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REVIEW

DNA methylation dynamics in response to abiotic and pathogen stress in plants

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Abstract
DNA methylation is a dynamic epigenetic mechanism that plays a significant role in gene expression and also maintains chromatin stability. The process is conserved in both plants and animals, and crucial for development and stress responses. Differential DNA methylation during adverse environmental conditions or pathogen attack facilitates the selective expression of defense-related genes. Both stress-induced DNA hypomethylation and hypermethylation play beneficial roles in activating the defense response. These DNA marks may be carried to the next generation making the progenies 'primed' for abiotic and biotic stress responses. Over the recent years, rapid advancements in the area of high throughput sequencing have enabled the detection of methylation status at genome levels in several plant species. Epigenotyping offers an alternative tool to plant breeders in addition to conventional markers for the selection of the desired offspring. In this review, we briefly discuss the mechanism of DNA methylation, recent understanding of DNA methylation-mediated gene regulation during abiotic and biotic stress responses, and stress memory in plants.

Keywords Epigenetics · DNA methylation · Abiotic stress · Biotic stress · Stress memory · Bisulphite sequencing · Methylation-sensitive amplified polymorphism

Introduction

Complex regulatory mechanisms exist for the adaptation of plant against various environmental stresses. DNA methylation is one such epigenetic phenomenon which regulates temporal gene expression in both plants and animals (Singh and Prasad 2021). DNA methylation is catalyzed by specific DNA methyltransferases at 5' position of the cytosine base resulting in 5-methylcytosine (5-mC). In plants, methylation can occur at specific cytosine sequence contexts, including CG, CHG and CHH, where H denotes A, T or C. Three distinct types of DNA methyltransferases are found in plants namely DNA methyltransferase 1 (MET1), domain-rearranged methyltransferase (DRM), and chromomethyltransferase (CMT). Irrespective of sequence contexts de novo methylation in plants is implicated by DRMs. Maintenance of hemimethylated DNA in CG context is carried out by MET1; CHG through plant-specific CMT3 and to some extent by CMT2; and CHH by DRM2 or CMT2 (Henderson and Jacobsen 2007; Stroud et al. 2014). Small RNA-directed nucleic acid methylation is also prevalent in plants (Singh et al. 2021). Demethylation of methylated cytosine is catalyzed by four enzymes including, REPRESSOR OF SILENCING 1 (ROS1), DEMETER-LIKE 2 (DML2), DEMETER-LIKE 3 (DML3) and DEMETER (DME) through a base excision repair pathway (Penterman et al. 2007; Ortega-Galisteo et al. 2008). Unlike mammalian DNA demethylation, oxidation of 5-mC is not essential for plant DNA glycosylases (Wu and Zhang 2017). Figure 1 represents the dynamics of DNA methylation in plants and underlying enzymes. Technological advancements coupled

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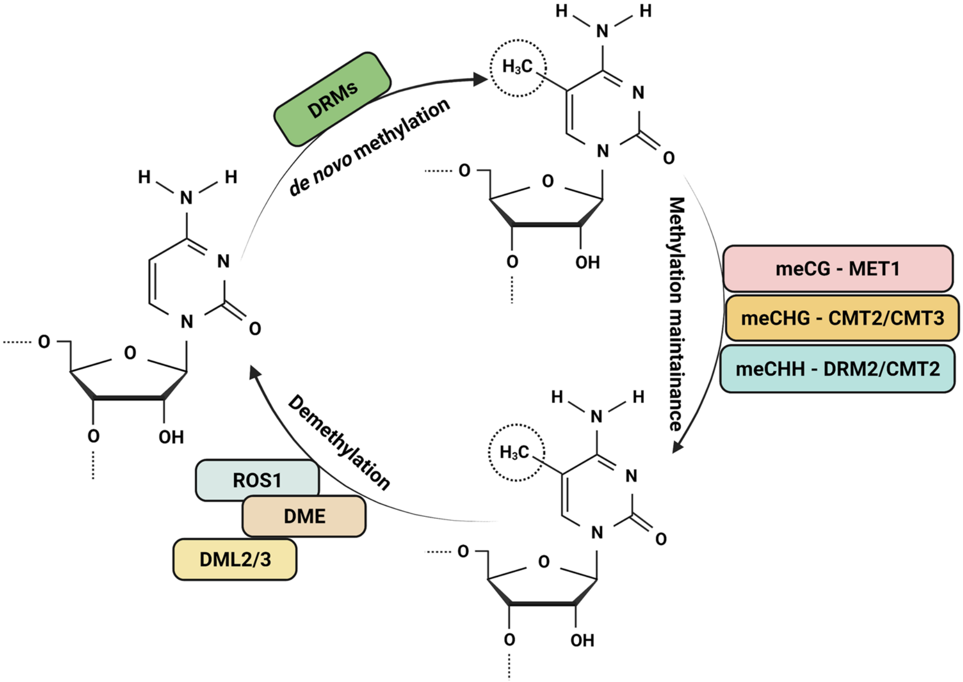
Fig. 1 Dynamics of cytosine methylation, demethylation, and methylation maintenance and enzymes responsible for them in plants



with next-generation sequencing platforms have enabled the detection of methylation dynamics at DNA region. Global DNA methylation status in plants is determined through Methylated DNA Immunoprecipitation (MeDIP) and bisulfite sequencing. Cytosine methylation influence the interaction of nucleic acid with the chromatin proteins and transcription factors thereby regulates gene expression. DNA methylation patterns are mostly stable and specific to particular cell types, persists throughout the lifetime, specifically altered during abiotic and biotic stress or particular developmental stage, and are heritable.

