Epigenetic mechanisms of plant stress responses and adaptation

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Abstract Epigenetics has become one of the hottest topics of research in plant functional genomics since it appears promising in deciphering and imparting stress-adaptive potential in crops and other plant species. Recently, numerous studies have provided new insights into the epigenetic control of stress adaptation. Epigenetic control of stress-induced phenotypic response of plants involves gene regulation. Growing evidence suggest that methylation of DNA in response to stress leads to the variation in phenotype. Transposon mobility, siRNA-mediated methylation and host methyltransferase activation have been implicated in this process. This review presents the current status of epigenetics of plant stress responses with a view to use this knowledge towards engineering plants for stress tolerance.

Keywords DNA methylation · Epigenetics · Environmental stresses · Cytosine methylation

Introduction

Adverse environmental cues distort the growth, development and productivity of crop plants. As a defence, plants have evolved sophisticated mechanisms to respond and acclimatize to these stresses by prompt and harmonized changes at transcriptional and post-transcriptional levels of whole gene complexes (Golldack et al. 2011). Various genes belonging to diverse transcription factor families have been shown to regulate stress-responsive genes, thus playing a pivotal role in stress signalling. Recently, epigenetic mechanisms have been implicated in the regulation of the expression of stress-related genes (Chinnusamy and Zhu 2009). These mechanisms regulate almost all the genetic functions including transcription, replication, DNA repair, gene transposition and cell differentiation. Modifications in chromatin and generation of small RNAs have been recently shown to be involved in transcriptional and post-transcriptional control of gene expression, critical for stress responses (Madlung and Comai 2004; Angers et al. 2010). These modifications include both DNA and histone methylation which are tissue-, species-, organelle- and age-specific (Vanyushin and Ashapkin 2011). In this phenomenon, expression of genes can also be altered by transgenerational or heritable modifications in chromatin. DNA methylation is controlled by hormonal fluxes, which are in turn influenced by various abiotic and biotic factors (Zhang et al. 2012) resulting in plant adaptation (Mirouze and Paszkowski 2011) Thus, deciphering how methylation-based epigenetic machinery acts towards tackling the environmental stresses in plants is important. It will also provide a valuable platform for potential applications including genetic manipulation of plants towards enhanced tolerance to environmental stresses.

Mechanisms of establishment of DNA methylation

Cytosine methylation refers to the covalent enzyme-catalysed transfer of methyl group from S-adenosyl methionine to the 5’ position of cytosine, thus converting cytosine to 5-methylcytosine (5mC). Plants have comparatively high levels of 5mC, ranging from 6 to 25 % of total cytosines,
depending on the species (Steward et al. 2000). DNA cytosine methylation in plants appears in three sequences, CpG, CpNpG and CpNpN, where N stands for A, C, or T. The CpG and CpNpG methylation can simply be copied after DNA replication because of their symmetrical nature, but non-symmetrical CpNpN methylation has to be established de novo subsequent to each DNA replication cycle (Karlsson et al. 2011). This epigenetic memory accumulated by plants during their vegetative phase under environmental influence is passed on to the next generation by germline cells, which get established later during development. DNA is methylated at promoter as well as gene body regions, thus allowing the gene to exist in a repressed state. Thus, a decline in the level of methylation is likely to lead an increase in gene expression (Finnean et al. 1998).

The epigenetic mechanism involves the post-translational modifications of amino acids in histone proteins, the methylation of cytosine or chromatin remodelling proteins (Angaji et al. 2010). For instance, exposure of Arabidopsis ddm1 (Decrease in DNA Methylatation, a chromatin remodelling protein) to methyl methane sulphonate (MMS) and NaCl has resulted in chromatin structure alterations, thus substantiating their vital roles in abiotic stress adaptation (Yao et al. 2012). Further, enzymes participating in cytosine methylation are grouped into three distinct categories: Methyltransferase1 (MET1), Chromomethylase3 (CMT3) and Domains Rearranged Methylase (DRM). MET1 is a homologue of mammalian Dnmt1 [DNA (cytosine-5-)methyltransferase 1] and is predominantly involved in upholding symmetric (CpG) cytosine methylation. Plants defective for MET1 lacks wide spread CpG methylation (Lindroth et al. 2001). CMT3 is a plant-specific enzyme which methylates CpNpG sequence particularly at centromeric repeats and transposons (Lindroth et al. 2001; Tompa et al. 2002). These methyltransferases transmit the symmetric methylation imprints on the parental DNA (Chan et al. 2005). DRM includes two DNA methyltransferases, DRM1 and DRM2 which catalyse de novo methylation at asymmetrical cytosine methylation in CpNpN site (Ramsahoye et al. 2000; Gowher and Jeltsch 2002; Cao and Jacobsen 2002). Various reports have highlighted the functional redundancy of CMT3 and MET1 in methylating CpNpG sites (Cao et al. 2003). Similarly, Kato et al. (2003) reported activation of a normally silenced CACTA transposon in the met1 and cmt3 single and double mutants implying existence of a CMT3 functional redundancy for CpG and CpNpG methylation.

