



Dynamics of DNA Methylation and Its Functions in Plant Growth and Development

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Epigenetic modifications in DNA bases and histone proteins play important roles in the regulation of gene expression and genome stability. Chemical modification of DNA base (e.g., addition of a methyl group at the fifth carbon of cytosine residue) switches on/off the gene expression during developmental process and environmental stresses. The dynamics of DNA base methylation depends mainly on the activities of the writer/eraser guided by non-coding RNA (ncRNA) and regulated by the developmental/environmental cues. *De novo* DNA methylation and active demethylation activities control the methylation level and regulate the gene expression. Identification of ncRNA involved in *de novo* DNA methylation, increased DNA methylation proteins guiding DNA demethylase, and methylation monitoring sequence that helps maintaining a balance between DNA methylation and demethylation is the recent developments that may resolve some of the enigmas. Such discoveries provide a better understanding of the dynamics/functions of DNA base methylation and epigenetic regulation of growth, development, and stress tolerance in crop plants. Identification of epigenetic pathways in animals, their existence/orthologs in plants, and functional validation might improve future strategies for epigenome editing toward climate-resilient, sustainable agriculture in this era of global climate change. The present review discusses the dynamics of DNA methylation (cytosine/adenine) in plants, its functions in regulating gene expression under abiotic/biotic stresses, developmental processes, and genome stability.

Keywords: DNA methylation, DNA modification, environmental stress, epigenetics, gene regulation, 5-methylcytosine, N⁶-methyladenine, plant growth

INTRODUCTION

Methylation of DNA bases at different positions (e.g., fifth carbon of cytosine and N⁶ of adenine) plays significant roles in epigenetic regulation of gene expression in both plants and animals (Zhang et al., 2006; Xiang et al., 2010; Kumar et al., 2018). Epigenomic changes such as methylation of DNA bases, modification of histone proteins, and changes in the biogenesis of non-coding RNAs (ncRNAs) influence chromatin structure (accessibility of the genetic information to transcriptional machinery), thus gene expression, and genome integrity/stability. Methylation of DNA bases is known to be an important regulator of biological processes, and interruption in DNA methylation homeostasis leads to several developmental abnormalities in plants

(e.g., *Arabidopsis thaliana*) and animals (e.g., mice; Slotkin and Martienssen, 2007; Lang et al., 2017). While DNA methylation is catalyzed by different methyltransferases (using S-adenosyl-methionine as a methyl group donor), active DNA demethylation uses enzyme-catalyzed base excision repair (BER) pathway (Penterman et al., 2007; Kumar et al., 2018; Li et al., 2018a). Although the RNA-directed DNA methylation (RdDM) pathway is vital for *de novo* DNA methylation in plants, it is not so important in mammals (Matzke and Mosher, 2014). Active DNA demethylation initiates with deamination and/or oxidation of 5-methylcytosine (5-mC) in mammals, but in plants, direct excision of 5-mC takes place using methylcytosine DNA glycosylase (Law and Jacobsen, 2010; Li et al., 2018a). Besides, covalent but reversible posttranslational histone modifications and interaction with DNA play important role in regulating chromatin condensation and DNA accessibility (Ooi et al., 2006; Wei et al., 2017). Various mechanisms involved in site-specific DNA base modifications and their functions in the regulation of gene expression are being deciphered in model plants like *Arabidopsis* (Wang et al., 2016; Pecinka et al., 2019). N⁶-methyladenine (6-mA) is another important modified DNA base (comparatively less abundant in plants) playing regulatory functions in animals and plants. It is considered to be essential for growth and development in *Arabidopsis* and rice (Liang et al., 2018; Xiao et al., 2018; Zhang et al., 2018b). Generally, a mutation in the gene encoding for component of DNA (de) methylation machinery or a regulatory factor does not cause lethality of the individual. Though *Arabidopsis* has been used as a model plant to understand the basic epigenetic machinery, the gathered information is validated and variations are being mapped in crop plants like rice (*Oryza sativa* L.). Efforts are also being made to identify the epigenetic marks associated with a trait of interest so that they can be utilized in crop improvement programs toward the development of climate-smart crops (Varotto et al., 2020). Nevertheless, DNA modifications appear to be crucial for developmental processes and protection from environmental stresses. Recent findings are unraveling the components (readers, writers, erasers, etc.) involved in DNA modification in plants. Such a recent understanding includes the necessity of a methylation-sensing genetic element in maintaining DNA (de)methylation homeostasis (Lei et al., 2015; Williams et al., 2015), the contribution of ncRNA in triggering *de novo* DNA methylation (Ye et al., 2016), and the role of increased DNA methylation protein in targeted DNA demethylation (Duan et al., 2017). The present review discusses the dynamics of DNA base methylation and its functions, particularly in controlling the activity of transposable elements (TEs), genome stability, regulation of gene expression during plant growth, development, and environmental stress.

DYNAMICS OF DNA METHYLATION

Variation in DNA methylation has been detected in many organisms, including viruses, prokaryotes, and eukaryotes (Berdis et al., 1998; Bell and Felsenfeld, 2000; Hoelzer et al., 2008). Methylation of DNA plays important roles in the

regulation of gene expression, growth, development, and protection from environmental stresses, as well as in stabilizing the genome (Zilberman et al., 2006; Mendizabal and Yi, 2016; Kumar et al., 2017a, 2018). DNA base modification in a context- and genomic region-specific manner is catalyzed by different enzymes through distinct pathways. Methylcytosine (5-mC), also known as the fifth base of DNA, was discovered long before the DNA was recognized as genetic material in a living cell. Although more attention is given to the conventional 5-mC, recent findings on additional base modifications [e.g., hydroxymethylcytosine (5-hmC), formylcytosine (5-fC), carboxylcytosine (5-caC), and N⁶-methyladenine (6-mA)] have resulted in overwhelming interest in epigenomic studies. In plants, cytosine methylation can occur in all contexts of cytosine (CG, CHG, and CHH, where H=A, C, or T; Lister et al., 2008; Wang et al., 2016). In *Arabidopsis* as well as in other plants, the heterochromatic regions are enriched with methylcytosines, generally in the repetitive sequences and TEs. However, TEs and 5-mC are also found to be interspersed in the euchromatic regions (Zhang et al., 2006; Rathore et al., 2020). The dynamics of DNA base methylation depends on the reversibility of the processes, which also controls switching on/off the gene. Diversity and complexity of epigenetic changes (DNA/histone modifications and ncRNA biogenesis) in different organisms are being discovered continuously, and the potential combinatorial interactions of epimarks indicate that epigenetic codons would be considerably more complex than it is thought today (Kumar et al., 2018).

Cytosine Methylation

Establishment, maintenance, and removal of cytosine methylation in different contexts/genomic regions in the plant genome occur through various pathways. While *de novo* cytosine methylation involves the RdDM pathway, maintenance of cytosine methylation in different sequence contexts depends on various DNA methyltransferases. Removal of 5-mC might occur either due to the malfunction of methyltransferase, scarcity of methyl donor (S-adenosylmethionine, AdoMet) during passive DNA demethylation, or by the active DNA demethylation process. In active DNA demethylation, a family of enzymes [bifunctional 5-methylcytosine DNA glycosylases–apurinic/aprimidinic lyase (APE1L)] initiate the demethylation process *via* BER pathway (Almeida and Sobol, 2007; Li et al., 2018a). While promoter methylation is generally associated with switching-off/downregulation of the gene, methylation of the coding sequence may have negative or positive effects on gene expression (Takuno and Gaut, 2013; Williams et al., 2015; Kumar et al., 2017a).