Abiotic stress responsive alteration in DNA methylation

Heat and drought stress

Heat and drought are one of the major climatic constraints causing a severe reduction in crop productivity worldwide. It is an estimate that global temperatures will rise by approximately 4–5 °C before the end of twenty-first century (Tollefson 2020). Prolonged heat stress and inadequate rainfall generally result in drought. Combined effects of heat and drought cause more damage to crop production than heat or drought occurs alone (Zandalinas et al. 2018). Both these stresses are reported to induce hypermethylation or demethylation in coding or intergenic regions. In response to heat stress the tolerant rapeseed (*Brassica napus*) genotype, Huyou2 was found to be dominated by hypomethylated DNA content than the sensitive genotype, Fengyou 1 (Gao et al. 2014). A naturally stress-tolerant crop, foxtail millet (*Setaria italica*) displayed enhanced CHG and CHH methylation on both upstream and coding region regions of heat shock protein (HSP)-encoding genes under ambient conditions. High-temperature stress induces hypomethylation at most of the *SiHSP* genes of CHG and CHH context in tolerant cultivar resulting in their higher expression (Singh et al. 2016). A total of 325 differentially methylated genes (DMGs) have been found in MethylRAD libraries constructed from leaves of heat-treated maize inbred line B73 (Qian et al. 2019). The DMGs associated with RNA splicing possess lower methylation status were the highly expressed genes suggesting enhanced spliceosome activity during heat stress. Nuclear proteome analysis during high-temperature stress of *Pinus radiata* was observed with a reduced abundance of S-adenosylmethionine (SAM) synthase and S-ADENOSYL-l-HOMOCYSTEINE HYDROLASE (SAHH) enzymes resulting in hypomethylated DNA content (Lamelas et al. 2020). S-adenosylmethionine is an essential cofactor and donor of the methyl group, while SAHH is required for regeneration of S-adenosylmethionine during methylation-mediated gene silencing. Regulation of transposable elements during heat stress was also found to be mediated through DNA methylation in plants. The combination of histone H1 variant and DNA methylation redundantly suppress the release of heterochromatin-associated transposable element during heat stress (Liu et al. 2021a).

Similar to heat stress, drought also triggers dynamic methylation status which is inversely correlated with gene expression. In *Polygonum persicaria* it has been demonstrated that drought-responsive transgenerational plasticity in next generation is mediated by inherited DNA methylation status (Herman and Sultan 2016). Progenies of drought-treated parents exhibited deeper root architecture in water-deficient soil, and extended roots at a faster rate, than offspring of the same inbred genetic lines whose parents were grown under well-watered conditions. These stress memories allow a primmed offspring to respond more swiftly and vigorously to recurring drought or any other abiotic stress than the non-primed plants. The role of phytohormone responses in modulating DNA methylation during drought stress was evident in poplar (Lafon-Placette et al. 2018). Majority of the hormone-responsive genes undergo hypomethylation state during the recovery process. The reduced DNA methylation of *Medicago sativa* var. Lamia primed with two phases of drought exposure leads to improved tolerance than the single drought exposure (Ventouris et al. 2020). Drought-induced differential DNA methylation was detected through amplified methylation polymorphism-polymerase chain reaction (AMP-PCR) technique in ABA-deficient maize mutant vp10 (Sallam and Moussa 2021). Differential methylation has abundantly occurred at intragenic regions of zinc finger containing proteins, DNA repair enzymes, amino acid catabolic enzymes, genes implicated with plant survival, and demethylated transposable elements.

Salinity stress

Salt stress is another major abiotic stress limiting plant development and crop productivity. About 20% of the global agricultural lands which makes approximately 45 million hectares of arable lands are severely affected by soil salinization (Singh 2021). The high salt concentrations in soil restrict water uptake and mineral absorption in the plants. Salt stress immediately induces osmotic stress and then ion toxicity, which ultimately causes oxidative stress leading to impaired physiology in the plant system (Liang et al. 2018). Methylation-sensitive amplified polymorphism (MSAP) analysis in contrasting cultivars of foxtail millet revealed the hypomethylated genome of tolerant cultivars facilitates the expression of stress-related genes up on induction of salinity stress (Pandey et al. 2017). Further, bisulphite sequencing of three rice cultivars under water-deficient and salinity stress revealed that CG methylation in the gene body and CHH methylation in distal promoter regions were positively correlated with gene expression (Rajkumar et al. 2020). Most of the differentially expressed and differentially methylated genes were cultivar-specific, suggesting genotype-specific epigenetic response against abiotic stress in rice. DNA methylation pattern is also affected by the ploidy level of plants, as lower sodium uptake in tetraploid rice is correlated with the epigenetic regulation of jasmonic acid-related genes (Wang et al. 2021). Hypomethylation conformation at several loci harboring stress-responsive genes and transposable elements is prevalent in tetraploid rice cultivars. Therefore, the expression of salt-responsive genes including those associated with the jasmonic acid pathway was relatively more rapid and higher in tetraploid than diploid rice, conferring salinity tolerance. Ploidy (both auto- and allopolyploidy) induces genomic stress and can cause an internal 'priming state' through hypomethylation which in turn facilitates a swift response to external stress stimuli.

DNA methylation as the plant strategy to fight against pathogens

Plants are a great source of nutrients and therefore are infected by several pathogens. The innate immune system of host plants is stimulated by perceiving pathogen-associated molecular patterns (PAMPs) by specific pattern recognition receptors (PRRs) (Yuan et al. 2021). Further, PAMP-triggered immunity (PTI) activates different cellular defense pathways such as signaling cascades, defense-related gene expression, and Hypersensitive Response (Bigeard et al. 2015). Moreover, pathogens override PTI by expressing virulence effector proteins, avirulence (*Avr*) genes, which suppress this defense mechanism. As a counter-counter defense, host plant receptors (Nucleotide-binding Leucine-rich Repeat (NLR) receptor genes; NLR genes) associate with these Avr proteins (either directly or indirectly, in association with a 'guardees' protein) and activate effector-triggered immunity (ETI; Yuan et al. 2021). Apart from ETI, the pathogen attack leads to an immense variation in gene expression which is might be regulated through epigenetic modifications. Therefore, in host plants, pathogens confront ubiquitous mechanisms of defense which include: (a) identification of pathogen-associated effectors by the corresponding *NLR* genes, and (b) the RNA silencing mechanism (also called RNA interference; RNAi). RNA silencing includes two branches: Post-Transcriptional Gene Silencing (PTGS) and Transcriptional Gene Silencing (TGS). PTGS inhibits gene expression by either inhibiting the translation of transcript or by initiating its degradation. On the other hand, epigenetic modifications/TGS inhibits transcription of the gene by promoting its chromatin modifications (Prasad et al. 2019).

Epigenetic processes including chromatin remodeling, DNA methylation, and, histone modifications are critical for the regulation of plant defense responses against pathogen infection. The correlation between DNA methylation and gene expression is highly complex. It depends on multiple factors such as the type of the tissue, sequence context, activity of the transposons, and the genomic region (Tirnaz and Batley 2019).

