Epigenome Landscape in *Capsicum* Genome

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Abstract

At DNA level, methylation is one of the most studied epigenetic marks and plays an important role in plant growth and development via regulating gene expression, integrity, and mobility of genome as well as transposons. The epigenetic studies especially the DNA methylation have been investigated only in a few members of the Solanaceae family like tomato and potato. So far, cytosine methylation landscape in *Capsicum*, a diploid, self-pollinating crop of the Solanaceae family grown worldwide for fresh and processed products, is far less documented. In our research study in the laboratory, we found the overall high cytosine methylation in *Capsicum* fruit as compared to other plants. The *Capsicum* fruit shows at an average 89.1% of CG, 84.85% of CHG, and 24.9% CHH cytosine methylation globally. The variation in genome size reflects the variations in the global cytosine methylation across different species. The *Capsicum* genome which is 3–4-fold larger than that of tomato and potato is found to have ~1.2–2.7-fold higher cytosine methylation at all methylation contexts. The abundance of repetitive elements (REs) generally affects the variations in genome size across species and generally has dense cytosine methylation. The intraspecific variations in cytosine methylation as well as the miRNA-regulated methylation are unexplored in *Capsicum*, which could provide plausible evolutionary relationship between different species of Solanaceae family. DNA methylation is considered as one of the requisites for various developmental and transcriptional gene expression regulation, while it is also important for reprogramming of various biological processes and transcriptional gene regulation by trimming down their methylation profiles. Therefore, the collaborative role of methylation and demethylation phenomenon in DNA results in the global dynamic nature of cytosine methylation.

11.1 DNA Methylation: An Overview in Plants

Heritable alterations which are not due to variations in underlying DNA sequences include DNA methylation and histone modification resulting in alteration of chromatin structure and DNA accessibility, thereby ultimately modulating expression of several genes, and are termed as epigenetic

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N. Ramchiary and C. Kole (eds.), *The Capsicum Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-319-97217-6_11

modifications (Bonasio et al. 2010). DNA methylation, the most studied epigenetic mark, includes the formation of 5-methylcytosine via inclusion of methyl group to the 5th carbon of sugar residue of nucleotide bases and plays an vital role through regulation of different aspects of development in plants via influencing the gene expression, integrity, and versatility of genome and transposons, respectively (Richards and Elgin 2002). Cytosine methylation in animals generally occurs at CG context representing methylated cytosines (mCs) at both strand making it symmetrical, while plants have mCs at both symmetric (CG and CHG) and asymmetric (CHH) context, where H is considered as other than guanine (Law and Jacobsen 2010; Matzke and Mosher 2014). The cytosine methylation is mainly maintained by three well-distinguished DNA methyltransferases classes: (i) the *methyltransferase 1* (*MET1*), (ii) *chromomethylases* (*CMT*) *methyltransferases*, and (iii) domain rearranged methyltransferases (DRM) family containing *DRM1* and *DRM2*. The first two classes maintain symmetric methylation, whereas the last class (*DRM1* and *DRM2*) maintains asymmetric methylation context via siRNA-facilitated RNA-directed DNA methylation (RdDM) pathway. Moreover, RdDM pathway can also maintain cytosine methylation at all sequence contexts by de novo approach (Aufsatz et al. 2004; Law and Jacobsen 2010). These DNA methyltransferases ubiquitously showed a collaborative methylation activity at all methylation contexts in *RPS* locus of *Petunia hybrida* (Singh et al. 2008). The level and pattern of cytosine methylation in all contexts varies across plants due to size variation at genomic level. The overall cytosine methylation in *Arabidopsis thaliana* genome was observed in all contexts that is 22.3% for CG, 5.92% for CHG, and 1.51% for CHH, while in rice genome with 3-fold larger genome size has 59, 21 and 2.2% mCs of CG, CHG, and CHH, respectively (Feng et al. 2010). Furthermore, in *Zea mays* genome cytosine methylation was found to be 86% for CG, 74% for CHG, and 5.4% for CHH context, respectively (Gent et al. 2013). Likewise, our finding in *Capsicum annuum* suggests that cytosine at an average of 89.1% of CG, 84.85% of CHG, and 24.9% of CHH context was methylated. Further, levels of DNA methylation have been shown tissue-specific characteristic throughout the developmental phase (Gehring and Henikoff 2007). In *Solanum lycopersicum*, methylation level in mature tissues like leaf, fruit, and seed was higher than immature stem, leaves, protoplasts, and roots (Messeguer et al. 1991; Teyssier et al. 2008).