RdDM pathway is responsible for *de novo* methylation of DNA which utilizes small-interfering RNAs (siRNAs), scaffold RNAs, and many accessory proteins (**Figure 1**). Present understanding of the RdDM pathway in *Arabidopsis* (Law and Jacobsen, 2010; Matzke and Mosher, 2014; Zhang et al., 2018a) suggests that RNA polymerase IV (Pol IV) initiates the production of 24 nt siRNA (noncoding P4 RNA) which serves as the template for RNA-dependent RNA polymerase 2-mediated generation of double-stranded RNAs (dsRNA). Sawadee Homeodomain Homolog 1

(Gao et al., 2010; Law et al., 2010; Zhong et al., 2012; Liu et al., 2014). The DDR complex also interacts with the suppressor of variegation 3-9 homolog protein 2 (SUVH2) and SUVH9 which bind together to the preexisting methylcytosine and help recruiting Pol V (Zhong et al., 2012; Johnson et al., 2014). SUVH2 and SUVH9 recognize methylcytosine through their RING finger-associated and SET domains which are needed for genome-wide chromatin binding of Pol V through preexisting DNA methylation. The binding of SUVH9, also having zinc finger, even to the unmethylated DNA was reported to be sufficient enough to recruit Pol V for methylation of DNA and silencing of the gene (Johnson et al., 2014). Pol V can produce ncRNAs with different 5' ends from a locus, which indicates that it can start transcription without a promoter (Wierzbicki et al., 2008). The Pol V-generated scaffold RNAs are long enough to be detected by PCR and lack polyadenylation at 3' end; thus, they differ from mRNA (Wierzbicki et al., 2008).

Methylation of cytosine in hemimethylated CG dinucleotide, created due to DNA replication, is performed by methyltransferase 1 (MET1), an orthologue of DNA methyltransferase 1 in mammals. It adds methyl (CH₃) group at fifth carbon of cytosine in daughter strand of the replicated DNA (Figure 2A).

Recruitment of MET1 to the hemimethylated CG is mediated by variant in methylation proteins, which are UHRF1 orthologs (Woo et al., 2008). Methylation at CHG context in the daughter DNA strand is catalyzed mainly by chromomethylase 3 (CMT3) and to some extent by CMT2 (Stroud et al., 2014). SUVH4, SUVH5, and SUVH6 bind to the methylated CHG domain and facilitate the CMT3/CMT2 function (Du et al., 2012, 2014; Stroud et al., 2013). Mutation in SUVH4, SUVH5, and SUVH6 was reported to reduce CHG methylation in *Arabidopsis* (Ebbs and Bender, 2006; Stroud et al., 2013). Moreover, methylation at asymmetric CHH context is performed by DRM2 or CMT2 depending on the nature of the genomic region. At shorter transposons and repeat sequences in euchromatic regions, as well as at longer transposons in heterochromatin, DRM2 causes CHH methylation through the RdDM pathway (Zemach et al., 2013; Liu et al., 2014). Mutation in decreased DNA methylation 1 (DDM1), a chromatin-remodeling protein, causes impaired methylation by CMT2; DRM2 and CMT2 can also methylate cytosine in other contexts (Zhang et al., 2018a).

Demethylation of 5-Methylcytosine

Replacing 5-mC with cytosine (unmethylated) is an equally important phenomenon in the regulation of gene expression

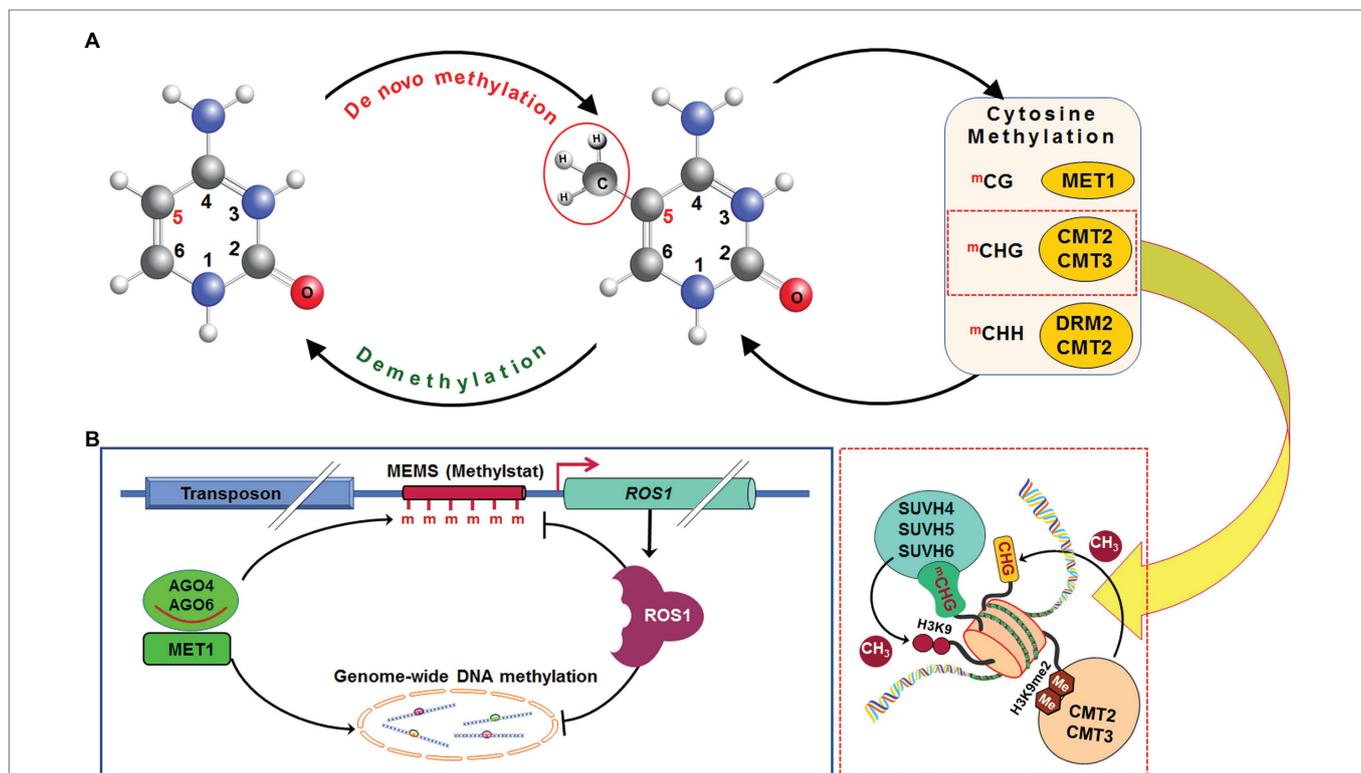


FIGURE 2 | Dynamics of DNA methylation in plants. *De novo* DNA methylation occurs in all (CG, CHG, and CHH; where H=A, C, or T) cytosine contexts. After replication of DNA, methylation in the CG context is maintained by methyltransferase 1 (MET1), while methylation in CHG context is maintained by chromomethylase 2 (CMT2) or CMT3, and methylation in CHH context is maintained by CMT2 or by DRM2 via RdDM pathway. Methylated CHG (mCHG) attracts histone H3 lysine 9 (H3K9)-specific suppressor of variegation 3-9 homolog protein 4 (SUVH4), SUVH5, and SUVH6 and generates dimethylated H3K9 (H3K9me2), which enables CMT2 and CMT3 (A). Methylation of methylation monitoring sequence (MEMS), also known as “methylstat” present in the promoter of the Repressor of silencing 1 (*Ros1*) is necessary for transcription of the *Ros1* gene. Cytosine methylation at MEMS is controlled by MET1/RdDM and *Ros1* itself. This helps to sense/monitor the level of methylation and regulate DNA (de)methylation homeostasis (B). CH₃, methyl group, Me/m, methylation (Redrawn from Zhang et al., 2018a).

through DNA methylation. Thus, methylation level is dynamically maintained by DNA (de)methylation. Passive (non-enzymatic) DNA demethylation occurs due to the loss of methylase activity during DNA replication (Mayer et al., 2000; Li et al., 2018a). Passive demethylation (reduced expression of MET1) was proposed to be responsible for demethylation in the central cell of female gametophyte, which develops into endosperm in seed after fertilization (Jullien et al., 2008; Kawashima and Berger, 2014). However, Park et al. (2016) recently reported maintenance of the methylation level in the central cell of *Arabidopsis* and rice. Calarco et al. (2012) reported maintenance of methylation in CG and CHG contexts during microsporogenesis which might be responsible for epigenetic inheritance in *Arabidopsis*. The vegetative nucleus in pollen shows very high methylation in CHH context, while the sperm cells show reduced CHH methylation due to reduced RdDM activity.

