Asian Journal of Biochemistry, Genetics and Molecular Biology

11(3): 1-15, 2022; Article no.AJBGMB.88476
ISSN: 2582-3698

Regulatory Role of DNA Methylation and Its Significance in Plants

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2022/v11i330267

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/88476

Review Article

Received 17 April 2022
Accepted 29 June 2022
Published 01 July 2022

ABSTRACT

DNA methylation is a well-known epigenetic modification that is essential for gene regulation and genome stability. Anomalies in plant development can result from aberrant DNA methylation patterns. DNA methylation is much more important in plants with more complicated genomes when it comes to growth and abiotic stress tolerance. Dynamic regulation via de novo methylation, maintenance of methylation, and active demethylation, which are catalysed by diverse enzymes that are targeted by different regulatory mechanisms, results in a unique DNA methylation state. We explain DNA methylation in plants, including methylating and demethylating enzymes and regulatory changes, as well as the coordination of methylation and demethylation activities by a mechanism known as the methylstat. We also explain the roles of DNA methylation in regulating transposon silencing, gene expression, and chromosome interactions, as well as the intervention of DNA methylation in plant responses to biotic and abiotic stresses.

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Keywords: Transposon silencing; epialleles; DNA demethylases; epigenetic alterations; RNA-directed DNA methylation; gene expression.

1. INTRODUCTION

To maintain genomic stability and to regulate nuclear gene expression epigenetically, DNA methylation at the 5' position of cytosine plays a role in this process [1]. Chromatin structure and the accessibility of genetic information are affected by epigenetic alterations, including DNA methylation, histone variations and histone modifications, and a few non-coding RNA (ncRNA) modifications. Therefore, DNA methylation is essential to many biological functions, and interrupting DNA methylation may bring developmental problems in plants and animals, such as abortion of tomato fruit and embryo mortality in mice [2].

When it comes to plants and animals, DNA methylation is a conserved trait, and the particular patterns of genomic DNA methylation are vital for sustainable development. While S-adenosyl-l-methionine serves as the methyl donor for DNA methyltransferases in both plants and animals, the nucleotide excision recovery mechanism is responsible for DNA demethylation during active cell division and cell division during rest [2,3]. De novo methylation in plants requires an RNA-directed DNA methylation process, but its role is limited in animals [4,5]. When it comes to DNA demethylation, plants use 5-mC DNA glycosylases rather than oxidation or deamination to remove the 5-mC base [3,6].

Recent findings, as well as current knowledge of DNA methylation synchronization and its role in plants, are discussed in this Review. *Arabidopsis thaliana* is an excellent model plant for studying DNA methylation and demethylation pathways since changes in the plant DNA methylation and demethylation machinery along with regulatory changes often do not cause death. DNA methylation, on the other hand, is much more important in plants with more complicated genomes when it comes to growth and abiotic stress tolerance. The methylation of DNA in plants is regulated by several systems, some of which have just recently been discovered. Like the initial activation by ncRNAs of de novo methylation of DNA, the new multiprotein IDM (increased DNA methylation) targets active demethylation of DNA, and the methylation-sensing genetic factor controls the balance between DNA demethylation and methylation. The discussion also covers their functions in chromosomal interactions, gene function, plant growth, transposon targeted silencing, and plant tolerance to abiotic and biotic stresses. We examined the methylation of DNA patterns and their importance in the regulation of fruit development, nodulation of roots, and several other physiological changes.

2. DYNAMIC REGULATION OF DNA METHYLATION IN PLANTS

Methylation patterns in certain regions of DNA are indicative of how actively established, maintained, and eradication processes are controlled. Several enzymes, each directed to a different region of the genome through a different approach, carry out these functions. Cytidine sequence circumstances CHH, CG, and CHG are all subject to plant-specific methylation of DNA (H denotes A, T, or C) [5,7,8]. Transposons and other repeating nucleotide sequences are found in high concentrations in the chromatin structure (the area outside the nucleus where DNA replication takes place) in the genetic makeup of *Arabidopsis thaliana* [9-11]. The methylation of DNA is also found in the euchromatic chromosomal arms of transposons [7,12].

