

Chromatin-Based Epigenetic Regulation of Plant Abiotic Stress Response



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ARTICLE HISTORY

Received: July 01, 2015
Revised: December 10, 2015
Accepted: December 13, 2015

DOI:
10.2174/138920291766616052010
3914

Abstract: Plants are continuously exposed to various abiotic and biotic factors limiting their growth and reproduction. In response, they need various sophisticated ways to adapt to adverse environmental conditions without compromising their proper development, reproductive success and eventually survival. This requires an intricate network to regulate gene expression at transcriptional and post-transcriptional levels, including epigenetic switches. Changes in chromatin modifications such as DNA and histone methylation have been observed in plants upon exposure to several abiotic stresses. In the present review, we highlight the changes of DNA methylation in diverse plants in response to several abiotic stresses such as salinity, drought, cold and heat. We also discuss the progresses made in understanding how these DNA methylation changes might contribute to the abiotic stress tolerance.



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Keywords: Abiotic stresses, DNA methylation, Epigenetic, Histone methylation, Plant adaption.

1. INTRODUCTION

Plants constantly face diverse kinds of stresses and have to respond to a variety of biotic and abiotic challenges. Abiotic stress involves unfavorable environmental factors such as temperature extremes, salinity, UV irradiation and lack of water. These abiotic stresses affect several cellular processes in plants leading to molecular, biochemical, physiological, and morphological changes affecting plant growth, development and reproduction [1]. Plants being sessile organisms needed to develop adaptation strategies to initiate defense mechanisms for the acquisition of stress tolerance. These involve changes in the expression of genes encoding stress-responsive transcription factors or functional proteins required for mechanical defense [2].

Several reports revealed a correlation between the regulation of gene expression and changes in chromatin modifications in plants during stress exposure. Epigenetic processes are crucial adaptive mechanisms which change the expression of genes in a heritable way without accompanying changes in DNA sequences [3]. Thus, heritable, but simultaneously reversible alterations in the transcriptional potential of cells are possible [4]. In eukaryotic cell, the structure and function of chromatin depends upon several regulatory epigenetic mechanisms, including DNA methylation and histone modifications [5].

Epigenetic mechanisms can modulate the chromatin structure and thus regulate the level of mRNA accumulation at the transcriptional level. Studies on epigenetic regulation in plants upon abiotic stress have focused on the examination of chromatin modulations at particular loci and the identification of proteins involved in it under stress conditions [6].

In plants such as *Arabidopsis thaliana*, *Oryza sativa* and *Zea mays*, it was recently reported that hyper- or hypomethylation of DNA induced by abiotic stimuli can modulate the expression of stress responsive genes [7-9]. In this review, we have focused on epigenetic changes in plant during salinity, drought, metal, cold and heat stress.

2. EPIGENETIC CHANGES IN PLANT

Epigenetic mechanisms involve among other modifications the methylation of cytosine in DNA and lysine residues of histones. Along with this, histone acetylation also can lead to altered transcription. Here, we focus on the mechanism of DNA methylation in plant. In (Fig. 1), we have depicted key players in the mechanisms of epigenetic regulation in plants as identified in *A. thaliana*.

2.1. DNA Methylation

DNA methylation involves the addition of a CH₃- group to the 5th carbon atom of cytosine or the 6th nitrogen atom of adenine nucleotides. In plant genomic DNA, cytosine methylation is mainly associated with transcriptionally silent sequences and can appear on symmetric context CpG and CpHpG (where H stands for A, C, or T) as well as asymmetric context CpHpH sites [10]. DNA methylation at CpG and CpHpG sites can be maintained in DNA replication by using hemi-methylated sites as substrate to re-establish methyl-