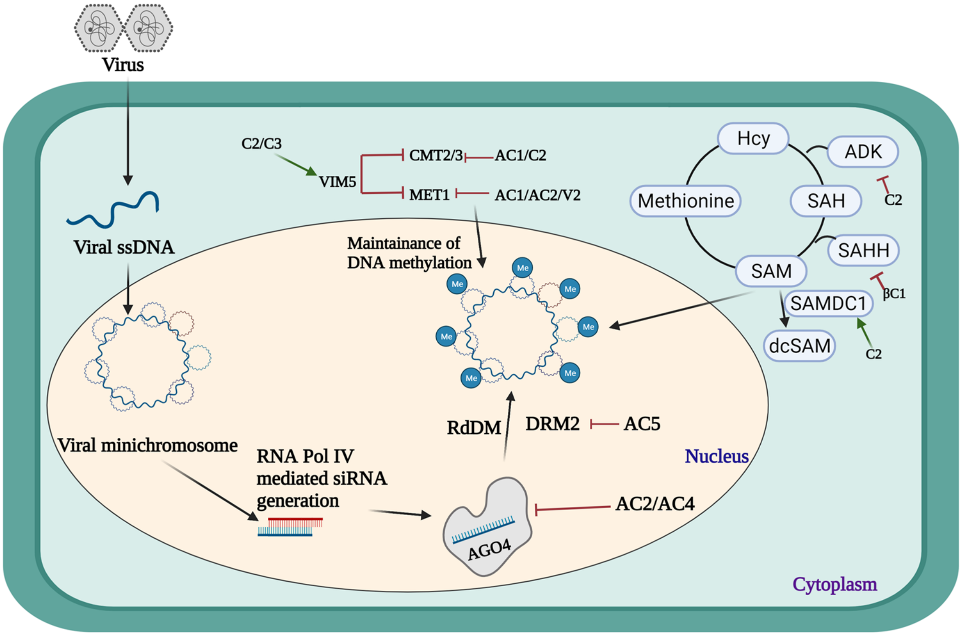


Fig. 2 Schematic illustration of DNA methylation in plants and interaction of suppressors viral proteins with methylation pathway components. Upon virus infection plant gene silencing machinery is activated. RdDM of the viral genome is mediated by Pol IV- dependent transcription for the synthesis of 24 nt siRNAs which are incorporated into AGO4. This siRNA-AGO4 complex causes virus DNA methylation via DRM2 (Domains rearranged methyltransferase 2). The methylation pattern on the viral genome is maintained by MET1 (Methyltransferase 1), CMT2/3 (Chromomethylase 2/3). The activity of enzymes involved in de novo DNA methylation and its maintenance is inhibited by different viral proteins. *Cotton leaf curl Multan virus* (CLCuMuv) AC2 and *Tomato leaf curl New Delhi virus* (ToLCNDV) AC4 interacts with AGO4, *Mungbean yellow mosaic India virus* (MYMIV) AC5 interacts with DRM2 and inhibits de novo methylation. MET1 is targeted by *Tomato Golden Mosaic virus* (TGMV) AC2, *Tomato yellow leaf curl Sardinia virus* (TYLCSV) AC1/AC2 and precoat protein (V2) of *Tomato yellow leaf curl virus* (TYLCV). The activity of CMT2/3 is reduced by TYLCSV AC1 and *Tomato leaf curl Taiwan virus* (ToLCTWV) C2. Transcriptional activator protein (C2) and Replication enhancer (Ren/C3) of *Beet severe curly top virus* (BSCTV) interacts with VIM5 (E3 ligase) and activates ubiquitin-26S proteasome-mediated degradation of MET1 and CMT3. Virus-encoded proteins also regulate the activity of methyl cycle proteins. The synthesis of donor for methyl group for cytosine methylation, SAM (S-adenosyl methionine) is inhibited by several viral proteins. The host kinase protein ADK (Adenosine kinase) is suppressed by C2 of *Beet Curly Top Virus* (BCTV) and SAHH (S-adenosyl homocysteine hydrolase) that catalysis reversible hydrolysis of SAM to Hcy (homocysteine) and adenosine is inhibited by βC1 of *Tomato yellow leaf curl China virus* (TYLCCV). C2 of BSCTV inhibits ubiquitin-26S proteasome-mediated degradation of SAMDC1 (S-adenosyl-methionine decarboxylase 1) thus increasing its expression. SAMDC1 catalysis decarboxylation of SAM (dcSAM; competitive inhibitor of SAM) and reduces virus genome methylation. The viral protein-based suppression is indicated in the red colour arrow within the figure. Green arrow indicates activation by virus proteins. Virus genes encoded by the genome component ‘A’ for the bipartite viruses are depicted as AC/AV and by only C/V for the monopartite viruses

In the subsequent section, a detailed description of plant defense mechanism with emphasis on DNA methylation against pathogens is provided.

Viruses

The role of gene silencing has been well studied in plant-pathogen interaction. Both PTGS and TGS arms of gene silencing have been found to function as an innate defense response against virus attacks (Jin et al. 2021; Zarreen and Chakraborty 2020; Wang et al. 2019; Prasad et al. 2019; Sharma et al. 2018, 2013). Involvement of epigenetic modification in antiviral defense is evident from the fact that the resistance genes *Ty1* and *Ty3* show similarity to RNA-dependent RNA polymerase 3 (RDR3) and are responsible for providing resistance against *Tomato yellow leaf curl virus* (TYLCV; Geminiviridae family) in tomato by increasing DNA methylation of the viral genome (Butterbach et al. 2014; Prasad et al. 2020).