DNA methylation is also erased by active (enzymatic) DNA demethylation. While the active DNA demethylation process requires a family of enzymes, only one enzyme (methyltransferase) can accomplish the methylation process. In mammals, active DNA demethylation occurs through the BER pathway deploying DNA glycosylase wherein a 5-mC gets removed by TET dioxygenase-mediated oxidation of 5-hmC (Wu and Zhang, 2017). But in plants, a family of bifunctional DNA glycosylases—APE1Ls initiates the process through the BER pathway (Li et al., 2018a). Plant DNA glycosylase binds to 5-mC and removes it directly by breaking the glycosylic bond between the base and deoxyribose sugar. Subsequently, it acts as APE1Ls and breaks the DNA backbone producing an abasic site. APE1L and ZDP (a DNA polynucleotide 3'-phosphatase) generate 3' OH; later on, the gap gets filled by the actions of DNA polymerase and ligase (Martinez-Macias et al., 2012; Lee et al., 2014; Li et al., 2015). In *Arabidopsis*, four known bifunctional DNA glycosylases include Repressor of silencing 1 (Ros1), Demeter (DME), Demeter-like protein 2 (DML2), and DML3 (Ortega-Galisteo et al., 2008). These glycosylases can remove 5-mC from any sequence context (Morales-Ruiz et al., 2006; Penterman et al., 2007; Zhu et al., 2007). DME is preferentially expressed in the companion (vegetative) cell of male and central cell of female gametes (Huh et al., 2008).

DME-favored demethylation of AT-rich TEs in euchromatin leads to changes in the expression of the nearby genes (Gehring et al., 2009; Hsieh et al., 2009; Ibarra et al., 2012). ROS1 demethylates TEs, which affects transposon activity and transcriptional silencing of the nearby gene (Tang et al., 2016). ROS1 also demethylates the RdDM-independent regions (He et al., 2009; Gao et al., 2010). The genomic regions targeted for ROS1-mediated demethylation are characterized by reduced H3K27me and/or H3K9me2, and enhanced H3K18Ac and/or H3K27me3 epimarks (Tang et al., 2016). At certain ROS1 target sites, chromatin environment legitimate for ROS1 active DNA demethylation is founded by the binding of histone acetyltransferase increased DNA methylation 1 at methylated DNA, which acetylates H3 particularly at the sites deprived of H3K4me2 and H3K4me3 (Qian et al., 2012).

The promoter of *ROS1* contains a 39 bp cytosine methylation monitoring sequence (*MEMS*), which has decreased methylation

in *met1* and *RdDM* mutants (**Figure 2B**). Since hypomethylation of *MEMS* is accompanied by repression of *ROS1*, it indicates *MEMS* to function as a sensor/indicator of RdDM and MET1 activities. Thus, *MEMS* coordinates the methylation and demethylation processes through *ROS1* expression (Lei et al., 2015). *ROS1* promoter also contains a Helitron transposon upstream of the *MEMS*, which attracts cytosine methylation factors, and thus makes the promoter reactive according to the methylation level. In *ros1* mutants, hypermethylation of *MEMS* is accompanied by increased *ROS1* expression (Lei et al., 2015). Thus, like a thermostat, *MEMS* is considered to be a “methylstat” that senses and maintains ROS1-dependent methylation in plants (Lei et al., 2015; Williams et al., 2015). Regulation of demethylase gene by sensing methylation level has also been reported in maize (Erhard et al., 2015). Hence, the presence of such “methylstat” is considered to be an essential feature for cytosine methylation dynamics not only in plants but also in animals (Jones et al., 2015; Baylin and Jones, 2016).

Adenine Methylation

Like cytosine, adenine in DNA can also be methylated by the addition of a CH₃ group at the N⁶ or N¹ position (Ratel et al., 2006; Kumar et al., 2018). Methylation of adenine at exocyclic NH₂ on the sixth position (C⁶) of the purine ring forms N⁶-methyladenine (6-mA). Similarly, methylation of the cyclic N at the first position (N¹) results in the formation of N¹-methyladenine (1-mA) due to the presence of endogenous or environmental alkylating agents (Sedgwick et al., 2007). The 6-mA has become a common and well-known player in the regulation of gene expression and defense against phage among the prokaryotes. *AlkB* gene of *E. coli* is considered to be an inducible factor for adaptive response to the environment. An *AlkB* homolog in humans performs a similar function and exhibits significant functional roles (Westbye et al., 2008); therefore, similar factors are expected to be present in plants also. Interestingly, N⁷-methylguanine is also created in the presence of endogenous/environmental alkylating agents. A review by Law and Jacobsen (2010) suggested a certain degree of conservation in the mechanisms for the establishment and maintenance of DNA methylation between animals and plants. While conservation of some of the mechanisms has been confirmed including the role of siRNA in targeted DNA methylation and the role of methylated DNA-binding proteins, several questions regarding adenine methylation/demethylation homeostasis in plants remain to be answered.

Being detected in the lower eukaryotes at the beginning of this century, 6-mA was difficult to be detected in higher eukaryotes probably because of its lesser abundance; hence, earlier considered to be absent in most of the eukaryotes. However, recent advances in high-throughput, highly sensitive techniques, such as deep-sequencing and liquid chromatography coupled with mass spectrometry (LC-MS), have resulted in the detection of 6-mA, its localization in the genome followed by understanding its epigenetic functions in animals and plants (Huang et al., 2015; Liang et al., 2016). Even highly sensitive techniques like mass spectrometry could detect only a few

6-mA per million nucleotides in the genome of animals and plants, which suggests that the turnover (demethylation) rate of 6-mA might be faster. With more intensive studies on modified DNA bases, distribution patterns and possible functions of 6-mA in the animal system are becoming clear day by day; such information is still less known in plants.

The enzymes responsible for conversion of adenine into 6-mA in bacteria (DAMT), *C. elegans* (DAMT-1), *Bombyx mori* and mammals (METTL4), and human (N⁶AMT1) have been well reported (Vanyushin, 2005), but only a little is known about adenine methyltransferase in plants (Li et al., 2019). In green algae *Chlamydomonas*, 6-mA plays an important role in the transcription of genes and nucleosome positioning. In *Chlamydomonas*, adenine-methylome was reported to contain ~85,000 6-mA in AT context mostly in the promoter and linker regions. However, it possesses a low level of 5-mC (Fu et al., 2015). A Mg²⁺/Ca²⁺-dependent N⁶ adenine DNA methyltransferase (wadmtase) purified from wheat coleoptiles showed its potential in generating 6-mA. Wadmtase recognizes TGATCA hexanucleotide, but not GATC tetranucleotide, to methylate adenine (Fedoreyeva and Vanyushin, 2002). This stimulates examination of the presence and the potential role of 6-mA in higher eukaryotes. Analysis of the genomic DNA of *Arabidopsis* revealed the presence of 0.006% (lowest) 6-mA in root and 0.138% (highest) in rosette leaves (Liang et al., 2018). The general distribution of 6-mA in the *Arabidopsis* genome was observed to be near the transcription start site (TSS). The analysis also revealed that 6-mA, particularly those in the TSS region, positively correlates with the expression of the corresponding gene. Furthermore, the changes in 6-mA at different developmental stages of the plant were reported to be associated with gene activation. Although 5-mC and 6-mA both correlate with the transcription of genes in different manners, these epigenetic marks show a certain level of interdependence. Unfortunately, the proteins (readers and erases) that interact with 6-mA in eukaryotes have not yet been characterized.

Analysis of the rice genome revealed about 0.2% of 6-mA, a level similar to that reported in *C. reinhardtii* and *C. elegans* (Fu et al., 2015; Greer et al., 2015). Generally, 6-mA occurs in GAGG context and it was detected in 20% of the genes and 14% of TEs in rice (Zhou et al., 2018). The occurrence of 6-mA was also identified earlier in the GAGG context in *C. elegans*; however, the occurrence of 6-mA in GAGG context in rice is not palindromic, indicating its occurrence only in one strand of DNA (Zhou et al., 2018). While the presence of 6-mA in the promoter causes silencing of the gene, its occurrence in the coding region correlates with activation of the gene. Different possible functions of 6-mA include transcriptional silencing/activation, regulation of transgenerational chromatin functions, and stress response (Liang et al., 2020), as well as in other biological activities like DNA replication and mismatch repair in *E. coli* (Pukkila et al., 1983; Campbell and Kleckner, 1990; Kumar et al., 2018). However, the studies conducted so far report contrasting functions of 6-mA in different eukaryotes.