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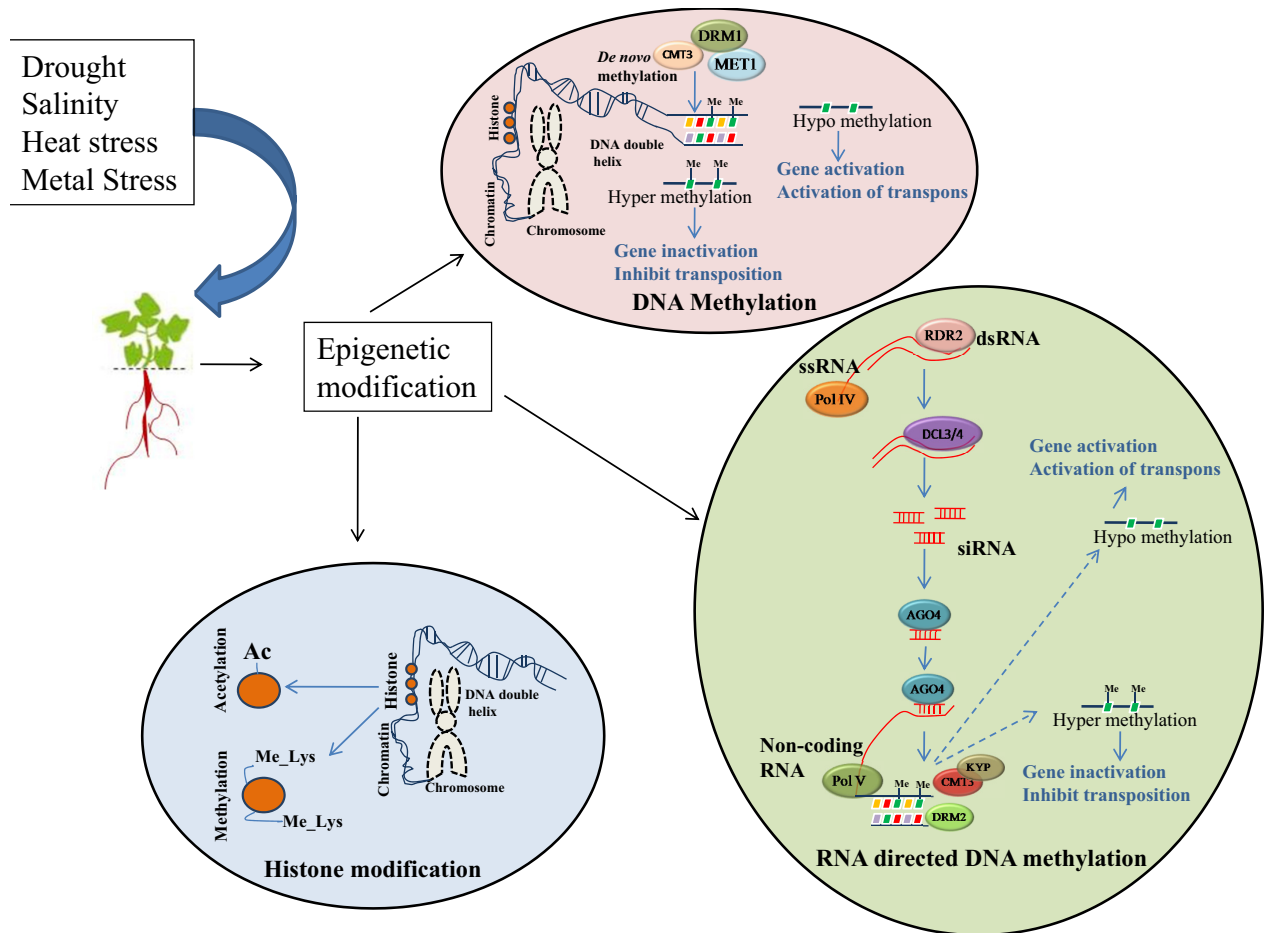


Fig. (1). A schematic diagram showing the involvement of epigenetic mechanism in plant cell under abiotic stresses. Plant can cope with these harsh environments by activating epigenetic switch. These modifications involve DNA methylation, RNA directed DNA methylation and histone modification events in the plant cell. Hypomethylation can lead to activation of gene/transposon while hypermethylation reduces the activity of gene/transposon. Histone methylation and acetylation also alter the gene expression to regulate abiotic stress response of a plant. CMT3, Chromomethylase; DCL3, Dicer-like 3; DRM, domain rearrangement methyltransferase; MET1, methyltransferase; Pol IV/V, DNA-dependent RNA polymerase; RDR2, RNA-dependent RNA polymerase 2.