The host plants use RNA-dependent DNA methylation (RdDM) as a counter-attack mechanism against virus infection (Fig. 2). It causes de novo methylation of coding and promoter regions of the virus and represses the transcription/replication of virus minichromosome (Yadav and Chattopadhyay 2011; Raja et al. 2014; Lewseya et al. 2015; Xie and Yu 2015; Deuschle et al. 2016; Basu et al. 2018; Piedra-Aguilera et al. 2019; Omae et al. 2020). It was found that in tomato the tolerance against *Tomato leaf curl New Delhi virus* (ToLCNDV; Geminiviridae family) was correlated with the hypermethylation of the virus promoter

Table 1 Viral suppressors of DNA methylation

Suppressor protein	Virus	Mechanism of suppression	References
AC1/C1/Rep	*Tomato yellow leaf curl Sardinia virus* (TYLCSV)	Reduces the transcript level of CMT3 and MET1	Rodríguez-Negrete et al. (2013)
AC2/C2/TrAP	*Mungbean yellow mosaic virus* (MYMV) *Cabbage leaf curl virus* (CaLCuV)	Decreases the activity of ADK and inhibits the synthesis of S-adenosyl-methionine (SAM)	Trinks et al. (2005) and Liu et al. (2014)
	BSCTV	Regulates DNA methylation by repressing degradation of SAMDC1 by 26S proteasome	Zhang et al. (2011)
	Tomato yellow leaf curl Sardinia virus (TYLSCV)	Decreases the expression of MET1	Rodríguez-Negrete et al. (2013)
	Tomato leaf curl Taiwan virus (ToLCTWV)	Down-regulates the activity of CMT3/2	Tu et al. (2017)
	Cotton leaf curl Multan virus (CLCuMuV)	Inhibits RdDM-mediated TGS by interacting with AGO4	Wang et al. (2019)
	Beet severe curly top virus (BSCTV)	Interacts with VIM5 and induces ubiquitin-26S proteasome-mediated degradation of MET1 and CMT3	Chen et al. (2020)
AC3/C3/REn	*Beet severe curly top virus* (BSCTV)	Interacts with VIM5 and induces ubiquitin-26S proteasome-mediated degradation of MET1 and CMT3	Chen et al. (2020)
	Pepper huasteco yellow vein virus (PHYVV)	TGS suppressor but mechanism not known	Rodríguez-Gandarilla et al. (2020)
AC4/C4	*African cassava mosaic virus* (ACMV)	Binds onto the ss-sRNA and inhibits its loading onto the RITS	Chellappan et al. (2005)
	ToLCNDV	Binds with AGO4 protein and inhibits RdDM	Vinutha et al. (2018)
	CLCuMuV	Associates with S-adenosyl methionine synthetase (SAMS) and reduces its activity	Ismayil et al. (2018)
	Tomato leaf curl Guangdong virus (ToLCGdV)	Interacts with BARLEY ANY MERISTEM 1 (BAM1) and inhibits cell-to-cell transmission of siRNAs	Li et al. (2020)
AC5/C5	*Mungbean yellow mosaic India virus* (MYMIV)	Represses the expression of DRM2	Li and Zhu (2015)
βC1	*Tomato yellow leaf curl China virus*	Binds to S-adenosyl Homocysteine Hydrolase (SAHH), and inhibits the synthesis of SAM	Yang et al. (2011)
V2/Pre-coat protein	TYLCV	Negatively regulates RDR1-mediated viral genome methylation Decreases viral genome methylation by interacting with histone deacetylase 6 and inhibits its binding to MET1	Basu et al.(2018) Wang et al. (2018b)

region (Intergenic region, IR) and coding region (AC1), leading to reduced expression of virus genes and thus providing resistance against virus infection (Sahu et al. 2014). Based upon this finding, artificial miRNA was expressed in susceptible tomato cultivar against the hypermethylated region of AC1 (Sharma and Prasad 2020). These transgenic tomato plants were found to have higher yields and reduced symptom development during ToLCNDV infection as compared to wild-type plants (Sharma and Prasad 2020). Further, the role of viroid in triggering the DNA methylation machinery has been studied in tomato and it was observed that *Potato spindle tuber viroid* (PSTVd: Pospiviroidae family), enhances the expression of vital genes involved in DNA methylation such as *DRM2, CMT3, MET1, AGO4,* and *HEN1* and cause hypermethylation of *Tomato yellow leaf curl Sardinia virus* (TYLCSV; Geminiviridae family) DNA which inhibits the virus replication and transcription (Torchetti et al. 2016). Another study in *Arabidopsis thaliana* highlighted the importance of RNA Polymerase V (PolV) in RdDM-mediated regulation of the virus infection. PolV initiates increased chromatin compaction of the *Beet curly top virus* (BCTV; Geminiviridae family) genome which causes symptom remission (Coursey et al. 2018). Simultaneously, PolV also targets TEs, and their hyper-suppression in the recovery phase helps in sustaining the host genome integrity in reproductive tissue (Coursey et al. 2018). However, the knowledge regarding the link between the regulation of TEs and its impact on virus infection is limited and needs to be further examined. Examination of the geminivirus genome through sequencing has established that virus minichromosomes are not randomly methylated (Deuschle et al. 2016; Piedra-Aguilera et al. 2019). However, the mechanism behind this preferential methylation is not known. Therefore, the changes in the pattern of viral DNA methylation during different stages of virus infection need to be investigated using the Single-base methylome assay.

It is noteworthy that virus infection has been found to shape the plant genome (Wang et al. 2018a; Sun et al. 2019; da Silva et al. 2020). Methylomes analysis has highlighted the role of DNA methylation in modulating the plant gene expression pattern upon virus infection. It was observed that during *Cucumber Mosaic Virus* (CMV; Bromoviridae family) infection in *Nicotiana tabacum* the level of methylation is reduced at the promoter region of Argonaute genes and pathogen defense genes, such as *phenylalanine ammonia lyase* (*PAL*), and *pathogenesis-related* (*PR*)-like proteins, thus highlighting the contribution of DNA demethylation to the resistance signaling (Wang et al. 2018a). Interestingly, *Beet severe curly top virus* (BSCTV; Geminiviridae family) C2 and *Tobacco vein banding mosaic virus* (TVBMV; Potyviridae family) helper-component protease (HC-Pro) interfere with DNA methylation in Arabidopsis and cause hypomethylation at the promoter region of *accelerated cell death 6 (ACD6), pathogenesis-related protein 5 (PR5)* and *nonexpressor of pathogenesis-related genes 1 (NPR1)* which ultimately enhances the expression of SA pathway genes and restraint virus accumulation (Yang et al. 2013, 2016). These epigenetic modifications of the host plant genome might provide a molecular memory in response to the attack by new pathogens (Wang et al. 2019). Recently, many new techniques (such as whole-genome bisulfite sequencing, Chromatin immunoprecipitation, Formaldehyde-Assisted Isolation of Regulatory Elements, and methylation-sensitive amplification polymorphism) have been used to study how the plant methylome is altered during virus infection (Lacatus and Sunter 2008; Coursey et al. 2018; Sun et al. 2019; da Silva et al. 2020), however, the dynamics of these changes in plant counter-defense needs to be exemplified.