In general, methylation in promoter region is directly related to the gene silencing. For instance, a natural epigenetic mutation or epiallele with the hypermethylated promoter in the tomato *colorless non-ripening* (*Cnr*) gene was responsible for gene repression. This has encouraged researchers to study more on controlling fruit ripening via targeted DNA methylation (Manning et al. 2006; Zhong et al. 2013). Methylation is one of the requisite for regulation of various developmental stages; however, a process involving removal of mCs and replacing it back with original cytosine is also equally important for rescheduling of many biological processes, known as demethylation (Zhang and Zhu 2012). The demethylation of mCs can be induced by *DEMETER* (DME), a DNA glycosylase (Choi et al. 2002); *DEMETER-LIKE 2* (*DML2*); *DML3* (Penterman et al. 2007); and *REPRESSOR OF SILENCING 1* (*ROS1*) gene (Gong et al. 2002) which replace the mCs with non-methylated cytosine. In *Capsicum annuum* L., the cytosine methylation in germinating seed has been reported using methylation-sensitive amplified polymorphism (MSAP) marker and observed that demethylation of mCs is an important factor for transcriptional gene activation during seed germination (Portis et al. 2004). In tomato, active DNA demethylation was suggested to supervise fruit ripening via tomato *SlDML2*; thereby suggesting importance of demethylation in regulation of ripening-specific and ripening-restrained genes (Liu et al. 2015; Lang et al. 2017). In an another instance, in tomato *rin* (ripening inhibitor) mutant line, promoter hypermethylation was observed at *RIN*-binding sites in fruit-ripening genes like in *Polygalacturonase* (*PG*), suggesting the role of *SlDML2*-mediated demethylation in phase transition during fruit-ripening process (Zhong et al.

2013; Lang et al. 2017). Thus collaborative activities between replication, maintenance of methylation and demethylation instances at DNA level, results the global dynamic nature of methylation at different context.

11.2 Global Cytosine Methylation in *Capsicum*

Capsicum ($2n = 24$), a member of the Solanaceae family, is one of the most important crops grown for spices and vegetables worldwide. Plants like tomato, potato, tobacco, eggplant, and *Nicotiana tabacum* are closely related to *Capsicum*. *Capsicum* fruits are rich source of various alkaloids, pigments, vitamins and nutrients and most commonly used as spices (Aza-González et al. 2011). Wide variations are observed for fruit morphology (size, shape, and color) and biochemical contents in *Capsicum* fruits. The transcriptome study of *Capsicum* fruits observed differential gene expression and improved our understanding of capsaicin biosynthetic pathway (Liu et al. 2006; Kim et al. 2014). Importantly, it was reported that the epigenetic modification, including cytosine methylation, regulates the expression of diverse genes during fruit development (Gallusci et al. 2016). So far, epigenetic studies, especially DNA methylation in *Capsicum* species, are far less documented. Stable epigenetic marks are maintained and inherited by *MET1* and *CMT3* at both strands of daughter DNA making it symmetrical, while asymmetric methylation as name suggests only occurs at either strand of daughter DNA and maintained de novo throughout the each cell cycle (Zhang and Zhu 2012). Alteration in the gene expression or genomic instability could be correlated with dynamics of global cytosine methylation at genome level, and often plant genomes are found to be densely methylated. Furthermore, in *C. annuum* L., ~15–16% increased level of global cytosine methylation phenomenon was observed in water-deficit as well as in drought-affected plants treated with 200 mM H_2O_2 compared to normal plants (Rodríguez-Calzada et al. 2017).