tion on the newly synthesized strand. In contrast, methylations at CpHpH sequences cannot be maintained as one of the two DNA copies does not have a methylated cytosine at the respective position after DNA replication. Accordingly, two types of DNA methyltransferases are present in plants, one for methylation maintenance and another for de novo methylation. The maintenance type includes DNA methyltransferase 1 (MET1) and chromomethylase 3 (CMT3). MET1 sustains methylation at symmetrical CpG sites and CMT3 at CpHpG sites. Simultaneously, CMT3 also mediates CpHpH DNA methylation at sites with certain histone modifications [11, 12]. During DNA replication, both of these enzymes are thought to pass the information from existing onto the newly synthesized strands. Further, domains rearranged methyltransferases (DRM1 and DRM2) are required for methylation at CpHpH sites [13, 14]. The information for the site of de novo methylation by DRM1 and more important DRM2 is provided by RNA-directed DNA methylation (RdDM). In a further study, it was determined that also a third gene family member, DRM3, plays an important role during RdDM by activating DRM2 [15].

2.2. RNA-directed DNA Methylation

RdDM involves 24-nt siRNAs which act as sequence-specific signals to direct de novo cytosine methylation in CpG, CpHpG and CpHpH to DNA regions sharing sequence identity [16]. Biogenesis of the 24-nt siRNAs involves DNA-dependent RNA polymerase Pol IV, RNA-dependent RNA polymerase 2 (RDR2), and dicer-like 3 (DCL3) [17]. One of the strands of the resulting 24-nt dsRNA fragments then loads onto argonaute 4 (AGO4) to form a silencing effector complex. *De novo* DNA methylation at target DNA sites having sequence homology to the siRNA requires another DNA-dependent RNA polymerase, Pol V, which transcribes non-coding RNAs. The chromatin remodeling protein defective in RNA-directed DNA methylation 1 (DRD1) is thought to facilitate Pol V transcription [17]. An adaptor protein, KOW domain transcription factor1 (KTF1), mediates binding of AGO4 and AGO4-bound siRNAs onto the transcripts generated by Pol V [18]. This recruitment of the silencing effector then serves as signal for DRM2 to introduce *de novo* methylation at the target locus [19].

2.3. Histone Modifications

Another process of epigenetic modification i.e., Histone acetylation and methylation, also alters the expression of genes [20-22]. These modifications of histones are linked to both active chromatin structure and transcriptional repressive marks. H3- and H4-acetylation is catalyzed by enzyme called histone acetyltransferases (HATs), while histone deacetylases (HDACs) involves in deletion of these acetyl groups for maintaining the level of gene expression. Histone methylation is a more stable form of modification occurring at lysine (K) residues 4, 9, 27, 36 and 79 on H3 and K20 residue of H4 [23]. Methylation of H3 lysine 4 (H3K4me), H3K36me, and H3K79me is associated with active transcription whereas H3K9me, H3K27me and H4K20me are correlated to chromatin condensation and heterochromatic marks [21]. H3K4 methylation has been shown to occur in di- and tri-methylated state where di-methylation represents both inactive and active state of genes, however tri-methylation exclusively signifies the active genes. Role of histone-mediated chromatin modification under various environmental stresses have summarized in the subsequent sections.

3. SALT STRESS AND CHROMATIN MODIFICATION

Environmental stresses result in hyper- or hypomethylation of DNA. Soil salinity is one of the major threats to some of the most important crops. Hence, cultivars with improved salt tolerance are a major goal of plant breeding approaches evaluating salt tolerance mechanism that are effective in different varieties of crops. In a study using salinity tolerant *Brassica* cv. 'Exagone' and salinity sensitive *Brassica* cv. 'Toccatà', DNA methylation assessed by methylation sensitive amplified polymorphism (MSAP) was found to be overall reduced in cv. 'Exagone' and overall increased in cv. 'Toccatà' under salt stress [24]. Polymorphic MSAP fragments involved genes related to stress responses such as *Lacerata* and *trehalose-6-phosphatase synthase S4* [24]. DNA methylation changes in response to salt stress are genotype, tissue and developmental stage dependent. When rice genotypes with different degrees of salt-responsive characteristics were checked, it was found that there was generally more pronounced hypomethylation in roots than in shoots. Gene expression showed variation within both, genotypes and tissue types, independent from the respective salinity tolerance [25]. Similarly, contrasting differences in cytosine methylation patterns were observed in salinity-tolerant wheat cultivar SR3 and its progenitor in coding and non-coding regions of salt responsive genes upon salinity stress imposition [26].

