tissue,

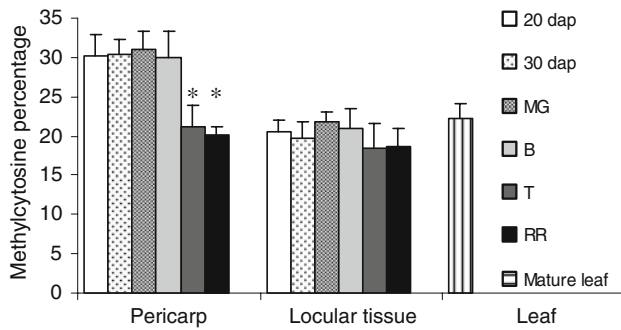


Fig. 3 Total percentage of methylcytosine during fruit development. Data are means \pm SE of four replicates using two independent genomic DNA preparations. *Indicates significant differences for a given tissue between developmental stages ($P \leq 0.05$) using 20 dap fruits as a reference for fruit samples

80% of the nuclei belong to the 32C and 64C classes in fruits of 30 dpa, with little changes during fruit development. Yet, the amount of nuclei with very high ploidy levels (above 64C) remained limited (less than 10% of the total number of nuclei) and no nucleus above 256C was observed. In contrast, the pericarp contains substantial amounts of cells with very different ploidy level ranging from 4C up to 512C. The ploidy profile changed progressively during pericarp fruit development and ripening: the amount of nuclei with DNA content above 64 C increased from 2.3% (20 dpa) to 27% (turning fruit).

These results indicate that locular tissue and pericarp contain highly endoreduplicated nuclei. However, analysis of ploidy profiles indicates significant differences between tissues and suggests tissue-specific control of endoreduplication in tomato fruit.

Global DNA methylation status

The overall cytosine methylation level of tomato genomic DNA prepared from the limb of fully expanded leaves and from pericarp and locular tissue was evaluated by HPLC (see [Materials and methods](#)). The 5mC content of tomato genomic DNA was significantly higher in pericarp (30.2 ± 2.7 to $31.1 \pm 2.1\%$) than in locular tissue ($19.6 \pm 1.8\%$ to $21.7\% \pm 1.2$) in fruits from 20 to 40 dpa (Fig. 3). There was no significant difference of the global methylation level of the pericarp DNA between the mature green stage and the breaker stage (Fig. 3). During fruit ripening the 5mC content of pericarp DNA decreased from $29.9 \pm 3.3\%$ at the breaker stage to $20.1 \pm 1.0\%$ in red ripe fruits, whereas no significant change was detected in locular tissue. The methylation level in the limb of fully expanded leaves was $22.3 \pm 1.4\%$.

Altogether, these results clearly indicate tissue-specific variations of the global DNA methylation level during fruit development.

DNA methylation status of repeated DNA sequences

To get further insight into DNA methylation events occurring during fruit development, the methylation profile was analysed at repetitive DNA sequences by Southern blotting using the methylation-sensitive enzymes *HpaII* and *MspI*. Both enzymes recognise the sequence $-C^1C^2GG-$: *HpaII* is inhibited when a single C or both are methylated, whereas *MspI* activity is blocked only when C^1 is methylated. *MspI* activity reflects CNG methylation level while the comparison between *HpaII* and *MspI* provides an evaluation of CG methylation. Three types of repetitive elements were analysed: the 5S rDNA located in pericentromeric heterochromatin (Xu and Earle 1996), a dispersed repetitive element, the TY3/gypsy-like retrotransposon (Su and Brown 1997) and a subtelomeric repetitive element, the 18S rDNA (Vongs et al. 1993).