2.1 Establishment of DNA Methylation by the RNA-directed DNA Methylation Pathway

It is the RdDM route, which also includes polypeptides (proteins) and small interfering RNAs (siRNAs), that mediates the de novo methylation of DNA in plants, and also many other molecules. Recognized RNA-directed DNA methylation (RdDM) in *Arabidopsis thaliana* is now considered to trigger the formation of 24-nucleotide small interfering RNAs (siRNAs) via RNA POLYMERASE IV transcription (POL IV) [9,13]. When the transcript is copied, it is transformed into a double-stranded RNA (dsRNA) and cleaved into small interfering RNAs (siRNAs) via RDRP2 (also known as RDR2)-dependent copying. POL V produces complementary scaffold RNAs, which couple with the siRNAs that are deposited into AGO proteins such as AGO4 and AGO6, before the RNAs are released into the cellular environment. It has been shown that AGO4 corresponds with the Domains Rearranged Methyltransferase2

(DRM2), an enzyme that catalyzes de novo methylation of DNA in an independent-sequence way [14]. Domains Rearranged Methyltransferase2 (DRM2) and AGO4 may help this process along if RDM1, which attaches single-chain methylated DNA, may help it as well [15-18].

Pre-existing chromosomal alterations may make it easier for POL IV and POL V to find RdDM candidate sites. Heterodimethylated histone H3 K9 (H3K9me2) attaches with SHH1, which then recruits POL IV by recruiting SHH1 [12,19]. The chromatin-remodeling protein SNF2 DOMAIN-CONTAINING PROTEIN CLASSY 1 (CLSY1), which is linked with the POL-IV transcription factor and necessary for POL IV-dependent small interfering RNAs generation, also binds with SHH1 in addition [20,21]. It is necessary for POL V-transcribed non-coding RNAs to stay on the genetic material to serve as scRNAs; this association appears to be assisted by RRP6L1 (RRP6-LIKE 1), which is homologous of the yeast and proteins found in human RRP6 that can participate in RNA persistence [22]. It is also possible that the IDN2–IDN2 PARALOGUE (IDP) complex, which binds with the SWI/SNF chromatin-remodeling complex and includes SWI/SNF COMPLEX SUBUNIT SWI3B, will stabilize the siRNA–scRNA mating, since this complex attach RNA and contributes to transcriptional suppression in POL V-mediated by modifying nucleosome location [23-27].

Pre-existing chromosomal alterations may aid in the translocation of POL-IV and POL-V to RdDM intended locus. Through its Tudor domain, SHH1 attaches to Dimethyl-substituted histone H3K9me2 (H3 lysine 9) and recruits POL-IV [7,28]. A histone modifications protein called SNF2 domain-containing PROTEIN CLASSY 1 (CLSY1), which is linked to POL-IV and necessary for POL-IV reliant siRNA synthesis, also binds with SHH1 [29,30]. As part of the process of producing scRNA, the histone modifications protein DRD1 (DEFECTIVE IN RNADIRECTED DNA METHYLATION 1), the presumed systemic prolongation protein DMS3 (DEFECTIVE IN MERISTEM SILENCING 3), and RDM1 are required to link POL-V to chromosome [31]. SuVH2 and SuVH9, two SUPPRESSOR VARIEGATION 3-9 HOMOLOG PROTEIN family proteins, interact with the DDR complex physically, but they lack histone methyltransferase function [20,32]. POL V is thought to be recruited to the chromatin by post methylation of DNA because of the roles of SUPPRESSOR VARIEGATION 3-9 HOMOLOG PROTEIN9 (SUVH9) and SUPPRESSOR VARIEGATION 3-9 HOMOLOG PROTEIN2 (SUVH2), which identify methylated cytosine via their SRA and SET sites [17,18]. POL V and gene silence may be established by anchoring the zinc-finger of SUPPRESSOR VARIEGATION 3-9 HOMOLOG PROTEIN9 to unmethylated chromosomes [33,34].

