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Chapter

Epigenetic Modifications in Plants under Abiotic Stress

Garima Singroha and Pradeep Sharma

Abstract

Plants face a plethora of biotic and abiotic stresses ranging from extreme temperatures to salinity, drought, nutritional deficiencies, chemical toxicity, and pathogen attacks. As a consequence, plants have acquired several sophisticated regulatory mechanisms that allow them to cope with such adverse conditions. Epigenetic regulation plays a key role in the mechanisms of plant response to the environment, without altering DNA sequences. Epigenetics refers to heritable alterations in chromatin architecture that do not involve changes in the underlying DNA sequence but alter gene expression through DNA methylation or histone modifications. The epigenetic regulation of the plant genome is a highly dynamic process that fine-tunes the expression of a pertinent set of genes under certain environmental or developmental conditions. Over the past two decades rapid advancements in the field of high throughput sequencing unveil epigenetic information at genome wide level in various plant species. In view of the adverse effects of global climatic change, utilizing epigenetic differences for developing improved crop varieties is of paramount importance.

Keywords: histone modification, DNA methylation, abiotic stress, chromatin

1. Introduction

Plants being sessile organisms are being constantly challenged by various biotic and abiotic stresses. In order to adapt themselves to the changing environments they need constant changes at molecular level. These efficient and effective controls are provided by epigenetic regulations which improve the survivability of plants by increasing their tolerance toward stress [1, 2]. It is now evident that heritable phenotypic variation does not need to be based on DNA sequence polymorphism [2, 3]. These epigenetic regulations involve different chemical modifications at molecular level that influence gene expression. Epigenetic as defined by Conrad Waddington, is "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" [4]. Today epigenetic refers mainly to the changes that do not relate to the changes in DNA sequence but to chemical modification that can be inherited from one generation to the next [5, 6]. Three types of epigenetic regulatory mechanisms viz. DNA methylation, histone modification and RNA interference (RNAi) are exploited by plants in order to survive adverse conditions.

DNA methylation is a chemical modification, catalyzed by cytosine methyltransferases which involves addition of a methyl group in a DNA sequence onto the

cytosine residue in a sequence specific manner, primarily within CpG dinucleotide [7, 8]. The added methyl group provides platform for attachment of various protein complexes that modifies the histone scaffolds resulting in altered gene expression.

In eukaryotic nuclei DNA is organized in the form of nucleosome where it is wrapped around by histone proteins. Histones comprise a family of highly conserved globular proteins whose N-terminal tails are exposed on the surface of the nucleosome octamer for chemical modifications. Histones offer a wealth of post-translational modifications (PTMs) that physically regulate the accessibility of the transcriptional machinery to certain genomic regions, making loci more or less permissive for transcription [9]. Histone modifications include acetylation, methylation, sumoylation, ubiquitination and phosphorylation of histone proteins. Acetylation and phosphorylation are mostly associated with induced gene expression while on the other hand modifications like sumoylation and biotinylation represses gene expression [10, 11]. Such modifications not only impinge on DNA accessibility, but also on the recruitment of specific proteins involved in several processes, including transcription, DNA replication and repair. Histone proteins are not only modified, but can also be replaced by histone variants with different physical properties, or released, in order to allow gene expression [12].

In epigenetic cross-talks diverse classes of noncoding RNA (e.g., small RNAs and long noncoding RNAs) can also modify chromatin structure and silence transcription through formation of RNA scaffolds mediating the recruitment of histone and DNA methyltransferases [13]. RNAi is a sequence specific gene regulation mechanism that acts as a barrier against viruses but also regulates gene expression. In plants RNA interference pathways are mediated by siRNA, miRNA and lncRNA (long non coding RNA). These RNAs are synthesized as 20–30 nucleotide, single stranded molecules from double stranded RNA precursors.