Demethylation of Methyladenine

To some extent, the mechanisms of adenine (de)methylation in animals have been understood. For example, a mutation in DNA

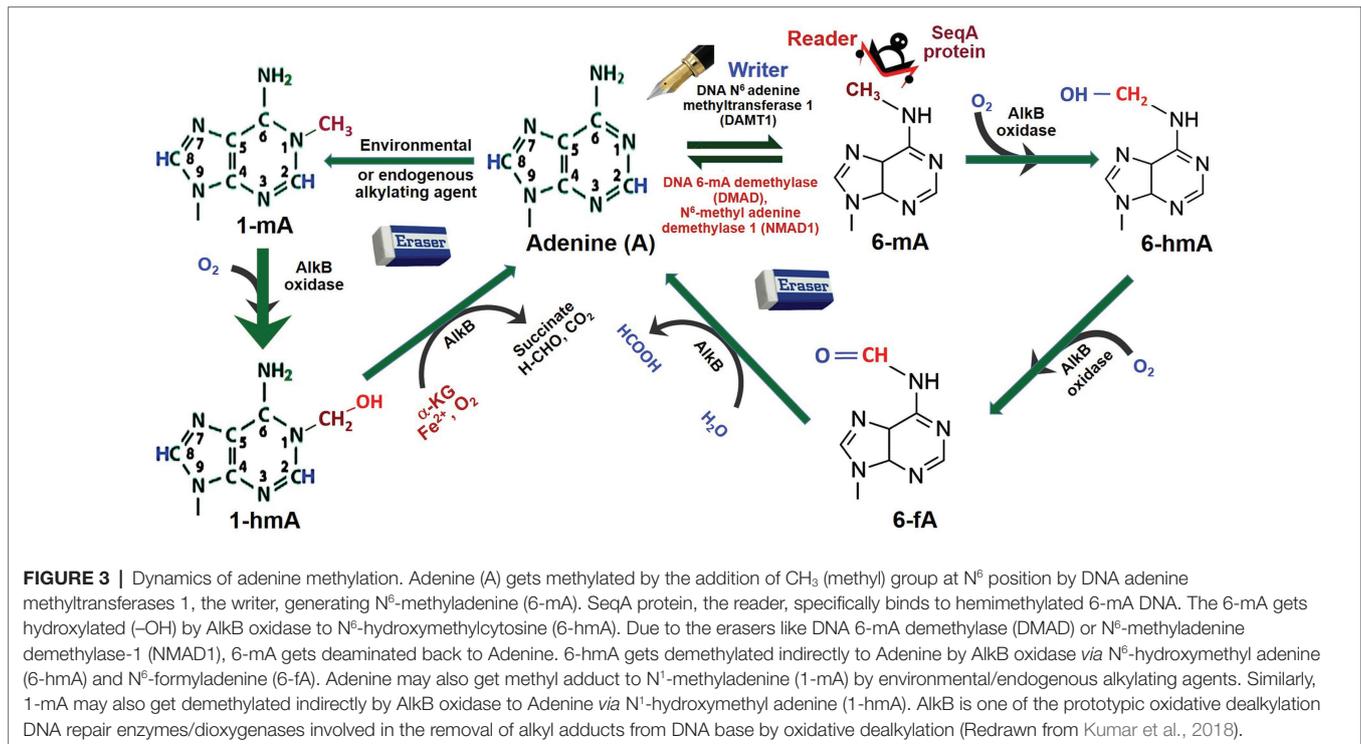
methyladenine demethylase (*DMAD*) resulted in the accumulation of 6-mA in *Drosophila*, which revealed its role in adenine methylation/demethylation homeostasis (Zhang et al., 2015). However, an adenine methyltransferase has not been identified in *Drosophila* (Shah et al., 2019). Moreover, oxidation of the attached methyl group at 6-mA by a demethylase (e.g., AlkB dioxygenase) results in its conversion to N⁶-hydroxymethyladenosine (6-hmA) and N⁶-formyladenosine (6-fA), and thus causes demethylation of adenine (Kumar et al., 2018; **Figure 3**). Studies suggest that AlkB family (Fe²⁺- and α -ketoglutarate-dependent dioxygenases involved in the removal of alkyl adducts from DNA bases by oxidative dealkylation) enzymes are important players in the demethylation of 6-mA (Fu et al., 2015; Iyer et al., 2016). Similarly, 1-mA may also get demethylated by AlkB oxidase and AlkB enzyme *via* N¹-hydroxymethyladenine (1-hmA).

Effects of Adenine Methylation

The presence of 6-mA in DNA is recognized by the binding of a specific effector molecule (reader) that may change chromatin conformation and/or transcriptional activity of the gene. Such readers, like SeqA protein, specifically bind to hemimethylated DNA with 6-mA. For example, polycomb proteins were reported as the coordinator between the accumulation of 6-mA and deactivated DMAD for transcriptionally repressing the gene (Yao et al., 2018). When present in the promoter, 6-mA generally represses the expression of the gene, but it may also function as an activator of the transcription process. These suggest that (de)methylation of adenine and cytosine takes place in a dynamic, coordinated, and context-specific manner. Therefore, it would be interesting to understand the interaction among the epimarks to investigate the complexity of epigenetic codons, which might help to answer several biological enigmas (Kumar, 2017; Kumar et al., 2018). A comprehensive understanding of such modifications and their functions in epigenetic regulation of gene expression would be essential for epigenetic manipulation of desirable traits in plants and animals (Kumar, 2019b).

FUNCTIONS OF METHYLATED DNA BASES

DNA base modifications, particularly cytosine methylation, were initially considered as a host defense mechanism in prokaryotes. Later on, it was found to play several vital functions in eukaryotes, mostly as a defense mechanism against jumping TEs to maintain genome integrity over the generations (Zhang et al., 2011). Over the last two decades, epigenetic changes in the plant genome have been reported during various developmental processes and environmental stresses (Bartels et al., 2018). Methylcytosine in the promoter region was reported to repress transcription of the gene by affecting the binding of TFs and by forming repressive-chromatin structures due to the interaction between methylated DNA-binding proteins (Bird, 2002). Regulatory flexibility is a characteristic feature of epigenetic mechanisms, particularly in response to environmental factors.



Similarly, gene/genome imprinting (preferential expression of the gene/genome coming either from male or female parents) is also regulated through epigenetic mechanisms. Imprinted genes are silenced by DNA and/or histone modifications. Demethylation of the maternal genome and activation of the genes in endosperm have been reported in *Arabidopsis*, rice (Hsieh et al., 2009; Luo et al., 2011; Rodrigues et al., 2013), and maize (Waters et al., 2011). Knockout of *ALKBH1* (a 6-mA demethylase) resulted in a higher 6-mA level and early flowering in rice (Zhou et al., 2018), suggesting that 6-mA plays an important role in reproductive development in rice. Nucleosome remodeler (e.g., DDM1) affects 5-mC content in plants (Tan et al., 2016). Decreased 6-mA content and no change in 5-mC level were reported in CRISPR/Cas9 *ddm1a/ddm1b* double mutants showing dwarfing and decreased seed-setting (Zhang et al., 2018b) suggesting that 6-mA is involved in vegetative and reproductive developments in rice. Thus, DNA base and histone protein modifications in combination with the nonhistone proteins define accessibility of the genes to help regulate their expression.