2.2 Mechanisms Responsible for the Maintenance of DNA Methylation in Plants

Each plant has its unique way of keeping methylation of DNA maintained via sequence context of cytosine and the use of methyltransferase enzymes as a catalyzer, which may be controlled through several processes. METHYLTRANSFERASE 1 (MET1) maintains methylation of CG cytosine. After the replication of DNA, it identifies semi-methylated CG dinucleotides and the unchanged cytosine methylates in the daughter strand, which are true homologs (orthologues) of the human DNA (cytosine-5) methyltransferase 1 (DNMT1) enzyme [17,35]. *Arabidopsis thaliana* MET1METHYLTRANSFERASE 1 (MET1) lacks the cysteine enriched CXXC sites, which is expected to aid DNA-methyltransferase 1 (DNMT1) identify non-methylated CGs from hemi-methylated CGs, in contrast to mouse and human DNMT1 [21,36]. The recruitment of DNA-methyltransferase 1(DNMT1) to DNA via the protein called E3 ubiquitin ligase UHRF1 has been postulated for METHYLTRANSFERASE 1 (MET1), which has been hypothesized to be attracted to DNA by METHYLATION VARIANT proteins, that are UHRF1 true homologs (orthologues) essential for the maintenance of methylation of CG in a way similar to that described for DNA-methyltransferase 1 [37].
CHROMOMETHYLASE 3 (CMT3) and CHROMOMETHYLASE 2 (CMT2) are required for the maintenance of methylation of CHG in *Arabidopsis thaliana*, albeit to only a limited degree [38]. MET2A, the maize CMT3 ortholog CHROMOMETHYLASE 1, was shown to link to H3K9me2 via its chromatic regions and Bromo-adjacent homology (BAH) [15,39]. In addition to avoiding CHROMOMETHYLASE 3 (CMT3) binding to nucleosomes, preventing the CHROMOMETHYLASE 3 (CMT3)–H3K9me2 association also results in a total loss of CMT3 functionality [21,27]. *Arabidopsis thaliana* H3K9 methyltransferase SUVH4 and its homologs, SUVH5 and SUVH6, completely eliminate

H3K9me2 and diminish methylation of CHG, resulting in a significant decrease in the number of methylated CHG sites [40,41]. In order to conduct methylation of H3K9, methylated CHG attaches to the SET and RING-associated (SRA) domain of SUVH4 [42]. Therefore, regulatory feedback mechanisms, the methylation of CHG and H3K9me2 encourage one another.

2.3 Enzymatically Induced DNA De-methylation

Submissive demethylation of DNA occurs when there is insufficient methyltransferase activity in DNA or a deficiency of a methyl donor after DNA replication, failing to sustain methylation, called passive demethylation of DNA [31,43]. Activated demethylation of DNA is another way to remove methylation of DNA from the genome. There are a number of enzymes such as DNA methyltransferase enzyme, involved in the demethylation process of DNA, which is distinct from methylation of DNA. The enzyme that initiates this process is called a DNA demethylator. Nucleotide excision repair starts the active demethylation of DNA in plants through a series of dual-functional 5-mC DNA apyrimidinic/glycosylases-apurinic lysases [6,12,44]. DNA glycosylases and nucleotide excision fixing are also involved in the active demethylation of DNA in mammals. While the DNA glycosylases in plants can identify and eliminate the 5-mC nucleotide without the need for oxidation, the DNA glycosylases in mammals need the 5-mC base to be oxidized in order to trigger nucleotide removal [45].

DEMETER-LIKE PROTEIN 3 (DML3), DEMETER-LIKE PROTEIN 2 (DML2), DEMETER (DME), and Repressor of Silencing 1 (ROS1) are all 5-mC DNA glycosylases in the *Arabidopsis thaliana* genome [46], which can remove 5-mC from all cytosine strand sequences [47]. When it comes to the DML3, DML2, ROS1, and reproductive tissues, are found in all of the cells, but TRANSCRIPTIONAL ACTIVATOR DEMETER (DME) is found only in the companion cells of the male and female gametes i.e., in the male gametophyte vegetative cell and in the female gametophyte central cell, respectively [48].

Bifunctional enzymes first hydrolyze the glycosylic connection between the nucleotide and the 2-deoxyribose before cutting the backbone of DNA and creating an abasic site during demethylation of DNA. There will be a gap left after the 5-mC base has been removed, and this gap will be filled with either a 3-phosphor-α, β-unsaturated (Pu) aldehyde or a 3-phosphate (P3), depending on the kind of removal procedure used. Following that, the apyrimidini/apurinic endonuclease DNA-(APYRIMIDINIC SITE OR APURINIC) LYASE (APE1L) and the DNA phosphatase POLYNUCLEOTIDE 3′-PHOSPHATASE ZDP operate downstream of the -removal and -removal processes, respectively, to form a 3OH group that allows the gap to be filled by DNA polymerase and ligase enzymes [44,49]. *Arabidopsis thaliana* species of the genus *Asparagus* Demethylation of the prenatally imprinted genes FLOWERING WAGENINGEN and MEA (MEDEA) in the endosperm is dependent on DNA LIGASE 1 (LIG1). This is supported by its colocalization with ROS1, ZDP, and APE1L, as well as its interpretation that LIG1 is crucial for demethylation and activation of these genes [50].