Activation of one or more of these pathways results into changes in chromatin architecture and impacts gene expression. Open chromatin form or closed

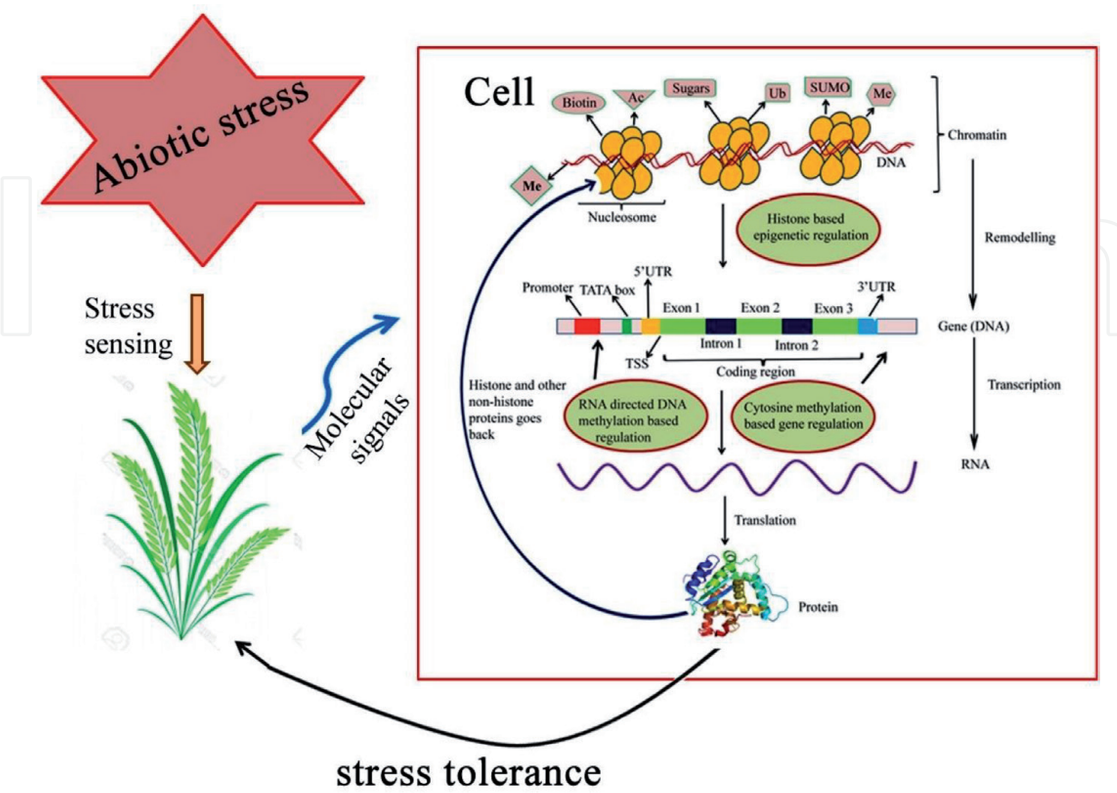

Figure 1.
Various types of epigenetic modifications under stress conditions.

chromatin conformation is associated with gene activation or silencing respectively and governs the onset of gene expression in cells under different developmental or environmental stimuli [14–16]. Transitions from open to close and crosstalk between different epigenetic mechanisms are vital to ensure proper cell function at different developmental stages and under abiotic stress conditions [17–19]. Different types of epigenetic modifications under abiotic stresses have been presented in **Figure 1**.

In the recent years, numerous studies performed toward the characterization of the epigenomic regulation of stress responses in plants have added to our understanding of how diverse abiotic stresses affect chromatin modifications, with their respective transcriptional and physiological implications.