### Regulation of DNA methylation

DNA methylation status in plants is regulated by various physiological, developmental and stress stimuli. DNA and histone methylation are inter-reliant processes. The loss of CpG methylation in met1 results in the loss of H3K9 methylation (Soppe et al. 2002; Tariq et al. 2003); however, the loss of H3K9 methylation in kyp (Kryptonite) histone methyltransferase does not necessarily affect the CpG methylation (Jasencakova et al. 2003). This suggests that H3K9 methylation acts downstream of CpG methylation and reinforces heterochromatin establishment. Conversely, DNA methylation at CpNpG sites appears to be partially dependent on KYP activity (Jackson et al. 2002).

The overall status of DNA methylation is controlled by both DNA methyltransferase and DNA demethylation enzymes. Demethylation occurs in two ways, passive and active. The passive loss of DNA methylation may occur due to the inhibition of de novo methylation or inability to maintain the parental imprint after DNA replication (Kankel et al. 2003). Alternatively, active demethylation may occur via the glycosylase activity by removing the methylcytosines from DNA (Zhu et al. 2000, 2007; Agius et al. 2006; Morales-Ruiz et al. 2006). It may play a critical role in preventing the formation of stable hypermethylated epialleles in plant genome (Penterman et al. 2007).

Small RNAs also play an important role in epigenetic regulation in response to abiotic and biotic stress, growth and developmental signal via transcriptional gene silencing through RNA-directed DNA methylation (RdDM). In this mechanism, production of transcripts required for siRNA biogenesis is mediated through RNA Pol II and Pol IV by the RNA interference pathway. Initially, single-stranded RNAs produced by Pol IV-mediated transcription of methylated DNA are converted into double-stranded RNA (dsRNA) by the RNA-dependent RNA polymerase 2 (RDR2). On the other hand, Pol II targets inverted repeat regions for generation of dsRNAs. These dsRNAs are further processed by Dicer-like 3 (DCL3) followed by HUA enhancer1 (HEN1; which deposits methyl group at 2'-OH of the 3'-terminal nucleotide by a methyltransferase activity) and loaded onto Argonaut 4 (AGO4). This complex in turn interacts with the largest subunit of Pol V through WG/GW repeats at the C-terminal domain of the Nuclear RNA Polymerase (NRPE1; El-Shami et al. 2007). This intact machinery including siRNA and associated proteins is recruited on the homologous DNA sequence, thus facilitating the DNA methylation by DRM2. In addition, AGO4 also binds to specific gene promoters facilitated by the Pol V-derived long non-coding RNAs (lncRNAs). Recruitment of this complex guides CpNpN-type asymmetric DNA methylation in the promoter regions which in turn regulates target gene expression (Zheng et al. 2013).

### DNA methylation-mediated stress memory

The transgenerational inheritance of DNA methylation pattern in plants growing under stress conditions has been
recently reported (Hauser et al. 2011; Feng et al. 2012). This inherent epigenetic plasticity plays an important role in the organisms’ immediate response and establishment of long-term adaption under stress (Mirozue and Paszkowski 2011). Such phenomenon was observed in contrasting genotypes of rice (Oryza sativa) under salt and alkaline stress treatments, revealing the persistence of altered DNA methylation levels in the selfed progenies (Feng et al. 2012). Recently, contrasting observations have also been highlighted in response to short-term adaptation processes. For example, the progenies of stress-treated plants showed increased global hypermethylation even in the absence of stress, but these transgenerational effects did not persist in successive generations during the absence of stress. In Arabidopsis, the level of cytosine methylated DNA was measured in progenies of treated and untreated plants for two generations (Boyko et al. 2010). Higher 5mC levels were maintained in the progenies of treated plants in response to stressed as well as unstressed conditions, relative to the progenies of untreated plants of the same generation, suggesting that DNA methylation decreases during the absence of stress. Viral infection in tobacco and exposure of UV-C and flagellin in Arabidopsis have shown to stimulate the transgenerational inheritance of stress tolerance even to the untreated progeny via increased homologous recombination frequency and global genome methylation (Boyko et al. 2010).