A recent genome-wide survey of methylation changes under the influence of salt stress in two contrasting rice lines also revealed that hypomethylation in response to salt stress may be correlated with altered expression of DNA demethylases. It was further confirmed that in lines carrying mutant alleles of these epigenetic modulators phenotypic variation associated with salinity tolerance was affected [27]. DNA methylation and histone modifications may have a combined effect on stress inducible gene as salinity stress was reported to affect the expression of various transcription factors in soybean [28].

Chromatin regulation facilitated through histone acetylation can also be dynamically altered to maintain genome activities. Under high salinity response, enrichment of two cell wall genes i.e., *ZmEXPB2* and *ZmXET1*, was linked with increased H3K9 acetylation particularly at promoter and coding regions of the Maize genome [29]. Similarly, mutational studies revealed that the transcriptional adaptor ADA2b (modulator of HAT activity) was responsible for the hypersensitivity to salt stress in Arabidopsis [30] interestingly, histone modifications are reversible process and cross-talk between histone acetylation and methylation makes it more complex. For example, histone deacetylase HDA6 is crucial for H3K4me3-mediated gene activation and it was evident that the mutation in HDA6 in Arabidopsis leads to hypersensitivity towards ABA and salt stress. Hence, both the processes are linked to each other and essential for the synchronized action during stress response. All these studies have revealed that salinity stress influences genome-wide DNA methylation as well as histone modification and hence potentially also expressions of stress responsive genes. Some of the key studies demonstrating the epigenetic regulation of salt stress adaptation and response in plant are summarized in Table 1.

DROUGHT STRESS AND CHROMATIN MODIFICATION

Drought is considered as an essential factor which limits a plant's growth and productivity. DNA methylation is also affected by drought stress. For example, a link between DNA methylation and drought stress tolerance has been implicated in lowland and drought-tolerant rice cultivars. Drought susceptible cv. 'IR20' showed hypomethylation, while the tolerant cultivars 'PMK3' and 'Paiyur' showed hypermethylation, which may contribute to differential expression of stress responsive genes [31]. Another study in rice illustrated that hypomethylation under drought stress plays a significant role in the drought tolerant attribute of the rice genotype [32].

Under drought stress, accumulation of transcripts of stress responsive genes was positively correlated with histone modifications H3K9ac and H3K4me3 as both are marks of an active state of gene expression in *A. thaliana* [33]. In *A. thaliana*, drought stress leads to genome-wide variations in H3K4me1, H3K4me2 and H3K4me3 [9]. DNA methylation decreased under drought stress in perennial ryegrass in up-regulated genes [34]. Drought-correlated methylation changes were genotype-, tissue- and developmental stage-specific. Genome-wide DNA methylation analysis in rice introgression line 'DK151' and its parent 'IR64' detected extensive DNA methylation variation upon drought stress that showed reversibility upon stress removal [35]. Further, in tomato, a methylated epialleles of *Asr2* gene was identified as a putative target for improved performance under restricted water availability [36]. However, despite that abiotic stress has been reported to be associated with DNA methylation changes, we still lack a model case demonstrating that a stress-specific epigenetic variation is passed on to successive generations [37]. Recently, transgenerational inheritance of variation in DNA methylation arisen under drought stress has been claimed in rice. A study performed in drought-sensitive rice variety 'II-32B' and drought-tolerant cultivar 'Huhan-3' revealed cumulative effects on DNA methylation in six succeeding generations under drought stress [38].

Table 1. List of studies on epigenetic regulation in diverse plant species under various abiotic stress conditions.