To overturn this defense response, viruses encode silencing suppressors, which manipulate and interfere with PTGS or TGS pathways by interacting with host proteins involved in the silencing process to ensure effective infection in the host plant (Fig. 2). Several viral proteins, with diverse structures and functions, have been associated with the inhibition of TGS machinery (Table 1). For example, AC4/C4 which is important for viral symptom development; was found to interact with S-adenosyl methionine synthetase (SAMS); a catalyst for S-adenosylmethionine synthesis. This interaction reduced cytosine methylation of the viral genome and enhanced virus accumulation in *N. benthamiana* (Ismayil et al. 2018). Single amino-acid mutation (R13A) in C4 abolished this interaction, leading to induced viral DNA methylation, decreased levels of symptoms, and viral DNA accumulation. Another viral protein, TrAP (AC2/C2/AL2/L2) which is required for the transcription of late virus genes, is a well-studied suppressor of TGS. It has been reported to interact with different components of the epigenetic pathway (Tu et al. 2017). AL2/L2 represses the activity of Adenosine kinase (ADK), which is required for the transfer of methyl group from S-adenosyl-methionine (SAM) onto the cytosine which causes reduced DNA methylation of the viral genome (Yang et al. 2013). Recently, a study in Arabidopsis revealed that BSCTV infection leads to increased expression of an E3 ligase, Variant in methylation 5 (VIM5). It causes ubiquitin/26S proteasome pathway-mediated degradation of MET1 and CMT3 due to which hypomethylation is observed at the C2-C3 promoter region of BSCTV. This study revealed how viruses recruit host genes for regulating the epigenetic modification of the virus genome (Chen et al. 2020).

Interestingly, two viral proteins, AC4 of ToLCNDV and precoat protein V2 of TYLCV were observed to interact with AGO4 (Wang et al. 2020; Vinutha et al. 2018). Recently, it was shown that V2 associates with AGO4 in Cajal bodies of *N. benthamiana* cells and hinders its binding to viral DNA thus inhibiting the silencing mechanism (Wang et al. 2020). This study provides a better understanding of the link between DNA methylation, Cajal body, and the antiviral defense. Simultaneously, V2 also interacts with histone deacetylase HDA6 which restricts the recruitment of MET1 onto the virus genome and reduces the methylation level of the virus genome (Woo et al. 2008; Wang et al. 2014, 2018b). Further, AC4 also interacts with and inactivates, DRM2, a pivotal component for de novo methylation, and suppresses methylation of the *Tomato leaf curl Yunnan virus* (TLCYnV; Geminiviridae family) genome in infected *N. benthamiana* plants (Mei et al. 2020). These studies furnish a new perceptive into the virus mechanisms through which viruses suppress RdDM. Therefore, repression of these suppressor proteins by methods such as CRISPR-Cas9, RNAi and artificially targeted DNA methylation might assist in enhancing the virus tolerance.

Bacteria

Cytosine hypomethylations induced by the bacteria *Pseudomonas syringae* were initially proposed by Pavet et al. (2006). Profiling of DNA methylome of plant genome

Table 2 The defense responses of the Arabidopsis mutants with hypomethylated and hypermethylated DNA to different bacterial, viral and fungal pathogens

Arabidopsis mutants	Pathogen	Phenotype	Defense response	References
DNA Hypomethylation				
drd1	*P. syringae pv. tomato DC3000* (*Pst*)	Resistant	Enhancement of SA-dependent defense	Dowen et al. (2012)
	P. cucumerina	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
ago4	*B. cinerea*	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
	Pst	Susceptible	RDR2 and DCL3 independent susceptibility	Agorio and Vera, (2007)
	Cabbage leaf curl virus (CaLCuV)	Susceptible	–	Raja et al. (2008)
	Beet curly top virus (BCTV)	Susceptible	–	Raja et al. (2008)
rdr2	*P. cucumerina*	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
	Pst	Resistant	Enhancement of SA-dependent defense	Dowen et al. (2012)
rdr6	*B. cinerea*	Susceptible	Loss of transfer siRNAs that target pathogen genes	Cai et al. (2018)
	Pst	Resistant	–	Dowen et al. (2012)
nrpd1	*Pst*	Resistant	Enhancement of SA-dependent defense	Dowen et al. (2012)
nrpe1	*P. cucumerina*	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
	B. cinerea	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
	Pst	Resistant	Enhancement of SA-dependent defense	López et al. (2011)
nrpd2	*P. cucumerina*	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
	B. cinerea	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
	Pst	Resistant	Enhancement of SA-dependent defense	López et al. (2011) and Yu et al. (2013)
	CaLCuV	Susceptible	–	Raja et al. (2008)
	BCTV	Susceptible	–	Raja et al. (2008)
nrpd1/nrpe1	*P. cucumerina*	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
	Pst	Resistant	Enhancement of SA-dependent defense	López et al. (2011)
drm1/drm2	*P. cucumerina*	Susceptible	Suppression of JA-dependent defense	López et al. (2011)
	Pst	Resistant	Enhancement of SA-dependent defense Primed state of defense response	Yu et al. (2013) and Cambiagno et al. (2021)
	CaLCuV	Susceptible	–	Raja et al. (2008)
	BCTV	Susceptible	–	Raja et al. (2008)
drm1/drm2/cmt3 (*ddc*)	*A. tumefaciens*	Susceptible	Enhancement of ABA-dependent response	Gohlke et al. (2013)
	Pst	Resistant	Enhancement of SA-dependent defense	Dowen et al. (2012), Luna et al. (2012), Yu et al. (2013) and Cambiagno et al. (2021)
	A. brassicicola	Susceptible	Suppression of JA-dependent defense	Luna et al. (2012)

Table 2 (continued)