Till now, variations in global cytosine methylation across different species are under-explored which could potentially reflect the evolutionary correlation between the species. To elucidate the global fruit methylome, we have performed whole-genome bisulfite sequencing (WGBS) in fruits of *C. annuum*. Fruit samples at different developmental stage (immature, breaker, and mature stages) were pooled together to get overall fruit methylome. Overall, 215,876,691 bisulfite sequencing reads were aligned to *C. annuum* reference genome (GCF). Almost 92.8% (200,317,410 reads) of total reads were aligned to reference genome out of that 88.7% (167,642,734 reads) of total aligned reads were uniquely mapped to the reference genome. Further, the status of methylated cytosine (mCs) was identified at CG, CHG, and CHH contexts. A sum of 5,143,414,121 cytosine was analyzed, of which 408,009,366, 624,328,406, 1,016,141,807 cytosines were found to be methylated at CG, CHG, and CHH contexts, respectively. In *C. annuum,* CHH (49.6%) methylation context has shown higher proportion of mCs followed by CHG (30.5%) and CG (19.9%) contexts which is approximately similar to tomato (Zhong et al. 2013). In *Capsicum*, the global cytosine methylation is the highest in all the methylation contexts (Fig. 11.1 and Table 11.1) compared to tomato (Zhong et al. 2013), potato (Wang et al. 2018), maize (Wang et al. 2018), rice, arabidopsis (Feng et al. 2010), soybean (An et al. 2017), and *Brassica rapa* (Chen et al. 2015). Further, it was found that plants show substantial variation at genome level due to the high abundance of REs which generally have highly dense regions of mCs regions in the genome (Rabinowicz et al. 2003; Fedoroff 2012); therefore, it was suggested that perhaps the genome size is related to global methylation level during the course of evolution (Alonso et al. 2015). This could be seen in view of *Capsicum* genome which has ~22-fold larger genome compared to *A. thaliana* and has shown significantly higher differences in global mCs at all contexts. As the genome size variation decreases, the variations in cytosine methylation

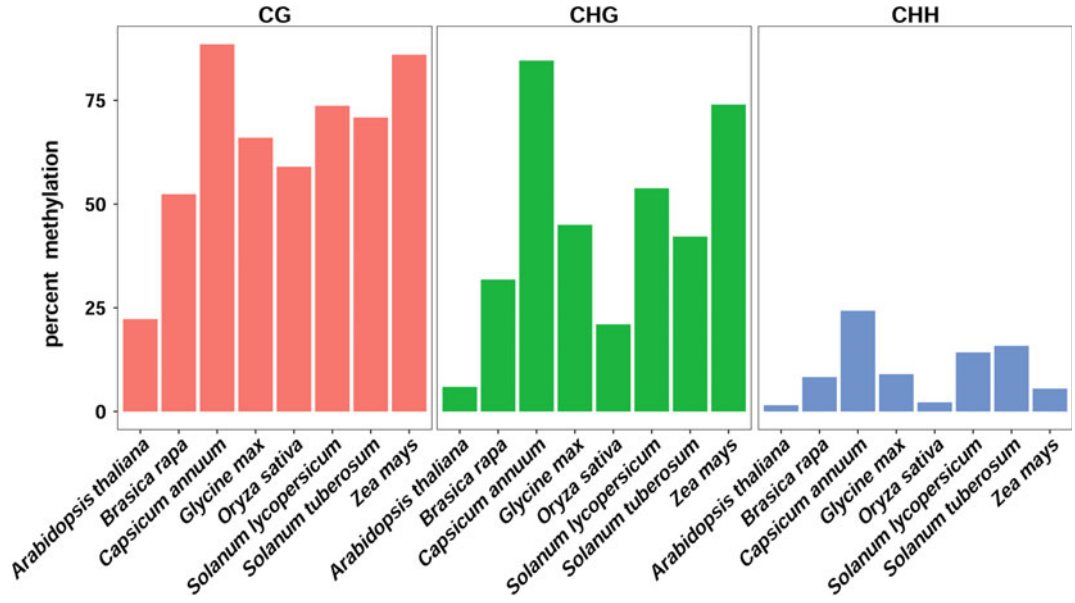

Fig. 11.1 Global cytosine methylation in *Capsicum annuum* compared to different plant species

Table 11.1 Global methylation level across different species along with their genome information at all cytosine methylation contexts

Species	Genome size	CG (%)	CHG (%)	CHH (%)
Capsicum annuum	2.8–3 gigabases	88.5	84.6	24.3
Arabidopsis thaliana	~135 Mbp	22.3	5.92	1.51
Brassica rapa	~485 Mbp	52.4	31.8	8.3
Oryza sativa	~500 Mbp	59	21	2.2
Solanum lycopersicum	~950 Mbp	73.7	53.82	14.26
Solanum tuberosum	~840 Mbp	70.9	42.19	15.84
Glycine max	1115 Mbp	66	45	9
Zea mays	2.4 gigabases	86	74	5.5

Table 11.2 Correlation of cytosine methylation variations at all methylation contexts compared to genome size variation with reference to *Capsicum annuum* genome