Abiotic Stress	Plant	Methodology	Response	References
Salt	Rice	ELISA-based calorimetric assay	Hypomethylation in tolerant cultivar	[27]
	Rice	MSAP	More methylation in shoot	[25]
	Rice	MSAP	Decrease in methylation in roots of sensitive cultivar	[51]
	Wheat	MSAP & HPLC	Lower methylation level in tolerant cultivar	[52]
	Wheat	MSAP	Demethylation in tolerant cultivar	[53]
	Arabidopsis	Methyl-DNA immunoprecipitation (MeDIP)	Hypermethylation in promoter and gene body, H3K9me2 abundance	[54]
	Maize	MSAP	Methylation of negative regulator, demethylation of positive regulator	[55]
	Brassica	MSAP	Hypomethylation in tolerant and hypermethylation in sensitive cultivar	[24]
	Arabidopsis	ChIP seq	Decrease in H3K27me3 and induction of sodium transporter gene	[56]
	Maize	ChIP	Elevation of H3K9 acetylation on the promoter and coding regions of cell wall genes	[29]
Drought	Arabidopsis	ChIP seq	H3K4me3 enrichment and transcriptional activation of genes	[9]
	Rice	MSAP	i) Tissue specific methylation/ Demethylation ii) reversal of Methylation/Demethylation on recovery	[35]
	Rice	Chromatin-immunoprecipitation (ChIP)	Increase in H3K4me3 level and up-regulation of associated genes	[57]
	Rice	MSAP	Continuous exposure for six generations leads to methylation/demethylation	[38]
	Rice	MSAP	Hypermethylation in susceptible and hypomethylation in tolerant cv.	[32]
	Tomato	Bisulfite sequencing	i) Removal of methylation mark in regulatory region of Asr2 gene ii) Loss of H3K9me2 on stress imposition	[36]
	Rice	Western blotting	Enhanced acetylation of H3K9, H3K18, H3K27, and H4K5	[58]
	<i>Physcomitrella patens</i>	ChIP seq	Changes in H3K4me3, H3K27Ac and H3K9Ac during drought stress	[59]
Heat	Brassica	MSAP	Hypermethylation in sensitive and hypomethylation in tolerant cv.	[41]
	Grapevine	MSAP	Transgenerational inheritance of methylation after removal of stress	[60]
	Cork oak	HPCE, MS-RAPD	Increase in global methylation	[61]
	Arabidopsis	ChIP-qPCR analysis, cytosine-extension assay	Decrease global genome methylation, elevated transposon expression	[62]
	Arabidopsis	Methylation-sensitive qPCR	Up-regulation of epigenetic- modulators	[43]
	Arabidopsis	ChIP assay, Western blotting	ASF1A/B mediated H3K56acetylation (H3K56ac), which regulates HsfA2 and Hsa32 expression	[63]
	Maize	ChIP assay, Western blotting	H3K9ac, H4K5ac and H3ac level increased, H3K9me2 decreased, no change in H3K4me2	[64]
	Maize	MeDIP	Genome-wide hypermethylation	[65]
	Rice	MSAP	Hypomethylation at CHG sites	[66]

(Table 1) contd....

Abiotic Stress	Plant	Methodology	Response	References
Heavy metal	Maize	Coupled restriction enzyme digestion-random amplification (CRED-RA)	Increase in methylation	[67]
	Poplar	MSAP	Hypomethylation at later stage of plant growth	[68]
	<i>Posidonia oceanica</i>	MSAP	DNA hypermethylation, as well as an up-regulation of CMT	[69]
	Radish	MSAP	Increase in <i>de-novo</i> methylation	[70]
	Brassica	MSAP	Selenium prevent changes in DNA methylation triggered by Cadmium	[71]
	Chickpea	MSAP	Hypomethylation in tolerant upon prolonged exposure	[72]
Cold	Arabidopsis	ChIP	Decrease in H3K27me3	[73]
	Maize	Methylation sensitive PCR	Demethylation of ZmMI1	[74]
	Maize	ChIP	Increase in H3K9ac and H4K5ac in promoter of cell cycle genes	[75]
	Maize	ChIP, Western blot analysis	Decreased level of H3K9Ac, H4K5Ac and H44Ac	[76]
	Rice	ChIP	Transcriptional activation of <i>OsDREB1b</i> by hyperacetylation H3	[77]

In the course of strong drought conditions, the histone methylation (H3K4me3) and acetylation (H3K9ac) have been correlated to the activation of *RD20* and *RD29A* [39, 40]. Moreover, moderate drought conditions resulted in the less effect on nucleosome loss, specifically at *RD29A* region [39] in comparison to strong drought conditions [40]. Fascinatingly, abundance of H3K9ac quickly declined during drought recovery and consequently alters the expression of *RD29A*, *RD20*. However, H3K4me3 activity decline during rehydration treatment, although it occurs slowly than H3K9ac. These outcomes highlighted that epigenetic reaction is influenced by the intensity of the drought stress.