2. Different types of epigenetic modifications

2.1 DNA methylation modification

DNA methylation arises as a result of addition of a methyl group to the nitrogenous base in the DNA strand in a sequence specific manner. DNA methylation occurs at the fifth carbon position of a cytosine ring. Methylation of cytosine leads to the generation of 5-methyl cytosine. On the basis of the target sequence, methylation is classified either as symmetrical or asymmetrical methylation. CG and CHG methylation are termed as symmetrical and CHH methylation as asymmetrical. In plants DNA methylation occurs in all three sequence contexts; the symmetric CG and CHG context and asymmetric CHH (H = A, C or T) context [20]. Plants methylate only some genes and this methylation is usually restricted to CGs located within the gene body while Transposable Element sequences tend to be methylated at most of their CG, CHG, and CHH sites. Methylation in transposable elements and promoter region of a gene leads to silencing on the other hand methylation inside gene body induce gene expression [21]. Thus DNA methylation results into following (i) methylcytosines in the gene body play an important role in regulating the gene expression and (ii) methylcytosines in repetitive sequences (transposable elements), are thought to prevent repetitive sequences from compromising normal genome function [20, 21]. Increased methylation of genomic DNA down regulates gene expression. Down regulated gene expression enable the plants to conserve energy for the sake of biotic or abiotic stress. In contrast, the reduction in methylation of resistance-related genes favors chromatin activation and the expression of novel genes, which provides long-term or permanent resistance for stress.

2.2 Histone modifications in plants

In addition to DNA methylation, histone N-terminal tail modifications constitute an attractive area in epigenetics [22]. Plants contain several histone variants and enzymes that posttranslationally modify histones and influence gene regulation. Application of chromatin immunoprecipitation followed by deep sequencing has given insight into the genome-wide distribution of histone variants and histones bearing different posttranslational modifications [22, 23]. Histone proteins are wrapped around DNA and forms a highly compact structure called nucleosome. Nucleosomes are composed of histone octamers that comprise two copies each of H2A, H2B, H3, and H4. A total of 147 base pair of DNA sequence is wrapped around the histone core. The N termini of histone proteins called N terminal tails undergo various chemical modifications like methylation or acetylation. Such histone modifications are associated with either gene repression or gene activation [24, 25].

In plants methylation and deacetylation of H3K9 and H3K27 results into gene repression whereas acetylation and methylation of H3K4 and H3K36 is associated with gene activation and thus induces gene expression [26]. These covalent modifications in response to various environmental stresses regulates the transcription of wrapped DNA sequence by altering the packaging structure which either activates the DNA for the transcription or makes the structure more condensed so that transcription machinery is unable to reach it.

2.2.1 Histone acetylation/deacetylation

Addition of acetyl group to the N terminal Lysine of histones results into transcriptional activation of a DNA sequence [27]. Acetylation of N terminal lysine causes reduction in the net positive charge of histone and as a result the electrostatic force of attraction between the negatively charged DNA and positively charged histone reduces which leads to the loosening of chromatin and transcriptional activation of DNA [28]. The addition of acetyl group to Lysine is catalyzed by histone acetyltransferases (HATs). Five types of HATs have been identified in eukaryotes viz. GNAT—GCN5-related N-terminal acetyltransferase; MYST—MOZ, Ybf2/Sas3, Sas2, and Tip60; CBP—CREB binding protein; TFII250—TATA binding protein-associated factors and the nuclear hormone-related HATs family. Only specific lysines in a histone protein are acetylated. In different histone proteins different lysine residues undergo modifications for instance, lysine residues of H4 (K5, K8, K12, K16, and K20) and histone H3 (K9, K14, K18, K23, and K27) are subjected to acetylation modifications [29, 30].

2.2.2 Histone methylation

Arginine and Lysine amino acids in histone proteins undergo methylation. Different arginine and lysine residues in different histones undergo different types of methylation (R3 of H2A, R3, K20 of H4 and K4, K9, K27, K36, R2, and R17 of H3 etc.) and these residues can be mono, di or tri methylated. Usually, arginine undergoes mono- and dimethylation only while lysine can undergo mono, di and tri methylation. Methylation can either activate or deactivate a gene depending on the nature of residues methylated for example H3K4 trimethylation activates transcription on the other hand K9 and K27 dimethylation in H3 acts as a repressor [31]. Methylation affects the hydrophobicity of the histone and hence may change histone DNA interactions or may create binding site for various proteins which restricts the binding of transcription machinery and prevents transcription. Histone lysine methyltransferases (HKMT) and protein arginine methyltransferases (PRMT) catalyze methylation of lysine and arginine residues respectively [32].