Dynamics of DNA methylation during abiotic stresses

Apart from normal developmental processes and functions, plants have evolved complex gene regulatory mechanisms to cope up with various environmental stresses (Fig. 1). It was shown that DNA methylation, chromatin remodelling and small RNA-based mechanisms are also involved in regulating the gene expression in response to the stresses, including climatic adaptations (Sabbah et al. 1995; Grati-vol et al. 2012; Table 1). This was substantiated by a recent report on natural epigenetic variations observed in mangrove plants flourishing at the riverside and salt marsh neighbourhood habitat (Lira-Medeiros et al. 2010). They showed a contrasting morphological difference from the riverside plants that are much taller and thicker when compared with the salt marsh plants. Methylation-sensitive amplification polymorphism (MSAP) analysis revealed that DNA molecules of riverside plants were considerably hypermethylated when compared with those of salt marsh plants, suggesting a pivotal role of natural epigenetic variations in a plant population towards environmental adaptation. Similarly, genome-wide study by means of MSAP analysis performed in diverse rice genotypes differing in their salt-responsive characteristics highlighted differential methylation and expression of salt stress-related genes, retrotransposons and chromatin modifier genes (Karan et al. 2012). In tobacco cell-suspension culture, osmotic and salt stress-induced DNA hypermethylation at two heterochromatic loci, which was reversible when the cells were re-inoculated onto non-stress media (Kovarik et al. 1997). Genome-wide investigation of two contrasting rice lines revealed site-specific, reversible and recoverable variation in cytosines which was epigenetically regulated and associated with drought adaptation (Wang et al. 2011). Thus, natural genetic difference in abiotic stress tolerance could be self-regulatory in level and pattern of DNA methylation or accumulation during the natural selection process. Water deficit also led to specific cytosine hypermethylation (CCGG) in the pea genome (Labra et al. 2002). Similarly, epigenetic changes under water stress have also been identified in lowland rice cultivars and drought-tolerant rice cultivars (Suji and John 2010).

Other studies also highlight the involvement of epigenetic processes in modulation of N-deficiency stress in several plants. MSAP studies confirmed the occurrence of locus-specific methylation change in leaf tissues of N-deficient plants (Kou et al. 2011). Forest trees are highly adaptive to diverse growth environments and intense climatic changes (Correia et al. 2013). For the first time, epigenetic control of heat stress tolerance in forest trees was discovered when cork oak leaves showed interplay between specific DNA methylation and histone H3 acetylation for high-temperature adaptation (Correia et al. 2013). Global warming and nitrogen deposition-associated stresses may also alter DNA methylation and offer a molecular basis for stress adaptation in natural population as examined in Leymus chinensis Tzvel. for changes in cytosine methylation (Yu et al. 2013a). It was observed that during these stresses transposable elements were hypermethylated in comparison with other genomic regions.

In plants, various transposable elements are involved in environmental stress adaptation. A genome-wide demethylation study conducted in maize root tissues exposed to cold stress identified a fragment named ZmMI1. This fragment contains a partial putative protein coding sequence as well as part of a retrotransposon-like sequence, the latter remaining demethylated under cold stress (Steward et al. 2000). The cold-induced root-specific hypermethylation of Ac/Ds transposon region was caused by down-regulation of MET1 expression. In addition, Tam3, a transposon from Antirrhinum majus, showed alterations in DNA methylation status at CpNpN motifs during low-temperature stress (Hashida et al. 2006). In maize, a low-energy Nitrogen ion (N⁺) implantation was shown to reduce cytosine methylation in Mutator element MudR, thus increasing the expression of mudrA and mudrB (Qian et al. 2010). This directly indicates a direct plausible
association of environmental stress with transposon mobilization in plants.