The 5S rDNA could not be efficiently digested by *HpaII* in any DNA samples, indicating a high level of methylation of CCGG sequences (Fig. 4b). After long exposure, bands of low intensity were visible in DNA samples from flowers and fruits before 20 dpa but not from fruits at later stages of development (Supplementary data Fig. S1b). This demonstrates an increase in the methylation level at 5S rDNA CCGG sites in pericarp during fruit growth (from 10 dpa to MG stage). Digestion with *MspI* resulted in a ladder of bands in leaves, flowers and young fruits up to 20 dpa. A band of approximately 400 bp corresponding to the 5S rDNA monomer was visible (Fig. 4b). This result demonstrates a low CNG methylation level at the 5S locus and indicates that *HpaII* inhibition is due to CG methylation. From 20 dpa to the MG stage (40 dpa) an important increase in methylation at CNG motives was observed in the pericarp, which may account for the global increase of methylation at CCGG motives.

During fruit ripening, variations of methylation at the 5S locus appeared more limited than during fruit growth. However, a slight increase in *MspI* cutting efficiency could be seen at the turning and RR stages (Fig 4b). A similar observation was made with the *HpaII* digest of DNA from RR fruits when blots hybridized with the 5S rDNA probe were exposed for a long time (Supplementary data Fig. S1b). These results are indicative of a decrease in methylation at this locus at late ripening stages.

The methylation level at the gypsy loci shows similar changes: an increase in CG methylation and CNG methylation during fruit growth (Fig 4c), followed by a slight demethylation at CNGs at late ripening stages (Supplementary data Fig. S1c). Analysis of the methylation level at the 18S rDNA also suggests an increase in both CG and CNG methylation between 20 dpa and the MG stage (40 dpa, Fig 4d, Supplementary data Fig. S1d). Long exposure of the blots indicates a better efficiency of the *HpaII* digest at

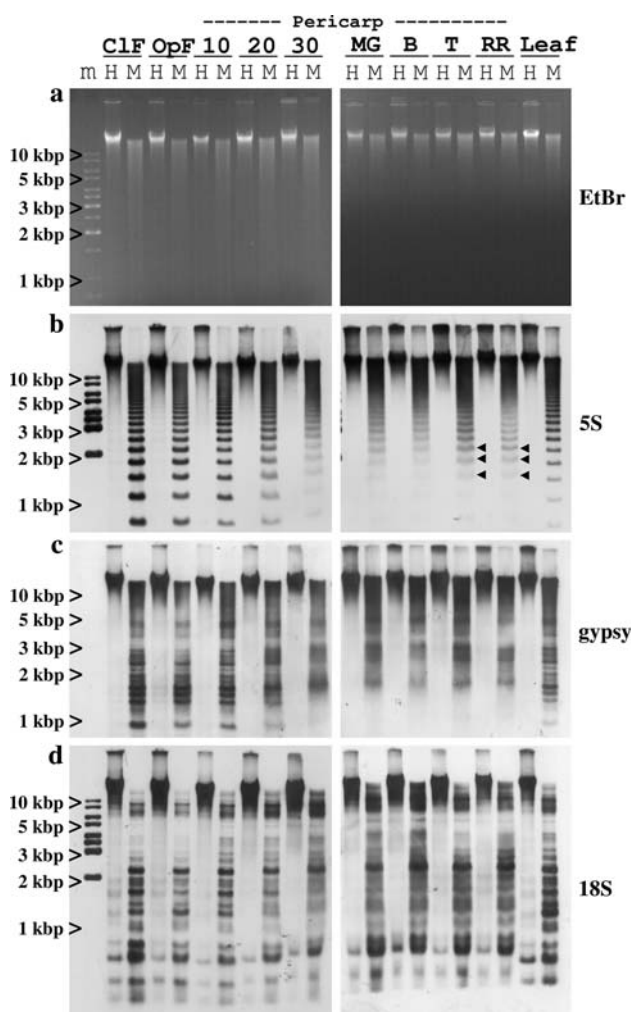


Fig. 4 CCGG methylation at repeated loci during pericarp development. Gels were stained with ethidium bromide (**a**), and blotted. The blot was successively hybridized with a 5S rDNA (**b**), a gypsy-like retrotransposon (**c**) and an 18S rDNA probe (**d**). Molecular weight is indicated. Flowers at two different developmental stages are presented: *Cl* closed flower, *Op* open flower. Developmental stage of fruits is indicated in days post anthesis (*dpa*) during fruit growth, and as ripening stages after the mature green stage (*MG*, 40 *dpa*); *B* breaker, *T* turning, *RR* red ripe. *Arrow heads* point out reduction of methylation during ripening

this locus in turning and red ripe samples consistent with a reduction of methylation (Supplementary data Fig. S1d).