Arabidopsis mutants	Pathogen	Phenotype	Defense response	References
dcl2/3/4	*B. cinerea*	Susceptible	Loss of siRNAs that move into fungal cells and suppress virulence genes	Cai et al. (2018)
	Pst	Resistant	Enhancement of SA-dependent defense	Dowen et al. (2012)
	CaLCuV	Susceptible	–	Raja et al. (2008)
	BCTV	Susceptible	–	Raja et al. (2008)
DNA Hypermethylation				
ros1	*Pst*	Susceptible	Methylation at the promoter of *RMG1* and *RLP43*	Yu et al. (2013) and Halter et al. (2021)
ros1/dml2/dml3 (*rdd*)	*F. oxysporum*	Susceptible	Suppression of defense-related genes	Le et al. (2014)

followed by bacterial infection shows that DNA methylation not only takes place in the promoter region and gene body but also in the transposable elements (Pavet et al. 2006; Dowen et al. 2012). A recent study shows that the regulation of pericentromeric TEs located at distal regions of *PRR/NLR* genes via RdDM pathway compromises plant defense against *P.syrinage* (Cambiagno et al. 2018). One of the earliest reports showed that hypomethylation in the promoter of a gene, *Xa21G* confers resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) infection in mutant rice (Akimoto et al. 2007). Wang et al. (2013) reported a reduction in methylation level in the promoter of *NPR1*, the master regulator of plant immunity upon infection of *Pseudomonas syringae* pv. tomato DC3000 (*Pst*). The *NPR1* DNA methylation modulator *ELP* prevents such hypomethylation and provides epigenetic resistance. DNA methylation by methyltransferases is reversed by the action of a class of enzymes called demethylases. Arabidopsis genome encodes four active demethylases. Mutation in one of these DNA demethylases, *ROS1* results in hypermethylation of the promoter of *RMG1*, flagellin–induced resistance gene, and *RLP43*, an orphan immune receptor. Such variations in methylation render the plant susceptible to *Pst* (Yu et al. 2013; Halter et al. 2021). These studies indicate that changes in the methylation pattern at the promoter region usually occur for defense-related genes. It regulates their expression, thus, deciding the host's response to bacterial infection.

The alteration of methylation patterns in TEs has been observed in response to the infection of *Pst* (Agorio and Vera 2007; Dowen et al. 2012). Zhang et al. (2016) reported the role of TEs-induced-siRNAs in regulating host–pathogen interaction. This study demonstrates that the presence or absence of methylated transposable elements in the promoter region of rice *WRKY45* decides opposite functions of two alleles of the same gene. The expression of the *WRKY45-1* allele (with no methylated TEs) results in the production of siRNAs that suppress the expression of resistance gene *siR815 Target 1* (ST1) with the help of RdDM rendering the plant susceptible to *Xoo*. While the other allele with methylated TEs provides resistance against the same pathogen. It has been reported that bacterial elicitors trigger epigenetic changes in the plant genome. Recently, it was observed that the bacterial PAMP flg22 is responsible for changing the methylation status of the plant genome. Whole-genome bisulfite sequencing revealed that flg22-induced global DNA hypomethylation occurs at the promoter region of defense-related genes which induces PTI-based plant defense responses against bacterial infection (Yu et al. 2013; Atighi et al. 2020). Such genomic modifications may sometimes show transgenerational defense priming and act as an epigenetic mark (He and Li 2018; Stassen et al. 2018; Sharrock and Sun 2020*)*.

Bacteria-induced systemic acquired response (SAR) may also be inherited through generation. SAR induced by *Pst* have been reported to enhance the expression of defense-related genes such as *PR1, WRKY6* and *WRKY53* (Luna et al. 2012). There are several mutation studies showing hypomethylation-based resistance from bacteria by inducing salicylic acid response (Table 2). For example, mutants like *met1*, *drm1/drm2/cmt3*, *nrpd1, nrpd2, nrpe1, drd1, rdr2, rdr6* and *dcl2/3/4* show resistance from *Pst* infection (López et al. 2011; Dowen et al. 2012; Yu et al. 2013; Cambiagno et al. 2021; Luna et al. 2012). On one hand, *drm1/drm2/cmt3* (*ddc*) triple mutant-based hypomethylation makes the plant resistant to *Pst*, while on the other hand, it makes the plant vulnerable to *Agrobacterium tumefaciens* (Gohlke et al. 2013). *ago4* mutant also shows such hypomethylation-based susceptibility to *Pst* (Agorio and Vera 2007) and facilitates the *A. tumefaciens* infection (Gohlke et al. 2013)**.** It is interesting to note here that the same pattern of methylation in two different genes alters the response to the same pathogen. However, what leads to such a difference is not yet clear.

Fungus

Fungal pathogens infect a wide variety of agronomically important plants. Upon fungus infection, several changes occur at the molecular level which includes chromatin modifications. Primarily, fungal infection modifies the DNA methylation status of the promoter and TEs thereby affecting the transcription of the genes (Li et al. 2011; Le et al. 2014; Deng et al. 2017). Generally, DNA methylation in the promoter region represses the expression of the gene. In rice, the methylation of TEs present in the promoter of resistance (*R*) genes regulate the defense response along with the yield penalty of the crop by controlling the tissue-specific expression of the gene (Deng et al. 2017). *PigmR*, one of the NLR genes present in the *Pigm* locus confers resistance against rice blast disease. *PigmS*, another NLR gene present in the *Pigm* locus is not expressed in the leaves due to the presence of TEs in its promoter that are highly methylated. However, there is a high expression of *PigmS* in the pollens. The expression of both *PigmS* and *PigmR* in pollens contributes to disease resistance with no penalty in the crop yield (Deng et al. 2017).