Further, it is imperative to elucidate the roles of stress-responsive genes to correlate their differential expression patterns with epigenetic mechanisms. This would also provide some clues for the downstream actions that govern stress adaptation. About 49 transcription factors, differentially expressed during salinity stress in soybean, were examined for their levels of expression and DNA methylation (Song et al. 2012). Methylation and expression profiles of MYB, b-ZIP and AP2/DREB transcription factor gene families were significantly correlated. A mutual progression of epigenetic mechanisms along with action of transcription factors in regulating the abiotic stress responses has been reported in rice (Santos et al. 2011). A novel insight into gene regulatory mechanism was presented by an epigenetic modification study of a tomato protein-coding non-transposon ASR1 (Abscisic acid stress, ripening 1) epiallele, demonstrating its role in DNA methylation during water deficit stress (González et al. 2011). An interesting approach to identify epigenetically controlled probable gene sets in response to drought stress was performed by Shaik and Ramakrishna (2012), where they conducted a genome-wide methylation analysis of >5,000 drought-responsive genes (DRGs) in rice to map the methylated regions to their genic and promoter regions. Cluster analysis of these DRGs (with respect to epigenetic and microRNAs attributes) has shown the enrichment of up-regulated cluster for drought tolerance mechanisms, whereas the down-regulated cluster was associated with drought resistance processes. This signifies a novel approach towards understanding regulatory switches of stress response through combination of multiple bioinformatic resources and sorting subsets of genes involved in key modules. Baek et al. (2011) reported that the hypersensitivity of met1-3 to salt has resulted because of a massive failure in cytosine methylation at a putative small RNA target site. This subsequently led to lower expression of a sodium transporter gene (AtHKT1), which is essential for salt tolerance. Several other reports suggest that the changes in DNA demethylation are required for stress protection. Various stresses including aluminium, salt and cold were reported to induce demethylation of the Glycerophosphodiesterase-like protein (NtGPDL) gene, consequently resulting in its upregulation in tobacco leaves (Choi and Sano 2007). Key regulatory elements (core DNA binding motifs) of the mevalonate pathway genes and a defence-related gene varied significantly in their methylation pattern.

Fig. 1 A schematic representation of the events taking place in a plant cell during environmental stress imposition. Exogenous genome (such as viral genome) is also subjected to viral DNA methylation by plant epigenetic factors. CMT3 Chromomethylase, DRM domain rearrangement methyltransferase, MET1 methyltransferase.
amongst agroclimatically different rubber clones. This diversity in methylation pattern of cis-regulatory elements pointed out at the direct impact of stress on the *H. brasiliensis* genome (Uthup et al. 2011).

Plants can also acclimatize and modulate the physiological processes such as reproductive organ development and photosynthesis at the onset of stress. The epigenetic action in the regulation of flowering time under stressed conditions has also been well reported (Yaish et al. 2011). DNA methylation profiles of dandelion (*Taraxacum officinale*) apomictic clones (genetically identical) subjected to environmental stresses revealed that the fraction of altered loci in stressed groups was much higher than that of the control group and DNA methylation changes further closely extended to their progeny (Verhoeven et al. 2010). An increase in CpNpG methylation was observed in nuclear genome of *Mesembryanthemum crystallinum* plants during high salinity-stress imposition (Dyachenko et al. 2006). This increase in methylation was associated with the switching of C3-photosynthesis to CAM metabolism.

### Table 1 Summary of gene, transposons, and promoter fragments of genome differentially regulated through DNA methylation as an epigenetic reprogramming