The methylation status of genomic DNA was also investigated in the locular tissue. As observed for the pericarp, *HpaII* weakly cut genomic DNA at the 5S rDNA locus, regardless of the developmental stage (Fig. 5b). On the contrary, genomic DNA from young fruits (20 *dpa*) was efficiently digested by *MspI* at this locus. Between 20 *dpa* and the *MG* stage (40 *dpa*) *MspI* activity was slightly inhibited, indicative of a limited increase in CNG methylation. Similar results were obtained using the tomato gypsy-like probe (data not shown).

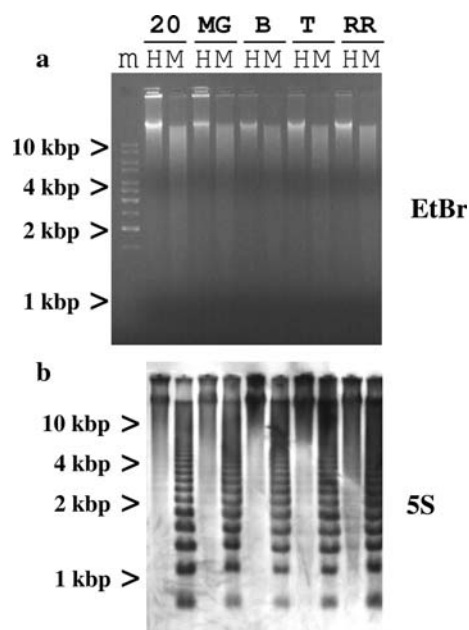


Fig. 5 CCGG methylation at repeated loci in locular tissue of developing fruits. Gel was stained with ethidium bromide (**a**), blotted and hybridized with a 5S rDNA (**b**). Molecular weight is indicated. Developmental stage of fruits is indicated in days post anthesis (*dpa*) during fruit growth, and as ripening stages after the mature green stage (*MG*, 40 *dpa*). *B* breaker, *T* turning, *RR* red ripe

Taken together, our results indicate an increase of DNA methylation at rDNA and gypsy like retrotransposon sequences in pericarp during fruit growth. This increase precedes a reduction of methylation at these repetitive loci in pericarp of late ripening fruits (turning and red ripe stages). In contrast, genomic DNA from locular tissue is not subjected to major methylation variations at any locus analysed. These results indicate tissue-specific control of DNA methylation during fruit development and ripening.

Expression analysis of tomato DNA methyltransferases

To determine whether pericarp preferential variations of DNA methylation could be correlated to the expression of genes involved in cytosine methylation, the expression of eight putative tomato DNA methyltransferase (DMT) genes was analysed. The DMT cDNA sequences identified in various tomato EST databases are listed in Table 1. They ranged into the three classes of DMTs described in *Arabidopsis*. Figure 6 presents the organ and tissue preferential expression of the different tomato DMT genes. *SIMET1* is expressed in all plant organs and fruit tissues analysed with a preferential expression in young plant organs. The genes encoding putative chromomethylases (CMT) are differentially expressed in tomato with a preferential expression of *SICMT2* in stems, and of *SICMT4* in flowers and young leaves. *SICMT3* mRNA is detected in all plant tissues,