The availability of DNA methylation profiling techniques and various studies on Arabidopsis mutants deficient in different steps of DNA methylation, DNA maintenance, and DNA demethylation, have contributed to understanding the regulatory roles of all three processes in the plant's response to fungal infection (Zhi and Chang 2021; Tirnaz and Batley 2019) (Table 2). For instance, Arabidopsis hypermethylated triple DNA demethylase mutant, *rdd* (*ros1dml2dml3*) showed increased susceptibility to *Fusarium oxysporum* (Le et al. 2014). The majority of the genes that were downregulated in *rdd* have known functions in defense response and contain multiple TEs in their promoters. For *F. oxysporum* resistance, DNA demethylase must target the TE sequences present in the promoter of defense response genes to enhance their expression. Interestingly, studies on the Arabidopsis mutants deficient in different steps of the RdDM process speculate inverse effects on the defense response against biotrophic and necrotrophic pathogens. Generally, RdDM-defective mutants exhibit enhanced susceptibility to necrotrophs and enhanced resistance to biotrophs. For example, different hypomethylated Arabidopsis mutants, as discussed in Table 2, showed increased disease susceptibility to the fungal necrotrophs *Botrytis cinerea* and *Plectosphaerella cucumerina* (López et al. 2011). Moreover, the Arabidopsis mutants *rdr6* and *dcl2/3/4*, with reduced synthesis of siRNAs, also showed increased susceptibility to *B. cinerea*. The susceptibility is caused due to the loss of the siRNAs that suppress the virulence genes of the fungus (Cai et al. 2018). On the contrary, knockdown of an RdDM component *DRM2* homolog in wheat progenitor *Aegilops tauschii* enhanced plant resistance to wheat powdery mildew caused by the biotrophic pathogen *Blumeria graminis* f. sp. *tritici* (Geng et al. 2019). Arabidopsis triple mutant *ddc* (*drm1drm2cmt3*) deficient in the methylation maintenance were highly susceptible to necrotrophic fungus *Alternaria brassicicola* (Luna et al. 2012). In some cases, DNA hypermethylation in promoters results in increased expression of genes. For instance, *Pib*, an NLR class of R gene that confers resistance to rice blast disease caused by *Magnaporthe grisea* was highly expressed in the infected leaves in spite of its heavily methylated promoter. Moreover, partial demethylation of the promoter results in disease susceptibility due to the reduction in the Pib expression (Li et al. 2011). Therefore, a more extensive investigation is required for a better understanding of the mechanisms that mediate plant immune responses toward fungal infection including studies on the dynamics of the cross-kingdom transfer of siRNA and RdDM pathway at different stages of infection.

Stress memory response in plants

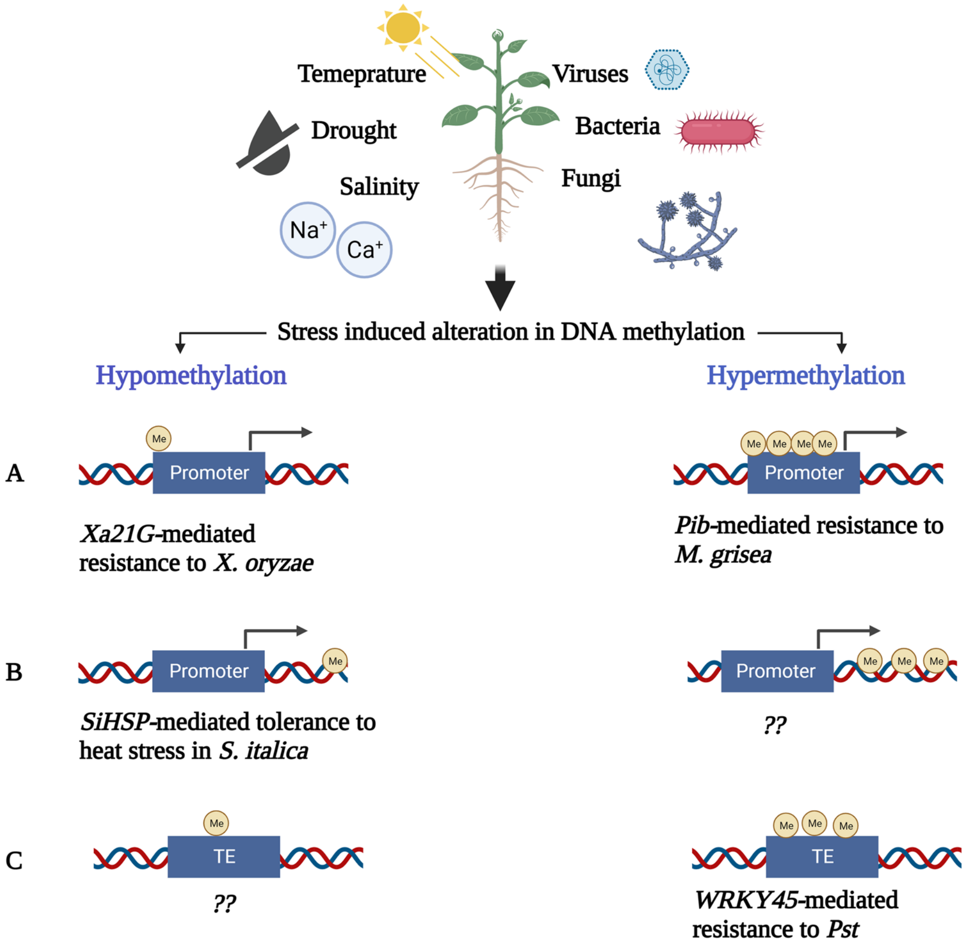
Plants are constantly subjected to various environmental stresses in natural conditions. Being immobile they must respond to challenges of pathogenic microbes, pests, and adverse climatic conditions. Magnitude of these environmental fluctuations is expected to further increase in future with global climate change. This will lead to some basal changes in germplasm composition of cultivated as well as wild-type plants. To cope up with these difficulties, plants activate various signalling cascades to reach the effect in the gene transcriptional network. One such mechanism in recent years that has emerged as a powerful tool to combat stresses is "stress memory" (Ashapkin et al. 2020; Bruce et al. 2007; Villagómez-Aranda et al. 2022). This mechanism involves molecular changes like DNA methylation, chromatin remodelling, histone marks etc. which helps plants to remember past stress affairs for the long or short term (Liu et al. 2014). Subsequently, this knowledge is used by plants to adapt to new or successive encounters. Exactly like immunization priming this stress memory becomes a defensive trait for the plant surviving in unfavourable conditions. Concept of short-term epigenetic memory called as "stress memory" was observed for the first time in Arabidopsis plants which were subjected to repeated dehydration stress which consequently showed enhanced transcription of stress-responsive genes (Ding et al. 2012). These plants exhibited higher water retention as compared to the ones which were given dehydration stress only once. Change in gene expression in these plants was hypothetically categorized into two classes namely stress-responsive (non-memory) genes and stress memory genes. Stress-responsive genes are expressed at parallel levels but stress memory genes have a characteristic increase in transcript levels upon recurring stress event as compared to the first event. Different marks including H3K4me3 and phosphorylation at 5th serine residue on RNA Pol II were found to be associated with the memory genes and stress-responsive genes. These marks stayed until the transcriptional memory of stress-related genes proceeded in the first category of genes but again came to basal levels during the recovery period given after stress. Physiological response of stress memory also heavily depends on the type of stress, the duration for which it was given and its intensity (Liu et al. 2021b). In a study, very high diversity in stress memory genes was observed in RNA-Seq analysis of Arabidopsis before dehydration stress and after the first and third encounter of dehydration stress, respectively (Ding et al. 2013). This further adds a new dimension to the molecular understanding of stress memory as an adjustment or defence response against stresses.