	Capsicum genome size (fold larger)	Increase in CG methylation in *Capsicum* (in fold)	Increase in CHG methylation in *Capsicum* (in fold)	Increase in CHH methylation in *Capsicum* (in fold)
Arabidopsis thaliana	~22	3.95	14.19	15.89
Brassica rapa, *Oryza sativa*	~6–7	1.5–1.7	2.7–4	2.8–10
Solanum lycopersicum, *Solanum tuberosum*, *Glycine max*	~3–4	1.2–1.4	1.6–1.8	1.6–2.7
Zea mays	~1.4	1.02	1.14	4.36

The genome size of *Capsicum annuum* is compared to genomes of different species, and increment in cytosine methylation (in fold) at all contexts is compared to methylation in different species

also decrease. *Capsicum* genome which is ~1.4-fold larger than the maize genome has shown less variation at cytosine methylation at CG and CHG contexts, while the cytosine methylation in CHH context has significantly higher variations (~4.36) compared to maize, suggesting its potential role in the *Capsicum* fruit development. Compared to *S. lycopersicum*, *S. tuberosum,* and *Glycine max*, the *Capsicum* genome is 3–4-fold larger and has 1.2–2.7-fold cytosine methylation variations across all contexts (Table 11.2).

11.3 Genebody Methylation Distribution

The term genebody methylation is self-evident, which indicates toward the enrichment of mCs within the transcribed regions of protein-coding genes itself and was first narrated in *A. Thaliana* (Tran et al. 2005). Cytosine methylation in intragenic region is mainly considered as genebody methylation, primarily occurring at euchromatic regions with high number of methylated CpG sites (Lister et al. 2008; Feng et al. 2010; Regulski et al. 2013). Additionally, context of cytosine methylation, methylation density, and methylation location within the gene could shed light on essential information on the enzymatic pathways and their functional consequences responsible for controlled regulation of methylation instances (Takuno and Gaut 2012, 2013). Methylation at genebody level is mainly characterized by enrichment of CG cytosine methylation (mCG) confined to the transcribed region along with reduction in cytosine methylation at transcriptional start site (TSS) and transcriptional termination site (TTS; Bewick and Schmitz 2017). The overall genebody methylation in *Capsicum* is higher as compared to genebody methylation in tomato and potato (Wang et al. 2018) of same family, and it was observed that gene body has shown similar pattern of mCs level at CG and CHH contexts to that in tomato and potato, but the overall average cytosine methylation was found to be highest in *Capsicum* than both tomato and potato. Another interesting observation is that in *Capsicum* genebody regions in mCHG context, after the TSS and before the TTS, were highly differentially methylated, while in tomato as well as in potato the regions to the genebody vicinity, i.e., prior to TSS and following to TTS, were mainly observed to have high level of differential mCs. This suggests that higher genebody methylation at CHG context is potentially responsible for the maintenance of genebody methylation in *Capsicum* species.

Furthermore, an overall high average cytosine methylation at genebody level across all contexts might be indicative of transcriptional repression of REs or activation in *Capsicum* (Fig. 11.2a–c). Further, it was noted that genebody methylation genes frequently come under the category of housekeeping genes with dense cytosine methylation and very often the pattern and type of cytosine methylation in gene body reflects their expression status. Further, genebody methylation is found to be common feature among interspecific transcriptionally active orthologous genes, indicating functional conservation (Feng et al. 2010; Takuno and Gaut 2013; Bewick et al. 2016). It was noted that the gene which is developmentally regulated or whose expression is regulated at specific developmental stage predominantly lacks the CpG genebody methylation (Coleman-Derr and Zilberman 2012). Further, chromosomal distribution of average methylation across all *Capsicum* chromosomes was mentioned in Fig. 11.2d where CG and CHG methylation is comparatively higher than CHH methylation at all chromosomes. Also, it was observed that chromosomes 1, 3–6, 8, and 12 preferentially have less methylation at their both ends compared to rest of the regions (Fig. 11.2d). Moreover, it was hypothesized that the genebody methylation has positive correlation with gene expression and may potentially regulate the alternative splicing by precisely improving the intron–exon definition (Maunakea et al. 2010; Regulski et al. 2013). Notwithstanding to their wider presence in the genome, the biological functions of genebody methylation largely remain unclear. It was also suggested that highly dense mCs at genebody region can silence the