Source of Diversity and Heritable Variations

Some of the epigenetic changes may persist even after the reversal of the conditions that caused such changes, and some of them may inherit to the next generation as epigenetic alleles (epialleles). Such heritable epialleles are being considered as an additional source of diversity, which may be utilized in breeding programs, particularly in those crops where genetic diversity is reported to be scarce. The creation of natural

epialleles is much faster than that of alleles due to natural genetic mutation; however, the reversal rate of epialleles is also higher. Even then, epigenetics is considered to create more heritable epialleles and helps in the evolution process. Reports suggest that environmentally induced epigenetic changes in plants may be mitotically stable and meiotically inherited. Therefore, the emphasis is now given to such epigenetic changes as a source of variation. Transcriptional activation of Tos17 retrotransposon (RT) during tissue culture in rice was reported earlier, which gets repressed on plant regeneration (Liu et al., 2004). Studies demonstrate that activation of the transcription process and transposition of the RT in tissue-cultured calli are controlled through DNA hypomethylation (Cheng et al., 2006; Ding et al., 2007). Later on, it was reported that RT is demethylated by DNA glycosylase/lyase which promotes its movement during tissue culture in rice (La et al., 2011).

RdDM pathway was reported to respond to the environmental stimuli, which triggers epigenetic changes at particular loci toward the generation of heritable epialleles (Manning et al., 2006; Verhoeven et al., 2010). However, the importance of epialleles in crop breeding would require determining the extent of variation in epimarks among the individuals, the extent to which the epimarks affect the phenotype, and the heritability of the epimark-linked superior phenotypes. Moreover, several technical challenges in estimating the epigenetic variations and the level of epimark-associated phenotypic diversity do exist. With the continuously increasing understating of epigenomics, it is expected that our efficiency of exploiting epigenomic variability and deploying epigenome editing in crop improvement would become better and will have a significant impact on food security.

Regulation of Gene Expression

DNA modification in different cells/tissues is dynamically regulated during plant growth, development, and under varying environmental conditions. This indicates the important roles of DNA modifications in the regulation of gene expression and physiology. Base modifications occurring in a promoter, in the nearby regions, and/or within the gene-body, might affect the gene expression. Generally, DNA base modifications repress transcription of the gene; however, in certain cases, this may also promote transcription of the gene. Such an example is cytosine methylation in the promoter of *ROS1* which enhances its transcription in *Arabidopsis* (Williams et al., 2015). Base modification may strengthen the binding of certain transcription activators, or it may inhibit the binding of transcription repressor. However, the exact mechanisms of regulating gene expression by DNA methylation in the promoter region and the gene-body are not yet clear. Since, only 5% of the genes in *Arabidopsis* are methylated in the promoter region, which indicates that DNA methylation is not the sole epigenetic regulatory mechanism for controlling the expression of genes (Zhang et al., 2018a; Kumar and Mohapatra, 2021). Crop plants with a large genome size possess a higher number of TEs, and many of them are closer to genes affecting their expression. Thus, DNA modification plays a significant role in controlling the expression of the gene in crop plants compared to that in *Arabidopsis* which contains a limited number of TEs. However, DNA demethylase targets TEs present in the promoter to regulate stress-responsive genes (Le et al., 2014). That is why DNA methylation mutants in crop plants have been reported to have severe growth/developmental defects or lethal effects (Wei et al., 2014; Liu et al., 2015; Lang et al., 2017).

In *Arabidopsis*, about one-third of the genes are methylated in the gene body (Zhang et al., 2006). In general, TEs and repeat regions are heavily methylated in all cytosine contexts, but gene body methylation sparsely occurs in non-CG context (Zhang et al., 2006; Cokus et al., 2008; Takuno and Gaut, 2013). Gene body methylation occurs in the transcribed/coding region between the transcription start and termination sites (Bewick and Schmitz, 2017). Some of the introns in a gene may harbor TE or repeat elements, which are hypermethylated in all cytosine contexts and regulate mRNA processing. Loss of DNA methylation from a retrotransposon present in an intron of the homeotic gene was reported to cause alternate splicing and premature termination of the transcript. The role of 6-mA in the regulation of gene expression appears to be conserved among the plants. 6-mA occurs in the gene-body of a transcriptionally active gene (Liang et al., 2018; Zhang et al., 2018b; Zhou et al., 2018). 6-mA and 5-mC sites might show overlap, and 6-mA-containing genes may show a high degree of nucleosome arrays (Zhou et al., 2018). This indicates that 6-mA provides an additional layer of the epigenetic regulatory mechanism of gene expression or it works along with the other epigenetic markers.

Transposon Silencing and Genome Stability

Active TEs threaten genome stability/integrity due to the jumping of transposons or repeated insertion of retrotransposons.

Heterochromatins as well as transposon-or repeat-containing euchromatic regions in *Arabidopsis* are hypermethylated in all cytosine contexts (Cokus et al., 2008). CHH methylation in smaller transposons and at the ends of long transposons is established by the RdDM pathway, while it is taken care of by DDM1 and catalyzed by CMT2 at the internal positions of heterochromatin and long transposons (Zemach et al., 2013; Stroud et al., 2014). The active genes and inactive transposons in the maize genome are separated by RdDM-dependent hypermethylated CHH islands. Any loss of methylated CHH island leads to transcriptional activation of the transposon, suggesting that RdDM is needed to keep the transposons silenced (Li et al., 2015). Transposon reactivation due to DNA demethylation was observed in only a few *Arabidopsis* mutants, whereas *met1-cmt3* double mutants or *ddm1* mutants showed hypomethylation in CG and CHG contexts, and increased transposition of TEs (Mirouze et al., 2009; Tsukahara et al., 2009). DNA glycosylase/lyase 701 (a *ROS1* homolog) controls the movement of retrotransposon *Tos17* in rice, which indicates that DNA modification regulates transposon activity (La et al., 2011). DNA methylation also influences chromosomal interactions. In *Arabidopsis*, all the five chromosomes were reported to interact with each other forming a structure known as KNOT (Grob et al., 2014). Moreover, the chromosomal regions involved in the formation of KNOT are comprised of interactive heterochromatin islands (IHIs) containing several transposons (Feng et al., 2014; Grob et al., 2014). The KNOT engaged element represents a preferred landing site for TEs, which may be a part of the defense mechanisms for genome stability. Increased chromosome interaction between the Pol V-dependent methylation and the genes repressed by RdDM was recently reported (Rowley et al., 2017). This indicates that even chromosomal interactions might have regulatory functions in gene expression. Several studies suggest a wide variation in DNA methylation among different cells, tissues, organs, and species. The variation in DNA methylation level, GC content, and chromatin architecture among different species do not correlate with the genome size and thus serve as the source of diversity.

Genome Imprinting and Heterosis

FIS2, *FWA*, and *MEA* are some of the well-characterized genes responsible for genome imprinting in plants. While the allele from one parent is expressed, the allele from the other parent is silenced. This is known as genome imprinting (Gehring et al., 2006; Jullien et al., 2006). In flowering plants, megaspore mother cell (MMC) undergoes meiosis to form female reproductive organs. Similarly, the microspore mother cell (MiMC) undergoes meiosis to form male reproductive organs. Both MMC and MiMC undergo large-scale chromatin changes, including heterochromatin decondensation, during cell specification indicating a highly active transcriptional activity (She and Baroux, 2015). During MMC specification, CHH methylation transiently decreases and then gets restored but CG methylation remains stable. While methylation at CG and CHG contexts is maintained by *MET1* and *CMT3*, respectively, CHH methylation is maintained by *CMT2* or by the RdDM pathway (Gehring, 2019). Recently, DNA methylation was

profiled in the MiMC of *Arabidopsis*, wherein high levels of CG and CHG methylation but low level of CHH methylation were reported (Walker et al., 2018).