5. HEAT STRESS AND CHROMATIN MODIFICATION

Global warming possesses serious threats to the flora and may have deep and diverse effects on plants. Thus, it has become essential to study heat sensing and tolerance in plants. Genetic analysis of heat stress response has established diverse mechanism; however, epigenetic regulation in heat stress mediated responses in plants still deserves more investigations. Recently, a wide range of genes has been shown to be induced by heat stress, suggesting important roles of these genes in response and adaption to heat (Table 1). For example, changes in DNA methylation in response to heat stress differed between heat-tolerant cultivar ‘Huyou 2’ and heat-sensitive cultivar ‘Fengyou 1’ of *Brassica napus* [41].

Epigenetic mechanisms repress the transcription of DNA repeats and transposons in plants. Nevertheless, this repression can be released transiently under heat stress. In *A. thaliana*, the imprinted gene suppressor of DRM1 DRM2 CMT3 (SDC) is suppressed by DNA methylation throughout

the vegetative growth phase. Tandem-repeats within its promoter are activated by heat, which potentially contributes to recovery from heat stress [42]. Up-regulation of epigenetic modulators DRM2, nuclear RNA polymerase D1 (NRPD1) and NRPE1 might contribute to increased genome methylation in *A. thaliana* upon exposure to heat stress, suggesting a role of RdDM pathway in response to heat [43]. The methylation status of loci important for development may also change in response to heat stress. For example, in developing rice seeds, transcription of rice methyltransferase *OsCMT3* is reduced by heat, which may be affecting methylation of *fertilization-independent endosperm1* (*OsFIE1*), a member of *Polycomb repressive complex 2* [44]. Heat stress also can modulate the transcriptional activation characteristics of transgenes [45, 46].

Histone modifications through acetylation have also been reported to be associated with the heat stress response. In general, at high temperatures, a histone variant H2A.Z (facilitate the thermo-sensory response) has been shown to be detached by chromatin remodeling complex and subsequently cause transcriptional alterations in stress responsive genes [47]. Recent studies also highlights that mutation in *A. thaliana* GCN5 (histone acetyltransferase) resulted in thermo-susceptibility due to impaired transcriptional activation of some heat stress responsive genes HSAF3 and MBF1c [48].

Finally, activation of TEs can be associated with epigenetic changes during heat stress, as in the case of the Ty1/copia retro-transposons of the ONSEN group in *A. thaliana* [49]. Cytosine methylation changes were profound around a transposable element in *Leymus chinensis* upon exposure to abiotic stresses associated to global warming and nitrogen deposition [50].

6. METAL STRESS AND CHROMATIN MODIFICATION

Heavy metals play a crucial role in each stage of the plant life, but unbalanced doses may be toxic and damage various cellular components. Undeniably, excessive doses of heavy metals lead to changes in DNA methylation (Table 1). Poplar clones inoculated with arbuscular mycorrhizal fungi that were grown on soil polluted by various metals such as copper and zinc showed little changes in cytosine methylation at early stages but extensive hypomethylation at later stages of plant growth. Hypomethylation affected genes involved in RNA processing, cell wall formation, and amino acid metabolism [68]. A study on rice leaf tissue exposed to heavy metal stress, DNA methylation patterns were found to alter in a way that CHG type hypomethylation was predominant. In the successive generation, enhanced tolerance to heavy metals was observed, suggesting a transmission of epigenetic modifications [66]. Another study highlighted altered DNA methylation in *Vicia faba* in excess of cobalt [78]. More recently, a study on maize seedling highlighted the alteration of DNA methylation by zinc stress in a concentration-dependent manner [67].