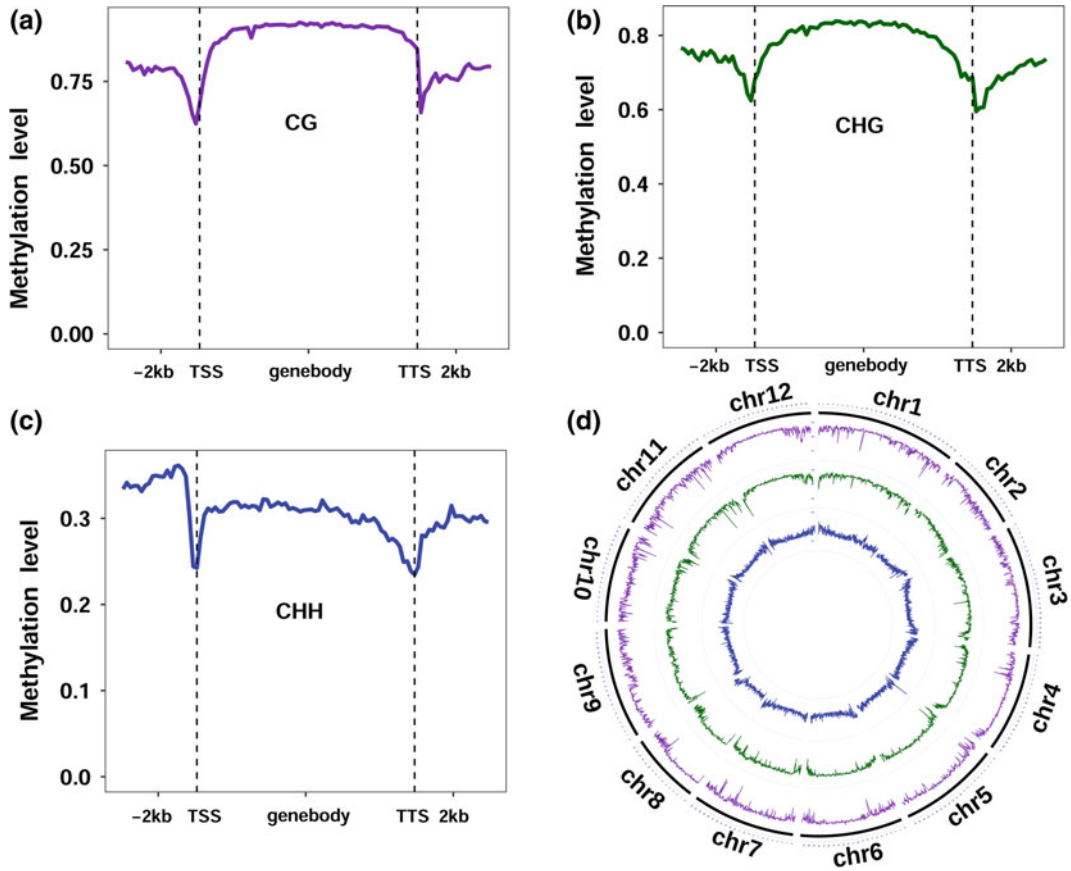

Fig. 11.2 Average cytosine methylation at CG, CHG, and CHH contexts in 2 kb upstream promoter region before TSS site, gene body region, and 2 kb downstream region after TTS site (**a**–**c**); circos representing average methylation distribution at all 12 *Capsicum* chromosomes. From outside to inside, the first lane represents chromosomes with their length information, 2–4th lane represents average methylation at CG, CHG, and CHH contexts, respectively (**d**)

repetitive DNA elements occurring within the gene body (Yoder et al. 1997). Moreover, methylation at genebody level is mainly maintained by the CHG methyltransferases of the chromomethylase gene family (Bewick and Schmitz 2017).