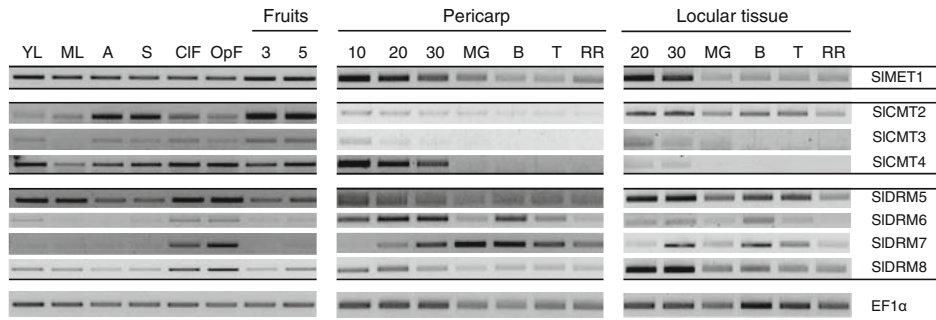


Fig. 6 Expression of tomato DNA methyltransferase genes. Semi-quantitative RT-PCR was performed on total RNA of the indicated organs and tissues using gene-specific primers (Table 1). YL, young leaves; ML, mature leaves; A, shoot apex; S, stems; CIF, closed flowers; OpF, open flowers. Fruits at 3 and 5 dpa were not dissected before RNA preparation. Fruits of 10 dpa and those of higher dpa were

dissected and RNA were prepared from pericarp and locular tissue. Developmental stage of fruits is indicated in days post anthesis (*dpa*) during fruit growth, and as ripening stages after the mature green stage (MG, 40 dpa). B breaker, *T* turning, *RR* red ripe. The constitutively expressed *EF1α* gene is shown as a control

including fruits, at a low level. The three genes are preferentially expressed in young fruits (3 and 5 dpa) with a progressive decrease during development. *SICMT2* and *SICMT4* are mainly expressed in locular tissue and in pericarp, respectively.

Among the four domains rearranged methyltransferase (DRM) like genes, *SIDRM5* and *SIDRM8* are expressed in all plant tissues with a preferential expression in leaves and flowers. *SIDRM6* and *SIDRM7* mRNA were detected predominantly in flowers. In fruits, *SIDRM5* and *SIDRM8* mRNAs abundance was higher in locular tissue than in pericarp of young fruits and decreased during fruit development in both tissues. *SIDRM6* and *SIDRM7* mRNAs were detected in pericarp and locular tissue at most stages, with a preferential accumulation in the pericarp of developing fruits.

Thus, although DMT genes present complex expression profiles in the different fruit tissues, members of the CMT and the DRM gene families show some pericarp-preferential expression.

Discussion

DNA methylation is a major epigenetic mark which participates to the control of gene expression, genome replication and genome protection. In plants, methylated cytosines are mainly found in a CG context, but also at CNG and at non symmetrical CNN sites (Gruenbaum et al. 1981). The overall content in 5mC of plant genomes depends on genome size and content in repetitive DNA sequences. Yet, the *Arabidopsis* genome (120 Mb) contains only 6% of 5mC, whereas the maize genome (2,500 Mb) contains more than 25% (Ergle and Katterman 1961). Consistent with previous findings (Messeguer et al. 1991), the 5mC content of the tomato genome (cv *Ailsa craig*) (950 Mb, Peterson et al. 1996), ranged between 20 and 30% and depends both on tissues and developmental stages (Fig. 1).

In contrast with the general view that methylation increases during plant development (Finnegan et al. 1998), a significant reduction of the total 5mC content (30%) was observed in fruit pericarp between the breaker stage and the RR stage. This reduction of methylation of pericarp DNA follows the fourfold increase in plastids number per pericarp cell that has been shown to occur between the MG and breaker stage during tomato fruit ripening (Cookson et al. 2003). However, no change in 5mC content was observed between the MG and breaker stages. This makes it unlikely that the reduction of 5mC content is primarily due to a dilution of the genomic DNA following an increase in plastid number per cell. Furthermore, our results indicate that the rDNA loci and gypsy-like retrotransposon sequences are partly demethylated during ripening concomitantly to the general decrease in 5mC content (Fig. 4 and supplementary data Fig. S1). Thus, loss of methylation at these repetitive sequences may be part of a general reduction of 5mC content observed at the turning and red ripe stages. These results argue in favour of a controlled loss of methylation of pericarp genomic DNA during fruit ripening at least in the *Ailsa craig* variety. Consistent with a developmentally controlled process the global and locus-specific reduction of methylation was only observed in pericarp and not in locular tissue. The loss of methylation occurred at a stage when most of the cell divisions have stopped (Joubes et al. 1999) and endoreduplication is mostly achieved which makes unlikely a dilution effect after DNA replication. Specific mechanisms might therefore be triggered that determine the process of demethylation during fruit ripening and may involve specific enzymes such as DNA glycosylases as recently shown in *Arabidopsis thaliana* (Zhu et al. 2007; Morales-Ruiz et al. 2006).