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Genome region</th>
<th>Plant</th>
<th>Methylation status</th>
<th>Stress</th>
<th>Mode of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transposons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>TAM3</td>
<td><em>Antirrhinum majus</em></td>
<td>Hypomethylation</td>
<td>Low temperatures stress</td>
<td>Methylation at CHH motifs</td>
<td>Hashida et al. (2006)</td>
</tr>
<tr>
<td>2</td>
<td>MuDR</td>
<td>Maize</td>
<td>Hypomethylation</td>
<td>N+ implantation</td>
<td>Increases the expression of <em>mudrA</em> and <em>mudrB</em></td>
<td>Hashida et al. (2006)</td>
</tr>
<tr>
<td>3</td>
<td>Ac/Ds transposon</td>
<td>Maize</td>
<td>Demethylation</td>
<td>Cold stress</td>
<td>Cold-induced root-specific demethylation</td>
<td>Steward et al. (2000)</td>
</tr>
<tr>
<td><strong>Gene/coding segment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>ZmMII</td>
<td>Maize</td>
<td>Demethylation</td>
<td>Cold stress</td>
<td>Cold-induced root-specific demethylation</td>
<td>Steward et al. (2000)</td>
</tr>
<tr>
<td>2</td>
<td>Nuclear genome</td>
<td><em>Mesembryanthemum crystallinum</em></td>
<td>Hypermethylation</td>
<td>High salinity</td>
<td>CpNpG methylation</td>
<td>Dyachenko et al. (2006)</td>
</tr>
<tr>
<td>3</td>
<td>Sodium transporter gene (AtHKT1)</td>
<td><em>Arabidopsis</em></td>
<td>Hypermethylation</td>
<td>Salt tolerance</td>
<td>Loss in cytosine methylation in a putative small RNA target region</td>
<td>Baek et al. (2011)</td>
</tr>
<tr>
<td>4</td>
<td>Non-transposon Asr1</td>
<td>Tomato</td>
<td>Asymmetric CNN methylation</td>
<td>Water stress</td>
<td>Drought conditions brought about higher CG methylation levels in the first exon</td>
<td>González et al. (2011)</td>
</tr>
<tr>
<td>5</td>
<td>NtAlix1</td>
<td>Tobacco</td>
<td>Hypomethylation</td>
<td>Tobacco mosaic virus</td>
<td>Altered DNA methylation</td>
<td>Wada et al. (2004)</td>
</tr>
<tr>
<td><strong>Promotor</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Glyma11g02400</td>
<td>Soybean</td>
<td>Hypomethylation</td>
<td>Salinity stress</td>
<td>−518 to −274, most of the cytosines were demethylated following exposure to salinity stress for 1–24 h</td>
<td>Song et al. (2012)</td>
</tr>
<tr>
<td>2</td>
<td>Glyma16g27950</td>
<td>Soybean</td>
<td>Hypomethylation</td>
<td>Salinity stress</td>
<td>Hypomethylation at transcription start codon (+24 to +233)</td>
<td>Song et al. (2012)</td>
</tr>
<tr>
<td>3</td>
<td>Glyma20g30840</td>
<td>Soybean</td>
<td>Hypomethylation</td>
<td>Salinity stress</td>
<td>Hypomethylated cytosines at promoter region 1 (−87 to +163)</td>
<td>Song et al. (2012)</td>
</tr>
<tr>
<td>4</td>
<td>RMG1 promoter</td>
<td><em>Arabidopsis</em></td>
<td>Demethylation</td>
<td>Pseudomonas syringae</td>
<td>RMG1 is targeted by RdDM and ROS1-Dependent DNA demethylation</td>
<td>Yu et al. (2013b)</td>
</tr>
</tbody>
</table>
some studies also have shown that ABA controls histone modification, which in turn can regulate DNA methylation (Chinnusamy et al. 2008; Yaish et al. 2011).

**Dynamics of DNA methylation during biotic stresses**

Upon exposure to biotic stresses, plants’ immune system, such as recognition of pathogen-associated molecular patterns (PAMPs) and the basal defence machinery, gets activated as a process of defence priming (Muthamilarasan and Prasad 2013). Plants have evolved the DNA methylation approach as a major defence strategy against biotic stress. A study on progenies obtained from diseased *Arabidopsis* resulted in enhanced resistance towards biotrophs. This mode of action, termed as transgenerational systemic stress. A study on progenies obtained from diseased *Arabidopsis* revealed that met1, a gene named *Elongator complex subunit2* (ELP2) regulated the DNA methylation in *Arabidopsis* antibacterial defence has been reported (Dowen et al. 2012; Yu et al. 2013b). *Pseudomonas syringae pv. tomato* DC3000 infection in *Arabidopsis* revealed that met1 and ddc were unable to develop the typical infection symptoms and showed the resistant phenotype because of their depleted cytosine methylation capability. Moreover, several pathogen-responsive genes were differentially regulated in these mutants, signifying the role of DNA methylation in establishing plant defence against bacterial pathogen (Dowen et al. 2012). A gene named *Elongator complex subunit 2* (ELP2) regulated the genomic DNA methylation pattern in *Arabidopsis*, and subsequently stimulated the pathogen-induced DNA methylation alterations (Wang et al. 2013). Hypomethylation of DNA during pathogen infection has been shown to influence the defence-related gene expression. The rice R gene *Xa21G*, which was demethylated chemically inherited resistance to *Xanthomonas oryzae pv. oryzae* (Akimoto et al. 2007). Another interesting study in the course of crown gall tumour development in *Arabidopsis* demonstrated that ABA-dependent drought stress defence regulates crown gall tumour formation is controlled by DNA methylation (Gohlike et al. 2013).