11.4 Promoter Methylation

In plants, cytosine methylation in promoter region is found to play a crucial role in managing the diverse developmental process by controlling the genes via repressing their expression. Unlike the mCs at gene body in CG context which shows positive regulation with transcribing genes, the promoter with dense mCs generally negatively controlled the expression of transcribing genes (Zemach et al. 2010; Wang et al. 2015). The CpG islands (CGIs) in promoter regions are generally unmethylated, thereby facilitating smooth binding between promoter region and proteins, and in arabidopsis, most of the endogenous genes were observed with less frequency of mCs in their promoter regions (Zhang 2008). Hitherto, several studies have been reported promoter methylation in its association with transcriptional gene silencing (Bewick et al. 2016; O'Malley et al. 2016; Lang et al. 2017). Till date, there is no direct study reported on dynamic of cytosine methylation in

Capsicum fruit and its development. We are working on categorizing the global cytosine methylation in *Capsicum* at different genomic levels. Our study suggested that compared to genebody methylation, the upstream 2 kb promoter region is less methylated in CG and CHH contexts, while in CHH methylation context, it is preferentially highly methylated than gene body, suggesting potential role of CHH cytosine methylation in regulation of gene expression as compared to CG and CHG contexts (Fig. 11.2a–c). Recently, it was found that promoter methylation is also responsible for regulation of different transition phases from early fruit to ripen fruit during its development and ripening. Manning et al. (2006) identified an epiallele in colorless non-ripening (*Cnr*) genes of tomato representing natural epigenetic mutation and whose hypermethylated promoter causes gene repression. In an another instance, promoter hypermethylation in *PcMYB10* gene which is responsible for anthocyanin accumulation in pear fruit skin drew attention toward plausible role of methylation in regulation of different aspects of development and ripening process in fruits (Wang et al. 2013).

11.5 Methylation of Transposable Elements

The mobile genetic elements aka transposable elements (TEs) are present in almost every genome and generally considered as 'parasitic' or 'selfish' elements. Mostly plant genome is enriched with long terminal repeat (LTR) retrotransposons and miniature inverted transposable elements (MITEs) among the diverse type of TEs (Casacuberta and Santiago 2003). TEs are integral part of constitutive heterochromatin and play a significant role in genome expansion and genome evolution (Slotkin and Martienssen 2007; Vicient and Casacuberta 2017). Due to larger in size, the LTR retrotransposons are predominant in almost all plant genomes, and in *Capsicum*, excess of single type of LTR retrotransposons could shed light on the genome expansion.

The *Capsicum* genome lacks whole-genome duplication, and ~81% of its genome comprises various transposable elements, while ~61% of tomato as well as potato (Park et al. 2012) genome were composed of TEs. Both LTR retrotransposons (70.3%) and DNA transposons (4.5%) were most abundant among the all plant TEs categories in *Capsicum* and LTR retrotransposons accorded more to genome expansion as compared to tomato (50.3%) and potato (47.2%; Park et al. 2012; Qin et al. 2014). The level of mCs acts as a key epigenetic signal which could repress the activation and transcription of TEs, controls the gene expression, and thereby can impact on the phenotypic variations (Zakrzewski et al. 2017). Moreover, the cytosine methylation pattern in transposable element is similar in *Capsicum*, tomato, and potato at both CG and CHG contexts which potentially provides mechanism of TE silencing and its inheritance. But the overall cytosine methylation at transposable element is highest than both tomato and potato, suggesting that the TEs were preferentially methylated in *Capsicum* genome. In contrast to CG and CHG methylation, CHH methylation at transposable element showed slight opposite pattern as those in tomato and potato and overall CHH methylation higher than both tomato and potato, suggesting potential maintenance of CHH methylation in transposable elements by de novo during the course of genome expansion (Fig. 11.3). Generally, active TEs target transcribing genes for potential insertion and can cause chromosomal breakage, genome rearrangement as well as illicit recombination. Like promoter methylation, cytosine methylation in TEs can suppress the expression of neighboring genes through posing as enhancers or promoters (Girard and Freeling 1999). Further, it was hypothesized that methylation of TEs is negatively correlated with diminishing expression of neighboring genes. Afterward, in *A. thaliana*, it was found that high amplitude of methylated TEs along with the abundance of TEs can reduce the expression of their neighboring genes which is independent of its chromosomal location (Hollister and Gaut 2009).