2.4 Integrated Functioning of DNA Methylation and De-methylation

All known RdDM mutants have lower ROS1 expression of genes because ROS1 works in opposition to RdDM to suppress hypermethylation of DNA at some locations [11,51]. These findings show that the processes of methylation of DNA and active demethylation are closely linked. Over 2,000 chromosomal sites in *Arabidopsis thaliana* DNA methylome were shown to have ROS1 expression working against RdDM, according to recent research. Hypermethylation of these areas was seen in ros1-4 mutant plants, although not in the dual mutated plants with ROS1 and the biggest subunit of POL IV, DNADIRECTED POL IV SUBUNIT 1, which exhibit both POL IV and ROS1 dysfunction (NRPD1) [37,45,52]. The expression of ROS1 gene and levels of hypermethylation of DNA in the nrpd1-3 mutant was significantly reduced. Hypermethylation of genome in the nrpd1-3 mutant seems to be at least in part owing to reduced ROS1 expression, according to methylome analyses.

ROS1 gene expression was also significantly reduced in both RdDM and met1 mutants [53,54]. There is a 39-bp region of the ROS1 promoter that is reduced in met1 in methylation and RdDM mutants of the gene. ROS1 gene suppression and hypomethylation in this specific pattern sequence that the so-called DNA methylation monitoring sequence (MEMS) may

serve as an indicator of RdDM and MET1 activity and, as a result, could allow ROS1 transcriptional regulation to regulate both active and passive RdDM and MET1 demethylation of DNA. As predicted, ROS1-induced hypermethylation of DNA of MEMS was seen in ros1-deficient mutants, confirming this concept [41-45]. ROS1 activity is enhanced in ros1 mutants that have hypermethylated MEMS [55]. A transposon helitron in the ROS1 promoter region may assist attract DNA-methylation factors when making the promoter sensitive to methylation of DNA. Until yet, the exact transcription factors that enhance ROS1 transcription through methylation of DNA have not been discovered.

3. CELLULAR FUNCTIONS OF DNA METHYLATION IN PLANTS

Methylation of DNA determines the shape and availability of chromosome in conjunction with chromatin structure modification and non-histone proteins. Thus, methylation of DNA regulates the inheritance of traits (Supplementary Box 1), chromosomal connections, transposon silencing, and the expression of genes.

3.1 Gene Expression

It is possible for plants to have gene-associated methylation of DNA both in the transcript and in the promoter. It is common for methylation of DNA to suppress gene transcription, such as in the ROS1 gene of *Arabidopsis thaliana* and in various genes in tomato which prevent the ripening process [56]. Restricting transcription catalysts or promoting the adhesion of transcription suppressor is a one-way promoter DNA methylation suppresses transcription directly, while simultaneously inhibiting permissive histone modifications such as acetylation while simultaneously promoting repressive histone modifications such as H3K9me2 [57]. Gene transcription is activated by promoter methylation, although the mechanism is not well known. Some transcription activators and some transcription repressors may be inhibited or enhanced in their binding by DNA methylation, it is conceivable. Genes transcription is activated by promoter methylation, although the mechanism is not well known. If methylation of DNA may improve the binding of certain transcription catalysts and can hinder the engagement of several transcription inhibitors, then this may be a possible mechanism. At promoters, methylation of DNA is often caused by the dispersion of methylation machinery from neighboring transposons and some other repeats. Machines of demethylation of DNA also aim at transposons and repeats that are close to the genes in order to prevent transcriptional silence [41,58]. It is possible to silence genes triggered by methylation of DNA by demethylating the promoter DNA [22-24,59].

Only around 5 percent of the genes in *Arabidopsis thaliana* are methylated in the promoter region. Several genes transcription cannot be controlled by methylation of DNA, hence mutants with reduced or enhanced methylation of DNA often do not have serious growth or developmental problems [60,61]. There are more genes methylated in plant species with broader genomes because they have a relatively high transposon subject matter and a greater number of transposons that are near genes [48,55]. Methylation of DNA thus plays a more significant role in the regulation of a gene in various agricultural species than in *Arabidopsis thaliana*, and methylated DNA mutants in these plant species are often either dead or have serious growth and development abnormalities [50,62].