DME expression in the central cell before fertilization was reported to cause extensive demethylation of the maternal genome (Gehring et al., 2009; Hsieh et al., 2011) which causes the expression of the genes from the maternal genome. Zhang et al. (2011) carried out MSAP analysis and reported hypomethylation in the endosperm of *Sorghum bicolor* because of demethylation in the CG context. Genome-wide demethylation of TEs was also reported in the endosperm (Gehring et al., 2009; Hsieh et al., 2009). During male gametogenesis in plants, the transposons present in vegetative/companion cells are derepressed by transcription activator DME-mediated DNA demethylation and downregulated expression of a chromatin remodeler DDM1 (Lippman et al., 2004; Zhang et al., 2016). In the vegetative cell, the transcripts generated from TEs are processed into siRNAs and enter sperm cells to reinforce transposon silencing *via* DNA methylation (Martínez et al., 2016). In a double-fertilization system, one of the two sperm cells of pollen fertilizes the central cell of the female gametophyte (first fertilization) while the other fertilizes the egg cell (second fertilization), which produce endosperm and embryo, respectively. A sperm cell fertilizes with a central cell of the female gamete to form the endosperm wherein global demethylation, but reinforced CHH methylation at TEs, is observed (Ibarra et al., 2012). Another sperm cell fertilizes the egg cell to produce the embryo where the RdDM pathway maintains methylation (Figure 4). In *Arabidopsis* as well as in rice, the endosperm displays global DNA hypomethylation compared to that in the embryos (Gehring et al., 2009; Hsieh et al., 2009; Ibarra et al., 2012). Imprinting of endosperm is schemed by differential DNA methylation of the maternal and paternal genomes together with the polycomb group of genes (Hsieh et al., 2011). The maternal genomes of endosperm are less methylated (particularly in CG context) compared to that of the paternal genome (Gehring et al., 2009; Hsieh et al., 2009; Zhang et al., 2014; Klosinska et al., 2016). Certain maternally expressed genes (MEGs), for example, MEDEA in *Arabidopsis*, from the paternal genome are silenced due to repressive histone (H3K27me3) modification rather than by DNA methylation (Gehring et al., 2006; Jullien et al., 2006). In maize, the endosperm-specific MEGs are associated with H3K4me3 modification, while paternal alleles are suppressed by hypermethylation near the TSSs (Dong et al., 2017). Thus, the set of imprinted genes show that imprinting is a major epigenetic phenomenon affecting endosperm development in plants. RdDM pathway was reported to regulate parental gene imprinting at several loci in *Arabidopsis* (Vu et al., 2013). Thus, manipulation in genome imprinting through epigenome editing might help to develop a superior endosperm for improvements in seed crops (Berger, 2003; Kumar, 2019a).

Evidence suggests that F_1 hybrids are hypomethylated compared to their parental inbreds (Kovacevic et al., 2005). RNA amount polymorphism and protein amount polymorphism data in maize indicate that quantitative variations in the expression of certain loci might be responsible for the heterosis

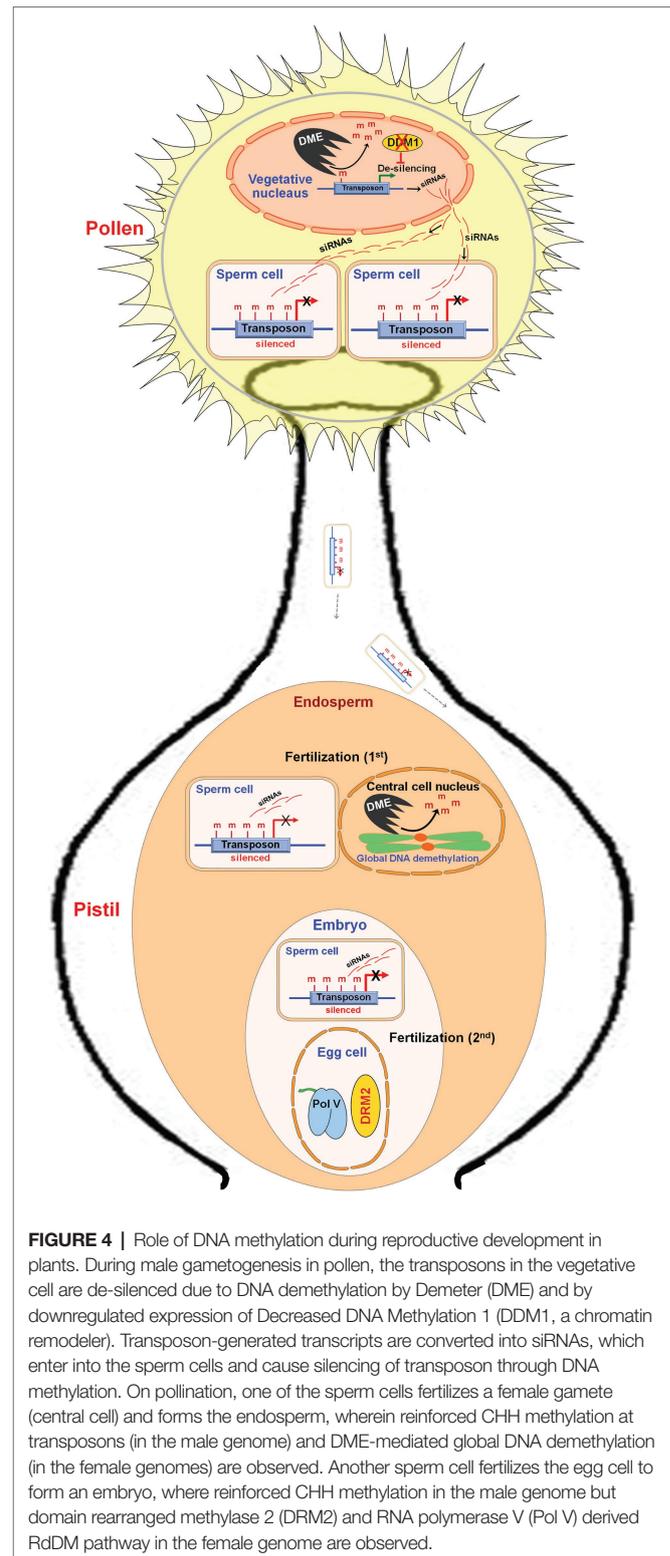


FIGURE 4 | Role of DNA methylation during reproductive development in plants. During male gametogenesis in pollen, the transposons in the vegetative cell are de-silenced due to DNA demethylation by Demeter (DME) and by downregulated expression of Decreased DNA Methylation 1 (DDM1, a chromatin remodeler). Transposon-generated transcripts are converted into siRNAs, which enter into the sperm cells and cause silencing of transposon through DNA methylation. On pollination, one of the sperm cells fertilizes a female gamete (central cell) and forms the endosperm, wherein reinforced CHH methylation at transposons (in the male genome) and DME-mediated global DNA demethylation (in the female genomes) are observed. Another sperm cell fertilizes the egg cell to form an embryo, where reinforced CHH methylation in the male genome but domain rearranged methylase 2 (DRM2) and RNA polymerase V (Pol V) derived RdDM pathway in the female genome are observed.

observed in the F_1 hybrid. Repeated selfing during the development of inbreds, giving more emphasis on combining ability, might result in a gradual accumulation of methylated loci (Kumar et al., 2017b). This gets repatterned and/or released

when the inbreds are crossed to develop a hybrid. Further, understanding the epigenetic regulation of embryo development might uncover the mysteries of apomixis (asexual reproduction through seeds) in a plant (Koltunow and Grossniklaus, 2003; Kumar, 2017; Rathore et al., 2020). If apomixis is successfully incorporated in commercial seed crops, heterosis can be preserved over the generations to overcome the current limitations of hybrid seed production.

Fruit Ripening, Seed Development, and Germination

DNA hypomethylation is reported to be a general feature at the promoter of many fruit ripening-associated genes as they contain binding sites for the ripening-associated transcription factors (Zhong et al., 2013; Lang et al., 2017). Ripening inhibitor binds to the methylated promoter of ripening genes to suppress the expression of the genes. The expression of DML2 (DNA demethylase) increases dramatically during the ripening of tomatoes. Active demethylation is required not only to activate ripening genes but also to suppress the ripening-inhibitor genes (Liu et al., 2015; Lang et al., 2017). Hypermethylation at CHH context in developing apple fruit, compared to that in the leaf, has been reported. A correlation between DNA hypomethylation and the smaller size of the fruit has also been reported (Daccord et al., 2017). Anthocyanin content in apple fruits has been reported to be negatively correlated with DNA methylation level at the promoter of MYB10 gene (Telias et al., 2011; El-Sharkawy et al., 2015).