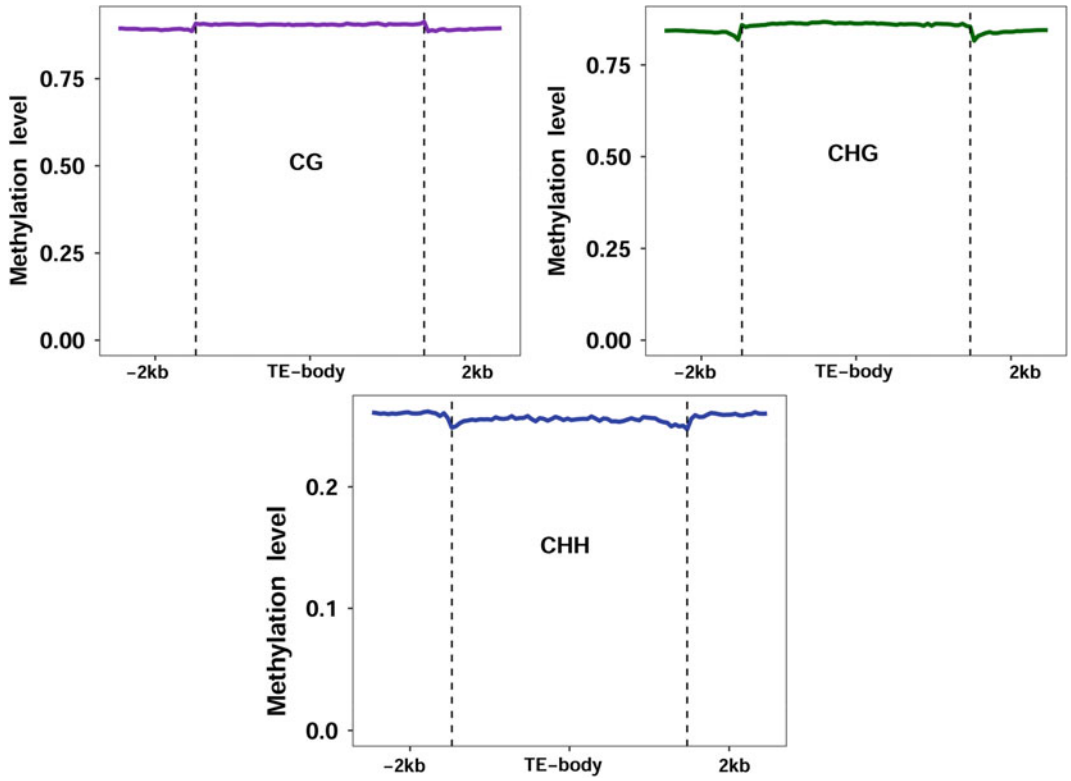

Fig. 11.3 DNA methylation patterns in *Capsicum annuum* across transposable elements (TEs). The metaplot shows cytosine methylation depicted in 2 kb vicinity of TEs at all three cytosine methylation contexts

11.6 Epigenetics of Cytosine Methylation in Hybrids

In plants, the status of cytosine methylation is easier to detect, which provided a wider scope in regulation of developmental process and tissue-specific expression of genes. Hybrid vigor or heterosis is an incident where hybrids from genetically different individuals show better traits in terms of enhanced phenotypic and functional features relative to their parents (Cheng et al. 2007). Till date, dynamics of mCs in hybrids and in their parent lines were reported substantially in *Capsicum* (Xu et al. 2015), arabidopsis (Kawanabe et al. 2016; Lauss et al. 2017), potato (Sanetomo and Hosaka 2011), *Zea mays* (Zhao et al. 2007; Lauria et al. 2014), rice (Xiong et al. 1999; Dong et al. 2006; Takamiya et al. 2008), and in sorghum (Yi et al. 2005; Zhang et al. 2007). These studies suggested notable variations in the level of mCs and their pattern in heterosis compared to parent plants (Zhao et al. 2008). The yield and quality of *Capsicum* has been improved through implementation of heterosis in *Capsicum* breeding, but the molecular and genetic bases of higher level of performance of heterosis relative to its parents remain elusive. Xu et al. (2015) analyzed the reciprocal hybrids with the help of MSAP from two genotypes of *hot pepper* having purple and green cotyledon and observed increased mCs level in hybrids. The overall observed DNA methylation in F1 hybrids of D85 × D34 and D34 × d85 was 67 and 64.36%, respectively, which was higher than mid-parent value (64.83%). Furthermore, in addition to the overall methylation, dynamic pattern of DNA methylation also varies during heterosis (Xu et al. 2015).