More than a third of *Arabidopsis thaliana* genomes have methylated gene bodies [43,47,50,63]. Non-CG methylation is rare in gene domains, unlike transposons and repeats, where methylation is common in all three environments. Methylation of DNA in gene bodies is quite low in comparison to transposons and repeats, which are typically extensively methylated in all 3 cytosine contexts [64]. Transcriptional start and stop sites are free of gene body methylation (gbM), which occurs more often at exons than any other region of the genome [65]. Genes containing gbM seem to be longer and more often transcribed in angiosperms, which is a common trait [20,66]. DNA damage caused by CMT3 in both *Conringia planisiliqua* and *Eutrema salsugineum* led to genome-wide depletion of gbM [33,67,68]. Histone H3.3, which suppresses histone H1-dependent chromatin packing and hence enhances the accessibility of chromatin to DNA methylases, lowered gbM levels in *Arabidopsis thaliana* with decreasing histone H3.3 levels [39,58].

3.2 Transposon Silencing

By moving DNA transposons or inserting new retrotransposons, transposons may put the genome at risk of instability. At least some of the

euchromatin areas that include transposon or repeat-containing transposons are highly methylated in *Arabidopsis thaliana* [69]. Methylation of CHH is maintained by RdDM in compact transposons and at the borders of elongated transposons, whereas methylation of CHH is maintained by DDM1 and catalyzed by CMT2 in the interior regions of heterochromatic, elongated transposons [62-64,70]. There are functional genes and dormant transposons throughout the maize genome, which are commonly divided by RdDM-dependent methylation of CHH islands, brief areas of enhanced CHH methylation. Loss of methylated CHH islands commonly results in transcription activation in surrounding transposons, which suggests that RdDM in maize is essential to avoid silenced transposons from being triggered by euchromatin from adjacent promoter regions [71]. Suppression of the DNA transposons in sugar beets seems to be driven by asymmetric methylation rather than retrotransposons or genes [70,72]. *Arabidopsis thaliana* and tomato show biased CHH methylation in the pericentromeric areas, with low levels of methylated CCG and over-representation of methylated CTA, CAT, or CAA; in comparison, asymmetric methylation in euchromatin regions is context-independent in the two dicots [73]. The context-dependent CHH methylation bias in rice and maize is distributed across the genomes of both monocots [61].

In *Arabidopsis thaliana* variants deficient in methylation of DNA, transposon removal of repression is prevalent, although transposition has only been seen for several transposons, probably due to post-transcriptional processes. Hypomethylation of DNA in CHG and CG contexts and increased transposition levels were also associated with DDM1, MET1, and CMT3 double malfunction or mutations, however, these conditions were uncommon causes of transposon mobilization [74,75].

3.3 Chromosomal Interactions

Epigenetic states of DNA may impact relationships between genomes, and this is why methylation of DNA is important. All 5 chromosomes in the *Arabidopsis thaliana* nuclei form a structure known as KNOT [76]. Interaction heterochromatin islands (IHIs) generate the KNOT structure in chromosomal arms, which are repressive chromatin areas characterized by a high abundance of transposons and a substantial enrichment of short RNAs [61,62,77]. All cytosine contexts of DNA hypomethylated in ddm1 and met1 mutants, and also the H3K9 methylation-defective tri mutant suvh4–suvh5–suvh6, are unaffected by IHI linkages [22,78]. In other words, chromosomal interactions at IHIs may not require methylation of DNA or H3K9me2. IHI loci are found to be ectopically expressed in mutants of ddm1 and met1 [76,79]. Methylation of DNA seems to reduce the possibility of chromosomal contacts, although the processes causing the emergence of new IHIs remain obscure. While certain RdDM areas seem to have a higher frequency of chromosomal contacts when compared to wild-type species, this does not necessarily mean that RdDM prohibits particular genomic regions from interconnecting with one another [67-69,80]. The enhanced chromosomal connection involving POL-V-dependent methylated DNA sites and RdDM-repressed distal genes was also discovered. This suggests the existence of an expression-regulating role for chromosome interactions [78,81].

4. SIGNIFICANCE OF DNA METHYLATION IN PLANT GROWTH AND DEVELOPMENT

A plant level of methylation of DNA is closely regulated during growth and development and across its life cycle, indicating crucial functions for DNA methylation in the physiology of plants (Fig. 1).

4.1 Imprinting and Gametogenesis

Multicellular female and male gametes of *Arabidopsis thaliana* plants enable them to adopt a double-fertilization method. Male gametophytes produce the endosperm and the embryo, respectively, by fertilization of the central cell and the egg cell by two sperm cells found on pollen grains. In comparison to embryos, *Arabidopsis thaliana* and rice endosperms exhibit global hypomethylation of DNA [82]. Prior to fertilization, DME-dependent active demethylation occurs in the central cell (the female gametes companion cell) of *Arabidopsis thaliana* [82,83] (Fig. 1a). However, MET1 transcriptional suppression is also evident during female gamete formation, although it does not appear to have a role in causing substantial demethylation, since genome-wide CG hypomethylation was not detected in endosperm of the wild-type and methylation of DNA is nearly completely restored in the endosperm of the dme mutant [24,71,84].