Seed development is an essential process for food quality and productivity. During seed development in soybean, CHH methylation was reported to increase from 6% at the early stage to 11% in the late stage (An et al., 2017; Lin et al., 2017). Methylome analysis at the globular stage and seed germination in *Arabidopsis* and soybean showed a significant increase in methylation at CHH context (Lin et al., 2017). Twenty-five genes were observed to be differentially methylated during rice seed development, and endosperm cellularization was reported to be regulated by methylation dynamics (Xing et al., 2015). In *Brassica rapa*, a mutation in the RdDM pathway resulted in reproductive defects, which suggests that DNA modification is necessary for seed development (Grover et al., 2018). The maternal allele of components in the RdDM pathway was reported to be required for seed development in *Brassica rapa* (Grover et al., 2018). Demethylation of a retroelement (RE) Gy163 was observed to be associated with apomictic seed development in *Cenchrus ciliaris*. The RE Ty3-gypsy was found to be differentially methylated/expressed in the reproductive tissues of apomictic and sexual plants. Hypomethylation was observed in CHH context in reproductive (aposporous initial and mature embryo sac) tissues of apomictic plants, which was directly correlated with the activity of the RE (Rathore et al., 2020). Thus, epigenetic regulation of seed development appears to be a common process in different plant species.

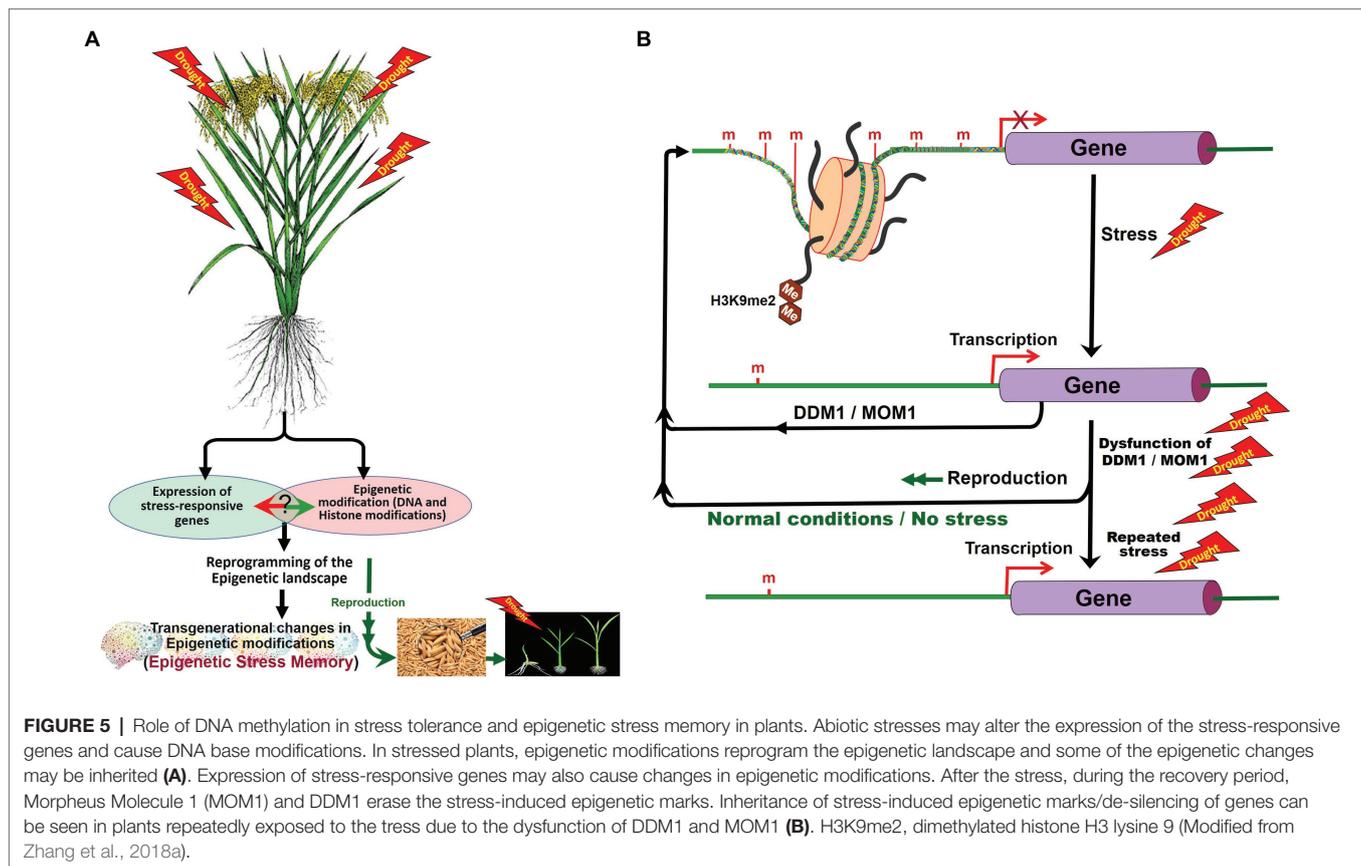
Tolerance to Abiotic Stress

Abiotic stresses have been reported to cause alterations in DNA methylation in plants. Heat stress-induced accumulation

of *ONSEN* retrotransposon was observed in *Arabidopsis* mutants impaired in the biogenesis of siRNAs (Ito et al., 2011). P5CS and δ -OAT genes were reported to be demethylated under osmotic stress in mother plants, but they restore methylation in the next generation under normal growth conditions (Zhang et al., 2013b), suggesting that epigenetic changes regulate the expression of the genes (Figure 5A). TEs have been reported to affect the expression of genes through their *cis-or trans*-regulatory elements, or even through serving as targets of epigenetic modifications (Seymour et al., 2014; Stuart et al., 2016; Wei and Cao, 2016). TEs being one of the targets of epigenetic machinery for controlling their activity as well as that of the nearby genes, they play important roles in the adaptation of plants to the changing global climate (Li et al., 2018b). Masuta et al., (2017) analyzed transcriptional and transpositional activation of *ONSEN* and observed heat stress-induced transposition of *ONSEN* during tissue culture. Heat stress response of soybean root showed a marginal (<10%) decrease in methylation; however, a significant change in the CHH context and TEs was observed (Hossain et al., 2017).

Several early studies on abiotic stresses indicate stress-induced DNA (de)methylation of stress-associated genes. Phosphate (Pi) starvation in rice was reported to cause more than 100 differentially methylated regions (DMRs) due to hypermethylation in CHH context mainly in the transposons near Pi-starvation-induced genes (Secco et al., 2015). Salt stress-induced changes in DNA methylation were reported to be partly inherited over the generations in *Arabidopsis*, especially through female gametes (Wibowo et al., 2016). Suppressor of DRM1, DRM2, and CMT3 (*SDC*) gene was reported to be silenced through DNA methylation of the promoter in vegetative tissues. Stress-induced activation of *SDC* due to repeated stress was also reported (Sanchez and Paszkowski, 2014). The RdDM pathway has been reported to alter TEs activity and gene expression involved in abiotic and biotic stress responses, plant development, and intercellular communication.

Recent studies suggest that 6-mA plays an important role in regulating gene expression in plants under environmental stress. 6-mA level showed dynamic changes in rice under heat, cold, and salt stress (Zhang et al., 2018b). Under heat stress, 6-mA level in the heat-tolerant rice genotype was 2.6-fold greater than that in the heat-sensitive rice genotype. 6-mA content in a heat stress master transcriptional regulator (heat shock transcription factor A1) exhibited a significant increase in heat-tolerant genotype than in heat-sensitive rice genotype. Decreased 6-mA level in HsfA1 repressor HSP70 in heat-tolerant genotype improves tolerance to heat stress (Zhang et al., 2018b). DDM1 and Morpheus molecule 1 (MOM1) in *Arabidopsis* were reported to be responsible for erasing stress-induced epigenetic marks after the stress. In double mutants for *ddm1-mom1*, stress-induced epigenetic marks are transmitted to the progeny, whereas in a single mutant either for *ddm1* or for *mom1*, stress-induced epigenetic marks are not inherited. This indicates that MOM1 and DDM1 function in checking the inheritance of stress-induced



epimarks (epigenetic stress memory; Iwasaki and Paszkowski, 2014). The stress-induced epigenetic memory may also be lost due to the passive demethylation process, if the progenies are grown under normal (without stress) conditions (Zhang et al., 2018a; Figure 5B).