The term 'graft hybrid' defined as genetically distinguishable plants which are produced through asexual combination of different plant species. The grafting has been considerably used to improve the production of crops. Hitherto, studies based on grafting revealed the interchange of nucleic acid molecules across the plants used as grafting partners, thereby indicating toward the molecular basis of genetic variations facilitated by grafting (Wu et al. 2013). Furthermore, reports in *A. thaliana* concluded that graft hybrid shows epigenetic variations at mCs level induced due to grafting process compared to normal seed plants (Molnar et al. 2010), in tomato, eggplant, and pepper of Solanaceae family (Wu et al. 2013) and *Cucurbitaceae* family (Avramidou et al. 2015). Furthermore, after reciprocal interspecies grafting, considerable variations at mCs were detected in grafted Solanaceae species at genome wide level using MSAP, while significantly altered global mCs level in tomato, eggplant, and pepper scion was observed at both CG and CHG contexts. Moreover, self-pollinated progeny of graft hybrid was observed to inherit the variations of mCs, suggesting potentiality of grafting to introduce stable epigenetic variations transferable to the progeny (Wu et al. 2013). Further, in *C. annuum* grafting results in fruit shape and size variations, and graft hybrid indicated toward the potential role of siRNA-mediated epigenetic regulation of genes responsible for maintenance of fruit shape (Tsaballa et al. 2013).

11.7 MiRNA-Mediated Methylation in *Capsicum*

As a conserved epigenetic mechanism, DNA methylation mainly regulates gene expression by epigenetic silencing of transcription. Both small and long ncRNA (lncRNA) are involved in epigenetic regulation of cytosine methylation and maintenance using RdDM pathway. Most of the instances of DNA methylation occurring through RdDM pathway are triggered by siRNAs and are involved in de novo maintenance of mCs at different contexts. Though in plants several studies have documented the DNA methylation events directed by miRNAs in plants, their regulation mechanism is not yet fully elucidated (Jia et al. 2011). Small RNAs (sRNAs) behave as indispensable triggers to regulate cytosine methylation at all mCs context, thereby regulating the transcriptional gene networks in most of the eukaryotes (Zilberman et al. 2003; Onodera et al. 2005; Teotia et al. 2017). In plant, primary miRNAs are transcribed by pol-II enzyme and are further cleaved into pre-miRNAs by dicer-like 1 (DCL1). RdDM is a major methyltransferase enzyme involved in maintenance and regulation of methylation phenomenon in plants. Hwang et al. (2013) identified miRNA-directed cleavage of *Capsicum* DRM methyltransferase which regulates and maintains cytosine methylation through de novo. In *Capsicum*, microRNA *Ca-mir-396* family regulates transcriptional silencing of REs and is responsible for de novo maintenance of mCs through targeting methyltransferases (Hwang et al. 2013). In spite of several research progresses in miRNA-mediated methylation regulation, still there is a need to focus on their mechanism for thorough understanding in plants, and especially in *Capsicum*. A lot of insides are yet to be explored to determine the more specific role of miRNA and other non-coding RNA-mediated DNA methylation for *Capsicum*.

11.8 Conclusion and Future Prospective

The epigenetic variations are much overlooked in most of the plant-breeding program dependent on DNA-based molecular markers. With the emerging evidence, so far the epigenetic landscape in *Capsicum* is under-explored. This work of DNA methylation profiling of fruit development in *Capsicum* could provide some insight about the overall epigenetic modification during fruit transition from unripe to ripe in *Capsicum* species. However, many more studies using different developmental stages of fruits separately and from contrasting genotypes will shed more light. Furthermore, recently developed

high-throughput methylome or histone sequencing using high-throughput sequencing technologies will tremendously help to study epigenetics mechanism of fruit development in *Capsicum* species. Therefore, plant engineering equipped with epigenetic variations might be informative in developing improved crop varieties with economically important traits. Till now, the discoveries of various mutants to demonstrate the epigenetic regulation in fruit development, such as *cnr* mutant, *rin* mutant, and *sldml2* mutant, have been performed on tomato fleshy fruit model which could facilitate the better understanding of controlled fruit ripening in *Capsicum* as well. Such information could help in improving the fruit quality and fruit harvesting for longer period. In the case of fruit development from unripe to ripe and quality of fruit, the differentially methylated regions kindred with various genes responsible for fruit ripening and fruit repressed ripening could manifest the targets for analysis of epigenetic differences across the fruit varieties. Thus, the assessment of epigenetic variation at different fruit developmental stages may help in improving and expanding the selection strategies, thereby helping in improving fruits traits like shelf life and quality across the agronomically important crops.

Acknowledgements This work was supported by the financial grant from the University Grants Commission (UGC), India, in the form of University of Potential For Excellence II grant to Jawaharlal Nehru University, New Delhi, India.

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