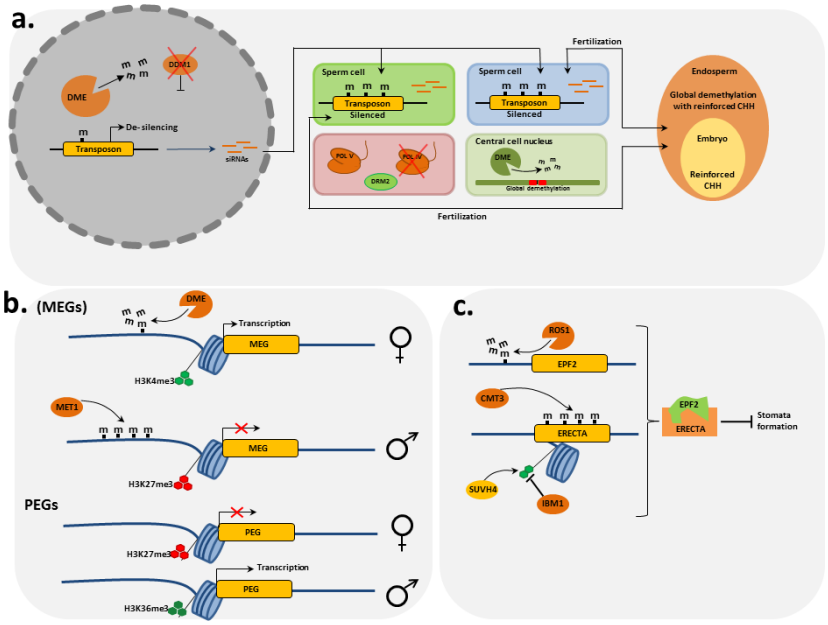

Fig. 1. Role of DNA methylation in plant development and growth

Male gamete companion cells (vegetative cells) are similarly affected by DME-mediated demethylation of DNA, which is accompanied by a significant dysregulation of DDM1 [85] (Fig. 1a). Thus, small interfering RNAs (siRNAs) are created from de-silenced and demethylated transposons, and they go to the sperm cells from the vegetative cell, where RdDM are strengthened [86]. Additionally, DRM2 and POL V were discovered in egg cells of *Arabidopsis thaliana*, except POL IV, which is required for small interfering RNAs (siRNA) generation in the normal RdDM pathway [87,88]. Thus, the accumulation of transposon small interfering RNAs (siRNA) in sperm cells may potentially contribute to the transposon silencing suppression after fertilization. There is evidence that methylation of DNA may play an important role in seed dormancy since the CHH methylation level rises later in development of seed and then decreases when the seeds germinate [81-84] (Fig. 1b). There were hundreds of RdDM-dependent hypermethylation sites in the male sexual lineage that were found to be necessary for meiosis despite the lower levels of CHH methylation than in reproductive cells [85,89] (Fig. 1b).

4.2 Pattern Formation and Vegetative Growth

Meristematic tissues in *Arabidopsis thaliana* have greater transcript levels of RdDM factors as compared to which develop primarily by cell differentiation, such as the differentiated leaves and hypocotyl [90]. Levels of methylation of DNA in root meristematic cells were compared across different cell types and found to be greatest in cells like columella, probably due to less compacted pericentromeric chromosomes in these cells, which makes RdDM factors more accessible [91]. RdDM mutants in *Arabidopsis thaliana* show no evident meristem problems, but maize and rice RdDM mutants show severe developmental deformity [87,90,92], and these variables are expected to play important roles in the meristem function.

Some *Arabidopsis thaliana* leaf epidermal cells pattern development depends on methylation of DNA. To understand how ROS1 malfunction affects stomatal cell lineage development, it is necessary to understand the role of EPIDERMAL PATTERNING FACTOR 2 (EPF2), which is an enzyme that suppresses stomata development [47,74,93]. H3K9 demethylase IBM1 dysfunction also leads to increased levels of CHG methylation of DNA and H3K9me2, as well as suppression of three ERECTA family genes encoding THREONINE-PROTEIN KINASE/LRR RECEPTOR-LIKE SERINE RECEPTOR 2, contributing to deformities in stomatal pattern formation similar to those observed in ros1 plants [55-57,94]. RdDM factor mutations in ros1 plants, H3K9 methyltransferase SUVH4 mutations in ibm1 plants, and CMT3 mutations in ros1 plants

may all help restore proper stomatal pattern development [82]. In *Arabidopsis thaliana* leaf patterning of epidermal cell is regulated by two different methods involving methylation of DNA (Fig. 1c).