It is clearly established that repetitive sequences are preferential sites for CG type DNA methylation in plants (Finnegan et al. 1998). We have shown that the 5S, 18S rDNA and gypsy-like retrotransposon are highly methylated

at CG sites in all plant organs tested. Inversely, cytosine methylation at CNG sites was low in leaves, flowers and young fruits at these loci. High CG methylation has been reported at the 5S locus in many plant species including *Arabidopsis* and tobacco, whereas the level of CNG methylation at this locus varies significantly between plant species (Fulnecek et al. 2002). In *Arabidopsis*, the global 5mC content of 5S rDNA increases during early seedling development with slight differences between plant tissues (Mathieu et al. 2003). In this study, increase in DNA methylation level was associated with variations of heterochromatin structure and of the composition in 5S rRNA species. However, variations of CNG methylation were not evaluated. We showed that during tomato fruit development, the increase of methylation of the 5S rDNA occurred predominantly at CNG sites in a pericarp-preferential manner. This increase was not restricted to the 5S rDNA and also occurred at gypsy-like retrotransposon sequences. It is unclear whether such an increase in methylation at repetitive loci is associated with chromatin conformational changes. Indeed variations of ploidy level may lead to a rearrangement of chromatin organization associated with variations of the epigenetic state of the fruit chromatin. Cytosine methylation did not increase significantly in locular tissue at the loci analysed, although nuclei were highly endoreduplicated in this tissue as well. Therefore endoreduplication does not seem to be accompanied by an increase in the methylation level of DNA at these loci in the locular tissue. On the other hand, results presented in Fig. 2 indicate that the control of endoreduplication is likely to proceed in different ways in pericarp and locular tissue. Thus, we cannot rule out that tissue-specific control of endoreduplication leads to tissue-specific changes of DNA methylation pattern. Consistent with this hypothesis, the proportion of nuclei of very high ploidy level (>64C) represents 58.2% of the genomic DNA in pericarp at 30 dpa, and only 16.6% in locular tissue at the same stage. The DNA methylation pattern may be determined by the ploidy level of given nuclei rather than by the endoreduplication process per se. Strategies aimed to enrich nuclei populations in nuclei of a given ploidy level should help determining whether methylation profile could characterize a particular ploidy level.

It is noteworthy that the increase in methylation described in pericarp tissue did not affect all repetitive loci to a similar extent. Significant increase of CNG methylation was observed at the 5S rDNA loci and at gypsy-like retrotransposon sequences, but the increase in CNG methylation appeared more limited at the 18S rDNA. This differential methylation of DNA sequences may explain why the local increase of cytosine methylation during fruit growth had no impact on the global 5mC content. The increase in methylation of pericarp DNA within CNG sequences may concern only a limited portion of the genome and could be compensated

by decrease in methylation at other loci or at other motives including non symmetrical (CNN) methylation sites (Vaillant et al. 2008). Additionally, the high level of CG methylation may buffer local variations of the methylation level in a CNG context.

So far, the mechanisms responsible for the tissue differential DNA methylation in fruits are not elucidated. Analysis of DMT gene expression has revealed that many of the DMT genes are expressed in young fruits which are composed of tissue enriched in actively dividing cells and cells undergoing endoreduplication. Additionally, some of the DMT genes present a tissue-preferential pattern of mRNA accumulation. Most notably, the *SICMT4* and *SIDRM6* genes are preferentially expressed in pericarp concomitantly with the increase in CNG methylation at repetitive DNA sequences. Chromomethylases have been shown to be involved in CNG maintenance methylation and together with the DRM class of DMTs in de novo methylation (Chan et al. 2005). This makes these genes possible candidates involved in pericarp-preferential methylation during fruit growth.

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