2.3 miRNA directed DNA methylation

RNA directed DNA methylation (RdDM) is *de novo* cytosine methylation primarily within the region of RNA-DNA sequence identity. Although RdDM pathway can methylate all sequence contexts, but it specifically methylates CHH sequences. The reason for this is that symmetrical methylation is maintained by maintenance methylation each time the DNA is replicated whereas the CHH methylation at many silenced loci is dependent on RNA-guided *de novo* methylation [33].

The 24-nt siRNAs are generated by DNA dependent RNA polymerase Pol IV enzyme, in association with RNA-dependent RNA polymerase 2 (RDR2), and processed by dicer-like 3 (DCL3) [34]. One strand of the resulting 24-nt dsRNA fragments is loaded onto argonaute 4 (AGO4) leading to generation of a silencing

effector complex. DNA methylation at sites having sequence homology to the siRNA is dependent on, Pol V, which is a DNA dependent RNA polymerase that transcribes non-coding RNAs. Transcription of Pol V is facilitated by a chromatin remodeling protein which is defective in RNA-directed DNA methylation 1 (DRD1) [35]. KOW domain transcription factor1 (KTF1) which is an adaptor protein, mediates binding of AGO4 and AGO4-bound siRNAs onto the transcripts generated by Pol V forming a silencing effector. This effector acts as signal for DRM2 to introduce methylation at target sites [35]. Development of stress tolerant crop has successfully been achieved by the use of RNAi technology. Transgenic rice plants with tolerance to drought were developed by silencing of activated C-kinase1 receptor [36].

3. Epigenetic changes in crops against abiotic stresses

Due to the unpredictable climate change, crop plants are frequently exposed to a variety of abiotic stresses resulting in reduced crop productivity. Analysis of the stress-associated genes and their regulation in response to the stress can be utilized to enhance understanding of the plant's ability to adapt under changing climatic conditions. DNA methylation and/or histone modifications are influenced by abiotic/biotic factors resulting in the better adaptability of the plants to the adverse environmental conditions. Such epigenetic modifications provide a mechanistic basis for stress memory, which enables plants to respond more effectively and efficiently to the recurring stress as well as to prepare the offspring for potential future assaults.

3.1 Salt-induced epigenetic changes in crop plants

Environmental stresses result in hyper or hypomethylation of DNA. Evidence implicates epigenetic mechanisms in modulating gene expression in plants under abiotic stress. Promoter and gene-body methylation plays important role in regulating gene expression in genotype and organ specific manner under salt stress conditions. Song et al. [37] observed that 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. Ferreira et al., [38] emphasized that hypomethylation in response to salt stress may be correlated with altered expression of DNA demethylases. In another report [39] contrasting differences in cytosine methylation patterns were observed in salinity tolerant wheat cultivar SR3 and its progenitor upon salinity stress imposition. The responses of contrasting wheat genotypes under salt stress could be attributed to the altered expression levels of high affinity potassium transporters (HKTs) regulated through genetic and/or epigenetic mechanisms [40]. It was found that the coding region of high affinity potassium transporters (HKTs) showed variations in 5-mC content in the contrasting wheat genotypes. Salt stress significantly increased methylation level in wheat genotypes. Cytosine residues in CG context were all methylated, whereas increase in 5-mC was observed in CHG and CHH contexts in the shoot of a salt-sensitive wheat genotype under the stress. Variations in chromatin structure (facilitated through histone modifications) also play important role in salt tolerance. Kaldis et al. [41] reported that in *Arabidopsis thaliana* the transcriptional adaptor ADA2b (a modulator of histone acetyltransferases activity) is responsible for its hypersensitivity to salt stress. However, histone modifications are reversible and cross-talk between histone acetylation and cytosine methylation makes the plant responses more complex. Thus, salt stress affects genome-wide DNA methylation as well as histone modifications and these processes are linked to each other for synchronized action against salt stress [42].