4.3 Fruit Development and Epialleles

The DNA methylome of the fruit pericarp is changed by around 1% during the growth of tomato fruits. Numerous genes involved in fruit ripening are subject to active demethylation of DNA because their promoter regions include RIN binding sites [95,96]. Many recognized ripening genes have been shown to have a negative correlation between their expression with the degree of methylation of DNA at the DNA-binding site. CNR (COLOURLESS NONRIPENING), a crucial RIN-targeted gene for fruit ripening, was hypomethylated and expressed after pretreatment with a chemical inhibitor of methylation of DNA. This resulted in early ripening of tomato fruits [44,97]. Demethylation of DNA in ripening fruits is mediated by the *Solanum lycopersicum* DNA demethylase DME-LIKE 2 (DML2), whose expression rises rapidly [98] (Fig. 1c).

Epialleles, which are alleles with various epigenetic alterations that are passed down through generations, may identify isogenic plants. Various crop species, such as cotton, tomato, and rice, contain natural epialleles that alter essential properties [86,99]. Genomic hypermethylation of DNA in the promoter results in the CNR gene being transcriptionally suppressed, leading to colorless and non-ripening fruits [57,100] (Fig. 1c). Promoter DNA hypomethylation in rice with the epiallele rav6 promotes ectopic expression of RELATED TO ABSCISIC ACID INSENSITIVE3/VIVIPAROUS1 6, which modifies leaf angle through altering brassinosteroid homeostasis. 1 Promoter DNA hypomethylation promotes ectopic expression of RELATED TO ABSCISIC ACID INSENSITIVE3/VIVIPAROUS1 6 in rice with the rav6 epiallele, altering leaf angle through influencing brassinosteroid levels [101]. Contrary to its epiallele in wild cotton, CONSTANS-LIKE 2D is hypomethylated in farmed allotetraploid cotton, promoting blooming [102].

5. STIMULATORY EFFECTS OF ENVIRONMENT ON DNA METHYLATION

When plants are exposed to diverse abiotic and biotic environmental stressors, their DNA is methylated. Plants may be able to remember their prior surroundings via methylation of DNA, which has sparked a lot of research in this area. Not only methylation-based epigenetic quantitative trait loci have been discovered in *Arabidopsis thaliana* population epigenome studies [100], but methylation of DNA is also connected to local adaptability, as shown by the discovery that geographical origin is linked to genome-wide gene expression and levels of DNA methylation differences induced by epialleles [103,104]. Additional research has shown that environmental stress may modify plant methylation of DNA at specific loci or throughout the whole genome, however it is yet unknown whether some of the changes in response to abiotic stress are adaptive. However, this research is still in its early stages.

5.1 DNA Methylation and Biotic Stress Responsive Changes

When plants are infected by pathogens or colonized by symbiotic microorganisms, they exhibit genome-wide changes in methylation of DNA. In *Medicago truncatula*, nodulation necessitates the presence of the demethylase DME [105]. In the course of nodule formation, a small fraction of nodule-specific symbiosis genes has their DNA methylation altered in a number of genomic locations [106]. Infected cyst nematodes caused widespread hypomethylation of DNA in the roots of soybean and *Arabidopsis thaliana* [77,94,107]. When *Arabidopsis thaliana* leaves are exposed to the bacterial pathogen *Pseudomonas syringae* pv. tomato strain DC3000 (Pst DC3000), modest but widespread differential methylation of DNA occurs; the differentially methylated cytosines are found mostly in CG and CHH contexts in gene-rich areas, particularly at the 5' and 3' ends of protein-coding genes. Pst DC3000-responsive methylation of DNA, on the other hand, has a negative correlation with the expression levels of neighboring genes throughout the whole genome [108], this finding suggests that methylation of DNA at gene borders is dynamically regulated and may contribute to differential gene expression in response to infections.

Demethylation of DNA in transcription activation and promoter regions of rRNA genes in cucumber leaves and pollen grains is caused by viroids, which are plant pathogenic non-coding RNAs (ncRNAs) [96,109]. Hypomethylation of DNA in the *Arabidopsis thaliana* pericentromeric areas was followed by a rise in 21-nucleotide

siRNAs from hypomethylated transposons, which is a key phytohormone for plant defense against pathogens [104-104,110]. Among the roughly 1,000 *Arabidopsis thaliana* accessions from throughout the globe, variation in methylation is most prevalent in genes producing receptors with binding-nucleotide and oligomerization domains [103], implying that plant epigenomes are shaped mostly by biotic environmental stimuli.