Cadmium is one of the most common heavy metals present in all environments [69]. Its toxicity has adverse effect on plant growth caused by reduced photosynthesis and metabolism. MSAP analysis was used to investigate cytosine methylation upon cadmium stress in radish, detecting hypermethylation at 5'-CCGG sites [70]. DNA hypermethylation was also observed in the sea grass *Posidonia oceanica* upon cadmium treatment [69]. There were various reports suggesting the involvement of DNA methylation-based epigenetic modification in plants, however no direct correlation between heavy metal stress and histone acetylation/methylation has been highlighted in recent years.

7. COLD STRESS AND CHROMATIN MODIFICATION

During cold stress, altered levels of histone modification and DNA methylation were found to be synchronized with modulation of gene expression. This process is initiated with the activation of various regulators of histone modification. Upon cold stress imposition, up-regulation of HDACs resulted in the deacetylation at H3 and H4 [79] and successively heterochromatic tandem repeats get activated. This consequently reduces the DNA methylation and H3K9me2 at the targeted region of the maize genome [80]. It has been observed that ~10% genes have been transcriptionally modulated in *Arabidopsis* under cold stress [81]. A WD40-repeat protein HOS15 (high expression of osmotically responsive gene1), play role in the repression cold-stress tolerance genes via histone deacetylation in *Arabidopsis* [82]. Moreover, a study claimed that upon cold stress, signaling pathways cross-talk with DNA methylation [72]. Epigenetic modifications responding to cold have also been highlighted in various studies (Table 1).

8. DNA METHYLATION AND STRESS MEMORY

Alterations in DNA methylation in response to environmental stress might either be reset after the stress ends or

might remain long term and that prepare plant for future stress adaptation. In a study conducted on somatal clones of grapevine, 40% of epigenetic modifications were found to vanish within a year following the termination of stress conditions [59]. However, 60% of DNA methylation variation between maternal plants and somatal clones persisted [60]. The three activating histone marks H3K4me3, H3K27Ac and H3K9Ac show significant changes during the juvenile to adult developmental transition as well as on imposition of drought stress in the moss *Physcomitrella patens*. This condition documented that drought-induced adult gametophore-expressed genes are prepared to respond to desiccation stress [59]. Inheritance of epigenetic variations triggered by stress can be envisioned as a way to develop more stress-tolerant crop varieties. As discussed above, drought stress for six generations had a cumulative effect on DNA methylation in sensitive as well as tolerant rice cultivars [38].

CONCLUSION

In conclusion, we have witnessed that epigenetic mechanisms potentially help plants to withstand extreme environmental conditions. Recent studies have encouraged the plant biologists to identify the in-depth mechanism of epigenetic-mediated stress response which might contribute to tolerance against adverse abiotic factors. Outcomes of the previous studies can be helpful and can be applied in the genetic manipulation of plants for superior abiotic stress tolerance. However, no direct evidences of DNA methylation and Histone modification in providing resistance/tolerance has been established in many crops, their applicability cannot be neglected. For this, the combined approach of modern technology with the genetics and molecular biology needs to be worked upon collectively. For this, existing data sets of stress-induced global DNA methylation can be used for the identification of the likely targets. Recently, a large number of novel and powerful epigenetic techniques have been developed such as single-cell restriction analysis of methylation (SCRAM), Single-cell Hi-C, ligation-free Hi-C, RNA antisense purification with mass spectrometry (RAP-MS), single nucleotide polymorphism and fluorescence *in situ* hybridization (SNP-FISH) and native elongating transcript sequencing (NET-seq).

Agronomically important traits are closely associated with epigenetic changes in the DNA methylation patterns. Genetic-QTLs which are differentially methylated to alter gene expression patterns, would also be beneficial for crop traits. Such, methylated-QTL would be considered 'epi-QTL' that could be targeted in crop breeding programs. More importantly, these epigenetic changes should be heritable, and transferred to subsequent generations. Thus, dissecting the epigenetic variation would help in understanding the molecular mechanism behind inheritance and could be promising in crop improvement program by the selection or creation of novel epialleles and regulation of transgene expression.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to the Director, National Institute of Plant Genome Research (NIPGR) for providing facilities. We gratefully acknowledge the financial support from NIPGR core-grant. GP and NS acknowledge the award of Senior Research Fellowship from University Grants Commission and Department of Biotechnology, Govt. of India, India, respectively.

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