Tolerance to Biotic Stress

In addition to the abiotic stresses, plants are also challenged by various biotic stresses like insect pests and diseases. Several studies have established the role of epigenetic variations in plant-microbe interaction mainly through gene regulation (Boyko and Kovalchuk, 2011; Diez et al., 2014; Zhu et al., 2015; Espinas et al., 2016). Another mechanism affecting the susceptibility of plants to pathogens has been reported which includes methylation of plant and viral genome (Wang et al., 2012; Sharma et al., 2013; Baulcombe and Dean, 2014). The importance of DNA methylation is emerging in transcriptional regulation of the virus-induced genes (Sahu et al., 2013; Ding and Wang, 2015; Wang et al., 2018a). Moreover, plants have evolved several defense mechanisms to cope up with viral infection mainly *via* siRNA-mediated antiviral silencing (Pumplin and Voinnet, 2013; Sharma et al., 2013). The siRNA cleaves/represses translation of the target mRNA and thus causes posttranscriptional gene silencing. Methylation of DNA base and/or histone protein causes gene silencing at the transcriptional level, which is known as transcriptional gene silencing (TGS). While DNA viruses are

targeted through TGS to restrict their proliferation (Rodríguez-Negrete et al., 2009), RNA viruses are not influenced by DNA methylation. However, methylation of RNA bases, e.g., N⁶-methyladenosine (m⁶A; Gokhale et al., 2016; Kumar and Mohapatra, 2021), controls viral replication as well as the interaction between virus and host (Brocard et al., 2017; Dang et al., 2019). Hundreds of DMRs were identified to be influenced by a viral infection in tobacco, several of which were reported to be associated with gene expression to regulate host antiviral defense (Wang et al., 2018b). Plants use methylation of viral genomic DNA to restrict its replication, while virus encodes viral suppressor proteins to protect it from getting methylated (Raja et al., 2010). The viral suppressors might interfere with the host methylation pathways to benefit the virus. A significant variation in methylation at CHH context was observed in *Arabidopsis* in response to different biotic stresses (Slaughter et al., 2012); however, the role of such alteration in DNA methylation in priming the plants against pests/diseases is not known (Wang et al., 2019). Interestingly, evidence shows that stress priming changes the epigenetic profile of plants which may improve the stress tolerance ability of the plant (Lopez Sanchez et al., 2016; Lämke and Bäurle, 2017; Varotto et al., 2020).

Stress Memory and Adaptation

It has been shown that plants can remember past environmental stress and use the memories to respond rapidly to the stress

when it recurs (Hilker and Schmölling, 2019). Plants have evolved various sensing and signaling mechanisms to respond appropriately to stress. Evidence gathered through various studies indicates that epigenetic variations are necessary as a part of the stress memories and adaptation in plants (Thiebaut et al., 2019). Moreover, plants have also evolved certain mechanisms by which they can memorize the events of past stress and trigger the responses to respond quickly/strongly to recurrent stress. Memorizing the events requires the storage of information, which might occur in plants (without a nervous system) in the form of chromatin architecture, transcription factor, posttranslational modifications, phytohormones, metabolites, etc., involved in the stress management (Xing et al., 2018; Hilker and Schmölling, 2019). The role of epigenetic variations in stress priming/memory is being studied (Crisp et al., 2016; Lämke and Baeurle, 2017; He and Li, 2018). Alterations to H3K4me3, which is a transcription activation mark, suggest that this epigenetic mark plays a role in transcription memory as it is enriched by drought stress and maintained at a higher level during the rehydration process (Kim et al., 2012). The small-RNA may also play a role in stress memory, as reported in the case of repeated drought stress to *Arabidopsis* (Ding et al., 2012). *Arabidopsis* plants with a mutation in *met1* were observed to be resistant to low humidity stress (Tricker et al., 2012). Moreover, a higher stomatal index was detected in *Arabidopsis* mutants for *dcl3* or *rdr6* under low humidity, indicating that RdDM pathway plays a role in remembering the stress. Transgenerational inheritance of epigenetic marks involves passing of the epigenetic changes through the germline without getting erased by the surveillance mechanisms (Lange and Schneider, 2010). Accumulating evidence indicates that short-term memory and transgenerational memories rely on epigenetic mechanisms; hence, they can be utilized in developing climate-smart crop varieties. However, many questions regarding the role of epigenetic marks in keeping the stress memory, their persistence, and stability during mitosis are still unanswered.

Many (~70%) stress-induced epigenetic alterations revert to the original state once the stress disappears, but a part of the epigenetic modifications might be carried forward as epigenetic stress memory (Crisp et al., 2016; Kumar, 2018). Therefore, utilizing such epialleles in breeding programs is still a challenging task because of the transgenerational stability of the environment-induced epigenetic alterations. Zheng et al. (2017) reported a high proportion of drought-induced DNA methylation in rice and maintenance of their pattern in successive generations under drought stress. Such findings suggested that epigenetic modifications can be utilized in improving stress responses of crop plants. Thus, epigenetic manipulation may become an efficient tool for crop improvement, as appropriate strategies are becoming available for the modulation of DNA methylation using chemicals or by genetic means, followed by the forward or reverse epigenetic approach. However, appropriate strategies would be required to ensure

retention of the transferred/introduced epialleles in the new genetic environment. Moreover, epigenome editing may help achieve the desired changes and adaptive advantages without entering into the controversy of genetic engineering (Gallego-Bartolomé et al., 2018; Kumar, 2019a).

CONCLUSION AND FUTURE PERSPECTIVES

Much progress has been made in our understanding of epigenetic regulation of gene expression, particularly in model plants like *Arabidopsis* and rice. Proteins and enzymes involved in DNA and histone modifications in plants are being characterized continuously. However, we still know a little about the components controlling targeted DNA (de) methylation during the developmental process and environmental stress. Does DNA modification interplay with other epigenetic marks and affect chromatin conformation are some of the enigmatic questions that need to be answered for a better understanding of epigenomics. Increasing focus on 6-mA as an additional epigenetic mark raises several questions like (1) which AlkB family protein acts as an eraser of 6-mA? (2) Whether/how does adenine methylation-and demethylation machinery interact with histone modification and transcription machinery? (3) Moreover, the readers of the 6-mA mark are yet to be discovered. DNA methylation pattern of different plant species varies because every species possesses a different set of DNA methyltransferases (MET1, CMT2, CMT3, DRM1, and DRM2), demethylases (DME, ROS1, DML2, and DML3), and RdDM pathway to (de) methylate the TEs, repeats, and genes to switch them on/off (Bartels et al., 2018).

Toward epigenetic manipulation, a catalytically inactive SpdCas9 fused with (de)methylase (SpdCas9-Tet1 and SpdCas9-Dnmt3a) was reported to be useful in epigenome editing in a site-specific manner in mammalian cells (Liu et al., 2016). In a recent study, on the development of the tool for targeted DNA demethylation in plants, Gallego-Bartolomé et al. (2018) reported efficient and targeted demethylation with minimal off-target effects in plant. This can also be used to answer some of the basic questions in epigenomics, to develop new strategies for modulating gene expression, and to create a new epiallele for the desired trait in plants. Gallego-Bartolomé et al. (2018) utilized fusion of the catalytic domain of human demethylase (TET1cd) with an artificial zinc finger targeting promoter of the *Flowering WAGENINGEN* (*FWA*) resulting in efficient and targeted demethylation, *FWA* upregulation, and heritable late-flowering phenotype in *Arabidopsis*. Recently (Taghbalout et al., 2016) used Casilio-ME for RNA-guided editing of 5-mC by targeting TET1 to specific genomic sites, and co-delivery of TET1 and other protein factors, to activate methylation-silenced genes. Similarly, Devesa-Guerra et al. (2020) fused the catalytic domain of ROS1 5-mC DNA glycosylase with dCas9 and reported that dCas9-ROS1 (but not the dCas9-TET1) can reactivate methylation-silenced genes by active demethylation.

With the advances in epigenomic tools and techniques, it can be expected that very soon we might be able to use epigenome editing to modulate phenotypic plasticity of plants (Kumar, 2019a) toward developing climate-smart crops for sustainable agriculture.

AUTHOR CONTRIBUTIONS

SK and TM conceived the review. SK prepared the preliminary draft. SK and TM revised the manuscript and approved the

final draft. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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