Advancements in epigenetic research have enabled researchers to unravel the molecular events in virus-infected plants. Plants systematically utilize the siRNA-mediated methylation strategy as defence mechanism towards various viruses, by methylating various viral genomic components such as intergenic and transcribed region (Bian et al. 2006; Tougou et al. 2007; Yadav and Chattopadhyay 2011; Emran et al. 2012; Sharma et al. 2012). Rodríguez-Negrete et al. (2009) correlated the symptoms of recovery after viral infection with a higher proportion of viral DNA methylation. Another study in soybean resistant to *Mungbean yellow mosaic India virus* (MYMIV) showed a higher level of Intergenic Region (IR)-specific DNA methylation (Yadav and Chattopadhyay 2011). Similarly, methylation of cytosine in the IR of *Tomato leaf curl virus* has also been reported in transgenic tobacco (Bian et al. 2006). Recently, we have evaluated the dynamics of defence-related components in *Tomato leaf curl New Delhi virus* (ToLCNDV) tolerant tomato cultivar (Sahu et al. 2010, 2012). We observed a heavy methylation at IR and part of replication-associated protein (rep) gene in ToLCNDV tolerant cultivar. Further, differential expression patterns of some key methyltransferase genes were detected showing that methylation in key regulatory regions reduces the expression of various ToLCNDV replication-related genes, thus providing tolerance against the virus infection (unpublished data).

Correlation between transcript abundance of defense-related genes and hypomethylation of genomic DNA in plants has been reported (Wada et al. 2004). The transcript induction and genomic methylation in tobacco plants infected with *Tobacco mosaic virus* (TMV) was assayed in order to elucidate the expression of a pathogen-responsive gene *NtAlix1* and correlate it with the DNA methylation status. At 24 h, the level of *NtAlix1* transcripts was found to be higher and changes in the methylation were also detected. This suggested that during pathogen infestation plants regulate the expression of the defense-related components by the action of DNA methylation. The virus-induced gene silencing which relies on generation of double stranded transcripts can be developed as an effective tool for introducing DNA methylation in any endogenous gene. *Cucumber mosaic virus* (CMV)-based gene silencing system has been demonstrated as an alternate approach for epigenetic switching in endogenous gene of plant, thus altering DNA methylation artificially (Kanazawa et al. 2011). Thus, epigenetic processes may prime a defence mechanism which allows plants to safeguard their offspring against repetitive biotic stresses without stable inherent trait fixation. An enhanced knowledge on this phenomenon will generate possibilities to moderate disease susceptibility in plants.

**Conclusions and future directions**

Though numerous reports substantiating the DNA methylation-mediated responses against biotic and abiotic stresses have been published, many unanswered questions including how plants sense the stresses, activate adaptive mechanisms and action of various components in methylation pathways remains elusive. Mutant analyses of regulatory components involved in methylation/demethylation pathways have provided insights into the mechanism part of these epigenetic processes, but a comprehensive investigation is
required for better understanding of these processes. With small size of genome and simple genetics of model plant Arabidopsis thaliana, it is much easier to study DNA methylation in this system. Understanding epigenetic mechanisms in response to environmental stress and its probable application in the genetic manipulation of plants is a major challenge for plant biologists. In this regard, available data sets of various plant methylomes can be used to select the differential epigenetic regions as probable targets for genetic manipulation. Transposons stimulate the regulation of neighbouring genes during distinct environmental stimuli, and their precise induction by mutating specific epigenetic regulatory pathways is still at the inventive phase. This may possibly become a motivational beginning to identify novel regulatory gene networks. Epialleles generated in the numerous studies can be exploited by different plant breeding programs. Initial steps involve the determination of methylation patterns between individuals in the selected population followed by examination of methylation patterns which significantly stimulate phenotypes. The final step could be the association of stably inherited superior phenotypes with the pattern of methylation in variants. Gene manipulation for stress-resistant/tolerant transgenic plant generation via DNA methylation pathway initially needs a proper selection of gene. For example, the differentially expressed genes identified from various techniques (such as subtraction library, MSAP/AFLP, microarray, etc.) can be subjected to promoter analysis. Evaluating promoter sequence of the target gene for RdDM-associated features can be done without any complexity through starPRO database (a 24nt siRNA-centered database). Further, artificially synthesized siRNA from the targeted parts can be introduced in the plants for stress tolerance. As host methylation machinery also plays a major part to direct the DNA methylation, overexpression or gene silencing approaches of methyltransferases and other key factors (such as host siRNA biogenesis components) can be combined with the siRNA generation, although these assumptions require further validation.

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