Envisioning priming of this stress memory in the whole plant or at least in seeds is thought to be an intelligent approach to combat stress in subsequent generations (Liu et al. 2021b; Villagómez-Aranda et al. 2022). Recently, it has also been shown that this memory during seed priming or changes that occurred at DNA and histone level can be inherited in next generation in plants (Sheteiwy et al. 2019). These transgenerational inheritance changes which can appear naturally during stress can also be artificially induced. This kind of carry forwarding of stress memory response ultimately helps the plants of subsequent generations to achieve an enhanced level of stress tolerance (Lämke and Bäurle 2017). One such example of caterpillar herbivory on Arabidopsis and tomato plants shows jasmonic acid-mediated transgenerational priming of defence response (Ashapkin et al. 2020). A phenotypic plasticity in the defence mechanism led by the changes in DNA methylation through the basic RdDM pathway credits a plant with elevated tolerance in successive generations. Transgenerational stress memory is meiotically stable and is independent of changes in DNA sequence. Similarly, stress priming in response to *Pseudomonas syringae* was shown to persist till the next generation (Ashapkin et al. 2020). Resultant, enhanced levels of salicylic acid-related stress responsive genes, enhanced priming and resistance to biotrophic pathogens were also observed. Although, the mechanistic basis of stress memory and priming still has many aspects to investigate but exploring new regulators of stress priming in concerted effect from gene-specific DNA methylation changes may lead to a better understanding. A brief study by Urrea Castellanos et al. (2020) reveals a novel gene *FORGETTER* (a type 2C protein phosphatase) playing a pivotal role in heat stress memory. *Arabidopsis thaliana* mutants of this gene (*fgt2*), though exhibited thermotolerance, still were completely defective in heat stress memory. Upregulation of defence-related hormones is also being reported to induce stress memory. Recently, when cold plasma-treated rice seeds were given combined plasma and salicylic acid, enhanced levels of salt stress tolerance at the seedling stage were achieved (Sheteiwy et al. 2019). Multi-omics analysis of cold stress primed Tartary buckwheat (*Fagopyrum tartaricum*) with repeated cold stress priming followed by severe cold shock reveals multiple memory-induced DNA methylation (Song et al 2020). These methylation patterns affect(s) various biochemical pathways such as pyrimidine metabolism and lysine degradation. This clearly suggests that cold stress memory particularly affects the metabolites which more often are involved in cold stress tolerance. These molecular mechanisms are potent in enhancing the stress tolerance levels of subsequent generations of plants. Seeds of crop plants can be primed with respective stresses before inoculating in the soil to reduce the yield penalty which comes with stress tolerance. Thermo-priming, which includes heat and cold stress priming is a 'cognitive ability' of plants which they can form and retain. This can be an excellent trait for plant scientists and breeders to design climate-smart crops with multi-trait advantages in future.

Conclusion

In the present review, we have discussed the impact of abiotic (temperature, drought and salinity) and biotic (virus, bacteria and fungus) stress in inducing alterations in the methylation status of chromatin, as part of the plant's defense mechanism (Fig. 3). Several studies have highlighted that the variation in the global methylation pattern of the plant genome is influenced by environmental factors. However, there are several unanswered questions which need to be addressed for a substantial understanding of the role of DNA methylation against abiotic and biotic stresses. Mutant analysis of enzymes associated with RdDM has highlighted the importance of this epigenetic mechanism in plant stress response. But how are these ubiquitously expressed components of DNA methylation recruited onto the specific loci of the genome remains elusive. Further, what are the signals and regulatory components which determine this specificity needs to be elucidated. TE-based DNA methylation of neighbouring genes has been reported to regulate plant response to changing environment but its initiation by epigenetic modifications needs in-depth investigation. Furthermore, gene body methylation has been reported in various methylome

Fig. 3 Schematic representation of stress-induced alteration in DNA methylation of plant genome affecting the transcription of the genes and plant response. Changes in the DNA methylation in (**a**) Promoter – hypomethylation in the promoter of *Xa21G* confers resistance to *Xanthomonas oryzae* pv. *oryzae* infection in rice whereas hypermethylation in the promoter of *Pib* confers resistance to *Magnaporthe grisea* (**b**) Gene Body – hypomethylation at CHH and CHG context of SiHSPs in foxtail millet provides tolerance to high-temperature stress in foxtail millet and (**c**) transposable elements (TEs) – hypermethylation of TEs in the promoter of *WRKY45* confers resistance to *Pseudomonas syringae* pv. tomato DC3000 (*Pst*). Encircled Me represents methylation and their number represents the methylation status



studies but the precise role it plays in gene expression needs to be defined.

Apart from this, there are several reports highlighting the impact of methylation of the viral genome on virus infection in plants, yet there is no clear understanding as to how DNA methylation of fungal and bacterial genomes regulate pathogenesis. Simultaneously, there are several viral suppressors known to repress the DNA methylation mechanism by inhibiting the activity of different DNA methylation enzymes but there are no such suppressors known in the case of other pathogens. In this regard, the study of epigenetic modifications in these pathogens and its effectors might provide new insights into the plant-pathogen interaction.

Methylome for several plants is available and a large number of epialleles have been identified which can be used by plant breeders for crop improvement. These epigenetic modifications in the host plant can be targeted through modern breeding or molecular approaches to develop stress-resistant plants. Still, there are several hurdles in its application, such as, (1) how to select the target region of DNA methylation (transposon or promoter) for breeding, (2) whether integration of such modification is stable and transferred to progeny as desired and (3) will these modifications provide broad-spectrum resistance against changing environment. Advanced research in the field of methylome study must be directed to fill in the gaps in our understanding of this crucial mechanism in plants.

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Declarations

Competing Interests Dr. Manoj Prasad is one of the editors of this journal and all other authors declare that they have no conflict of interest.

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