5.2 DNA Methylation and Abiotic Stress Responsive Changes

Pesticides, high salinity, laser irradiation, ultraviolet radiation stress, soil nutrient insufficiency, re-oxygenation and anoxia, drought, cold, heat, climate change, and hyperosmotic stress are just a few of the abiotic environmental stress factors that researchers have looked into. *Brassica rapa, Brassica napus, Arabidopsis thaliana, Quercus lobata*, winter wheat, maize, rice, barley, and *Populus trichocarpa* have all been used in this study [111]. Many early investigations of abiotic stress revealed stress-induced demethylation and/or methylation of DNA patterns either genome wide or at specific loci, similar to plant responses to biotic stress. Changes in methylation of DNA have been linked to transcriptional control of genes implicated in responses to plant stress in certain circumstances [112,113], this would support the hypothesis that methylation of DNA plays a role in modulating plant responses to abiotic environmental stimuli. In recent research, it has been suggested that long-term stress may be critical to the development of DNA methylation-dependent stress memory in plants [114].

Rice plants starved of inorganic phosphate (Pi) produce over 100 DMRs, the majority of which are hypermethylated CHHs and almost exclusively overlap with transposons near stress responsive genes known as Pi-starvation-induced (PSI) genes, these genes are characterized by their proximity to stress responsive genes [102,109,115]. These DMRs may be a result of PSI-gene activation and have no effect on responses to stress, according to time-course investigations that showed PSI-gene transcription occurred prior to local methylation of DNA changes. For this reason, after replenishing the plants phosphate supply with inorganic phosphate, the levels of methylation of DNA of most PSI DMRs progressively restored to inorganic-phosphate adequate levels. Since transmission of meiotic PSI DMRs was not found, rice PSI DMRs were shown to be temporary. Cold treatment may generate non-CG hypermethylation in the VERNALIZATION-A1 gene of wheat, which is passed by mitosis but not meiosis. DNA demethylases DML1 and DML2 are downregulated in tomato fruits when they are cold-treated, resulting in a decrease in promoter hypermethylation and gene silence [116]. This explains why the flavor of tomatoes diminishes when they are kept in the refrigerator.

6. FUTURE SCENARIO AND CONCLUSION

Recent studies on the function and regulation of plant methylation of DNA has resulted in a number of important findings, including the protein complex consisting of ASI1, AIPP1, and EDM2 that binds and promotes distal polyadenylation of mRNA, a protein complex called IDM that directs the targeting of the DNA demethylase, de novo DNA methylation primary trigger ncRNAs identification, management of the equilibrium between demethylation and methylation of DNA by MEMS methylstat element, Epigenomes and interactions between DNA methylomes in genetic hybrids and transcriptomes from the 1001 Genomes collection of natural accessions of *Arabidopsis thaliana*. Furthermore, these and other findings may provide light on how patterns of methylation of DNA are created in non-plant creatures, such as mammals, as well.

These recent findings have also led to a lot of new questions. How are AGOs loaded with the short P4 RNAs in DCL-independent RdDM methylated? Many trans-chromosomal methylation locations in *Arabidopsis thaliana* hybrids show a mutual increase in methylation of DNA between two alleles, suggesting allelic interactions in RdDM [117]. RdDM models now in use cannot explain these allelic interactions, hinting those substantial alterations may be necessary. POL IV is recruited to just a fraction of the classical RdDM target loci by SHH1; likewise, the IDM complex recruits ROS1 to only a subset of the ROS1-dependent demethylation target loci. Following from this, it is necessary to investigate alternate mechanisms for initial recruitment in both the ROS1-mediated demethylation pathway and the RdDM route. In part because DNA methylation plays a limited function in this plant and hence demethylated and methylated DNA mutants are often not fatal, *Arabidopsis thaliana* has served as and continues to serve as an ideal model system for studying the fundamental processes of

demethylation and methylation of DNA. In plants with more complicated genomes than *Arabidopsis thaliana*, DNA methylation seems to control many more critical genes for development and growth, and for stress responses. New functions for methylation of DNA in these plants, new ways to target DNA demethylases and methylases, and new mechanisms for generating, maintaining, converting, and erasing epialleles of methylation of DNA will definitely be discovered in future studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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