3.2 Heat induced epigenetic changes in crop plants

Naydenov, [43] reported that upregulated epigenetic modulators like DRM2, nuclear RNA polymerase D1 (NRPD1) and NRPE1 may be responsible for increased genome methylation in *Arabidopsis thaliana* under heat stress conditions. Heat stress related study in rice showed reduction in seed size which is controlled by OsFIE1 (fertilization independent endosperm). Folsom and coworkers [44] in their study reported that DNA methylation and histone (H3K9me2) methylation are the two major factors governing the expression of OsFIE1. It was found that under heat stress both DNA methylation as well as histone methylation showed a decline (DNA methylation declined by 8.8% and 6.6% with respect to CH and CHG context). Reduced methylation levels resulted into lower expression of OsFIE1 and lead to reduction in rice seed size. Histone modifications like acetylation have also been reported to occur under heat stress conditions. At high temperatures, a histone variant H2A.Z causes transcriptional changes in stress responsive genes [45]. Mutations in a gene *GCN5* that codes for histone acetyltransferase, resulted in impaired transcriptional activation of heat stress responsive genes like HSAF3 and MBF1c and lead to thermal susceptibility of *Arabidopsis thaliana* [46]. The duration of heat treatment also has diverse effects on the epigenetic mechanisms emphasizing complexity in the epigenetic regulation of heat stress [47].

3.3 Epigenetic modifications in response to drought

Drought stress conditions generally tend to increase demethylation. It is also observed that DNA methylation shows tissue specificity. In *Oryza sativa* drought induced a total of 12.1% methylation differences accounted across different tissues, genotype and developmental stages. The overall DNA methylation level at the same developmental stage was lesser in roots than in leaves indicating significant role of roots under water insufficiency [48]. Correlation between DNA methylation and drought stress tolerance has been shown in lowland and drought-tolerant rice cultivars. IR20, a drought susceptible variety, undergoes hypomethylation under drought conditions whereas the tolerant varieties "PMK3" and "Paiyur" showed hypermethylation. These changes in methylation pattern contributed to differential expression of stress responsive genes [49]. In another study conducted in rice it was illustrated that hypomethylation has significant role in the drought tolerant attribute of rice genotypes [50].

In several studies the abundance in transcript levels of drought responsive gene was correlated with changes in histone modification. Under drought conditions several histone alterations like acetylation, methylation, phosphorylation and sumoylation occurs [51]. Reports have documented that drought stress response is memorized through histone modification of various drought stress induced genes [52]. In a study in *A. thaliana* it was shown that an increase in H3K4 trimethylation and H3K9 acetylation on the promoter region and H3K23 and H3K27 acetylation on the coding regions is responsible for drought-induced expression of stress-responsive genes [53]. Under stress conditions, 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 [54].

3.4 Epigenetic modifications in response to cold

Upon imposition of cold stress HDACs are upregulated that results into deacetylation of H3 and H4 and successively heterochromatic tandem repeats get activated [55, 56]. This results into reduction of DNA methylation and histone (H3K9me2) methylation at the targeted region of maize genome [39, 57]. In a study conducted

on the effect of cold on maize seedlings it was found that cold stress induced genome wide DNA methylation in root tissues except only in a 1.8-kb segment designated as ZmMI1. Under normal conditions ZmMI1 segment is methylated but under chilling conditions it is demethylated. This segment is representative of a stress responsive gene that plays role under stress conditions [58].

Even after 7 days of recovery, cold induced hypomethylation was not reverted back. In a similar study conducted by Saraswat et al. of DNA methylation pattern in cold grown maize 28 differentially amplified fragments were obtained. *In silico* analysis of these fragments revealed their role in several processes like photosynthesis, hormone regulation and in cold response [59]. A recent study in apple highlighted the importance of epigenetic changes in response to dormancy caused by low temperature. High chilling conditions decreased total methylation that lead to reinitiation of active growth and subsequent fruit set in apple [60, 61].

4. Techniques for deciphering epigenetic changes in plants

4.1 Histone modifications

4.1.1 Chromatin immunoprecipitation (ChiP) techniques

This technique is used to assay DNA–protein binding under *in vivo* conditions. This involves shearing genomic DNA into smaller fragments through sonication to generate fragments ranging 200–800 base pairs. Gentle formaldehyde treatment is given to crosslink proteins with DNA. Antibodies raised specifically for protein of interest are used to precipitate the protein-DNA complex. Precipitated DNA thus obtained is released by acid treatment and amplified by PCR [62].

4.1.2 ChiP-Seq

Advancements in the field of next-generation sequencing have made it possible to combines ChiP with next-generation sequencing technology such as Solexa. ChiP-Seq combines Chromatin immunoprecipitation and sequencing technologies to decipher genome wide distribution of histone proteins [62].

4.1.3 ChiP PCR

Immunoprecipitated DNA is amplified and quantified by real time PCR (RT-PCR) using TaqMan or Syber Green Technologies with specified primers for analysis of specific genomic regions associated with particular histones.

4.2 DNA methylation profiling in plants

Earlier studies focused on determining methylation status of the gene of interest. With the use of microarray hybridization technology DNA methylation has been scaled up to genome wide level. Next generation sequencing platforms are now being used for the construction of genomic maps of DNA methylation at single-base resolution.

4.2.1 Genome-wide bisulfite sequencing

Bisulfite treatment converts unmethylated cytosines to uracil, allowing for the identification of methylated cytosines by comparing a treated sample to a reference

sample [63]. Bisulfite sequencing evaluates individual cytosines in a target sequence for essentially all cytosines in a genome (i.e. whole-genome bisulfate sequencing or WGBS).

4.2.2 Methylated DNA immunoprecipitation (MeDIP)

Genomic DNA is fragmented and precipitated with 5-methylcytosine-specific antibody. The precipitated DNA is then analyzed by PCR or whole genome tiling microarrays [64, 65].

4.2.3 Reduced-representation bisulfite sequencing (RRBS)

RRBS came into existence for the purpose of deciphering the mammalian methylome at low cost [66]. Bisulfite sequencing can be used for genomic fragments that are isolated with restriction enzymes thus providing single-nucleotide resolution of DNA methylation within each of the fragments. Availability of both sequence and methylation variation from same set of locus allows comparison of genetic and epigenetic differences. It is based on MspI restriction digestion and selection of (40 and 220 basepair) digested fragments for bisulfite conversion and sequencing [67]. RRBS has been adopted for plant population studies and can be applied to species for which no reference genomes are available [68, 69]. RRBS has also been used in oak populations [70] and *Brassica rapa* [71].

4.2.4 Shotgun bisulfite sequencing

This combines bisulfite treatment of genomic DNA with next generation sequencing technology such as Solexa sequencing. The converted sequences are mapped to the reference genome sequence to identify methyl-cytosines [63, 72].

5. Conclusions

In view of the increasing stress conditions experienced by plants due to global climatic changes, epigenetics is considered as an important regulatory mechanism that is influenced by environmental stimulus. This regulatory mechanism is of utmost significant importance in terms of its inheritance over generations. Advancements in the ultra-high-throughput techniques have revolutionized identification of epigenetic changes and improved our knowledge on effect of epigenetic changes on regulation of gene expression. Manipulation of DNA (de) methylation level at specific loci may allow us to regulate gene expression and the neighboring chromatin states, impacting cell physiology and biochemistry. Therefore, one of the possible, yet unexplored, ways to improve stress tolerance in crop plants may be to augment stress memory of the plants by targeted modification of the epigenome. Thus utilizing epigenetic variation for developing improved abiotic stress tolerant crop verities is an undertaking of paramount importance.

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Conflict of interest

The authors hereby declare that there is no conflict of interest.

Abbreviations

DNA	deoxyribonucleic acid
RNAi	ribonucleic acid interference
PTMs	post-transcriptional modifications
H3K9	histone 3, 9th lysine
H3K27	histone 3, 27th lysine
HATs	histone acetyltransferases
HKMT	histone lysine methyl transferases
PRMT	protein arginine methyl transferases
nt	nucleotide
RDR2	RNA-dependent RNA polymerase 2
siRNAs	small interfering ribonucleic acid
AGO4	argonaute 4
dsRNA	double stranded ribonucleic acid
HKTs	high affinity potassium transporters
NRPD1	nuclear RNA polymerase D1
OsFIE1	fertilization independent endosperm
PCR	polymerase chain reactions
WGBS	whole-genome bisulfate sequencing
MeDIP	methylated DNA immunoprecipitation
RRBS	reduced representation bisulfite sequencing

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